CHRONIC PHYSIOLOGICAL, GROWTH, AND PRODUCTIVITY EFFECTS OF PHOTOCHEMICAL OXIDANTS OR SO_2 ON TREES: (CITRUS SINENSIS)

Interim Report

to the

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ABSTRACT

The Statewide Air Pollution Research Center has a continuing mission to investigate the effects of air pollutants on agricultural crops, and to determine the amount of losses caused by these pollutants. To further this mission we continued and built upon the project to investigate the chronic physiological, growth, and productivity effects of ambient photochemical oxidants [(measured as ozone (O_3)] or sulfur dioxide (SO_2) on Valencia orange trees (Citrus sinensis). The exposures were initiated in May, 1984, with four chamber treatments: filtered air, filtered air plus 0.10 ppm SO_2 continuously, half-filtered and half-ambient air, ambient air. Outside control trees were used to determine chamber effects. There were seven trees per treatment. Tree response to air pollutants was documented in terms of fruit yield and quality, leaf physiology and biochemistry, and leaf biomass production per tree.

Ozone resulted in statistically significant reductions in fruit yield and alterations in fruit quality. Yield was reduced by 27% in ambient air, and by 10% in half-ambient air compared to filtered air. The reduction in yield with the three O_3 treatments (filtered, half-filtered, ambient air) fit a linear O_3 concentration vs. fruit weight per tree equation: yield = 69.997 - (O_3 concentration x 300.5). The O_3 concentration was the seven month (April-October) growing season average for 1986. Sulfur dioxide reduced yields by 35% compared to filtered air. The reduced yields with both O_3 and SO_2 were due primarily to reduced fruit number and not reduced fruit weight. Fruit quality effects due to SO_2 were reduced orange color, increased fruit circumference, and increased rind thickness. The chambers themselves did not affect yield, but did result in altered fruit quality based on comparisons between ambient chamber and outside trees.

Sulfur dioxide resulted in reduced leaf drop on occasional monthly as well as seasonal and yearly basis. Neither ozone nor the chambers effected leaf drop, and no treatment had any effect on individual leaf weights or fruit drop. In terms of physiology, ozone reduced stomatal conductance during summer months and on a whole year basis. Trees in ambient chambers tended to show indications of greater water stress than outside trees. This was indicated by the lower stomatal conductances for

ambient chamber trees during during summer months and on a whole year basis, and by the more negative leaf water potentials for nine dates, and on winter, summer, and a whole year basis. Sulfur dioxide did not affect physiology and no treatment affected photosynthesis. The primary biochemical effects were a trend toward higher chlorophyll content, and lower specific leaf area for ambient chamber compared to outside trees.

While the results collected to date clearly document the effects of air pollutants on Valencia oranges, additional research is needed to determine the impact of the chambers themselves on tree response and the mechanistic bases for the O_3 and SO_2 effects.

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DISCLAIMER

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SUMMARY AND CONCLUSIONS

California is a major United States producer of citrus and other tree fruit crops. Oranges are grown on about 175,000 acres of some of the State's most productive land. Counties with major production are Tulare, Fresno, Kern, Ventura, and San Diego counties with lesser amounts in adjoining areas. Total annual production is in excess of 2.0 million tons valued at $524 million (1985). Valencia oranges for juice account for approximately 50% of the volume produced. Most of the areas of production presently have photochemical oxidant (primarily O_3), and some areas have sulfur dioxide (SO_2) pollution which may be reducing yields. However, no studies have been available which indicate the amount of economic losses.

During the late 1950's and 1960's, Taylor, Thompson and co-workers studied the chronic (low level, long term) effects of photochemical oxidants which occur in the Los Angeles basin and/or fluoride on navel oranges and lemons. 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 of one-third to one-half of total production were recorded even though no easily observed injury occurred on the trees.

However, the sensitivity of the 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 the fact that there were only filtered and ambient air treatments, and little accurate air monitoring data from the exposed sites. Thus, it is not possible to produce accurate dose-response models to describe the relationship between O_3 dose and orange yield. Such models are necessary for interpreting current O_3 and orange yield data for different counties as part of the ARB Crop Loss Assessment Project. Furthermore, the susceptibility of oranges to long-term low level "chronic" exposure to SO_2 was not known.

Thus, to address the effects of air pollutants on citrus, the Air Resources board funded a study in early 1983 to investigate physiological, growth, and yield responses of Valencia orange trees (*Citrus sinensis*) to ambient ozone (O_3) or added sulfur dioxide (SO_2).

Since May, 1983, the chambers have been used with four 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, ambient air to represent conditions in southern California and filtered air plus 0.10 ppm sulfur dioxide continuously to represent potential conditions in the vicinity of industrial point sources. In addition, outside "control" trees were used for determination of the effect of the chamber itself on responses to air pollutants. Results obtained to date include two years of harvest, tree canopy growth, and fruit and flower part drop data; monthly leaf and fruit drop data for three years; weekly to biweekly stomatal conductance data for over three years; and biweekly to monthly photosynthetic and water potential data for over two years.

Specific research questions to be addressed in the 1986-87 portion of this project were:

(1) What are the effects of photochemical oxidants on oranges based on current levels found in the Sacramento 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 the citrus-producing areas of California?

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

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

(5) What parameters best indicate the physiological basis for injury to orange trees from air pollutants? There were seven trees per treatment. Tree response to air pollutants was documented in terms of fruit yield and quality, leaf physiology and biochemistry, and leaf biomass production per tree.

Ozone resulted in statistically significant reductions in fruit yield and alterations in fruit quality. The reduction in yield with the three O_3 treatments (filtered, half-filtered, ambient air) fit a linear O_3 concentration vs. fruit weight per tree equation: yield = 69.997 - (O_3 concentration x 300.5). The O_3 concentration was the seven month (April-October) growing season average for 1986. Sulfur dioxide reduced yields by 35% compared to filtered air. The reduced yields with both O_3 and SO_2

were due primarily to reduced fruit number and not reduced fruit weight. Fruit quality effects due to SO_2 were reduced orange color, increased fruit circumference, and increased rind thickness. The chambers themselves did not affect yield, but did result in altered fruit quality based on comparisons between ambient chamber and outside trees.

The changes in fruit quality due to air pollutants could have important commercial implications depending on complex relationships between the overall amount and quality of oranges available in a particular season. For example larger oranges could bear a higher price if smaller oranges predominated in the market.

Sulfur dioxide resulted in reduced leaf drop on occasional monthly as well as seasonal and yearly basis. Neither ozone nor the chambers effected leaf drop, and no treatment had any affect on individual leaf weights or fruit drop. In terms of physiology, ozone reduced stomatal conductance during summer months and on a whole year basis. Trees in ambient chambers tended to show indications of greater water stress than outside trees. This was indicated by the lower stomatal conductances for ambient chamber trees during during summer months and on a whole year basis, and by the more negative leaf water potentials for nine dates, and on winter, summer, and a whole year basis. Sulfur dioxide did not affect physiology and no treatment affected photosynthesis. The primary biochemical effects were a trend toward higher chlorophyll content, and lower specific leaf area for ambient chamber compared to outside trees.

While the results collected to date clearly document the effects of air pollutants on Valencia oranges, additional research is needed to determine the impact of the chambers themselves on tree response and the mechanistic bases for the O_3 and SO_2 effects.

Conclusions. All conclusions should be considered to be preliminary as one more year of data will be collected. However, a number of conclusions appear to be likely at this stage in the studies:

(1) Ozone results in a reduction in yield which can be defined by a linear ozone concentration-yield loss equation.

(2) Sulfur dioxide results in a reduction in yield.

(3) The reductions in yield with air pollutants are associated primarily with reduced numbers of fruit, with reduced fruit size playing a minor role.

(4) There appear to be some alterations in fruit quality with pollutant treatments which may be related to the reduced number of fruit.

(5) Physiological and biochemical affects primarily occur between ambient chamber and outside trees. However, there is no definite trend indicating consistent differences in pollutant sensitivity between these two treatments. The greater water stress for ambient chamber trees suggests less pollutant sensitivity than for outside trees. In contrast, the greater chlorophyll content and specific area indicate more succulent leaves with greater capacity to take up pollutants for chamber trees, and, hence, than for outside trees.

(6) The only consistent pollutant effects on physiology, biochemistry, and growth were lower stomatal conductance for ozone exposed trees and lower leaf drop for sulfur dioxide exposed trees.

(7) Future studies should focus on obtaining a third and final year of harvest data, and more physiological measurements to verify the ozone effect on stomatal conductance and chamber vs. outside differences in water relations. Additional research should be conducted at some point on the effects of ozone on individual leaf longevity and fruit retention.

RECOMMENDATIONS

(1) Obtain a third year of harvest data in 1988 for the three oxidant treatments and outside trees. This additional year will increase the available replications to determine significant ozone and chamber effects, and will provide valuable data for evaluating different statistical designs.

(2) Obtain additional physiological measurements focussing on the effects of ozone on stomatal conductance and the chamber effects in terms of leaf water stress.

(3) Obtain additional information on the effects of ozone on leaf drop. If possible, this could include specific measurements to determine the length of time leaves and fruit stay on the trees.

(4) Additional biochemical work to establish the metabolic basis for the reduction in yield due to ozone exposure. This should focus on starch reserves before flowering.

I. INTRODUCTION

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

California is a major United States producer of citrus and other tree fruit crops. Oranges are grown on about 175,000 acres of some of the State's most productive land (1). Counties with major production are Tulare, Fresno, Kern, Ventura, and San Diego counties with lesser amounts in adjoining areas. Total annual production is in excess of 2.0 million tons valued at $524 million (1985). Valencia oranges for juice account for approximately 50% of the volume produced. Most of the areas of production presently have photochemical oxidant (primarily O_3), and some areas have sulfur dioxide (SO_2) pollution which may be reducing yields. However, no studies have been available which indicate the amount of economic losses.

During the late 1950's and early 1960's, Taylor, Thompson and co-workers (19,20,21,22) studied the chronic (low level, long term) effects of photochemical oxidants which occur in the Los Angeles basin and/or fluoride on navel oranges and lemons. 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 of total production were recorded even though no easily observed injury occurred on the trees.

However, the sensitivity of the 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 the fact that there were only filtered and ambient air treatments, and little accurate air monitoring data from the exposed sites. Thus, it is not possible to produce accurate dose-response models to describe the relationship between O_3 dose and orange yield. Such models are necessary for interpreting current O_3 and orange yield data for different counties as part of the ARB Crop Loss Assessment Project.

Furthermore, the susceptibility of oranges to long-term low level "chronic" exposure to SO_2 was not known. Thomas (23) 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 found 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 2 hrs/day for 40 days caused no foliar injury. Later work showed Satsuma orange (Citrus unshiu) to have accelerated leaf drop after exposure with 5 ppm SO_2 for 2 hrs/day for 34 days. After spraying with Bordeaux mixture, leaf drop was accelerated in 13 days of exposure with SO_2. These studies also were done in closed greenhouses.

Thus, to address the effects of air pollutants on citrus, the Air Resources Board funded a study in early 1983 to investigate physiological, growth, and yield responses of Valencia orange trees (Citrus sinensis) to ambient ozone (O_3) or added sulfur dioxide (SO_2).

B. Valencia Orange Results to Date

A unique design open-top field chamber was developed, tested, and constructed for use with the orange trees (8). The primary component of the chamber, a vinyl plastic dome, recently had been developed for use in controlling air temperature over spas during the winter. The dome has many advantages over previous designs of open-top field chambers developed for herbaceous crops. This dome had characteristics which indicate its potential usefulness in constructing an exposure chamber for young trees especially: size, clarity, and light transmission characteristics of the vinyl plastic, sectional construction for ease of transportation and construction, commercial accessibility, and relatively low cost (7).

Since May, 1983, the chambers have been used with four 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, ambient air to represent conditions in southern California, and filtered air plus 0.10 ppm sulfur dioxide continuously to represent potential conditions in the vicinity of industrial point sources. In addition, outside "control" trees were used for determination of the effect of the chamber itself on responses to air pollutants. Results obtained to date include two years of harvest, tree canopy growth, and fruit and flower part drop data; monthly leaf and fruit drop data for three years; weekly to biweekly stomatal conductance data for over three years; and biweekly to monthly photosynthetic and water potential data for over two years (16).

Preliminary conclusions were based on preliminary statistical analysis, and may change based on the three years of data:

(1) Effects of O_3 on fruit yield in 1986. The difference was primarily between filtered and ambient trees based on analysis of variance, but when the analysis was based on a linear regression the previous growing season O_3 data (12 hour April-October growing season average) was correlated with yield reductions the following spring.

(2) The reductions in yield were associated primarily with reduced numbers of fruit and not directly altered on fruit size. Ozone also had some effects on fruit quality.

(3) Sulfur dioxide produced a reduction in yield which was associated primarily with reduced fruit size.

(4) Outside trees were behind chamber trees in terms of first year of significant bearing.

(5) Both the O_3 and SO_2 air pollutant treatments resulted in greater leaf drop than for filtered air trees in the fall-winter months of October - December, and less leaf drop in the spring, e.g., April.

(6) There were no consistent O_3 or SO_2 treatment effects on leaf physiology (photosynthetic rates, stomatal conductance, or water potential) within the measurement protocol for this study, i.e., weekly to monthly measurements for a limited number of leaves.

C. Statement of the Problem

Earlier field research indicated that citrus trees can suffer 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 actual outside trees. Only navel oranges were tested and not Valencia oranges. Furthermore, the studies used only the high oxidant exposure conditions of the Los Angeles Basin to determine tree response. There were no oxidant exposures representative of potential oxidant levels in the important orange producing areas in the San Joaquin Valley. Thus, there was an important need to carefully investigate the effects of oxidants on Valencia oranges using the best available current exposure and response measurement technology.

An investigation of the effects of oxidants was initiated in 1983. However, the two-year duration was not adequate for determination of all important tree responses to air pollution. The orange trees required two years after planting for the normal pattern of fruit set to occur. The first year of yield data indicated effects from ambient O_3 on yield. At least an additional year of study was required; not only to obtain additional growth and yield data, but also physiological and biochemical data to investigate the mechanistic bases for the pollution effects.

D. Objectives

Specific research questions to be addressed were: (1) What are the effects of photochemical oxidants on oranges based on current levels found in the Sacramento 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 the citrus-producing areas of California? (3) Are Valencia oranges as sensitive to oxidants (ozone) as navel oranges were in the previous work? (4) What growth parameters are most useful in indicating the effects of air pollutants on oranges? (5) What parameters best indicate the physiological basis for injury to orange trees from air pollutants?

II. METHODS

A. Pollutant Exposure

The pollutant exposures continued from April of 1984. The SO_2 and ozone analyzers were calibrated approximately quarterly using standard ARB procedures. The pollutant exposure system for SO_2 consisted of a temperature controlled liquid SO_2 tank, heatless air drier, mass flow controller, sample lines, scanning valve, and ThermoElectron Company Model 43 SO_2 analyzer. The exposure system for ozone consisted of sample lines, scanning valve, and Bendix ozone analyzer. The ozone treatments of filtered air, half-filtered air, and ambient air were achieved, respectively, by totally, partially, and not filtering the air entering the chambers. Electronic signals from both SO_2 and ozone 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.

The SO_2 treatments were terminated at the end of October, 1987, following consultation with ARB staff. The filtered, half ambient, ambient, and outside treatments are continuing and have been included in a proposed extension of this project.

B. Tree Culture

Valencia orange trees (*Citrus sinensis*) were used, which were growing on the experimental site at U.C. Riverside. The trees were planted during July of 1983, and have had experimental treatments since May 22, 1984. The trees were grafted on Troyer Citrans rootstocks. The trees were selected by the grower based on similar stem diameters of 0.032 m. Stem diameter is a useful covariate to account for some variability in statistical analysis of citrus growth and yield data (personal communication, Carol Adams, University of California Cooperative Extension).

The trees received a foliar spray with zinc when major flushes of growth had just expanded, possibly twice a year. The trees also received irrigation with a nutrient solution, 57 g nitrogen as urea, applied over six 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. The Irrometers® were checked periodically and recorded weekly as an indicator of water use by the trees. The trees were irrigated if both Irrometers® read over 50 centibars. All other management practices were as normally prescribed for Valencia oranges based on specific cultural practices, e.g., pesticide sprays, pruning, etc.

There was a fence around two sides of the site to provide extra security for the chambers. The north and east side 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. 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.

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.

Leaf Temperature. Leaf temperature was measured continuously using fine wire thermocouples attached to the undersides of leaves with surgical tape. Leaf temperature was measured for six trees; four in chambers and two for outside trees. The thermocouples were read by the Apple®-ISAAC® data acquisition system. Data were reported as the mean of two outside or one ambient chamber measurements.

Air Temperature. Air temperature was measured continuously using fine wire thermocouples. The thermocouples were shielded from the sun within tubes which are equipped with fans to draw air over the thermocouple junction. Air temperature also was measured for nine trees; seven in chambers and two outside using thermocouples read by the Apple®-ISAAC® data acquisition system. Data were reported as the mean of two outside or one ambient chamber measurements.

Relative Humidity. Relative humidity was measured by dewpoint sensors located in the air stream coming 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 two outside or two ambient chamber measurements.

D. Yield and Quality Measurements

Yield and quality parameters were approximately the same as those used in earlier citrus studies. They focused on economic yield.

Yield. Yield was determined as weight and number of marketable ripe fruit and immature unmarketable fruit per tree. The marketable fruit was picked over an approximate one-day harvest in May of 1986. The unmarketable fruit was determined as immature or ripe fruit falling from, or picked from, the trees out of season.

The fruit on the SO_2 trees will be picked in late November, 1987, and counted and weighed. The trees will then be cut down and removed from the chambers. These results will be included in the Final Interim Report.

Quality. Fruit quality was measured in terms of size, rind thickness, rind color, individual weight, sugar concentration, and acidity. Size was measured as the average of three circumferences per individual fruit. 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 insure uniformity in color evaluations all ratings were done by the same individual as in 1986 under similar light conditions. Sugar concentration was measured with a refractometer, and acid concentration was measured by titratration with sodium hydroxide.

E. Growth Measurements

Growth was determined by monthly measurement of leaf drop, and as flower and small fruit drop in the spring.

Leaf Drop. Leaf drop, a sensitive indicator of tree stress, was measured monthly over the year. 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 10th 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 30 leaves were determined. The 30-leaf weight was an indicator of the relative size of individual leaves per treatment.

Fruit and Flower Drop. All small fruit and flower parts dropped from the trees were retained and measured. The fruit and flower parts were swept into paper bags, cleaned, and weighed. The data were used as an indicator of the effects of the different treatments on flower production, fruit set, and premature fruit drop.

Larger fruit dropping from the trees were collected monthly. These indicated unseasonable loss of fruit which could have contributed to the final yield. The fruit was summed across May, 1986 - April, 1986 to represent extra fruit drop relevant to the 1987 harvest. The fruit also were summed across June 1 1987 - August, 1987, to represent drop relevant to the 1988 harvest.

F. Physiological Measurements

Physiological measurements were made to: (a) assess the responses of the three 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.

Physiologic 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 net photosynthesis were measured using the Lambda Instruments Company LI-6000 portable gas exchange unit. This is a closed system which detects water vapor in the air with a capacitance sensor and carbon dioxide in the air with an infrared analyzer. The system is computer controlled for calculation of gas exchange rates and processing of data. A LI-1600 steady state porometer was used on a few dates when the LI-6000 was not available, resulting in collection of only stomatal conductance data on those dates. Water potential, an indicator of tree stress, was measured with a pressure bomb at approximately the same time as the gas exchange measurements.

G. Biochemical Measurements

A number of biochemical parameters were measured to evaluate their potential as indicators of air pollutant stress in citrus. Chronic gaseous pollutant exposures have been reported to have both beneficial and detrimental effects on plant physiology. In the absence of significant amounts of leaf injury, it is important to determine what changes in leaf physiology precede or are concomitant with plant development in polluted environments. Presently, little is known with respect to whether chronic exposures can alter tree responses in the field, and if enclosing trees in chambers with open tops alters responses to gaseous pollutants. The objectives of the biochemistry measurements were to determine selected metabolite levels in Valencia orange leaves exposed to the different treatments. Methods used were as follows:

1. Leaf Collection

Leaves were collected seasonally in October 1986, and January, April and July, 1987. Fifty dark green, healthy, leaves were selected per tree for all measurements. The leaves were frozen immediately in liquid nitrogen. Samples were stored on dry ice until all plots were sampled. This material was used in all subsequent experimental protocols.

2. Chlorophyll and Carotenoid Assay

Four leaves 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 (8). Absorbences at 663, 646, and 470 nm were measured with a Beckman DB spectrophotometer.

3. Sulfite Assay

Three leaves per tree of the SO_2 and filtered treatments were homogenized in 0.1 m sodium tetrachloromercurate at 4°C. Sulfite concentrations were determined by measuring the formation of the pararosaniline-sulfite complex at 560 nm with a Beckman DB spectrophotometer (19).

4. Thiol Assay

Three leaves 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 paranitrothiobenzoic acid at 412 nm with a Beckman DB spectrophotometer (3).

5. Specific Leaf Area

Twenty leaves per tree of all treatments were dried to constant weight at 80°C, and discs weighed (Ainsworth Type 10 N Balance).

Leaves from the SO_2 and filtered chamber trees may be collected in mid-November, 1987, and sent out for total sulfur content analysis of feasible.

H. Statistical Analysis

Statistical analysis for all data sets was according to procedures described by Snedecor and Cochran (10) and Steel and Torrie (11). The basic design for statistical analysis was analysis of variance ANOVA with five treatments and seven observations (trees) per treatment.

For most parameters measured only once a completely randomized design was used with contrasts for treatment effects addressed as follows:

Source	df
Treatments	4
Linear Ozone (filt., half, amb.)	(1)
Quadratic Ozone	(1)
Filtered vs. SO_2	(1)
Ambient vs. Outside	(1)
Error	25
Total	29

If more than one leaf was sampled per tree a sampling error was added for that parameter.

The linear and quadratic terms from the analysis of variance gave some indication of the ozone treatment effect. However, regression analysis of ozone concentrations vs. yield was necessary to determine the quantitive relationship between response and ozone dose. The ozone concentrations corresponding to filtered air, half-ambient and half-filtered air, and ambient air were 0.01, 0.039, and 0.07 ppm respectively using the data described in Section III.A. below.

Physiological data for the dates of October 1986 through September 1987 included in this report were pooled to determine whether significant O_3 or SO_2 treatment affects could be determined over a long period of time. The data was further subdivided into winter (October-March) and summer (April-September) periods for analysis. This analysis was preliminary; further multi-year and seasonal analysis will be made for the final report in 1988.

Pooling of the data for multiple measurements was achieved through use of a multifactorial analysis of variance. The experimental design built upon the design for single measurements with the addition of factors for replicate trees, day of measurement, and day x air treatment interactions as shown below for a full year of photosynthesis measurements (19 days):

Source	df
Treatments	
Linear Ozone	4
Quadratic Ozone	(1)
Filtered vs. SO_2	(1)
Ambient vs. Outside	(1)
Replicate Tree	6
Error a	24
Day	18
Day x Treatment	72
Error b	540
Total	664

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. The numbers of days available were as follows on a whole year, summer, and winter basis, respectively: stomatal conductance-20, 12, 8; photosynthesis- 19, 12, 7; water potential- 19, 11, 8; leaf drop and weight- 10, 5, 5; and fruit drop- not analyzed, 7, 3. A replicate factor for tree was possible as multiple measurements per tree were available, i.e. one per month.

For the biochemical measurements the outside tree and ambient chamber, or filtered and SO_2 chamber treatments, were compared with a one-tailed, non-paired t-test. The three oxidant (ambient, half ambient, filtered) treatments were compared by regression analysis, for most measurements, except for a one-way analysis of variance for the pilot biochemical studies.

III. RESULTS AND DISCUSSION

A. Pollutant Treatments

Pollutant concentrations for the different treatments are shown in Table 1. The half-ambient and filtered treatments were successful at providing O_3 treatments that were near the targets of 50% and and 10% of the ambient chamber concentration: i.e., half-ambient and filtered trees had growing season O_3 averages 55.7 and 14.3% of ambient chamber trees, respectively. Weekly variation in the 12-hour O_3 and SO_2 concentrations over the past year is shown in Figures 1-3. The annual averages for O_3 were slightly higher for 1986-87 compared to 1985-86 even though the ambient O_3 concentrations were the lowest than in many years in early summer at Riverside. The averages for 1985-86 were 0.053, 0.049, 0.026, and 0.006 for the outside, ambient chamber, half-ambient chamber, and filtered treatments, respectively. The annual average for SO_2 in 1985-86 was 0.093 ppm.

B. Environmental Measurements

Quantum Intensity. Quantum (light) intensity varied considerably over the growing season based on weekly averages between 0800 and 2000 (Figures 4-6). Values for outside trees ranged from 362 to 1084 $\mu mol\ m^{-2}\ s^{-1}$ on a weekly basis. Quantum intensity averaged 20% lower for for ambient chamber compared to outside trees. This decrease was largely due to shading of the sensor by the canopy and not due to the chamber dome.

Leaf Temperature. Daily leaf temperature ranged between 16.8 and 32.7°C for outside trees between 0800 and 2000 (Figures 7-9). Leaf temperatures averaged 1.8°C higher in ambient chambers compared to outside trees over the entire period.

Air Temperature. Daily air temperature ranged between 17.0 and 33.2° for outside trees (Figures 10-12). Air temperatures averaged 1.2°C higher for ambient chamber compared to outside trees over the entire period.

Relative Humidity. Relative humidity ranged between 12 and 79% for outside trees (Figures 13-15). Relative humidity averaged 2.5% lower for ambient chamber compared to outside trees over the entire period. The decrease in relative humidity was proportional to the increase in air temperature for chamber vs. outside trees.

Table 1. Ozone and Sulfur Dioxide Treatments for Valencia Orange Study (In ppm)[a]

Treatment	Pollutant	Growing Season Average 4/4/86-10/31/86	Annual Avg. 8/9/86-8/7/87
Outside	O_3	0.076 ± 0.025	0.054 ± 0.029
Ambient Ch.	O_3	0.070 ± 0.022	0.051 ± 0.027
Half-Ambient Ch	O_3	0.039 ± 0.014	0.027 ± 0.014
Filtered Ch.	O_3	0.010 ± 0.003	0.008 ± 0.004
Filtered + SO_2 CH.	SO_2	0.098 ± 0.014	0.101 ± 0.010

[a]Values are means ± SD for all weeks over the growing season (n=28), or annual period (n=53). Data is for 0800-2000 PST. The SO_2 concentration in the non-SO_2 chambers and for outside trees was approximately zero. The O_3 concentration in the SO_2 treatment was assumed to be the same as in the filtered chambers.

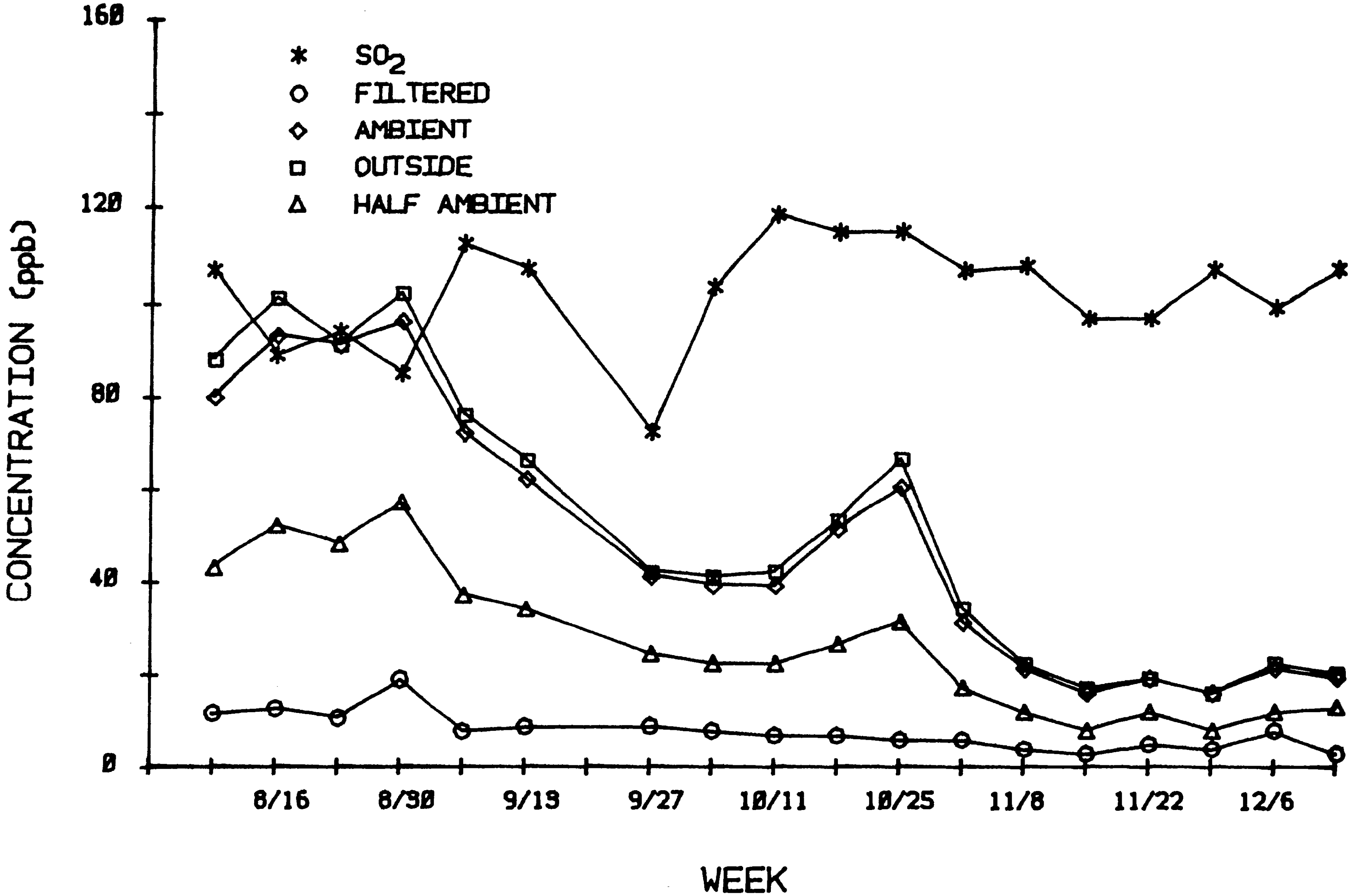

Figure 1. Air pollution concentrations for Valencia orange treatments for 8/3-12/13/86. Values are weekly means.

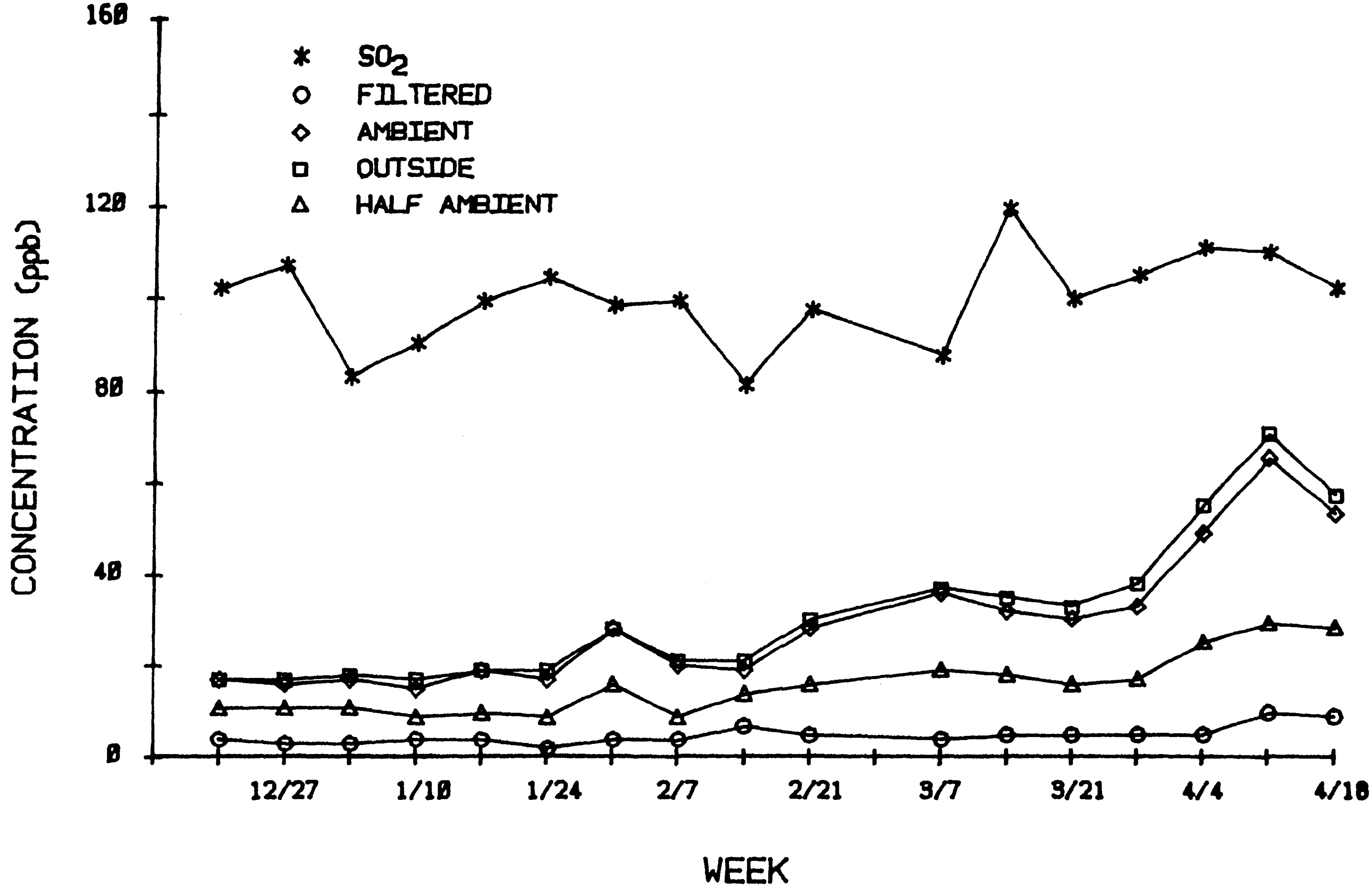

Figure 2. Air pollution concentrations for Valencia orange treatments for 12/14/86-4/18/87. Values are weekly means.

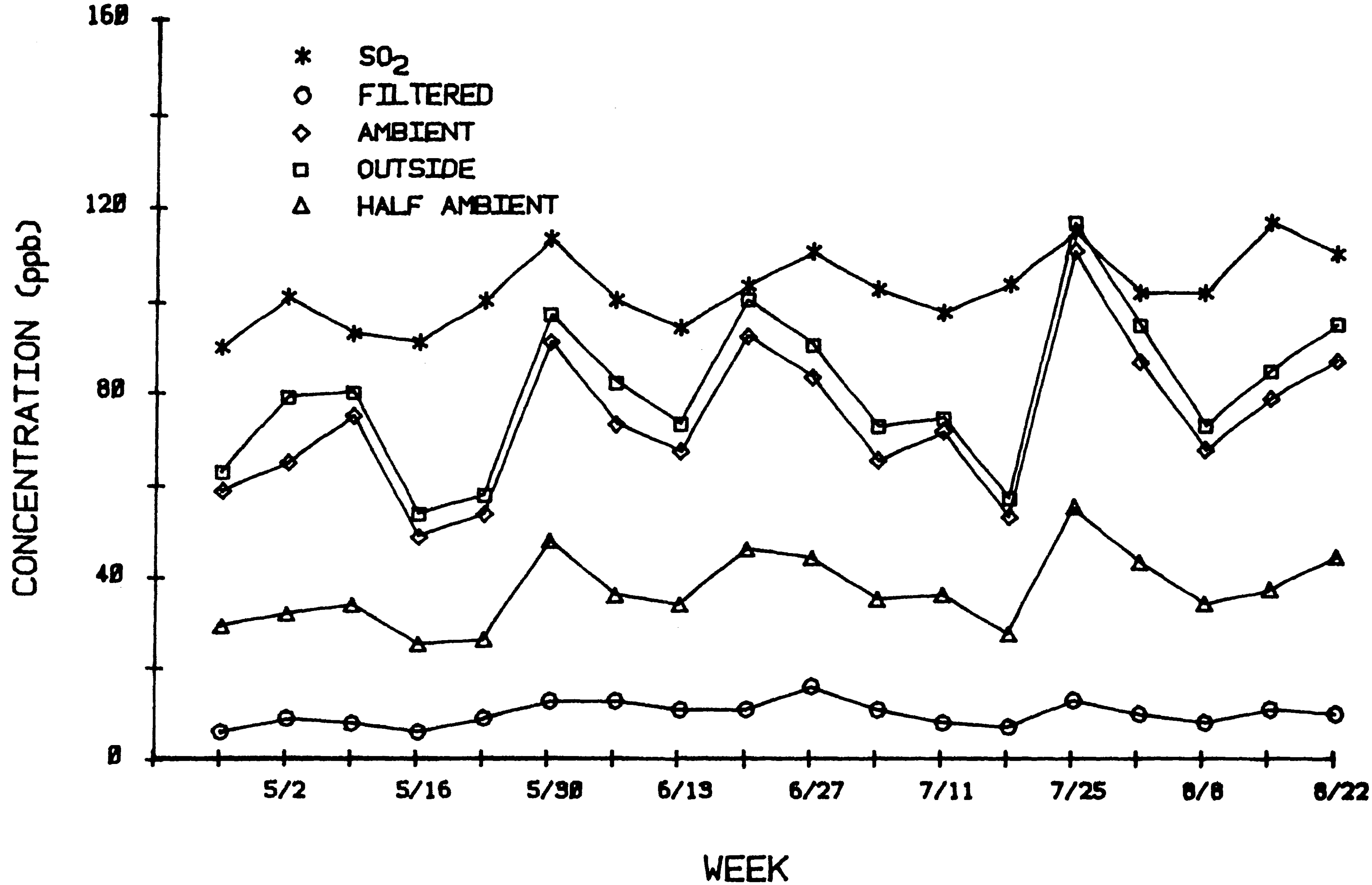

Figure 3. Air pollution concentrations for Valencia orange treatments for 4/19/86-8/22/87. Values are weekly **means**.

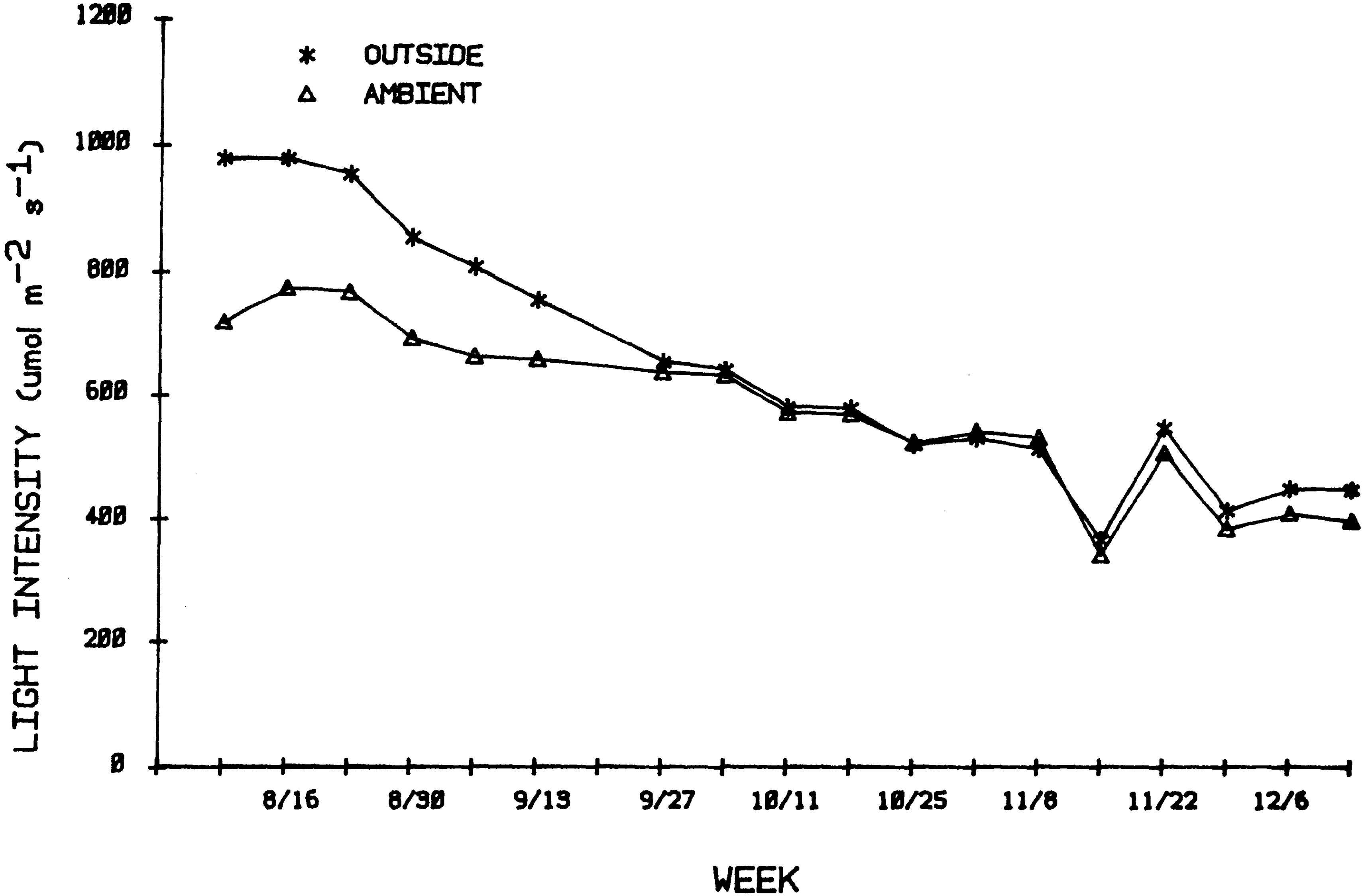

Figure 4. Quantum light intensity for 8/3-12/13/86. Values for ambient chamber and outside are weekly means for one tree, between 0800 and 2000 daily.

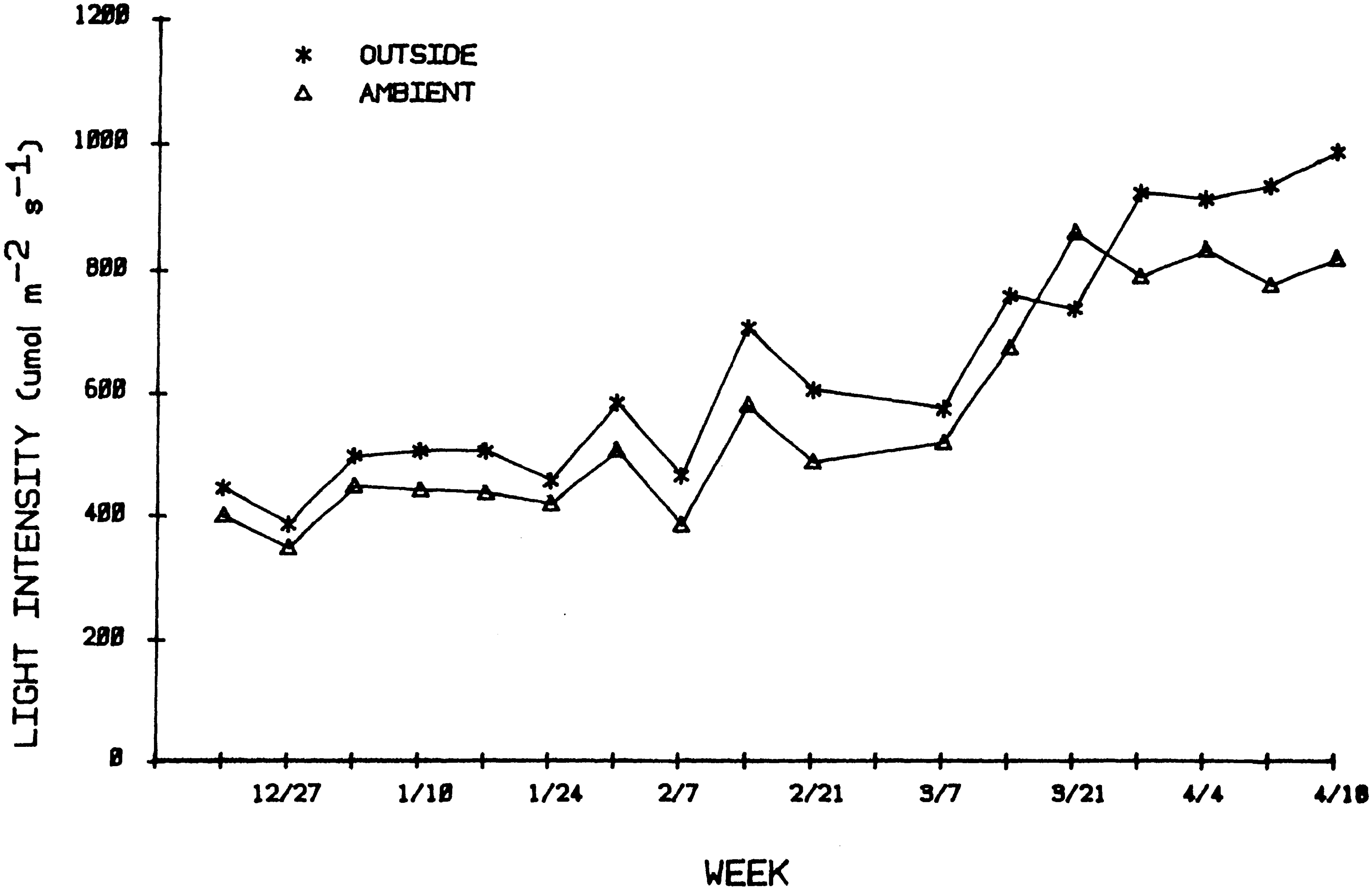

Figure 5. Quantum light intensity for 12/14/86-4/18/87. Values for ambient chamber and outside are weekly means for one tree, between 0800 and 2000 daily.

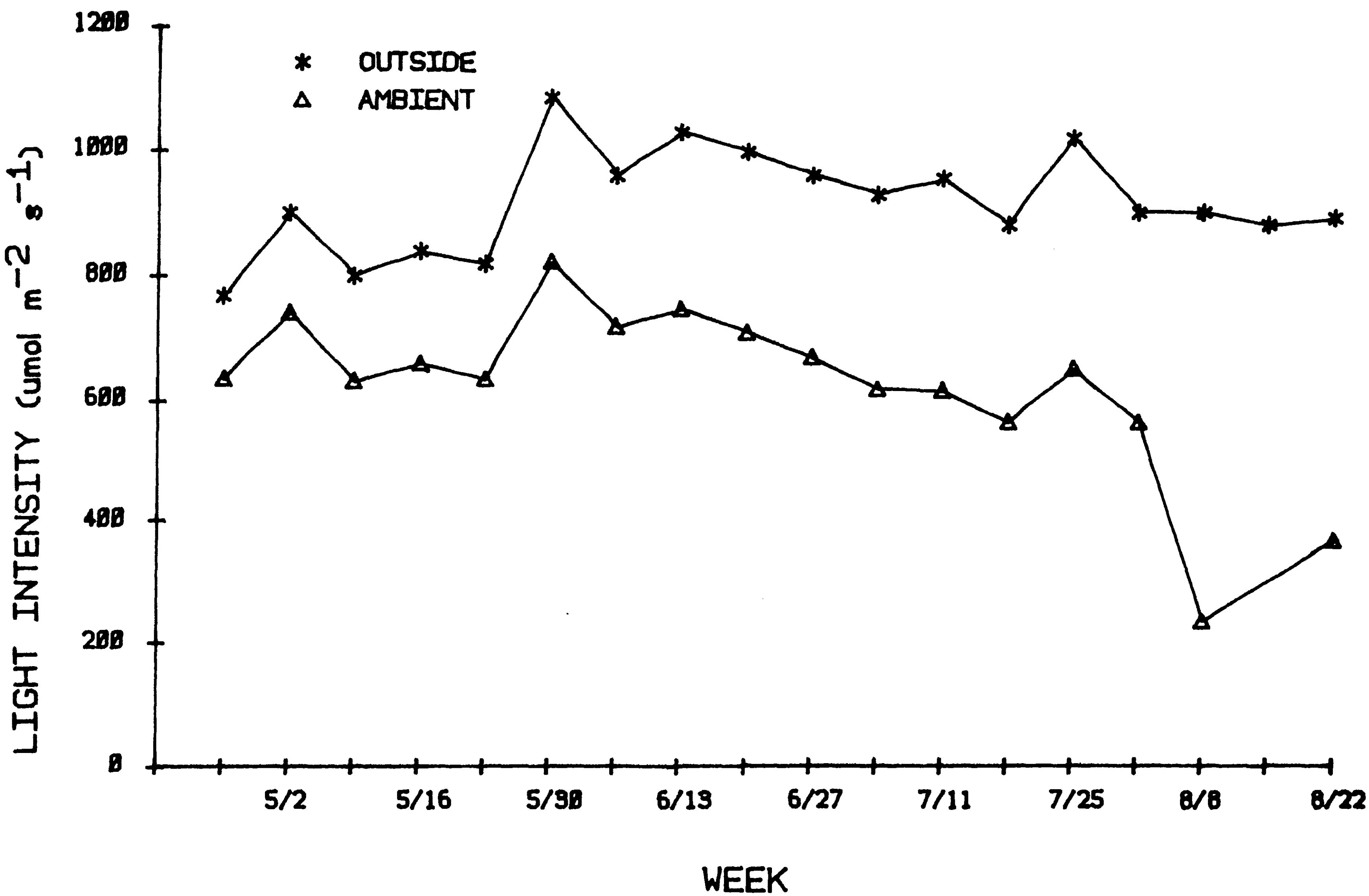

Figure 6. Quantum light intensity for 4/19-8/22/87. Values for ambient chamber and outside are weekly means for one tree, between 0800 and 2000 daily.

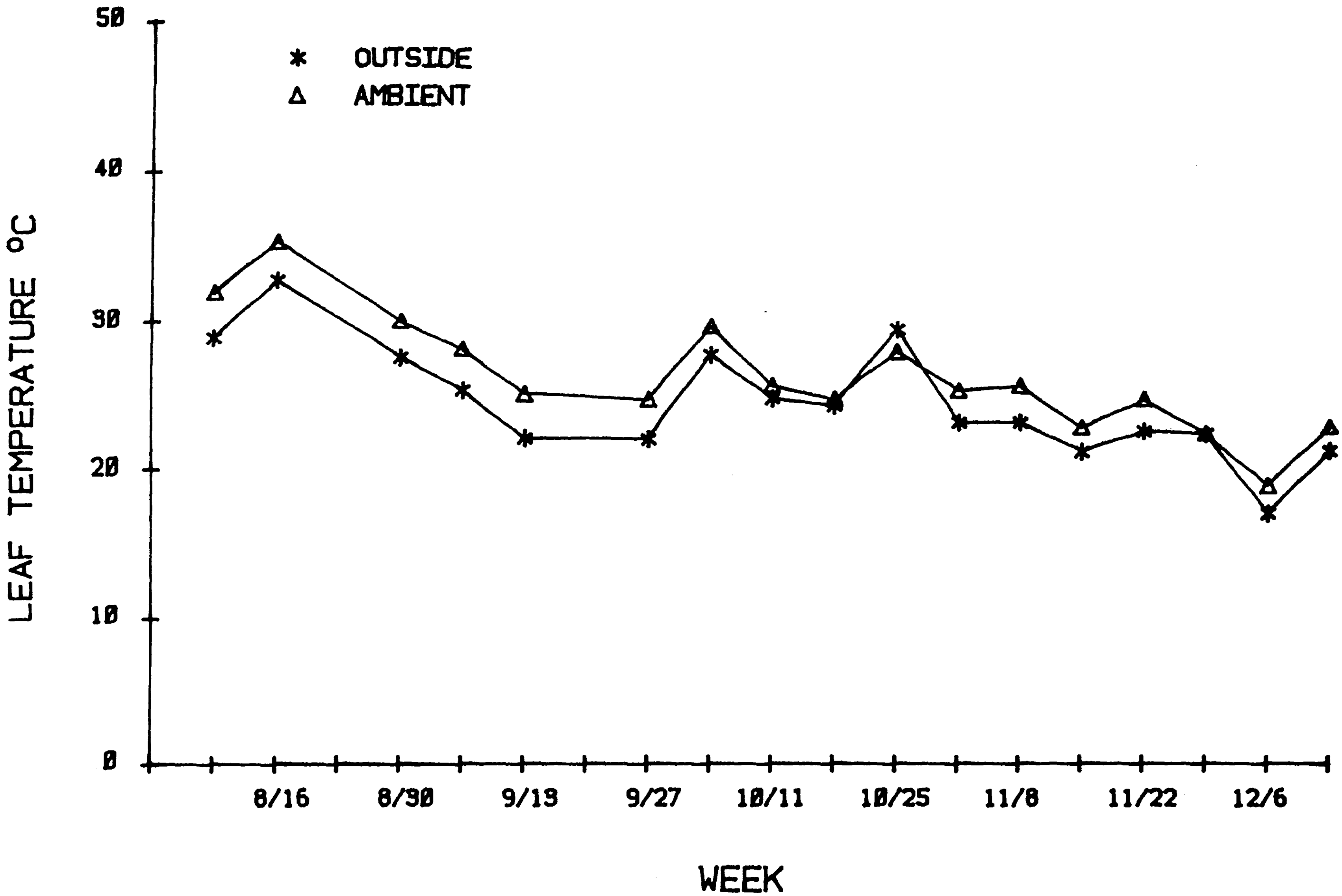

Figure 7. Leaf temperature for 8/3-12/13/86. Values for ambient chamber and outside are weekly means for two outside and one ambient tree, between 0800 and 2000 daily.

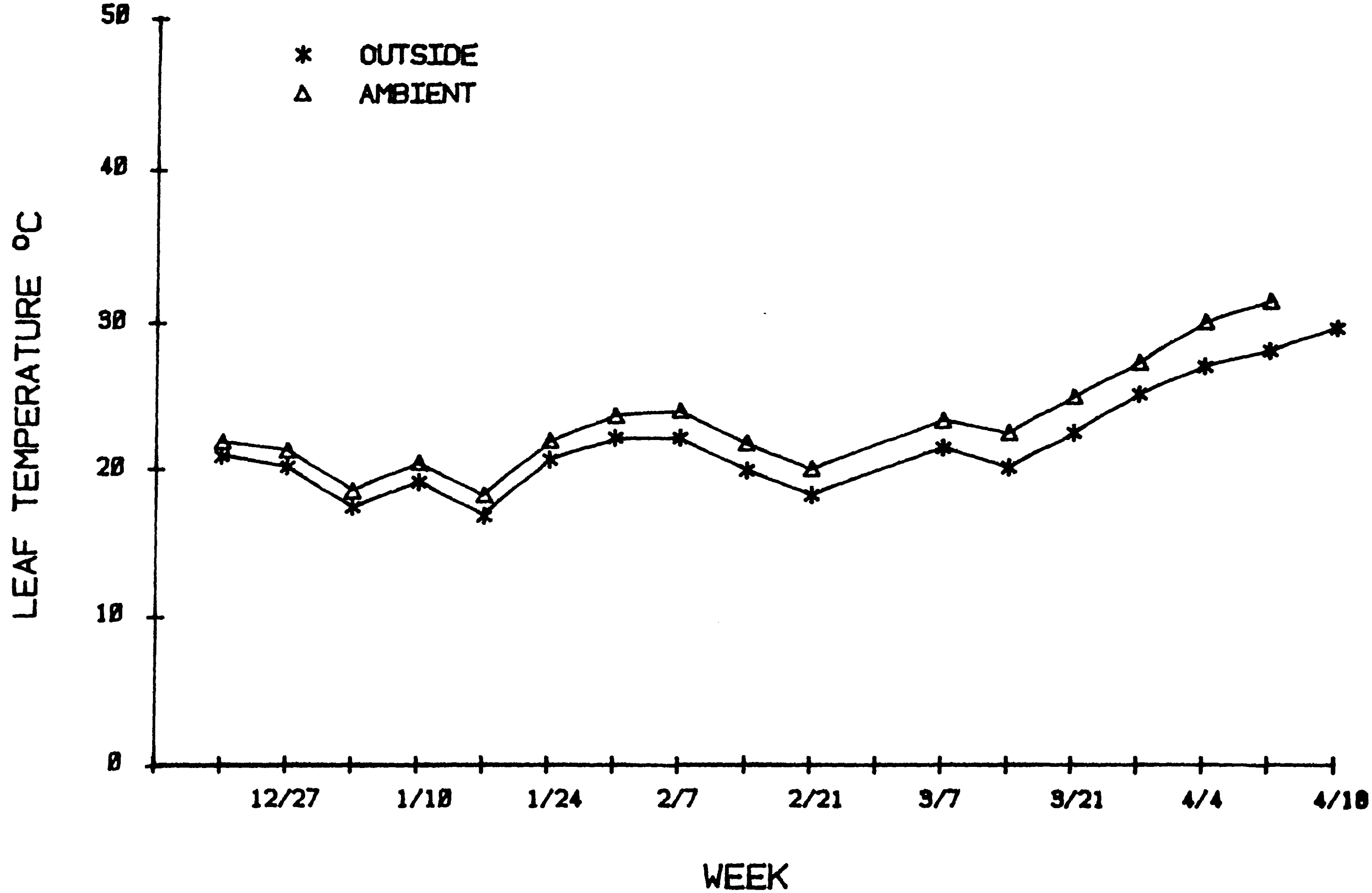

Figure 8. Leaf temperature for 12/20/86-4/18/87. Values for ambient chamber and outside are weekly means for two outside and one ambient tree, between 0800 and 2000 daily.

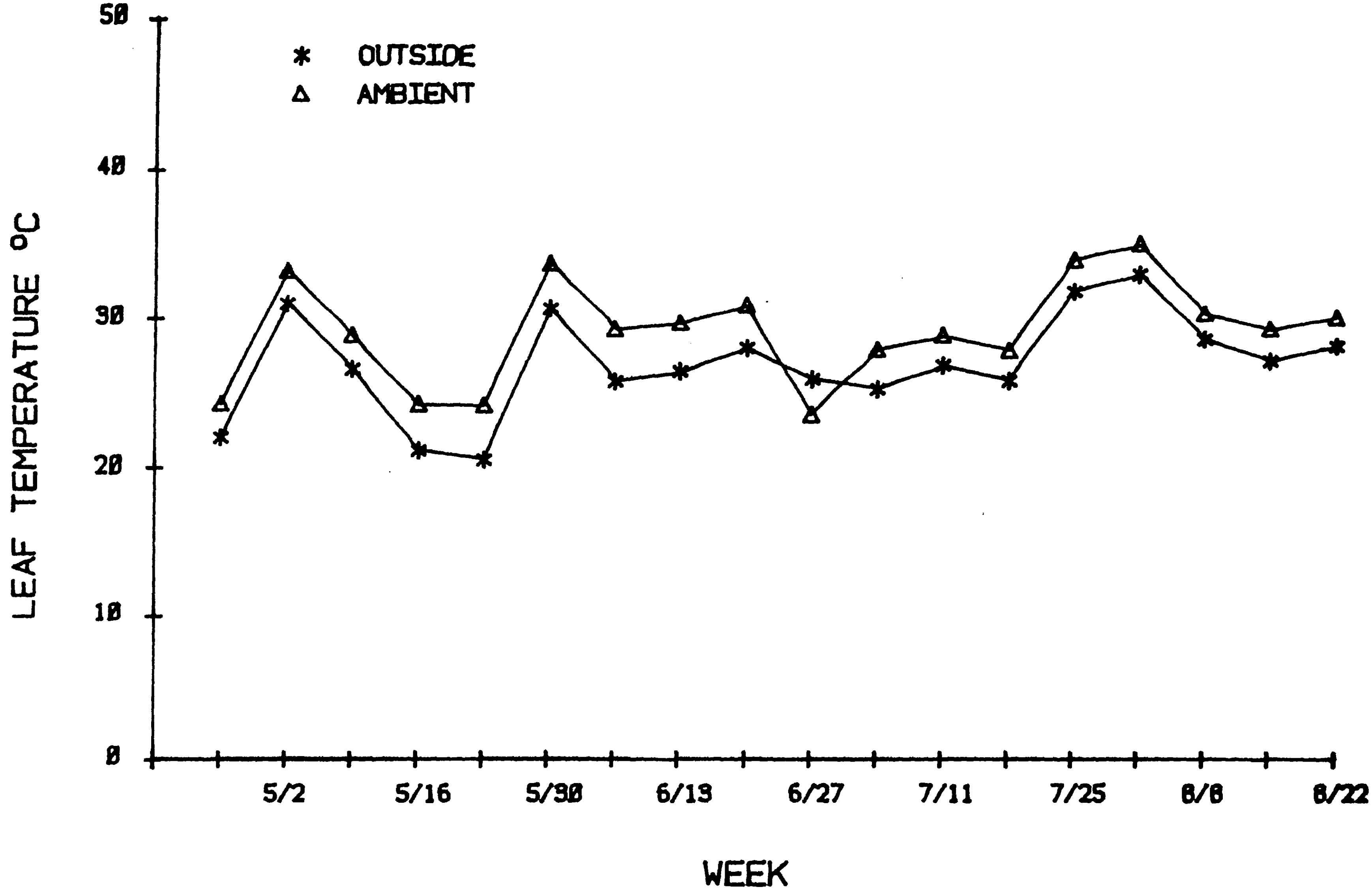

Figure 9. Leaf temperature for 4/19-8/22/87. Values for ambient chamber and outside are weekly means for two outside and one ambient tree, between 0800 and 2000 daily.

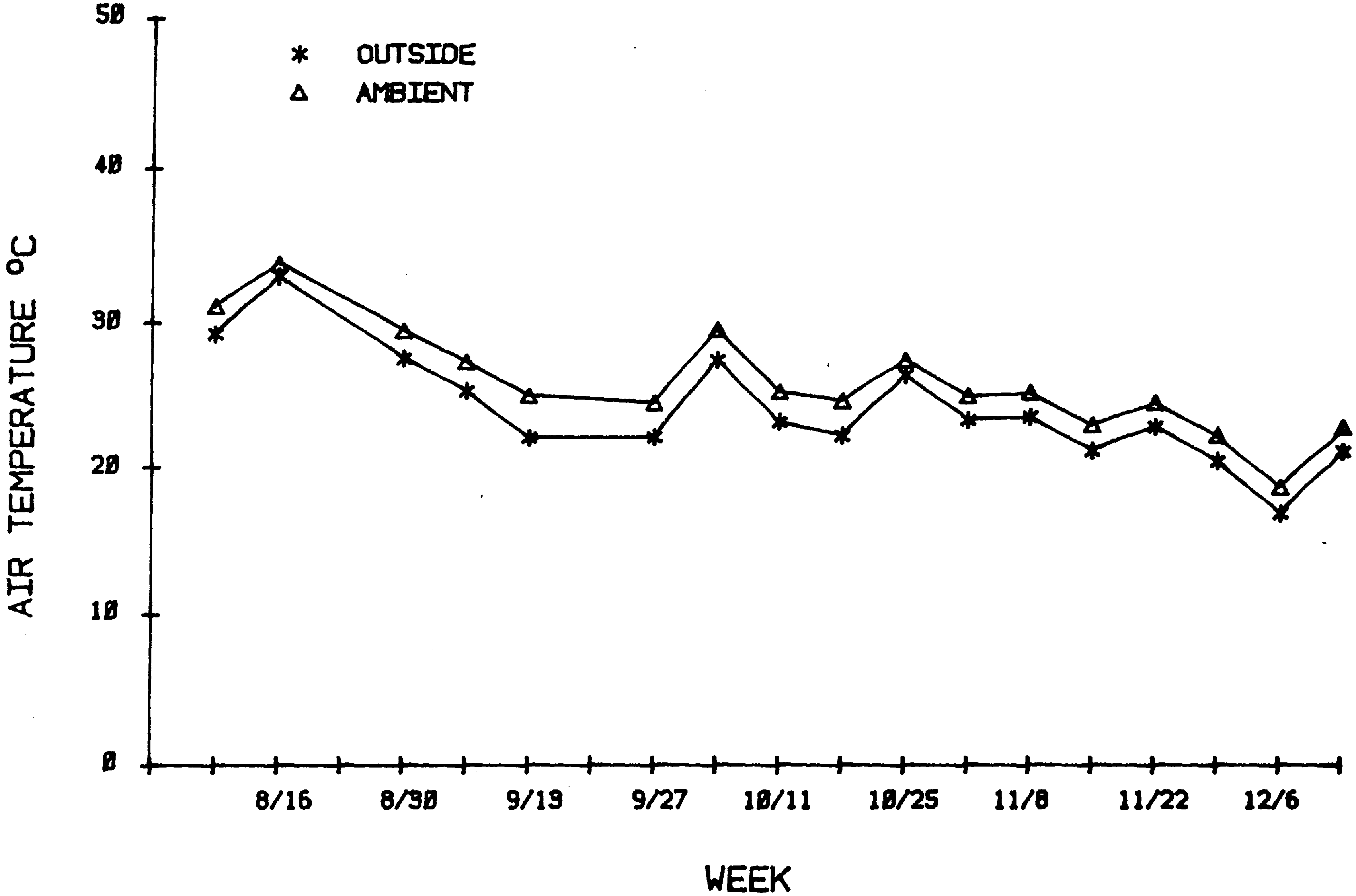

Figure 10. Air temperature for 8/3-12/13/86. Values for ambient chamber and outside are weekly means for two outside and one ambient tree, between 0800 and 2000 daily.

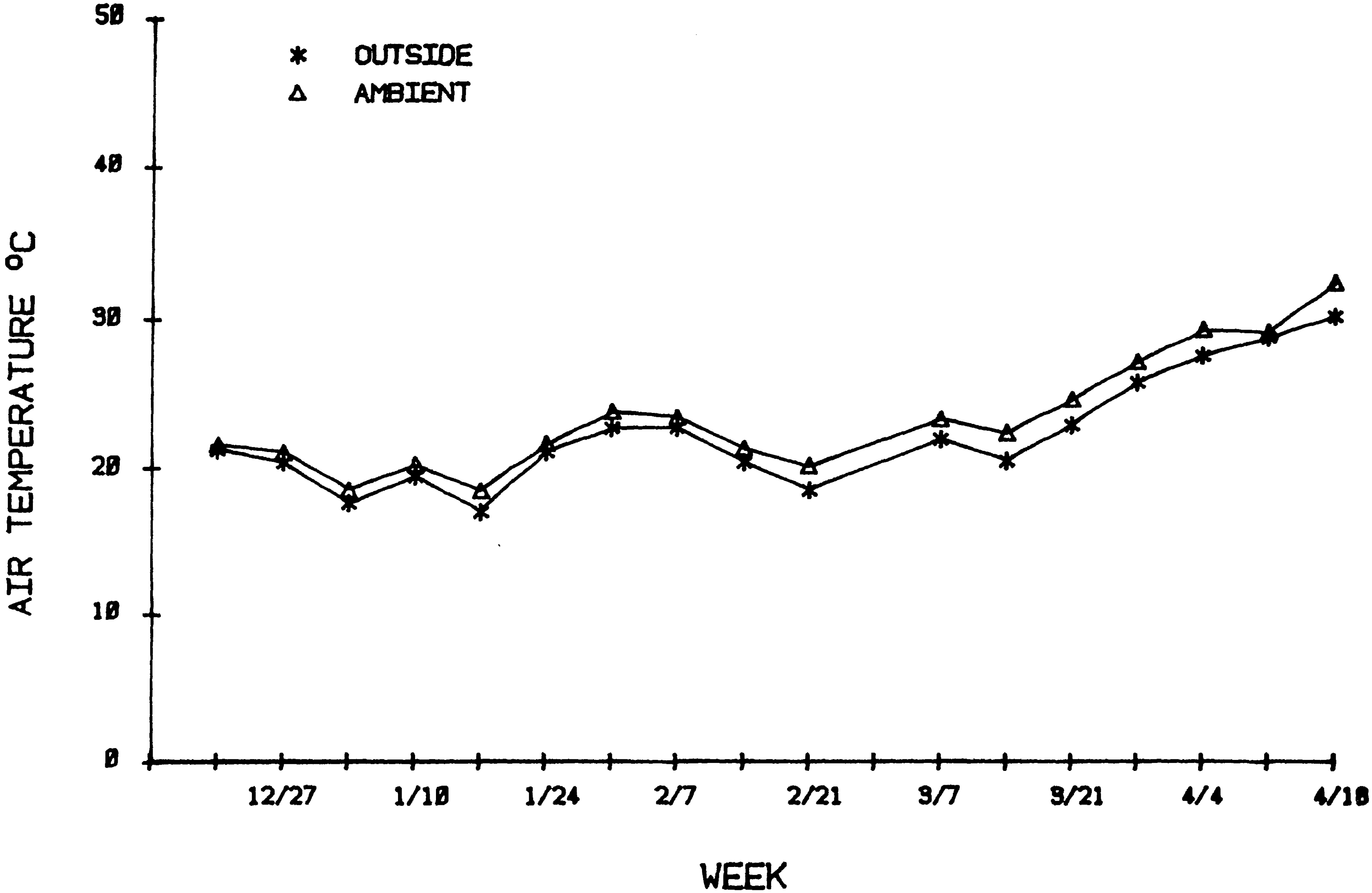

Figure 11. Air temperature for 12/14/86-4/18/87. Values for ambient chamber and outside are weekly means for two outside and one ambient tree, between 0800 and 2000 daily.

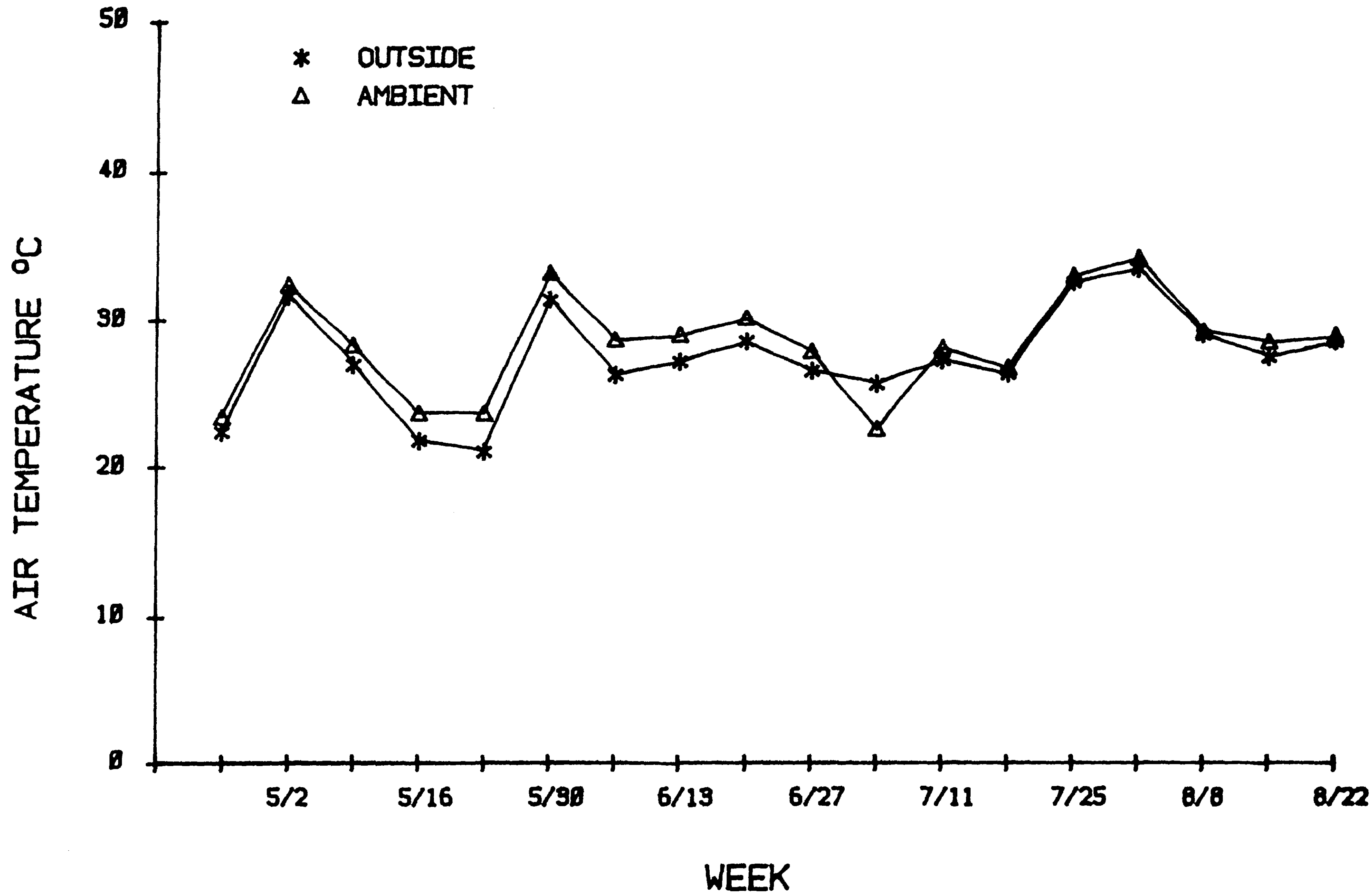

Figure 12. Air temperature for 4/19-8/22/87. Values for ambient chamber and outside are weekly means for two outside and one ambient tree, between 0800 and 2000 daily.

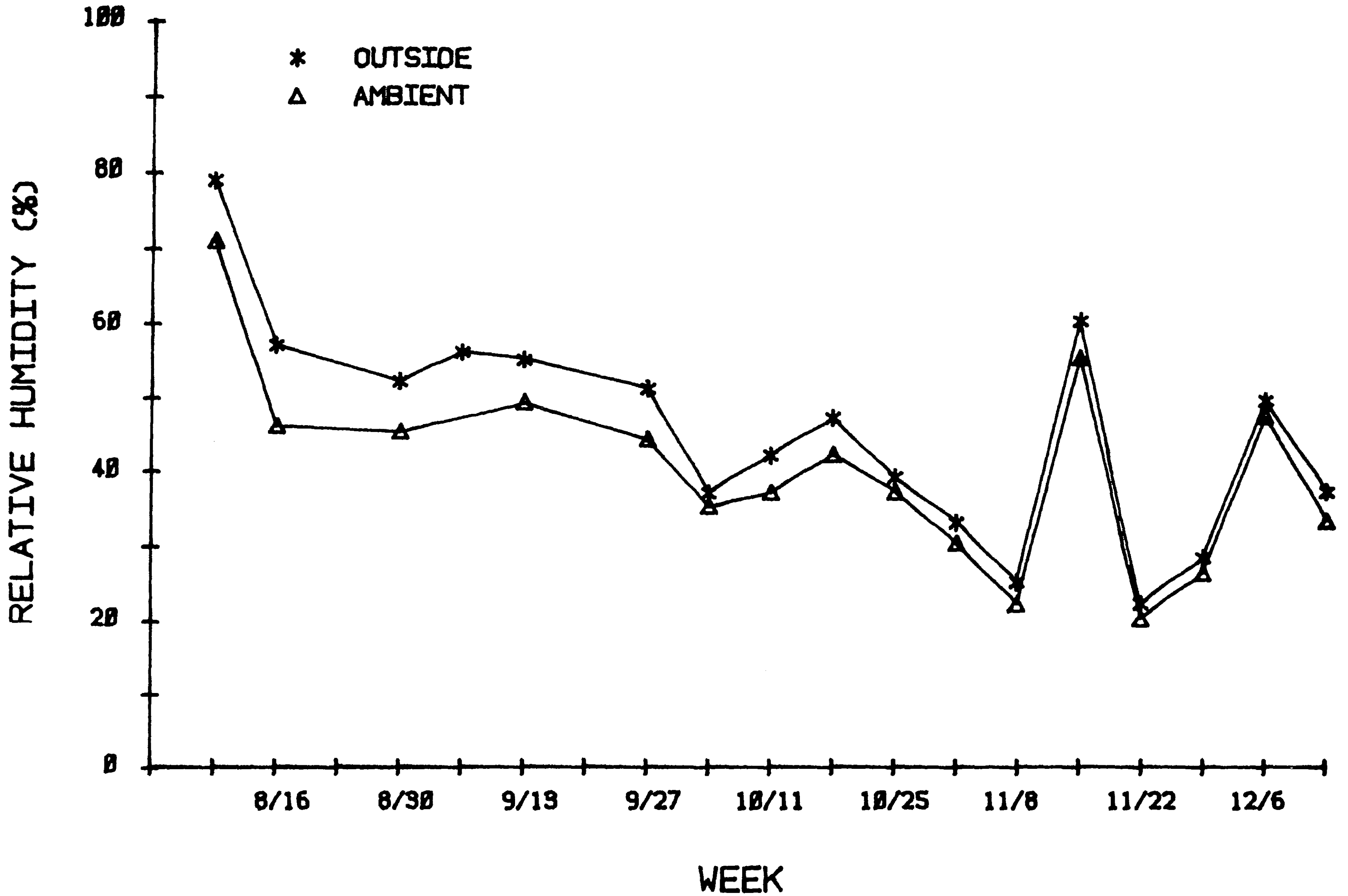

Figure 13. Relative humidity for 8/3-12/13/86. Values for ambient chamber and outside are weekly means for one tree, between 0800 and 2000 daily.

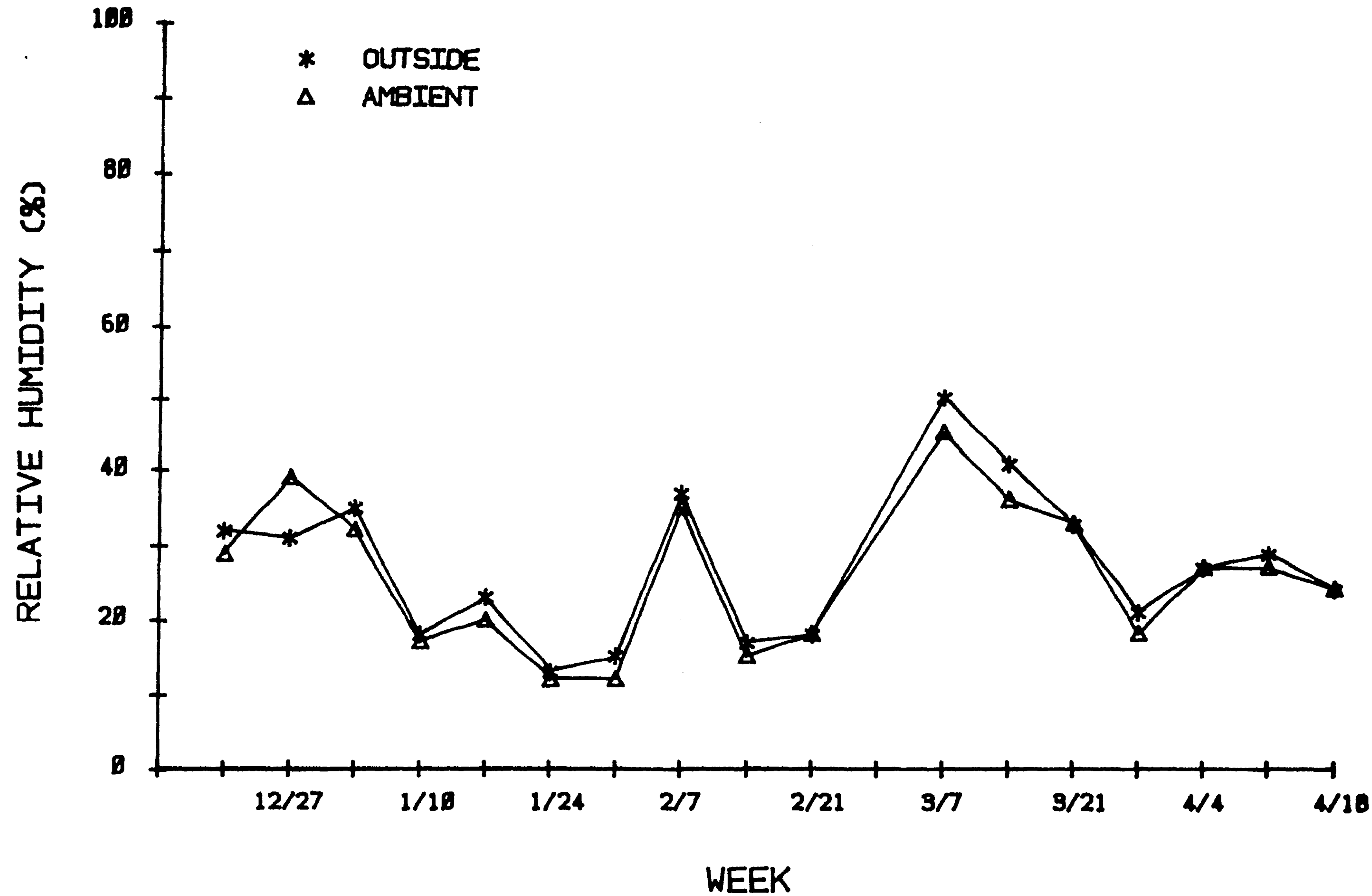

Figure 14. Relative humidity for 12/14/86-4/18/87. Values for ambient chamber and outside are weekly means for one tree, between 0800 and 2000 daily.

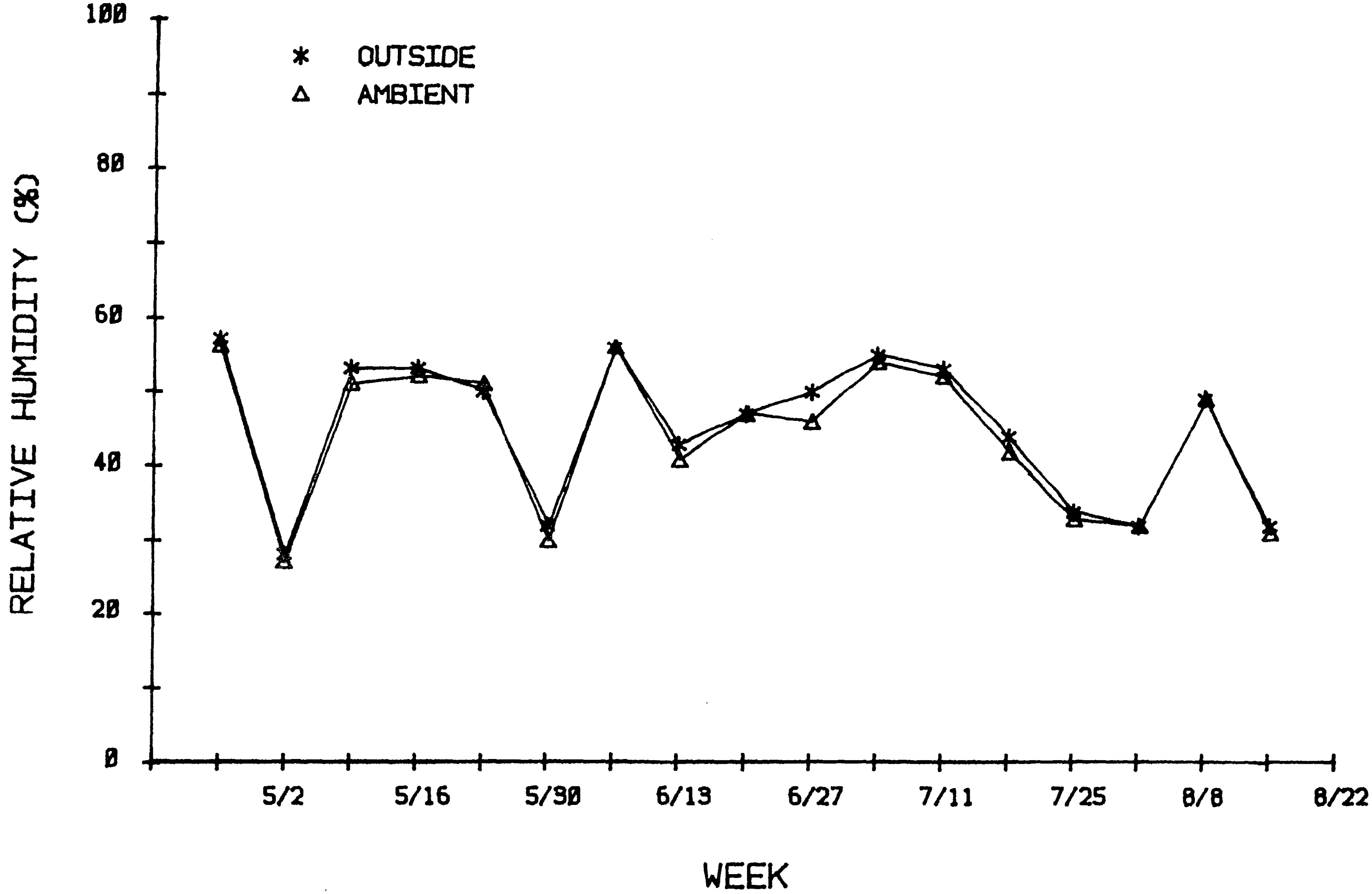

Figure 15. Relative humidity for 4/19-8/22/87. Values for ambient chamber and outside are weekly means for one tree, between 0800 and 2000 daily.

C. Ozone Effects

Yield and Quality. The oranges were picked to obtain the second experimental yield on May 19, 1987. The data for weight/tree, number/tree, and weight/fruit are shown in Table 2. Data for color, circumference, and rind thickness are shown in Table 3. Data for % sugar and % acid are shown in Table 4. The data indicated that ambient oxidants (measured as O_3) significantly reduced weight per tree when the data were analyzed with regression analysis comparing yield to growing season ozone concentration in 1986. The regression equation obtained fit the line: total weight = 69.997 - (300.5 x 12 hour O_3 average). The ozone effect was not significant if the analysis of variance with contrasts was used as the three ozone treatment were not quite equally spaced.

The yields from all treatments were much higher in 1987 vs. 1986, However, the slope of the regression line was similar in both years, indicating that the ozone response may have been similar. Further statistical analysis will be run on the yield data after the 1988 harvest has been made. This analysis will consider the year factor possibly as repeated measurements from the same trees over time.

The number of fruit per tree was dramatically affected by O_3 (Table 2). The number data also fit a statistically significant number vs. ozone concentration linear regression equation. The 28% reduction in number per tree for the ambient vs. filtered chamber trees was essentially the same as the 27% reduction in total weight per tree, indicating that a reduction in number and not weight per fruit was the likely cause of the yield reduction with O_3.

There was evidence that fruit quality parameters were affected by O_3. Fruit circumference and rind thickness were highest in the half ambient compared to either the filtered or ambient ozone treatments (Table 3). The analysis for these parameters was based on a subsample of fruit collected from each tree. However, since the number varied per tree, the statistical analysis was a one-way ANOVA with approximately 200 fruit per treatment. The altered fruit quality at the half ambient treatment was likely due to the moderate O_3 stress which apparently reduced the number of fruit per tree to the same extent as the in the ambient chambers

Table 2. Yield Data for Valencia Orange Trees Exposed to Photochemical Oxidants or Sulfur Dioxide[a]

Treatment	Weight/Tree (kg)	Number/Tree	Weight/Fruit (g)
Outside	34.8 ± 18.5	246 ± 113	141 ± 30
Ambient	48.3 ± 9.0	271 ± 40	179 ± 32
Half-Ambient	59.6 ± 17.3	279 ± 81	215 ± 16
Filtered	66.3 ± 18.3	376 ± 100	170 ± 29
Filtered + SO_2	42.9 ± 21.4*	229 ± 150*	220 ± 68

[a]Values are means ± SD for seven trees. Pairs of filtered vs. filter + SO_2 trees followed by * are significantly different at $p < 0.05$ using a non-paired, one-tailed "t" test. There were significant O_3 effects using regression analysis for total weight of fruit per tree (total weight = 69.997 - 300.5 x 12 hr O_3 ave., n=21, df=19, s_{yx}=15.078, r^2=0.454), and number per tree (total number = 377.690 - 1732.887 x 12 hr O_3 ave., n=21, df=19, r^2=0.491).

Table 3. Fruit Quality Data for Valencia Orange Trees Exposed to Photochemical Oxidants or Sulfur Dioxide[a]

Treatment	Color[b] (3-13 scale)	Circumference (m)	Rind Thickness (mm)
Outside	11.6 ± 1.9	0.324 ± 0.074	3.65 ± 1.10
Ambient	11.9 ± 1.2*	0.362 ± 0.077*	4.27 ± 1.20*
Half-Ambient	11.9 ± 0.9	0.447 ± 0.087	5.13 ± 1.21
Filtered	12.1 ± 0.4	0.388 ± 0.104	4.53 ± 1.19
Filtered + SO_2	11.6 ± 1.7*	0.420 ± 0.124*	4.84 ± 1.44*

[a]Values are means ± SD for all single fruit measurements from seven trees. Pairs of ambient vs. outside or filtered vs. filtered + SO_2 trees followed by * are significantly different a $p < 0.05$ using one-way analysis of variance. There were statistically significant ozone regression effects (quadratic) for circumference and rind thickness.

[b]The color scale corresponds to 3 as green and 13 as orange.

Table 4. Fruit Quality Data for Valencia Orange Trees Exposed to Photochemical Oxidants or Sulfur Dioxide-Continued[a]

Treatment	% Sugar	% Acid
Outside	13.3 ± 0.7	1.8 ± 0.2
Ambient	13.2 ± 0.7	1.4 ± 0.1*
Half-Ambient	12.3 ± 0.8	1.3 ± 0.2
Filtered	13.0 ± 1.1	1.4 ± 0.1
Filtered + SO_2	12.3 ± 1.2	1.4 ± 0.1

[a]Values are means ± SD for seven trees. Pairs of filtered vs. ambient vs. outside trees followed by * are significantly different at $p < 0.05$ using one-way analysis of variance.

(Table 2). However, since the stress was not that great, the fruit actually grew more and became larger in the half-ambient chambers compared to either the ambient or filtered chambers. The circumference and rind thickness effects are thus a secondary effect due to the increased fruit size in the half-ambient treatment. Neither fruit acidity nor sugar content was affected by O_3 at the 1987 harvest (Table 4).

The changes in fruit quality due to air pollutants could have important commercial implications depending on complex relationships in the market. Research personnel working on citrus fruit quality at UCR indicated that the importance of quality parameters is highly dependent on total production of oranges, relative abundance of large vs. small or thick vs. thin oranges, commercial acceptance of different colors of oranges, and even geographical differences in characteristics of oranges from the San Joaquin Valley vs. Southern California. The relative importance of these factors varies between and even within growing seasons. Thus, these is no one constant "standard" of quality for oranges, but, instead a fluctuating perception of the most marketable characteristics. For example, larger oranges may be worth more than smaller oranges if smaller oranges predominate in the market. Thicker rinds may be more desirable in Southern California where rinds are occasionally too thin, while thinner rinds may be more desirable in the San Joaquin Valley where rinds may already be too thick. Some yellow color may generally be less desirable than more orange color. However consumers recently have been encouraged to purchase oranges even if they have some green as this is a normal characteristic later in the production season and does not affect taste. Furthermore, changes in fruit quality may have different impacts for Valencia vs. navel oranges, such as a thicker rind being a advantage for navel oranges which are usually hand peeled.

Tree vegetative growth were omitted early in 1987 as the data did not appear to be important, even though stem diameter, crown volume, and height determinations were described in the original proposal. The vegetative growth data would have been highly subjective as the tree canopies filled the upper portion of the dome in most chambers, with some branches pressing on the sides and escaping from the top making accurate measurements of canopy volume difficult. In addition, in early 1987 we noted that chamber tree canopies were much less dense than for outside trees. Chamber trees normally and open space within the canopy especially near the tree trunk, whereas outside trees had compact canopies with leaves throughout. Thus, vegetative growth estimates based on tree size would have been essentially meaningless. Instead, during 1987 we concluded that total tree biomass measured at the end of the study would be most appropriate for determining treatment effects on growth. Therefore we have saved all branches broken off or pruned from the trees for addition to the final tree biomass weight.

Extra fruit drop data are shown in Table 5. Because of the lack of fruit drop in many months, statistical analysis was conducted only on the fruit dropped over the late summer to spring period before the 1987 harvest (9/86-4/87), and summer period which will help determine the 1988 harvest (5/87-8/87). There were no statistical differences in fruit drop between the three ozone treatments for either the winter or summer period.

Growth. The results from the statistical analysis of the leaf growth parameters are shown in Table 6. Monthly patterns of total leaf drop are illustrated in Figures 16 and 17, and patterns of average leaf weights are illustrated in Figures 18 and 19.

The only statistically significant ozone effect occurred for leaves collected in January, 1987 (Table 6). On this date, the greatest leaf drop was for trees exposed to ambient ozone compared to filtered air. There was also a trend for greater leaf drop with ozone exposure on a few dates, especially, in November, 1986; but none of these results were statistically significant. There were no statistically significant ozone effects on individual leaf weights. There was a possible trend toward reduced leaf weight only in March, 1987.

Physiology The results from the statistical analysis for the physiological response parameters are shown in Table 7. Patterns of leaf response for the different parameters are illustrated in Figures 20 and 21 for stomatal conductance, Figures 22 and 23 for net photosynthesis, and Figures 24 and 25 for water potential. There were no general ozone effects on any response parameter for any date.

Ozone had no general effect on leaf water potential. There was a statistically significant ozone effect only on one date, when the most negative potential (greatest) water stress) was for the ambient air treatment and least negative potential for the filtered air treatment (Figure 25).

Table 5. Fruit Drop for Different Treatments in 1986-87[a]

Treatments	9/86-4/87	5/87-8/87
	g	
Outside	303 ± 621	123 ± 337
Ambient	285 ± 398	100 ± 223
Half-Ambient	265 ± 386	142 ± 256
Filtered	329 ± 573	176 ± 322
Filtered + SO_2	355 ± 496	158 ± 266

[a]Values are means ± SD for seven trees. No significant differences between treatments.

Table 6. Results from the Statistical Analysis for the Growth Response Parameters for Valencia Orange Leaves Exposed to Air Pollutants.[a]

Date	Parameter	Effects		
		Ozone	SO_2	Chamber
9/10/86	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
10/10/86	Leaf Drop	NS	*[b]	NS
	Leaf Weight	NS	NS	NS
11/10/86	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
12/10/86	Leaf Drop	NS	***[c]	NS
	Leaf Weight	NS	NS	NS
1/10/87	Leaf Drop	*[d]	*[c]	NS
	Leaf Weight	NS	NS	NS
2/10/87	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
3/10/87	Leaf Drop	NS	***[c]	NS
	Leaf Weight	NS	NS	NS
4/10/87	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
5/10/87	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
6/10/87	Leaf Drop	NS	**[c]	NS
	Leaf Weight	NS	**[c]	NS
7/10/87	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
8/10/87	Leaf Drop	NS	NS	NS
	Leaf Weight	NS	NS	NS
Winter	Leaf Drop	NS	***[c]	NS
(11/86-3/87)	Leaf Weight	NS	NS	NS
Summer	Leaf Drop	NS	**[c]	NS
(4/87-8/87)	Leaf Weight	NS	NS	NS
Year	Leaf Drop	NS	***[c]	NS
(11/86-8/87)	Leaf Weight	NS	NS	NS

[a]Based on comparison within analysis of variance for all five treatments with seven trees per treatment. Each *,**, or ***, signifies a statistically significant difference at $p<0.05$, 0.01, and 0.005, respectively.
[b]Greater weight value with SO_2 than in filtered air.
[c]Smaller weight value with SO_2 than in filtered air.
[d]Greater weight value with greater ambient ozone in air, liner effect.

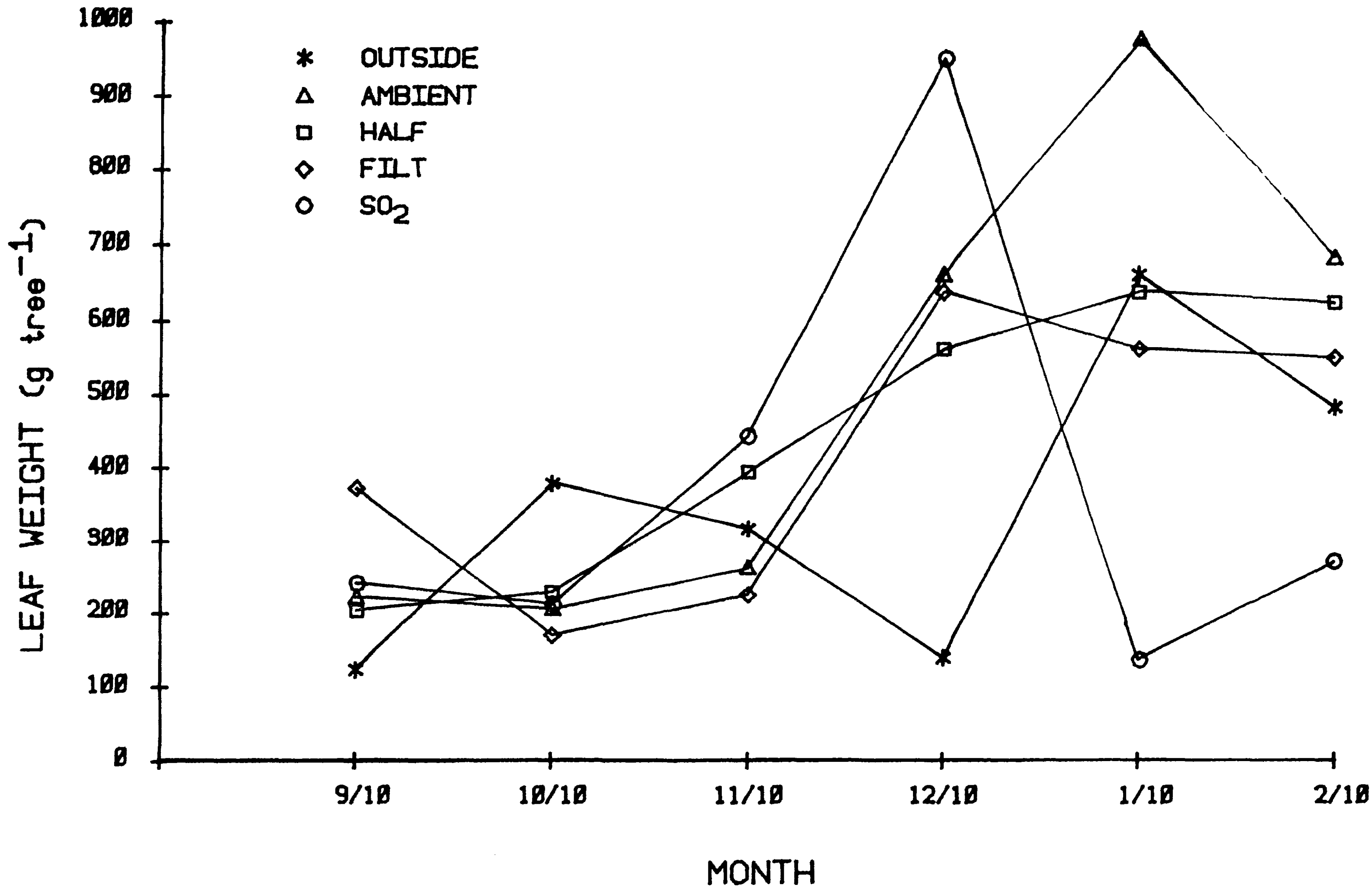

Figure 16. Total weight of dropped leaves for 9/10/86-2/10/87. Values are means for seven trees.

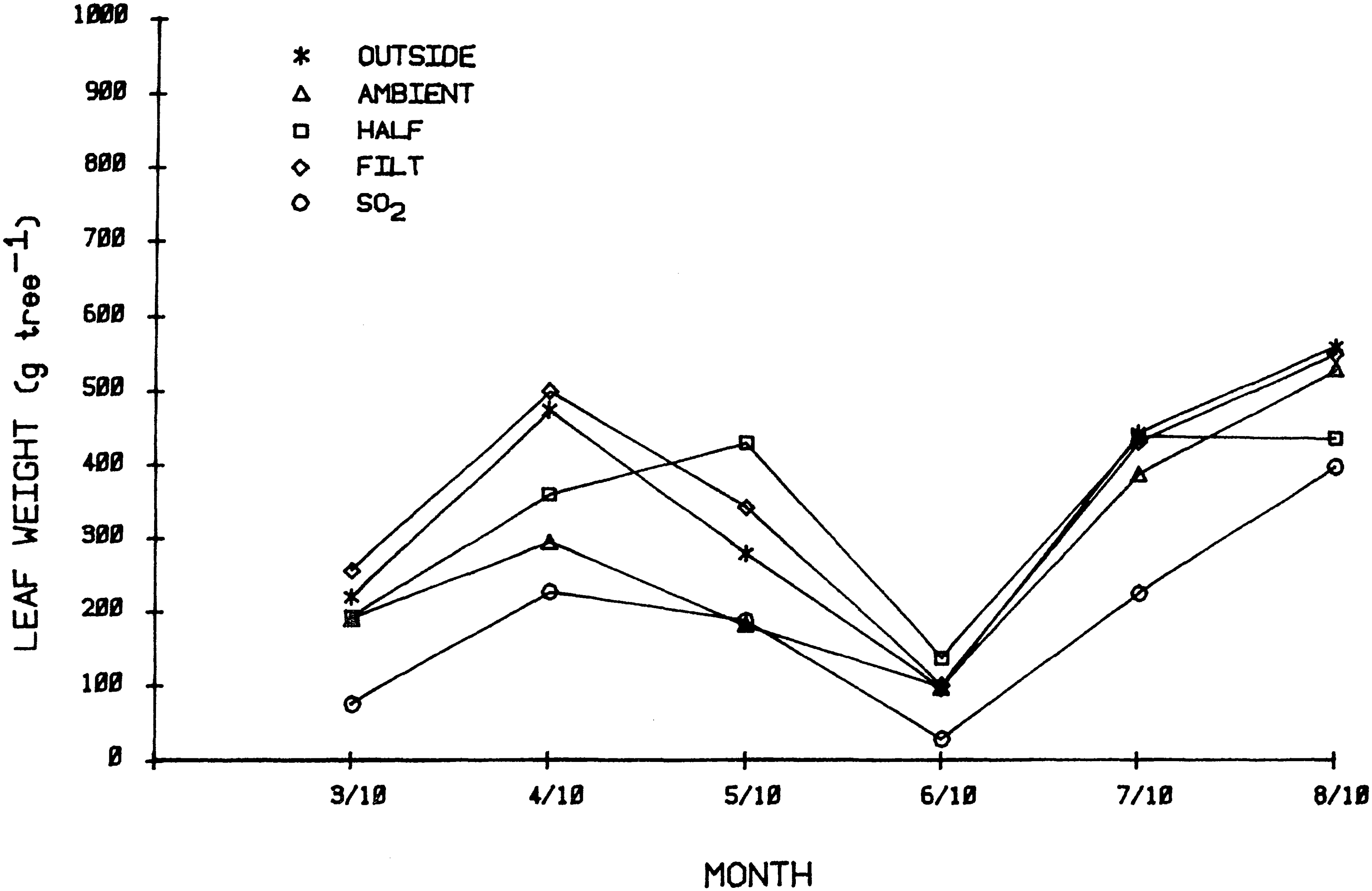

Figure 17. Total weight of dropped leaves for 3/10/87-9/10/87. Values are means for seven trees.

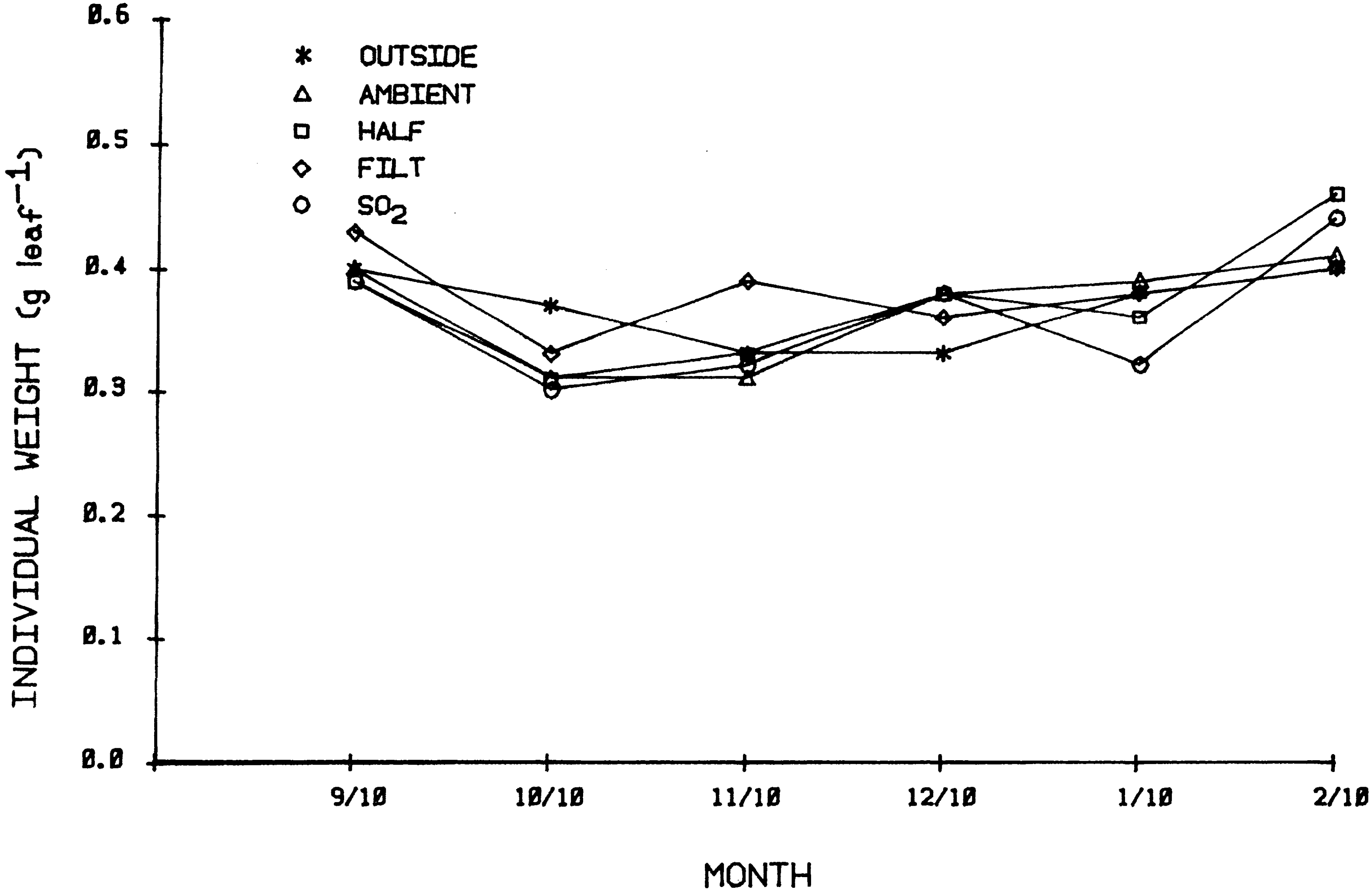

Figure 18. Individual leaf weight of dropped leaves for 9/10/86-2/10/87. Values are means for seven trees.

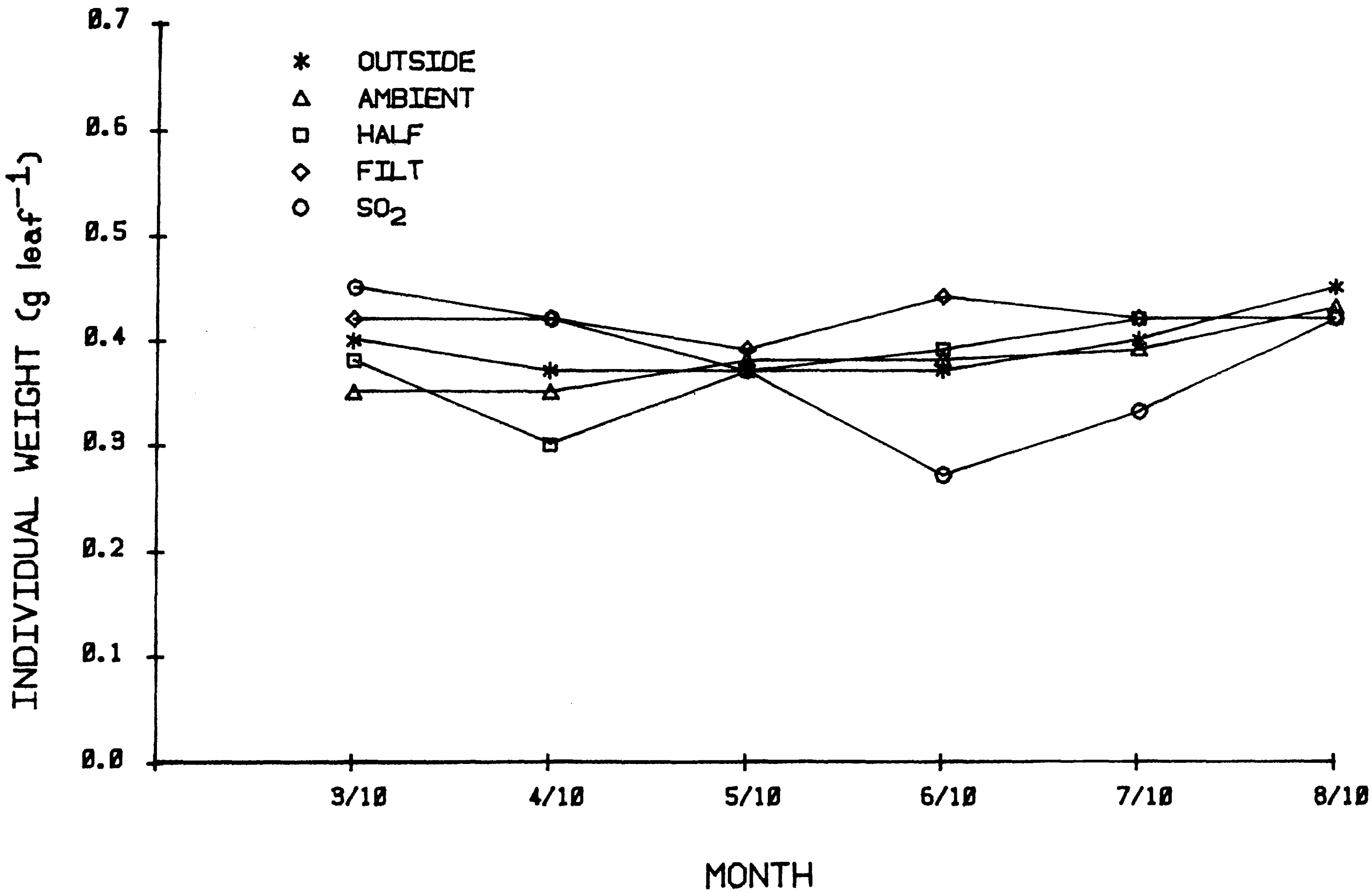

Figure 19. Individual leaf weight of dropped leaves for 3/10-8/10/87. Values are means for seven trees.

Table 7. Results from the Statistical Analysis for Physiological Response Parameters for Valencia Orange Leaves Exposed to Air Pollutants[a]

Date	Parameter	Effects: Ozone	SO_2	Chamber
10/13/86	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
10/29/86	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
11/12/86	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
12/03/86	Conductance	NS	*[b]	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
12/17/86	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
1/14/87	Conductance	NS	NS	NS
	Photosynthesis	--	--	--
	Water Potential	NS	NS	NS
1/30/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	*[c]	NS
2/11/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	**[d]
	Water Potential	*[e]	NS	***[f]
3/12/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]
3/26/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
4/9/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	*[f]

Table 7. (continued)

Date	Parameter	Effects: Ozone	SO_2	Chamber
4/29/87	Conductance	**[g]	**[h]	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
5/12/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	**[f]
5/29/87	Conductance	NS	NS	***[d]
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]
6/12/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	--	--	--
6/25/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
7/14/87	Conductance	NS	*[b]	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	**[f]
7/27/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]
8/7/87	Conductance	--	--	--
	Photosynthesis	--	--	--
	Water Potential	NS	NS	NS
8/19/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]
8/27/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	--	--	--
9/18/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	NS
10/8/87	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]

Table 7. (concluded)

Date	Parameter	Effects: Ozone	SO_2	Chamber
Winter (11/86-3/87)	Conductance	NS	NS	NS
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]
Summer (4/87-10/87)	Conductance	*[g]	NS	*[d]
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]
Year (11/87-10/87)	Conductance	*[g]	NS	**[d]
	Photosynthesis	NS	NS	NS
	Water Potential	NS	NS	***[f]

[a]Based on comparison within analysis of variance for all five treatments with seven trees per treatment. Each *, **, or ***, signifies a statistically significant difference at $p < 0.05$, 0.01, and 0.005, respectively.
[b]Higher rate with SO_2 than in filtered air.
[c]Less negative value with SO_2 than in filtered air.
[d]Lower rate in ambient chamber than outside air.
[e]More negative value with greater ambient ozone in air, linear effect.
[f]More negative value in ambient chamber than outside air.
[g]Lower rate with greater ambient ozone in air, linear effect.
[h]Lower rate with SO_2 than in filtered air.

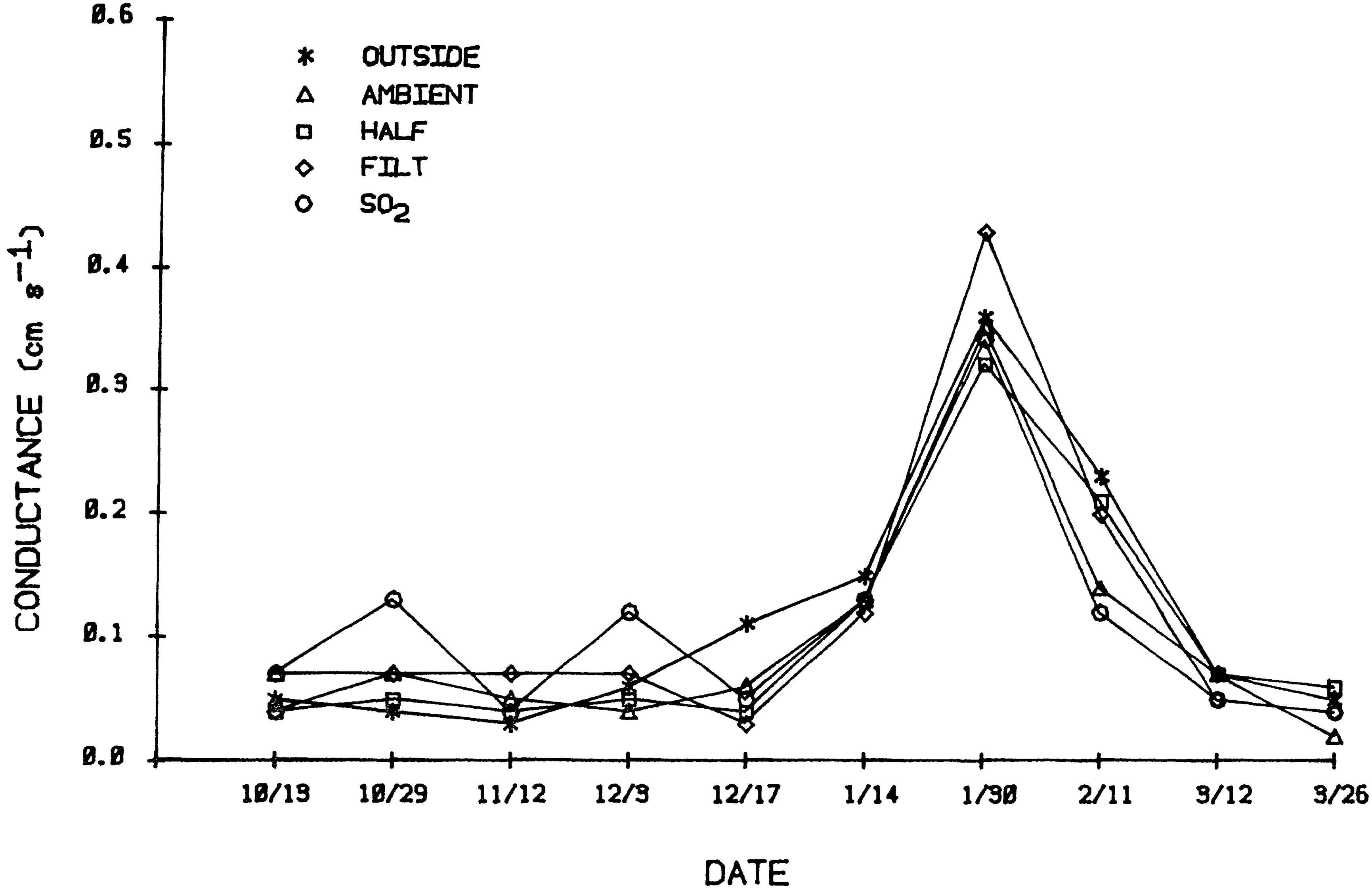

Figure 20. Stomatal conductance for 10/13/86 - 3/26/87. Values are means for seven leaves, one per tree, measured on one day every two to four weeks.

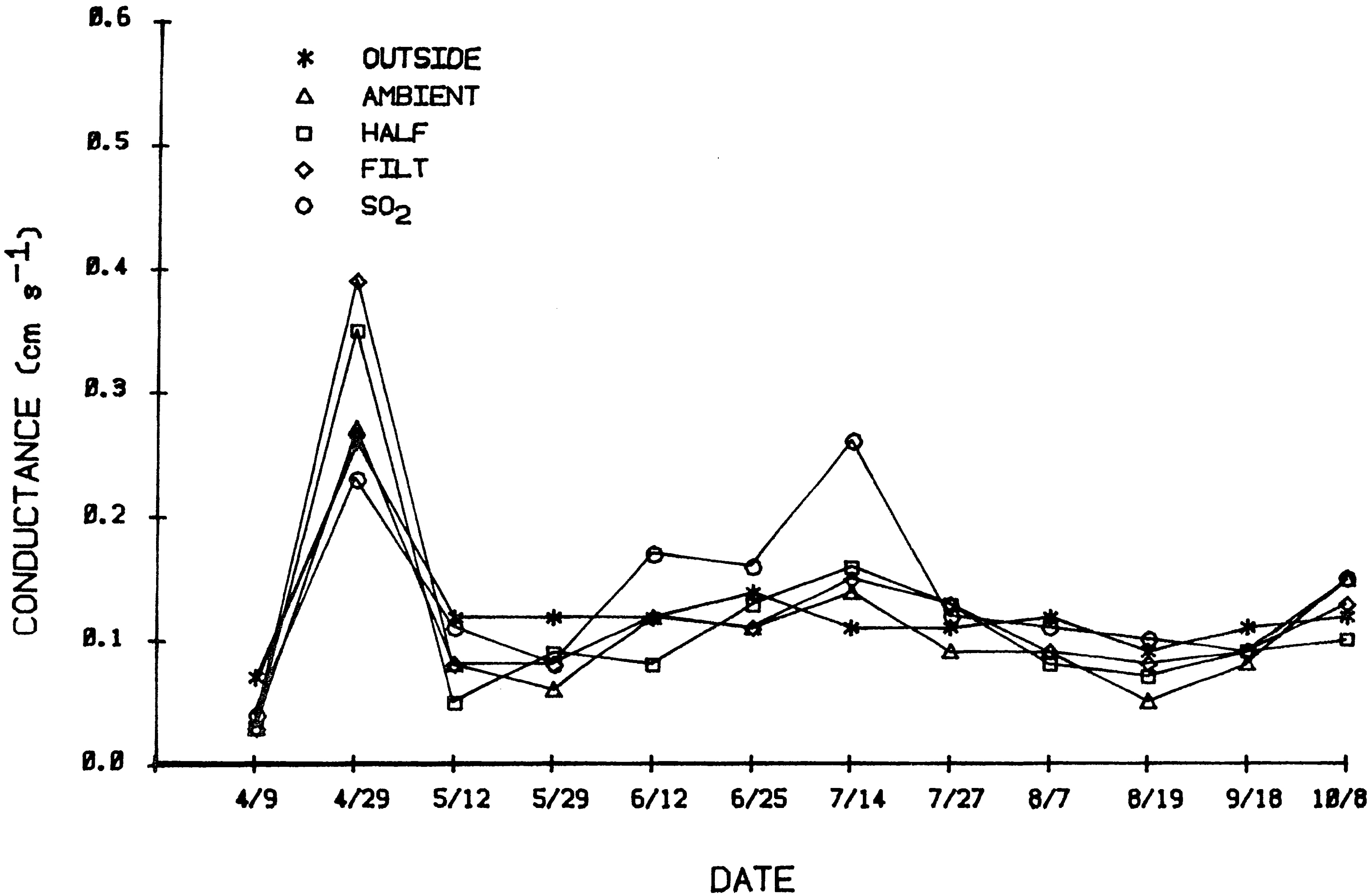

Figure 21. Stomatal conductance for 4/9/87 - 10/8/87. Values are means for seven leaves, one per tree, measured on one day every two to four weeks.

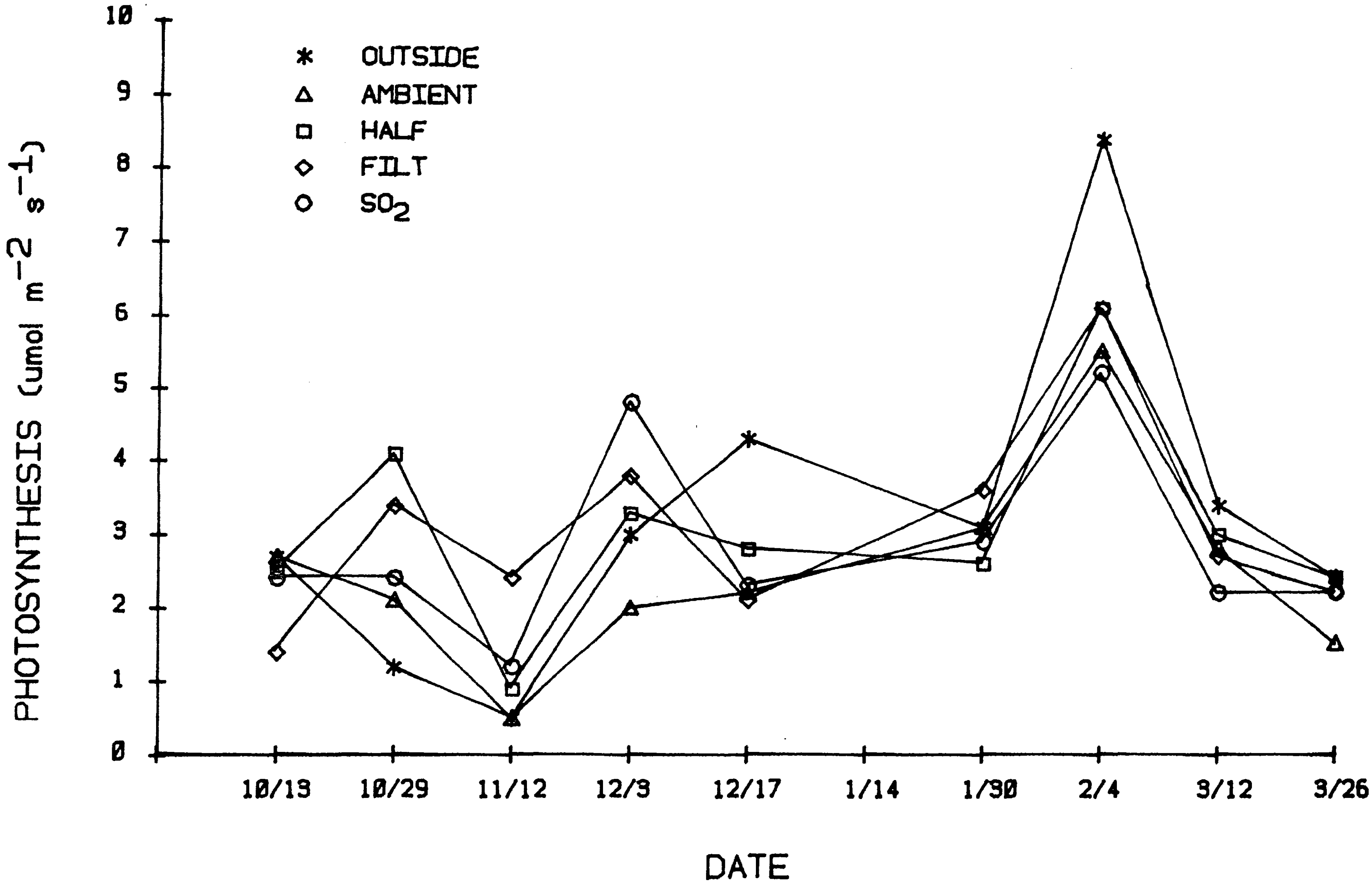

Figure 22. Net photosynthesis for 10/13/86 - 3/26/87. Values are means for seven leaves, one per tree, measured on one day every two to four weeks.

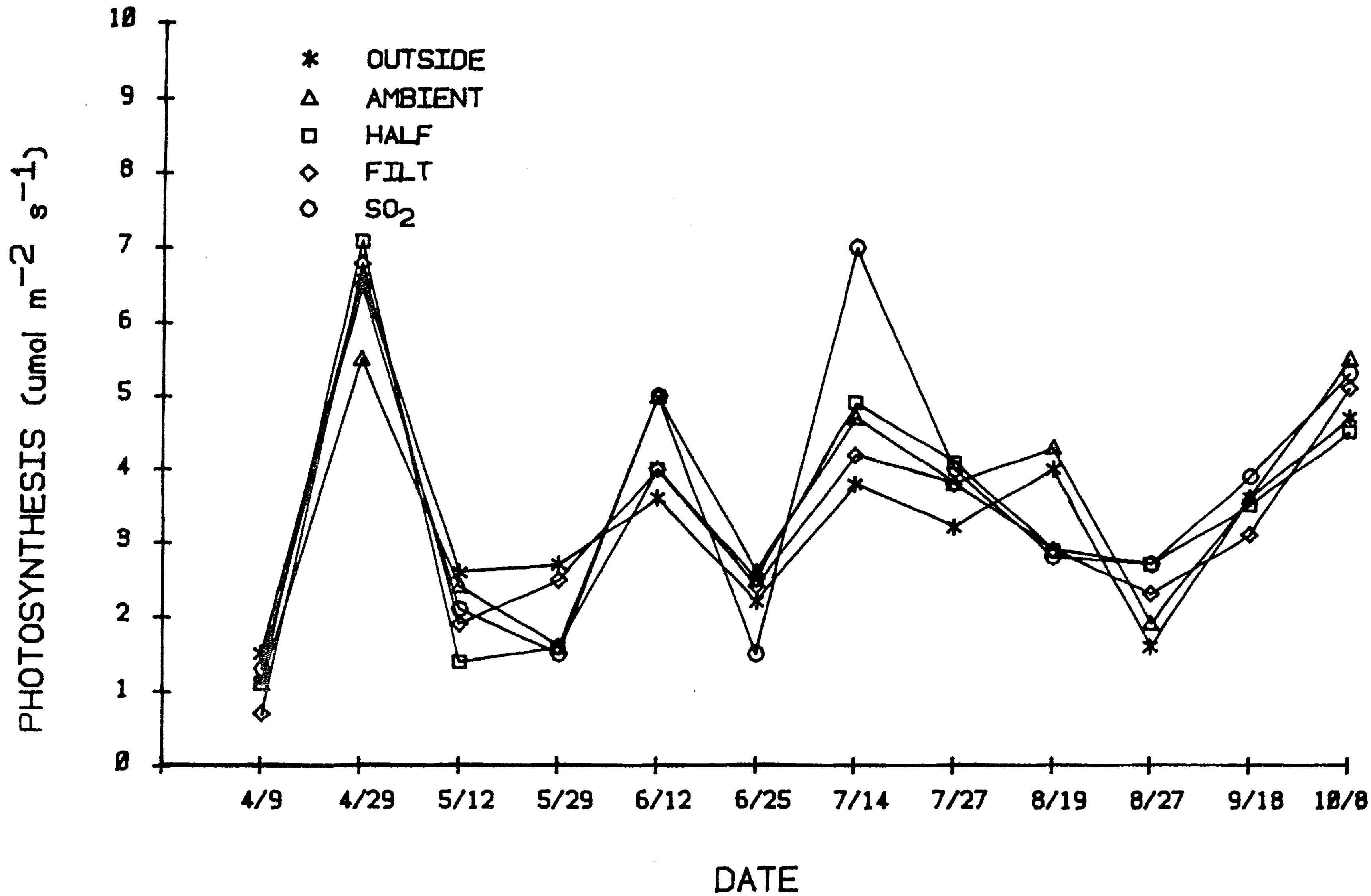

Figure 23. Net photosynthesis for 4/9/87 - 10/8/87. Values are means for seven leaves, one per tree, measured on one day every two to four weeks.

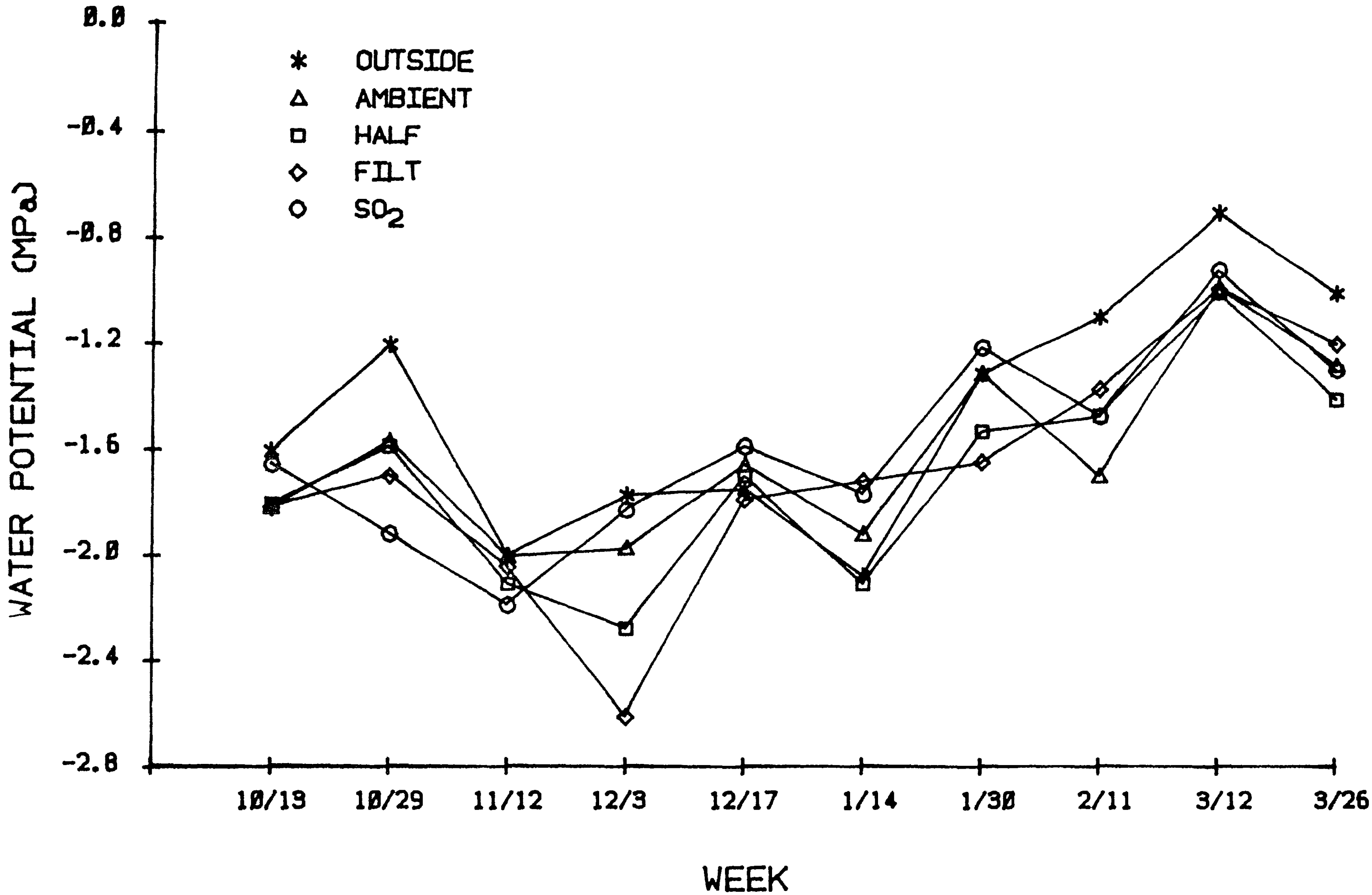

Figure 24. Water potential for 10/13/86 - 3/26/87. Values are means for seven leaves, one per tree, measured on one day every two to four weeks.

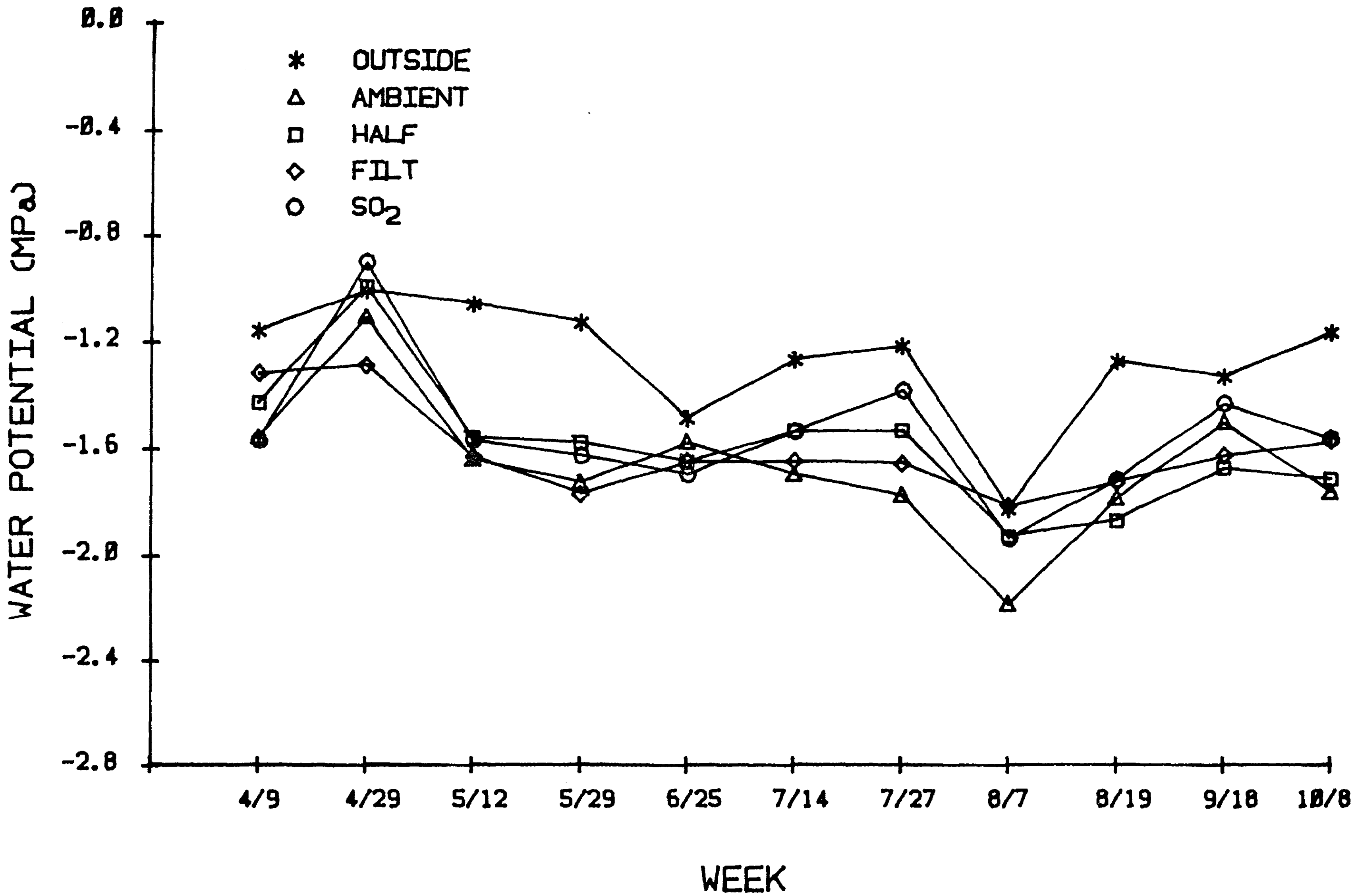

Figure 25. Water potential for 4/9/87 - 10/8/87. Values are means for seven leaves, one per tree, measured on one day every two to four weeks.

There were no other ozone effects either on a single day, seasonal, or yearly basis.

Ozone resulted in a significant decrease in stomatal conductance, with the lowest values for ambient chamber and the highest values for filtered chamber trees (Table 7). The ozone effect was statistically significant across the summer months and on a whole year basis. There was a significant ozone effect on a biweekly basis only for April. Ozone had no effect on photosynthetic rate.

Biochemistry. There was a trend toward an increase in leaf chlorophyll content with increasing ozone concentration in all months, and which was statistically significant in April (Table 8). This increase in chlorophyll content may represent a compensatory response to ozone injury (27). There were no other significant ozone effect for leaf leaf carotenoid content (Table 8), or specific leaf area (Table 9).

D. Sulfur Dioxide Effects

Yield and Quality. Sulfur dioxide produced at statistically significant reduction in orange tree yield (Table 2). The percentage reduction in weight (35%) was very close to the reduction in number (39%) for SO_2 vs filtered air trees, indicating that the was the primary determinant of the yield reduction. The yield reduction in 1987 was much larger than the 23% reduction in 1986, indicating that the continuous SO_2 stress may be having a cumulative effect on yield over years.

Fruit quality also was significantly affected by SO_2 exposure (Table 3). Fruit color was less orange and more yellow, circumference was greater, and rind thickness was greater for the SO_2 exposed compared to filtered air trees. These responses indicate that the fruit on the SO_2 trees had secondary changes in morphology and growth due to the reduced number per tree, and subsequently greater availability of reserves per fruit resulting in changes in fruit quality. This also was found for fruit on O_3 exposed trees, as described earlier. Neither fruit acidicity nor sugar content was affected by the SO_2 exposures.

Table 8. Results from Biochemical Analysis of Valencia Orange Leaves-Leaf Chlorophyll and Carotenoid Concentrations (mg g dry weight^{-1})[a]

Treatment	Month			
	October	January	April	July
		Total Chlorophyll		
Outside	2.16 ± 0.43	1.94 ± 0.24	2.13 ± 0.41	2.56 ± 0.55
Ambient Chamber	2.50 ± 0.54	2.64 ± 0.91	3.07 ± 0.81*	2.99 ± 0.33
Half Ambient Chamber	2.07 ± 0.38	2.31 ± 0.66	2.37 ± 0.33	2.96 ± 0.69
Filtered Chamber	2.09 ± 0.54	2.39 ± 0.90	2.00 ± 0.62	2.66 ± 0.58
SO_2 Chamber	2.30 ± 0.45	2.68 ± 0.59	2.33 ± 0.53	2.61 ± 0.64
		Carotenoids		
Outside	0.73 ± 0.10	0.46 ± 0.07	0.24 ± 0.11	0.14 ± 0.06
Ambient Chamber	0.78 ± 0.13	0.53 ± 0.14	0.19 ± 0.11	0.07 ± 0.02*
Half Ambient Chamber	0.68 ± 0.10	0.52 ± 0.12	0.21 ± 0.06	0.07 ± 0.04
Filtered Chamber	0.67 ± 0.12	0.51 ± 0.16	0.20 ± 0.06	0.09 ± 0.08
SO_2 Chamber	0.73 ± 0.12	0.56 ± 0.16	0.20 ± 0.06	0.09 ± 0.05

[a]Values are means ± SD for seven trees, and four leaves 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 chlorophyll for the April sampling at $p<0.05$ according to analysis of variance.

Table 9. Results from Biochemical Analysis of Valencia Orange Leaves-Specific Leaf Area (cm^2 g dry wt^{-1})[a]

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

[a]Values are means ± SD for seven trees, and 20 leaves 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.

Growth. There were no statistically significant differences in fruit drop between the sulfur dioxide and filtered air treatments for either the winter or summer period (Table 5).

Sulfur dioxide consistently resulted in decreased leaf drop (Table 6). The sulfur dioxide treatment had lower total leaf weight than the filtered air treatment for four of the 12 months, and on the seasonal and yearly basis. There also was a significantly higher leaf drop for sulfur dioxide compared to filtered air trees in October, 1986. These results can be interpreted as indicating that sulfur dioxide reduced leaf production and also increased the loss of leaves during the important fall leaf drop period. Thus, both the reduced amount of photosynthetically active leaf material on a whole tree basis, and the earlier senescence of leaves that were present likely contributed to the lower productivity of sulfur dioxide exposed compared to filtered air trees.

Sulfur dioxide had no clear effect on individual leaf weights. There was a significantly lower individual leaf weight for sulfur dioxide compared to filtered air trees in June, 1987; but no significant sulfur dioxide effects either on a monthly, seasonal, or yearly basis.

Physiology. Sulfur dioxide had no general effect on leaf water potential. There was a statistically significant SO_2 effect only on one date, when the less negative potential (less water stress) was for the SO_2 treatment and more negative potential for the filtered air treatment (Figure 25). There were no other SO_2 effects either on a single day, seasonal, or yearly basis.

Sulfur dioxide had no general effect on either stomatal conductance or photosynthesis on a seasonal or yearly basis (Table 7). The biweekly results for stomatal conductance were variable, with an increase with sulfur dioxide exposure on 12/3/86 and 7/14/87, and a decrease on 4/29/87 compared to filtered air.

Biochemistry. There were no statistically significant differences due to SO_2 exposure for any month or parameter. This was true for chlorophyll or carotenoid content (Table 8), specific leaf area (Table 9), or the SO_2 specific indicators of leaf sulfite or thiol content (Table 10).

E. Chamber Effects

Yield and Quality. There was no statistically significant effect of the chambers on yield at the 1987 harvest (Table 2). There was still a 28% lower average yield for the outside compared to ambient chamber trees, However, the variability between outside trees was larger than for chamber trees and some outside trees had yields similar to ambient chamber trees on an individual tree basis. Apparently the outside trees had increased production substantially over the 1986 yield when virtually no fruit were present on them.

The number of fruit was 9% lower and weight/fruit was 21% lower for outside compared to ambient chamber trees, However, these differences were not statistically significant (Table 2). Fruit quality was altered by the chambers for nearly all parameters. Color was slightly more orange, circumference was greater, and rind thickness greater for ambient chamber vs. outside trees (Table 3). Apparently, there was a trend for smaller fruit on the outside trees compared to chambers. This may be at least partially due to the larger amount of canopy available to put reserves into fruit for the chamber vs. outside trees - which resulted in larger fruit in chambers. Chamber fruit also were less acidic than outside fruit (Table 4).

Table 10. Results from Biochemical Analysis of Valencia Orange Leaves-Leaf Thiol and Sulfite Concentrations (μMoles per g dry $wt.^{-1}$)[a]

Treatment	Month			
	October	January	April	July
		Thiols		
Filtered Chamber	1.24 ± 0.42	1.27 ± 0.09	1.35 ± 0.39	1.42 ± 0.34
SO_2 Chamber	1.06 ± 0.30	1.29 ± 0.20	1.46 ± 0.21	1.34 ± 0.14
		Sulfite		
Filtered Chamber	1.39 ± 0.27	1.52 ± 0.31	1.95 ± 0.24	1.62 ± 0.36
SO_2 Chamber	1.57 ± 0.30	1.59 ± 0.31	2.03 ± 0.47	1.62 ± 0.29

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

Growth. There were no statistically significant differences in fruit drop between the ambient chamber and outside air treatments for either the winter or summer period.

The open-top chamber had no effect either on total leaf drop or individual leaf weights, as indicated by the similar values for ambient chamber and outside trees (Table 6). This indicated that the trees in open-top chambers had approximately the same growth of leaves over the past year as outside trees.

Physiology The chambers in general were associated with a more negative leaf water potential, i.e., more water stress than for outside trees. There were statistically significant differences between chamber and outside trees on eight of the 21 measurement dates, during both winter and summer months, and on a yearly basis (Table 6). In all cases the chambers had greater water stress. This was similar to the pattern observed in 1985-86 and indicates that on the basis of water stress, that the chamber trees may actually be less sensitive to O_3 than outside trees. Consequently, this data would suggest that the O_3 concentration-yield loss equation generated in the chambers may underestimate losses compared to actual outside field conditions.

The open-top chambers apparently produced a decrease in leaf stomatal conductance as shown by comparing the results form ambient chamber and outside trees (Table 7). This reduction in conductance in chambers was most evident on the summer and whole year bases. On a biweekly basis there was a significant reduction in conductance only on 5/29/87, and a distinct trend toward reduced conductance only on 4/9 and 9/18/87. The chambers had no effect on photosynthesis either on a seasonal or annual basis. However, there was a statistically significant reduction in photosynthetic rate for ambient chambers compared to outside trees on 2/11/87, and a general trend toward reduced photosynthesis in chambers during the winter months.

Biochemistry. There was a trend toward biochemical differences between ambient chamber and outside trees for all three measured parameters: leaf chlorophyll and carotenoid content (Table 8), and specific leaf area (Table 9). Leaf chlorophyll tended to be higher for the ambient chamber compared to outside trees, with a statistically significant higher chlorophyll content for the ambient chamber trees in April. Leaf carotenoid content tended to higher in ambient chamber compared to outside trees in October and January, and lower in ambient chamber trees in April and July, with the difference statistically significant in October. The higher specific leaf area for ambient chamber trees provided additional information to show that chamber tree leaves are less dense than outside tree leaves, as suggested by the lower weights for individual ambient chamber tree leaves.

F. Applicability of Results to Crop Loss Assessment

The study to date has documented the potential effects of O_3 and SO_2 on Valencia orange trees under the open-top chamber conditions necessary to control the concentrations of pollutants in the air. This was based on one year of data. The SO_2 treatment has produced large differences in yield due to the high SO_2 concentration.

A third harvest year with the ozone treatments would add to the potential of determining yield loss in a year with different environmental conditions and ozone concentrations, as ambient ozone concentrations and air temperatures have been lower than normal so far in 1987. Even if the conditions were similar to the two preceding years, the third harvest data would add greatly to the power of the ozone dose-yield loss equations to estimate potential losses based on different ambient ozone concentrations. A third harvest year is the maximum number of harvests possible for this study given the constraining size of the chambers compared to the tree canopy. With a limited amount of pruning of non-fruit producing shoots, the trees can be contained within the chambers to produce a yield in the spring of 1988.

However, the question remained whether the projected yield decreased from the pollutants accurately represent field growing conditions without chambers. Over three years of weekly to biweekly stomatal conductance data has indicated that the uptake of O_3 may be actually less for chamber vs. outside trees. This would indicate that the outside trees would be more sensitive to pollutants. Hence the projected yield losses based on chambers would be conservative, underestimating losses in the field. In contrast, the specific leaf area data suggested that chamber tree leaves were thinner, and possibly more sensitive to air pollutants than outside tree leaves.

One means of determining whether the sensitivity of chamber and outside trees to air pollutants is the same is by comparing the growth of the trees in the ambient chamber vs. outside treatments. Unfortunately, the outside trees did not grow as fast in the year after planting and produced very few fruit during the first harvest in 1986. During the next year the outside trees began to catch up to the chamber trees. In terms of productivity, the 1987 harvest showed no statistically significant difference in terms of yield, even though the outside trees still tended to have a lower yield than chamber trees. Continuing the experiment to a third harvest in the spring of 1988 would provide the needed information as to whether or not the outside trees do in fact attain a yield comparable to that in chambers. Comparable yields in 1988 would add evidence that after the initial chamber effect was compensated for, ambient chamber trees do indeed have similar susceptibility to air pollutants.

G. Mechanistic Basis for Air Pollution Effects on Orange Yield

The mechanistic basis for 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, as these instantaneous measurements show no general ozone or SO_2 treatment effects. As described by Sinclair (19), 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 years' 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 (3). The amount of flower bud formation, while in great excess to the number of fruit produced, still is an important determinant of the total fruit potentially carried to maturity as the proportion of flowers to fruit se may remain the same with different numbers of flowers. Carbohydrate reserves increase in leaves in late winter prior to flowering (2), and are especially important in determining the subsequent fruit production of the trees (5,7). These reserves and the amount of fruit produced are affected by the amount of crop and time of picking the previous year (4,6).

The interplay of vegetative growth and fruit production is even more complex than the flowering-fruit production relationship. Saurer (18) 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 (19). The relationship between leaf defoliation and fruit production is also very important as defoliation reduces fruit size (9), 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 affect on subsequent flowering and fruit production.

Thus, a number of factors may be involved in the mechanism by which air pollutants affect fruit number: amount and time of leaf defoliation, proportion of leafy to leafless influorescences, amount of flowers, and amount and timing of fruit set. All of these processes are tied to the carbohydrate reserves of the tree. Air pollutants have been shown to affect leaf defoliation both in previous years of this Valencia orange study (15), and in the previous Navel orange study (24). Some data also have been obtained in this study regarding flowering and fruit set. No data has been obtained as of yet regarding leaf starch reserves.

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