CALIFORNIA AIR RESOURCES BOARD
Contract A6-125-32

DOCUMENTATION OF OZONE AS THE PRIMARY PHYTOTOXIC AGENT IN PHOTOCHEMICAL OXIDANT "SMOG"

Final Report

February 23, 1987 - May 23, 1988

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May 1988

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Documentation of ozone as the primary phytotoxic agent in photochemical oxidant "smog"

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ABSTRACT

The Statewide Air Pollution Research Center has a continuing mission to investigate the effects of air pollution on agricultural crops, native vegetation, and forests; and to determine the amount of loss being caused by these pollutants. To further this mission we have conducted the pilot study: Documentation of Ozone as The Primary Phytotoxic Agent in Photochemical Oxidant "Smog". The study evaluated whether equivalent ozone concentrations in ambient oxidant "smog" and added ozone in filtered air produce the same physiological, growth, yield, and injury effects in plants.

The study used three treatments in open-top field chambers: charcoal-filtered air (CF), non-filtered air (NF), and filtered air plus added ozone to equal the ozone concentration in the ambient chambers (O_3). Outside (ambient air, i.e. AA) plots served as controls for the non-filtered chambers to evaluate any chamber effect on the plant response to ozone. The added ozone was dispensed from an ozone generator equipped with dry air or oxygen. Ozone was added according to the same temporal pattern as the ambient ozone via a computer feedback system. Alfalfa was the test plant with two cultivars in the chambers, the ozone susceptible cultivar "Mesa Sirsa", and tolerant cultivar "Eldorado." Physiological measurements for the alfalfa included pigment analysis (chlorophyll and carotenoids) and gas exchange (stomatal conductance and transpiration). Growth, yield, and injury measurements included height, fresh weight, dry weight, and percentage empty nodes per stem.

This study indicated that ozone is the primary agent in phytotoxic effects of photochemical oxidants. This was demonstrated by the similar plant responses in the O_3 and NF treatments including necrotic injury symptoms, enhanced lower leaf senescence, stomatal closure, leaf pigment degradation, and decreased growth and yield. For most parameters there were no statistically significant differences between the O_3 and NF treatments.

However, the study also indicated that in addition to the general similarities in response between the O_3 and NF treatments, detrimental effects were increased with generated ozone compared to ambient ozone. The O_3 treatment resulted in significantly greater leaf injury, and

chlorophyll concentrations, and a distinct trend toward a larger reduction in dry weight than for the NF treatment. There were no differences in stomatal conductance and transpiration between the O_3 and NF treatments.

These results also indicated that use of dry air to generate ozone may overestimate losses due to ambient ozone. Conversely, use of oxygen to generate ozone may underestimate losses due to ambient oxidants as other detrimental oxidants such as nitric acid vapor are not present as they would be in ambient air.

Both the ambient and added ozone treatments indicated that ambient oxidants produced yield and growth reductions, leaf injury, stomatal closure, and lower leaf pigment concentrations compared to CF air as seen in previous studies. Significant chamber effects on growth and yield also were observed, with less intense yield, growth, and injury effects from ozone in NF air than in ambient air (outside) plots.

This information will aid in the interpretation on past and current controlled studies where ozone is added to chambers to simulate different ambient oxidant concentrations, and studies where ozone in ambient air was assumed to be the phytotoxic air pollutant in the photochemical "smog" complex.

ACKNOWLEDGMENTS

The author thanks the following staff of the Statewide Air Pollution Research Center for their efforts in this project: Dr. Brent Takemoto and Ms. Wendy Hutton for physiological measurements; Dr. Robert Musselman for use of the LI-COR 1600 steady-state porometer; Mr. Gerrit Kats, Mr. Phil Dawson, Mr. Lynn Morrison, Mr. Guillermo Torres-Jardon, Mr. Adam Johnson, Ms. Joanne Wolf, and Mr. Ian Gocka for many types of technical assistance; and Ms. Chris LaClaire and Ms. Barbara Crocker for word processing assistance. Special thanks go to Dr. Homero Cabrera, Manager of this Project for the Air Resources Board Staff for his advice and encouragement.

DISCLAIMER

The statements and conclusions in this report are those of the University and not necessarily those of the State 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.

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SUMMARY AND CONCLUSIONS

California is the number one agricultural state in the country with over 50 crops with a total value of over $10 billion in 1984. California also has some of the most severe air pollution conditions in the United States, with areas both in the South Coast Air Basin, Central Valley, and other areas affected. Photochemical "smog" is by far the most important air pollution problem in the state in terms of vegetation, and ozone has been assumed to be the primary phytotoxic gas in the "smog" complex.

Studies to determine the effects of photochemical "smog" on vegetation generally have used one of three exposure protocols:

1) Exposure of plants to filtered or some percentage of non-filtered "ambient" air,
2) exposure to filtered or filtered plus some level of added ozone in the air, and
3) a hybrid of 1) and 2).

With all three protocols, ozone is considered to be the primary phytotoxic gas, and often is the only pollutant measured during the exposures, even if ambient air is used. Plant responses are then related to the ozone concentrations during the exposures. For field crop loss studies the ozone concentrations in the different treatments are used to generate an ozone dose-plant yield response equation. Dose-response studies such as this have formed the basis for recent crop studies in California including Air Resources Board sponsored research with alfalfa, grapes, and oranges; USEPA National Crop Loss Assessment Network (NCLAN) sponsored research with cotton, tomatoes, and lettuce; and California Department of Food and Agriculture sponsored research with many other crops. The crop dose-response studies have then formed the basis for economic analyses of the effects of ambient ozone concentrations on crops both in the South Coast Air Basin, the San Joaquin Valley, and other areas of the state.

Central to the analysis of all the field studies and economic loss projects is the hypothesis that ozone is the primary phytotoxic agent in photochemical "smog". Past evidence for the involvement of ozone includes

a large array of field, greenhouse, controlled environment, and laboratory studies which described the phytotoxic effects of ozone on plant injury, growth, yield, physiology, and biochemistry. The studies definitely documented that ozone at ambient concentrations was harmful to plants, and that the types of responses with ozone were similar to those found with ambient "smog" in the field. Ozone had the highest concentrations of all the measured pollutants in "smog". Thus, by inference, ozone has been described as the main phytotoxic agent in smog, and ozone exposures have been considered to be totally equivalent to exposures to ambient air.

However, few studies have actually included field exposures to both ambient oxidants and added ozone in filtered air. Thompson and colleagues sought to identify the phytotoxic component of ambient air pollution in a series of field studies with navel orange trees. A variety of pollutants including ozone, peroxyacetyl nitrate (PAN), hydrogen fluoride and nitrogen dioxide were added to filtered air at concentrations representing those in ambient outside air. Ambient air also was added to chambers to detect plant responses to the complex of pollutants in smog. Only the added ozone produced the reduction in fruit yield, increased leaf drop, and other detrimental effects also found on trees growing in ambient air and in outside air. Thus, by inference, ozone was reported as the primary phytotoxic agent at ambient levels. However, the extent of yield reduction was greater with ambient "smog" than with added ozone indicating that the complex of pollutants in ambient air either possibly was more toxic than ozone alone, or contained unknown pollutants which are phytotoxic at low levels.

Thus, there has been distinct need to specifically determine whether plant responses to photochemical oxidant "smog" are in fact equivalent to responses to ozone alone. Until recently, it has been very difficult to fully replicate the concentrations and diurnal pattern of occurrence of ambient ozone in an added ozone treatment in the field. However, with current computer control of exposures it is now possible to accurately replicate a real-time ambient exposure in a filtered air chamber. It is recognized that even filtered air chambers do contain a low level of ambient "smog" (10-20%), however, these "background" pollutants should have minimal effects on plant responses compared to the high added ozone concentration.

The primary objective of this study was to document plant response to ambient ozone vs. added generated ozone conditions.

Secondary objectives were:

1. To demonstrate the feasibility of using a computer feedback system to add ozone to the ARB chamber facility at UCR.
2. To document the efficiency of open-top field chambers in removing air pollutants other than ozone from the air.

Fulfillment of these objectives was intended to result in increased understanding of the role of ozone in injury to plants from the complexity of pollutants known as photochemical "smog". This understanding would aid in the interpretation of past ozone-plant response research, and assist in design of new studies. This study also would indicate the appropriateness of using ambient ozone data to describe plant response to ambient air in reconstructing ozone dose-plant response equations from past experiments.

The general approach to address the primary objective focused on exposing plants in open-top chambers to three treatments: charcoal filtered air (CF), nonfiltered air (NF), charcoal filtered with added ozone (O_3). There also were ambient air control plots (AA). Comparison of NF and O_3 treatments showed the effects of ambient ozone in nonfiltered air as compared to generated ozone in filtered air, where the concentrations of ozone were the same in both treatments. Comparison of NF and CF treatments showed the effects of charcoal filtered air without added pollutants for determination of the general effect of ozone on plant responses. Comparison of NF and AA treatments showed the "chamber effect" on plant responses. Standard exposure protocols common for many field studies were used: i.e., open-top field chambers of the standard NCLAN design. To address secondary objective #1, ozone was generated from either dry air or oxygen with proportional control of the added ozone concentrations. To address secondary objective #2 all major pollutants, i.e. ozone, nitric acid, nitrogen dioxide, and peroxyacetyl nitrate, as well as sulfur dioxide were monitored for all treatments.

The test species was alfalfa (Medicago sativa) which responds to ozone with a characteristic loss of lower leaves, yield reduction, and decrease in height. Two cultivars of alfalfa were used, one ozone susceptible and the other ozone tolerant in order to help verify that the plant responses were indeed associated with the pollutant treatments.

There were five harvests which represented a form of replication of the entire experiment over time. In addition to standard injury, growth, and yield parameters; gas exchange characteristics (stomatal conductance and transpiration) and pigment concentrations (chlorophylls and carotenoids) were measured in order to help elucidate the physiological mechanism for the pollutant effects.

Conclusions

1. These results indicated that ozone is the primary agent in phytotoxic effects of photochemical oxidants. This was demonstrated by the similar plant responses in the O_3 and NF treatments including necrotic injury symptoms, enhanced lower leaf senescence, and decreased growth and yield.

2. Adding ozone to filtered air produced more detrimental effects to vegetation than ambient ozone; significantly greater leaf injury and causing a distinct trend toward a larger reduction in dry weight. This indicated that the procedure used to generate ozone and type of air to which it is added are very important for producing the right type of pollutant mixture characteristic of oxidants.

3. The cause of the increased ozone injury in the O_3 treatment is open to question but may involve the enhancement of ozone effects through addition of pollutants, such as HNO_3 when dry air is used in the generator. Use of oxygen in the generator, (with subsequent lack of added oxidants) did not result in increased injury.

4. A computer-feedback system worked well to control ozone addition to the chambers to maintain the same concentration in ambient air. The system ran continuously for approximately five months and could add ozone even with very low ambient concentrations (<0.02 ppm).

5. The charcoal filtered open-top field chambers were effective in removing ozone (~70% removed vs. outside), nitrogen dioxide (65%), and peroxyacetyl nitrate (90%). The filters did not remove nitric oxide; in contast the concentration of nitric oxide actually was higher in filtered chambers than ambient air.

RECOMMENDATIONS

1. Conduct a larger study to determine the air quality basis for the increased effects of ozone in the filtered air + ozone (O_3) compared to ambient ozone (NF) treatment. The study would include treatments designed to test the following hypotheses:
   (a) Nitric acid vapor (HNO_3) produced from the ozone generator was responsible for the increased injury effects in the O_3 treatment. This would be tested by comparing the O_3 (generated from dry air) treatment with the NF treatment, while monitoring the air for nitric acid in both treatments. If possible a series of nitric acid vapor concentrations in CF air would be included to test the toxicity of this compound.
   (a) Ozone alone was responsible for the increased effects in the O_3 treatment. This would be tested by comparing O_3 (generated from oxygen) and NF treatments.
   (b) Nitrogen dioxide protects plants from injury in the AA treatment. This would be tested by comparing a treatment with O_3 plus added NO_2 (equal to the concentration in the NF treatment) , and the NF treatment.
2. Any additional experiment should use the following modifications in methodology to better detect treatment differences:
   (a) Use ozone purposely generated from oxygen or dry air to have a known potential for production of oxidants other than ozone.
   (b) Use clonal alfalfa plant material or other species which is genetically uniform in order to increase the power of the statistical tests to determine treatment differences.
   (c) Conduct the study in the warmest summer months to minimize chamber effects on plant response.
3. Couple the plant study with additional air quality measurements to determine if unusual oxidants are present, i.e. nitric acid, present in the NF treatment vs. O_3 (generated from dry air) treatment. These measurements could be based on continuous or periodic automatic monitoring, or spot sampling using bag sampling for wet chemical or other measurements in a laboratory.

I. INTRODUCTION

California is the number one agricultural state in the country with over 50 crops with a total value of over $10 billion in 1984 (CDFA 1985). California also has some of the most severe air pollution conditions in the United States, with areas both in the South Coast Air Basin, Central Valley, and other areas affected. Photochemical "smog" is by far the most important air pollution problem in the state in terms of vegetation, and ozone has been assumed to be the primary phytotoxic gas in the "smog" complex (Haagen-Smit et al. 1952, Heath 1975).

Studies to determine the effects of photochemical "smog" on vegetation generally have used one of three exposure protocols:

1) Exposure of plants to filtered or some percentage of non-filtered "ambient" air,
2) exposure to filtered or filtered plus some level of added ozone in the air, and
3) a hybrid of 1) and 2).

With all three protocols ozone is considered to be the primary phytotoxic gas, and usually is the only pollutant measured during the exposures, even if ambient air is used. Plant responses are then related to the ozone concentrations during the exposures. For field crop loss studies the ozone concentrations in the different treatments are used to generate an ozone dose-plant yield response equation. Dose-response studies such as this have formed the basis for recent crop studies in California including Air Resources Board sponsored research with alfalfa (Brewer 1982), grapes (Brewer 1983), and oranges (Kats et al. 1985); USEPA National Crop Loss Assessment Network (NCLAN) sponsored research with cotton (Temple et al. 1985a), tomatoes (Temple et al. 1985b), and lettuce (Temple et al. 1986); and California Department of Food and Agriculture sponsored research with many other crops (McCool et al. 1986). The crop dose-response studies have then formed the basis for economic analyses of the effects of ambient ozone concentrations on crops both in the South Coast Air Basin (Adams et al. 1982, Leung et al. 1982), the San Joaquin Valley (Rowe and Chestnut 1985), and other areas of the state (Howitt et al. 1984).

Central to the analysis of all the field studies and economic loss projects is the hypothesis that ozone is the primary phytotoxic agent in photochemical "smog". Past evidence for the involvement of ozone includes a large array of field, greenhouse, controlled environment, and laboratory studies which described the phytotoxic effects of ozone on plant injury, growth, yield, physiology, and biochemistry (USDHEW 1970, USEPA 1978). The studies definitely documented that ozone at ambient concentrations was harmful to plants, and that the types of responses with ozone were similar to those found with ambient "smog" in the field. Ozone had the highest concentrations of all the measured pollutants in "smog". Thus, by inference, ozone has been described as the main phytotoxic agent in smog, and ozone exposures have been considered to be totally equivalent to exposures to ambient air.

However, few studies have actually included field exposures to both ambient oxidants and added ozone in filtered air. Thompson and colleagues sought to identify the phytotoxic component of ambient air pollution in a series of field studies with navel orange trees (Thompson and Taylor 1969, Thompson et al. 1971, Thompson et al. 1972). A variety of pollutants including ozone, peroxyacetyl nitrate (PAN), hydrogen fluoride and nitrogen dioxide were added to filtered air at concentrations representing those in ambient outside air. Ambient air also was added to chambers to detect plant responses to the complex of pollutants in smog. Only the added ozone produced the reduction in fruit yield, increased leaf drop, and other detrimental effects also found on trees growing in ambient air and in outside air. Thus, by inference, ozone was reported as the primary phytotoxic agent at ambient levels. However, the extent of yield reduction was greater with ambient "smog" than with added ozone indicating that the complex of pollutants in ambient air either was more toxic than ozone alone, or contained unknown pollutants which are phytotoxic at low levels (Thompson et al. 1972).

The navel orange studies used exposures to constant levels of ozone and the other pollutants over the day. Recent research indicates that varying concentrations of ozone in exposures are more injurious to plants than continuous, i.e., "square wave" exposures (Musselman et al. 1983). Thus, the greater orange yield reduction in ambient air may have been due to naturally varying ozone concentrations over the day. Current NCLAN

studies in California have been designed to add ozone to background ambient ozone (Temple et al. 1985a), and do not include filtered plus added high ozone treatments. Most NCLAN ozone treatments therefore, also include "background" ambient concentrations of other components of photochemical smog.

Thus, there has been a distinct need to specifically determine whether plant responses to photochemical oxidant "smog" are in fact equivalent to responses to ozone alone. Until recently, it has been very difficult to fully replicate the concentrations and diurnal pattern of occurrence of ambient ozone in an added ozone treatment in the field. However, with current computer control of exposures it is now possible to accurately replicate a real-time ambient exposure in a filtered air chamber. It is recognized that even filtered air chambers do contain a low level of ambient "smog" (10-20%), however, these "background" pollutants should have minimal effects on plant responses compared to the high added ozone concentration.

A. Project Objectives

The primary objective of this study was to document plant response to ambient ozone vs. added generated ozone conditions.

Secondary objectives were:

1. To demonstrate the feasibility of using a computer feedback system to add ozone to the ARB chamber facility at UCR.
2. To document the efficiency of open-top field chambers in removing air pollutants other than ozone from the air.

Fulfillment of these objectives was intended to result in increased understanding of the role of ozone in injury to plants from the complexity of pollutants known as photochemical "smog". This understanding would aid in the interpretation of past ozone-plant response research, and assist in design of new studies. This study also would indicate the appropriateness of using ambient ozone data to describe plant response to ambient air in reconstructing ozone dose-plant response equations from past experiments.

B. General Approach

The general approach for this study focused on exposing plants in open-top chambers to either ambient ozone in non-filtered air as compared to generated ozone in filtered air, where the concentrations of ozone were the same in both treatments. Two other treatments were included: charcoal-filtered air without added pollutants for determination of the general effect of ozone on plant responses, and outside plots for comparison with the non-filtered chambers for determination of the "chamber effect" on plant responses. Standard exposure protocols common for many field studies were used: i.e., open-top field chambers of the standard NCLAN design, ozone generated from either dry air or oxygen, and proportional control of the added ozone concentration. The test species was alfalfa (Medicago sativa) which responds to ozone either with a characteristic loss of lower leaves, yield reduction, and decrease in height. Two cultivars of alfalfa were used, one ozone susceptible and the other ozone tolerant in order to help verify that the plant responses were indeed associated with the pollutant treatments. There were five harvests which represented a form of replication of the entire experiment over time. In addition to standard injury, growth, and yield parameters; gas exchange characteristics (stomatal conductance and transpiration) and pigment concentrations (chlorophylls and carotenoids) were measured in order to help elucidate the physiological mechanism for the pollutant effects.

II. METHODS

A. Chamber Maintenance

The study was conducted in the ARB open-top field chamber facilities at the University of California, Riverside. The 3.0 m diameter x 2.4 m high chambers have the capability to control the atmosphere around the plant canopy with minimal modification of the environment during summer exposures (Olszyk et al. 1986a). Nine chambers were used for this study; three with each pollutant treatment. There also were three outside non-chamber control plots. The chamber walls were routinely cleaned, blowers checked, and soil weeded under tasks provided for in the ARB Maintenance contract.

B. Ozone and Oxidant Exposures

The three pollutant treatments were as follows: 1) charcoal-filtered air (CF), 2) non-filtered air with ambient pollutants (NF), and 3) filtered air plus added ozone to equal the concentration in the ambient chambers (O_3) (Figure 1). In addition, there was a fourth outside plot treatment with ambient air (AA).

Ozone was generated with an electric arc, (Griffith® ozonator) and delivered to the chambers via underground teflon tubing. Production was controlled by altering the current to ozone generator according to an electronic feedback signal from a Cyborg ISAAC® data acquisition and control interface connected to an Apple IIe computer. The signal increased or decreased the ozone generation depending on the ozone concentration monitored in the ambient chamber via a Dasibi ozone analyzer and the interface/computer system. Thus, the ozone that was provided to the added ozone chamber was continuously changed to trace the concentration in the ambient chamber. The proportional control tended to overshoot the desired ozone concentration early in the study, but after continuous readjustment delivered the desired concentration by the initiation of the second harvest.

The proposal originally called for use of oxygen to generate the ozone. However, we decided to use dry air at least for the initial part of the study because many past field chamber studies and large scale open-air release system studies used dry air to generate ozone. We decided to use dry air as it was difficult to supply an adequate amount of pure

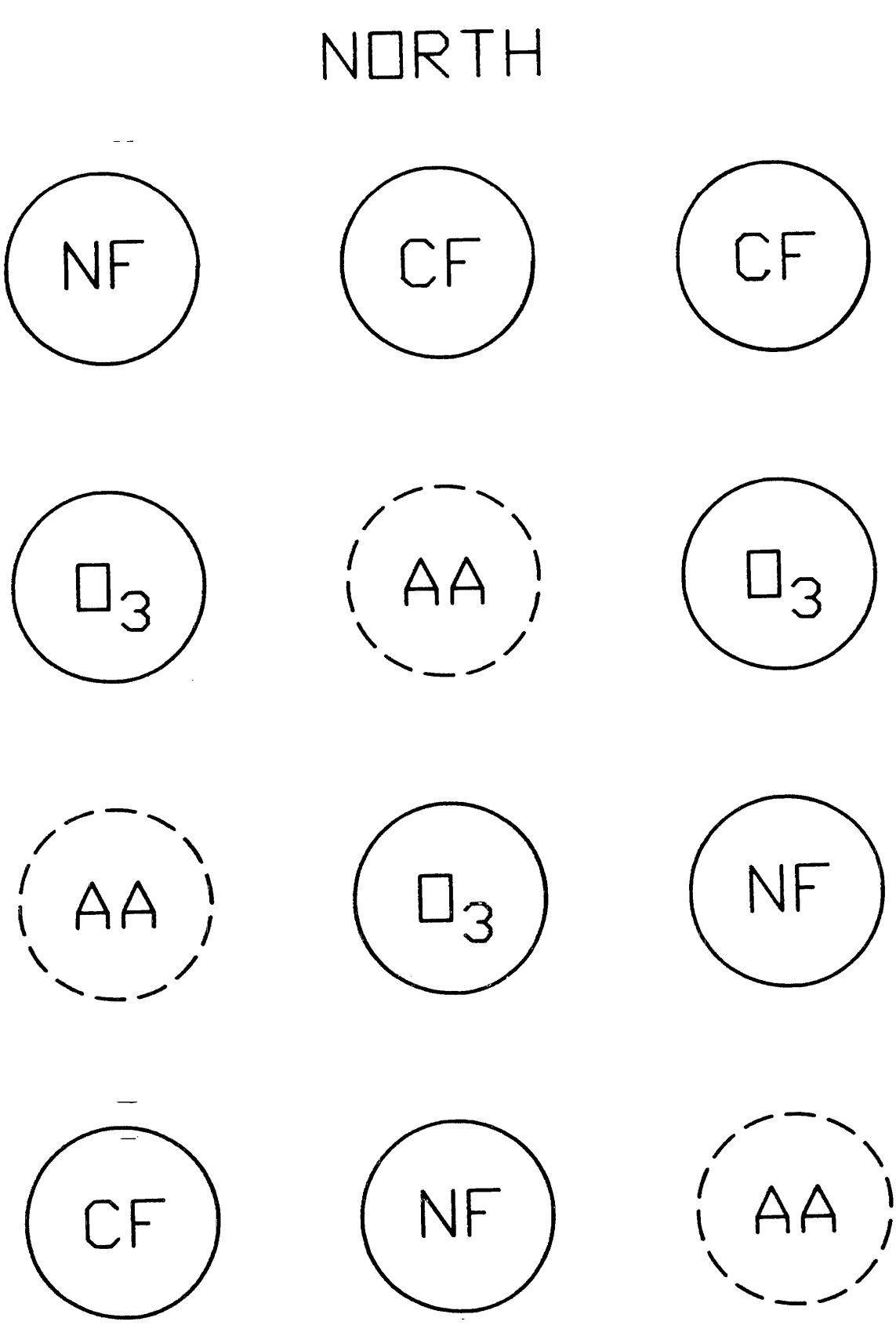

Figure 1. Plot diagram for oxidant study.

oxygen for the long-term, 24 hour per day exposures in this study where ozone had to be added at high concentrations to filtered air. Past studies which used oxygen such as some of the NCLAN studies added ozone into non-filtered air, a process which required less ozone. Furthermore, while it was possible that trace pollutants such as nitrogen dioxide or nitric oxide could be produced from dry air, we did not think that this would be a problem as the amounts would be relatively small. In addition, both gases were to be measured routinely and, thus, we would be aware of any problems and could switch to oxygen if necessary.

After the first harvest we noticed that there appeared to be dramatically greater effects from the generated ozone than from ambient ozone. Thus, the protocol of using dry air to generate ozone was continued for three more harvests to verify this response and to provide more data as to whether nitrogen oxide pollutants were produced by the generator. This information was especially critical as we were considering use of dry air as the only practical means of generating ozone in large scale studies such as for seedling or mature trees at remote sites. However, we made plans for a fifth harvest where pure oxygen would be used to generate ozone in order to help evaluate whether trace compounds could be at least theoretically associated with the increased injury in the O_3 treatment.

The chambers were on for 24 hours per day to insure that pollutant levels was controlled continuously in the chambers. The exposures were over a period of five months from approximately 6/10/87 through 11/13/87. This period included high ambient ozone concentrations for the Riverside area. During the study the following pollutants in addition to ozone were measured in both filtered and ambient chambers: sulfur dioxide (SO_2) with a Teco Inc. pulsed fluorescent analyzer, nitrogen dioxide/nitric oxide (NO_2/NO) with a Monitor Labs chemiluminescent analyzer, and peroxyacetyl nitrate (PAN) with a gas chromatograph. These pollutants were measured to determine the efficiency of open-top chambers in removing gases other than ozone from the air.

The CF and NF conditions began on 6/10/87 when the plants were first put in the chambers. At this time the plants had some alfalfa growth which was cut and discarded on approximately 6/25/87. The first exposure period began on 6/26/87 and continued until the first harvest on 7/16/87. The second exposure was 7/17/87 until 8/6/87. The third 8/7/87 until 9/3/87

and the fourth 9/4/87 until the final harvest on 10/1-2/87. The exposure period began on 10/3/87. Use of pure oxygen in the system did not begin until 10/8/87, however, it was not believed that this short five day lag had much affect on plant response as few new shoots had grown back during this time. The fifth exposure period lasted until harvest which began on 11/10-13/87.

Environmental parameters were routinely monitored during the study to determine the conditions which might encourage differential removal of the gases by the chambers. The environmental parameters would also indicate whether the plants are sensitive to air pollutants during any particular period of time. The environmental conditions include quantum (light) intensity, air temperature, and relative humidity.

C. Plant Culture

Alfalfa (Medicago sativa) was the primary test species for this study. Alfalfa was useful because it has been shown to be sensitive to photochemical smog both in the South Coast Air Basin (Olszyk et al. 1986b), and San Joaquin Valley (Brewer 1982). Two cultivars of alfalfa used were 'Mesa Sirsa' which is susceptible to ambient oxidants, and 'Eldorado' which is relatively tolerant based on previous research (Thompson et al., 1976). The plants were transplanted to 3.8 L paper pulp pots in the field and allowed to become established over three months prior to placement in the chambers. Clonal alfalfa was not used due to difficulty in obtaining enough shoots and the short time period between implemation of the contract and start of the exposures. Enough plants were established of each cultivar for 10 pots per chamber or outside plot (120 pots). An initial harvest was made in June for the plants, and than the pots were placed within plastic liners in the soil in the chambers. The plants were maintained in the chambers for five exposure harvests over approximately five months.

The final harvest was made in mid-November when the plants were beginning to flower. The weather had been cool and wet and it was anticipated that ambient oxidant concentrations would be low for the remainder of the fall and winter. Four days were needed for the harvest due to the size of the plants and lodging that was occurring in some treatments. However, the plants were harvested by block and cultivar so that any

harvest date effects would be evenly distributed over the replicate plants.

On July 30, 1987, seeds of pinto beans (Phaseolus vulgaris) were planted in order to provide a second species for study. Pinto beans are a good bioindicator of ozone stress exhibiting a characteristic oxidant stipple, especially on the unifoliates which are the first leaves to emerge from the plants. The bean plants were transplanted to 3.8 L paper pulp pots on August 4, 1987, which transfered to the chambers on August 5, 1987. There were five pots per plot. The bean plants were harvested at the time of the fourth alfalfa harvest on October 6, 1987. This resulted in a total 62 days of exposure corresponding approximately to the third and fourth alfalfa harvests.

D. Plant Measurements

1. Physiology

Stomatal conductance and transpiration were measured as an indicator of ozone stress on plant gas exchange. Measurements were made using the LI-COR LI 1600 steady state porometer, on three plants per cultivar

Pigment concentrations in leaf tissue was measured before each harvest as an indicator of ozone induced leaf senescence. The pigments were extracted by a modified version of the ethanol extraction method of Knudson et al. (1977) using a Beckman DB spectrophotometer. Results were expressed on a unit of mg pigment per g dry weight basis. Five leaves were taken per chamber, per cultivar, for pigment analysis. Each leaf was from the fourth or fifth node of one stem from an individual plant. The leaf at this node was at a critical stage of development, where senescence was just beginning to develop on a stem. Lower leaves were already turning yellow and senescing in the polluted treatments, higher leaves were still green. Each leaf was measured independently for pigment concentrations, with two absorbence readings taken per leaf. These two readings were averaged to determine the pigment concentrations.

Physiological measurements made either the day before or on the day of harvest for growth and yield measurements. Physiological and biochemical measurements were not made for the fifth harvest as the personnel were not available and the cool, cloudy conditions reduced the likelihood of finding ozone effects on any parameter. The fifth harvest was extra

test the hypothesis that use of oxygen to generate ozone would produce differences in nitrogen oxide concentrations in the chambers.

2. Growth, Yield, and Injury

Plant growth was determined with height and number of node measurements. Yield was determined as fresh weight, dry weight, and % dry weight [(dry/fresh) x 100]. Injury was determined as defoliation by measuring the total number of nodes and number of empty nodes per plant, to indicate percentage empty nodes [(empty/total) x 100]. Growth, yield, and injury measurements were made at each harvest. Plants were cut off approximately 0.02 m above the crown to avoid damage to new shoots. Total fresh weight was measured immediately, total dry weight was measured after drying in ovens for several weeks. Three stems per plant were taken as subsamples to determine length of stems (height of plant), number of nodes, and number of empty nodes with a node counted as empty if the leaf was missing or chlorotic. The number of empty nodes divided by total nodes indicated leaf injury as senescence.

E. Statistical Analysis

A completely randomized experimental design was used as described by Steel and Torrie (1960). Comparison between treatment means for all parameters was by analysis of variance. Each harvest's data was analyzed independently, and the results of the first four harvests also were analyzed together. There were four treatments: charcoal filtered (CF), filtered plus added ozone (O_3), and non-filtered chambers (NF); and outside (ambient) plots (A). There were three replicate chambers or outside plots per treatment as the experimental unit (considered in Error a). Two cultivars also were included in the experiment as an independent factor. The analysis of variance table considering only treatment means at each harvest is shown below. The approximately 10 pots per cultivar in each chamber or outside plot were considered to be a separate sampling error. All pots of both cultivars were randomly located in each chamber.

Source	df
Air Pollutant	3
Contrast Between NF and CF	(1)
Contrast Between NF and O_3	(1)
Contrast Between NF and AA	(1)
Error a	8
Cultivar	1
Cultivar x Air Pollutant	3
Error b	218
Total	233

Additional analysis of yield and physiology data included harvest and harvest x air pollutant interaction terms. Only the first four harvests were included in the analysis, as the fifth harvest period used oxygen as a source of ozone and had cooler, more overcast weather conditions than the other harvests. The analysis of variance table considering harvest date is shown below.

Source	df
Air Pollutant	3
Contrast Between NF and CF	(1)
Contrast Between NF and O_3	(1)
Contrast Between NF and AA	(1)
Error a	8
Cultivar	1
Cultivar x Air	3
Error b	704
Harvest	3
Harvest x Air	9
Error c	24
Harvest x Cultivar	3
Harvest x Cultivar x Air	9
Error d	2084
Total	2851

III. RESULTS

A. Air Quality

Tables 1, 2, and 3 indicates the average hourly concentrations for ozone, sulfur dioxide, nitric oxide, and nitrogen dioxide for the five exposure periods. Data were averaged (± SD) across the three replicate chambers or outside plots. Data were averaged both for the 12-hour daylight period (0800-2000 PST) and for the entire 24-hour period as the treatments were continuous.

1. Ozone

In general the ozone proportional controller worked quite well in insuring the same ozone concentration for the filtered air + O_3 as for ambient chamber treatments. For the first exposure period the average ozone concentration in filtered + O_3 was 110% and 107% of ambient chambers, for 24 hours and 12 hours, respectively. This slightly higher ozone concentration in the filtered + O_3 was due to overshooting of the concentration by the proportional controller early in the period. For the second exposure period the average ozone concentration in filtered + O_3 was 100% and 99% of ambient chambers, for 24 hours and 12 hours, respectively. The results from the third through fifth harvests were similar. This is as exactly as predicted for the proportional controller.

Of particular interest was the relative filtering efficiency of the activated charcoal filter for the different pollutants. Table 4 indicates the percentage filtration for the filtered chambers vs. outside air. The filtering efficiency for ozone was approximately 70% (range of 67 to 78%) for filtered chambers vs. ambient air (outside) plots, which was similar to the 70 to 80% reported from other studies (Heck et al. 1982, Olszyk et al. 1986a).

Table 1. Average Hourly Concentrations of Air Pollutants for the Oxidant Study in ppb for the First and Second Harvests

Pollutant	Time	Treatment			
		CF	O_3	NF	AA
		First Harvest (6/26 - 7/16/87)			
O_3	12 HR[b]	21 ± 2	77 ± 3	72 ± 2	77 ± 1
	24 HR	13 ± 2	46 ± 2	42 ± 1	45 ± 1
SO_2	12 HR	1 ± 0[d]	1 ± 0	1 ± 0	1 ± 0
	24 HR	1 ± 0	1 ± 0	1 ± 0	1 ± 0
NO	12 HR	9 ± 0	7 ± 1	6 ± 0	7 ± 1
	24 HR	13 ± 2	13 ± 0	12 ± 1	13 ± 0
NO_2	12 HR	12 ± 0	14 ± 1	25 ± 1	25 ± 1
	24 HR	11 ± 1	12 ± 1	28 ± 0	28 ± 1
PAN[c]	12 HR	0.4 ± 0.6	0.4 ± 0.6	1.3 ± 1.4	1.3 ± 1.3
	24 HR	0.2 ± 0.5	0.2 ± 0.5	0.7 ± 1.2	0.7 ± 1.2
		Second Harvest (7/17 - 8/6/87)			
O_3	12 HR	22 ± 2	81 ± 9	82 ± 2	89 ± 2
	24 HR	13 ± 2	48 ± 5	48 ± 1	51 ± 1
SO_2	12 HR	0[e]	0	0	0
	24 HR	0	0	0	0
NO	12 HR	19 ± 1	19 ± 1	11 ± 6	11 ± 6
	24 HR	41 ± 1	39 ± 1	38 ± 0	38 ± 1
NO_2	12 HR	14 ± 2	19 ± 1	33 ± 1	33 ± 1
	24 HR	13 ± 2	15 ± 1	40 ± 6	41 ± 1
PAN	12 HR	0.3 ± 0.7	0.3 ± 0.5	2.2 ± 1.7	2.2 ± 1.7
	24 HR	0.2 ± 0.5	0.2 ± 0.4	1.2 ± 1.6	1.2 ± 1.6

[a]Values are means ± SD for three replicate chambers or outside plots.
[b]0800-2000 PST.
[c]PAN monitoring only during last six days of study.
[d]Standard deviation less than 0.05.
[e]Not detected.

Table 2. Average Hourly Concentrations of Air Pollutants for the Oxidant Study in ppb for the Third Harvest[a]

Pollutant	Time	Treatment			
		CF	O_3	NF	AA
		Third Harvest (8/7-9/3/87)			
O_3	12 HR[b]	21 ± 3	78 ± 5	74 ± 3	79 ± 1
	24 HR	13 ± 2	46 ± 3	42 ± 1	45 ± 1
SO_2	12 HR	0	0	0	0
	24 HR	0	0	0	0
NO	12 HR	14 ± 1	10 ± 1	9 ± 1	8 ± 1
	24 HR	27 ± 2	24 ± 1	24 ± 0	24 ± 1
NO_2	12 HR	13 ± 5	25 ± 1	41 ± 1	42 ± 2
	24 HR	15 ± 0	19 ± 1	45 ± 1	46 ± 1
PAN	12 HR	0.2 ± 0.5	0.3 ± 0.6	2.0 ± 1.7	2.0 ± 1.7
	24 HR	0.1 ± 0.4	0.1 ± 0.5	1.1 ± 1.5	1.1 ± 1.5

[a]Values are means ± SD for three replicate chambers or outside plots.
[b]0800-2000 PST.

Table 3. Average Hourly Concentrations of Air Pollutants for the Oxidant Study in ppb for the Fourth and Fifth Harvests [a]

Pollutant	Time	Treatment			
		CF	O_3	NF	AA
		Fourth Harvest (9/4-10/1/87)			
O_3	12 HR[b]	17 ± 2	82 ± 3	75 ± 3	78 ± 1
	24 HR	12 ± 1	48 ± 2	44 ± 2	45 ± 1
SO_2	12 HR	0	0	0	0
	24 HR	0	0	0	0
NO	12 HR	18 ± 1	10 ± 1	8 ± 1	9 ± 1
	24 HR	44 ± 1	39 ± 1	39 ± 1	40 ± 2
NO_2	12 HR	15 ± 1	26 ± 2	42 ± 1	42 ± 2
	24 HR	13 ± 1	20 ± 2	45 ± 1	44 ± 1
PAN	12 HR	0.1 ± 0.4	0.2 ± 0.5	2.0 ± 2.0	2.0 ± 2.0
	24 HR	0.1 ± 0.3	0.1 ± 0.4	1.2 ± 1.8	1.2 ± 1.7
		Fifth Harvest (10/2-11/10/87)			
O_3	12 HR	10 ± 1	39 ± 1	38 ± 1	38 ± 2
	24 HR	8 ± 0	25 ± 1	24 ± 0	24 ± 1
SO_2	12 HR	0	0	0	0
	24 HR	0	0	0	0
NO	12 HR	38 ± 2	32 ± 2	28 ± 1	29 ± 1
	24 HR	68 ± 2	65 ± 2	62 ± 0	63 ± 2
NO_2	12 HR	17 ± 1	24 ± 3	47 ± 1	46 ± 2
	24 HR	15 ± 1	20 ± 2	45 ± 0	44 ± 2
PAN	12 HR	0.1 ± 0.3	0.1 ± 0.4	1.1 ± 1.8	1.0 ± 1.6
	24 HR	0.1 ± 0.3	0.1 ± 0.3	0.7 ± 1.4	0.7 ± 1.3

[a] Values are means ± SD for three replicate chambers or outside plots.
[b] 0800-2000 PST.

Table 4. Filtering Efficiency of Open-Top Field Chambers for Different Pollutants as Indicated by Difference Between Filtered Chambers and Outside Plots[a]

Period	Time	Filtering Efficiency (%) for O_3	NO	NO_2	PAN
First	12 HR	73	+29[b]	52	69
	24 HR	71	0	61	71
Second	12 HR	75	+73	58	86
	24 HR	75	+8	68	83
Third	12 HR	73	+75	69	90
	24 HR	71	+13	67	91
Fourth	12 HR	78	+100	64	95
	24 HR	73	+10	71	92
Fifth	12 HR	74	+31	63	90
	24 HR	67	+7	66	86

[a]Based on means for three chambers and outside plots.
[b]Values preceded by a '+' indicate that the filtered chamber value actually was this percentage higher than outside plots.

2. Nitrogen Dioxide

Approximately 65% of the ambient nitrogen dioxide was removed from the air by charcoal filtration (range of 58 to 71%), indicating that the removal was nearly as efficient as for ozone (Table 4). The filtering efficiency for NO_2 had not been routinely reported for open-top chambers, but had been expected to be similar to that for ozone. The reductions in nitrogen dioxide and nitric oxide concentrations are similar to those recently reported for open-top chambers in the proceedings of a workshop held for the European Community (CEC, 1987), which reported nitrogen dioxide reductions (vs. ambient air) of 50-100% for seven types of chambers. Thus, the reduction reported in this study was in the range found in Europe. Nitric oxide reductions were estimated at 0-22% for six types of chambers, which also was similar to the general lack of removal by filtered chambers observed in our study.

The nitrogen oxide concentrations found in this study were apparently high compared to those in agricultural areas of the state. It is difficult to compare the harvest period averages shown in Tables 2-4 with hourly peak or annual mean values as reported to the ARB from ambient air monitoring stations. Thus, a comparison of published data for Riverside (Rubidoux station) with agricultural stations would better indicate the relative nitrogen dioxide concentrations in the different areas. For example, in 1985 the maximum hourly peak value for nitrogen dioxide in Riverside was 169 ppb compared to 43 ppb at Fresno (Herndon site), 42 ppb at Visalia, and 62 ppb at Bakersfield (Oildale site). The annual average nitrogen dioxide concentrations were somewhat closer at the sites at 35 ppb for Riverside, 17 ppb for Fresno, 22 for Visalia, and 26 ppb for Bakersfield.

3. Nitric Oxide

The charcoal filters did not reduce nitric oxide concentrations in the chambers compared to outside air. On the contrary, nitric oxide was higher in filtered chambers than in ambient air, especially during the 12 hour daylight period (Table 4). The source of the nitric oxide was not determined, but was likely re-emitted from the charcoal. Increased nitric oxide also occurred in the O_3 chambers, but at slightly lower concentrations than in filtered chambers. This difference is likely due to oxidation of at least part of the nitric oxide by the added ozone to produce nitrogen dioxide. Evidence of this was seen by the slightly higher nitrogen dioxide concentrations in the O_3 compared to filtered chambers, with the decrease in nitric oxide approximately equal to the increase in nitrogen dioxide on a ppb basis (Tables 1-3).

4. Peroxyacetyl Nitrate

The charcoal filters removed PAN to a greater degree than any other pollutant. Filtering efficiency ranged from 86 to 95% for periods two through five for which the data is relatively complete (Table 4). The removal of PAN was slightly less efficient during the first period when the data was only for 16 days of measurement (data collection did not begin until July 1, 1987) and PAN concentrations were very low. The PAN concentrations in CF and O_3 chambers ranged from only 0.1 to 0.4 ppb on a 12 hour, and 0.1 to 0.2 ppb on a 24 hour basis; whereas PAN concentrations in AA plots and NF chambers were from 1.0 to 2.2 ppb on a 12 hour, and 0.7 to 1.2 ppb on a 24 hour basis (Tables 1-3).

The low 12 and 24 hour averages are due largely to the high number of zero values. In addition, there were no periods when ambient oxidants were at very high levels which would encourage PAN formation. The highest hourly PAN values were 8 or 9 ppb for the NF and AA treatments and 4 or 5 ppb for the CF and O_3 treatments. This was considerably lower than the maximum hourly observations of up to 42-58 ppb reported for Riverside in the past (Temple and Taylor, 1983).

5. Sulfur Dioxide

There was no measurable sulfur dioxide for most periods and treatments. The only evidence for SO_2 was the 1 ppb detected in most treatments during the first harvest period (Table 1). This low value was just at the detection limits of the analyzer, thus, no differences could be detected between treatments.

B. Non-filtered vs. Added Ozone Effects

1. Growth, Yield, Injury

This study indicated that ozone is the primary agent in phytotoxic effects of photochemical oxidants. This was demonstrated by the similar plant responses in the O_3 and NF treatments including necrotic injury symptoms, enhanced lower leaf senescence, stomatal closure, leaf pigment degradation, and decreased growth and yield. For most parameters their were no statistically significant differences between the O_3 and NF treatments (statistics in Tables 5-10, treatment means in Tables 11-13, 15-17).

However, the study also indicated that in addition to the general similarities in response between the O_3 and NF treatments, detrimental effects were increased with generated ozone compared to ambient ozone. There was a distinct trend toward greater effects from the O_3 than NF treatment, as shown by the percentage reduction in dry weight vs. CF air for the two polluted treatments (Table 14). At the first four harvests and across the four harvests, there was a greater yield reduction for the O_3 than the NF treatment (Table 17). Only at the fifth harvest was the percentage reduction similar for O_3 or NF vs. CF air.

At the second harvest and across the first four harvests only the O_3 treatment was significantly different from CF air for dry weight, a fact which is not obvious from the contrasts shown in Tables 5-10. This

indicated that only the difference in yield between O_3 and CF plants was great enough compared to the variability between plants in order to be statistically significant. The average reduction in dry weight for O_3 vs. CF plants across the first four harvests (both cultivars) was 38% compared to 25% for NF vs. CF plants (Table 14).

A primary reason for the inability to statistically detect O_3 vs. NF differences may be the amount of variability associated with each mean. For example, the CV (SD as a % of the mean) for dry weight rose from 10-20% at the first harvest to over 50% by the fifth harvest for many treatments (Tables 11-13,15-17). The CV's were especially large for the two chamber-polluted treatments, O_3 and NF, which may be at least partially due to the variation in pollutant sensitivity between individual plants. Thus, the potential to detect statistically significant effects was lowest for the contrast between the two treatments of greatest interest in this study. Therefore, the comparison of O_3 vs. CF and NF vs. CF differences may provide the only statistical information indicating whether the O_3 or NF treatment is more detrimental to plants.

The only growth parameter which was significantly affected by the O_3 vs. NF treatment was height at the fourth harvest and across the first four harvests (Tables 8 and 9). In this case, O_3 resulted in lower heights than NF air.

Added ozone had a greater impact on leaf injury than the NF air. There were statistically significant differences between these two treatments for both number of empty nodes and percentage empty nodes of the first four harvests and across the fourth harvest. The difference was especially dramatic for the first and second harvests where the O_3 treatment had 10 to 20% more leaf drop (% empty nodes) than the NF treatment (Tables 11-12).

Generated ozone had a much greater effect on pinto bean plant growth and pod yield than ambient ozone. The difference between the O_3 and NF treatments was statistically significant for two parameters: total plant dry weight and number of pods (Table 19). The difference in growth between the two air pollutant treatments was especially noticeable when comparing their results to CF air. Plant and pod fresh and dry weights were over 60% lower for O_3 treated vs. CF plants, whereas weights were only 30-50% lower for NF vs. CF plants.

Table 5. Results from Statistical Analysis of Yield, Height, and Injury Data from the First Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)	Dry Weight (g)	Dry/ Fresh (%)	Height (m)	Nodes (#)	Empty Nodes (#)	Empty Nodes (%)
Air: CF vs NF	NS	NS	NS	NS	NS	***	***
Air: NF vs O_3	NS	NS	NS	NS	NS	***	***
Air: NF vs AA	NS	NS	NS	NS	NS	*	**
Cultivar	**	*	NS	NS	***	*	***
Air x Cultivar	NS	NS	NS	NS	NS	NS	**

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at $p < 0.05$, 0.01, and 0.005 levels, respectively.

Table 6. Results from Statistical Analysis of Yield, Height, and Injury Data from the Second Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)	Dry Weight (g)	Dry/ Fresh (%)	Height (m)	Nodes (#)	Empty Nodes (#)	Empty Nodes (%)
Air: CF vs NF	NS	NS	NS	NS	NS	***	***
Air: NF vs O_3	NS	NS	NS	NS	NS	**	**
Air: NF vs AA	NS	NS	NS	***	NS	*	NS
Cultivar	***	***	***	***	***	***	***
Air x Cultivar	NS	NS	NS	NS	NS	*	**

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at $p < 0.05$, 0.01, and 0.005 levels, respectively.

Table 7. Results from Statistical Analysis of Yield, Height, and Injury Data from the Third Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)	Dry Weight (g)	Dry/ Fresh (%)	Height (m)	Nodes (#)	Empty Nodes (#)	Empty Nodes (%)
Air: CF vs NF	NS	NS	NS	NS	NS	**	*
Air: NF vs O_3	NS	NS	NS	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS	NS	NS	NS
Cultivar	NS	*	***	NS	*	NS	NS
Air x Cultivar	NS	NS	NS	NS	NS	***	***

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at p <0.05, 0.01, and 0.005 levels, respectively.

Table 8. Results from Statistical Analysis of Yield, Height, and Injury Data from the Fourth Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)	Dry Weight (g)	Dry/ Fresh (%)	Height (m)	Nodes (#)	Empty Nodes (#)	Empty Nodes (%)
Air: CF vs NF	NS	*	NS	NS	NS	***	***
Air: NF vs O_3	NS	NS	NS	*	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS	NS	*	NS
Cultivar	NS	NS	NS	NS	***	***	***
Air x Cultivar	NS	NS	NS	NS	NS	NS	**

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at p <0.05, 0.01, and 0.005 levels, respectively.

Table 9. Results from Statistical Analysis of Yield, Height, and Injury Data Across the First Four Alfalfa Harvests.[a]

Treatment	Fresh Weight (g)	Dry[b] Weight (g)	Dry/ Fresh (%)	Height (m)	Nodes (#)	Empty Nodes (#)	Empty Nodes (%)
Air: CF vs NF	NS	NS	NS	NS	NS	***	***
Air: NF vs O_3	NS	NS	NS	*	NS	**	**
Air: NF vs AA	NS	NS	NS	*	*	*	NS
Cultivar	NS	**	***	NS	***	***	***
Air x Cultivar	NS	NS	NS	NS	NS	***	***
Harvest	***	***	***	***	***	***	*
Harvest x Air	NS	NS	*	NS	NS	NS	NS
Harvest x Cultivar	***	**	***	**	***	*	***
Harvest x Air x Cv.	NS	NS	**	NS	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at $p < 0.05$, 0.01, and 0.005 levels, respectively.
[b]There was a significant difference between the CF and O_3 treatments at $p < 0.05$.

Table 10. Results from Statistical Analysis of Yield, Height, and Injury Data from the Fifth Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)	Dry[b] Weight (g)	Dry/ Fresh (%)	Height (m)	Nodes (#)	Empty Nodes (#)	Empty Nodes (%)
Air: CF vs NF	*	*	NS	NS	NS	***	**
Air: NF vs O_3	NS	NS	NS	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS	NS	NS	NS
Cultivar	NS	NS	*	***	***	NS	NS
Air x Cultivar	NS	NS	NS	***	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at $p < 0.05$, 0.01, and 0.005 levels, respectively.

Table 11. Treatment Means for Yield, Height, and Injury Data from the First Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air: CF	144 ± 21	137 ± 23	33.2 ± 4.3	33.2 ± 5.7	23 ± 2	24 ± 3
Air: NF	141 ± 24	131 ± 25	32.1 ± 5.2	30.7 ± 7.2	23 ± 2	23 ± 3
Air: O3	126 ± 14	117 ± 23	29.5 ± 3.4	27.1 ± 6.2	24 ± 1	23 ± 3
Air: AA	147 ± 22	134 ± 40	33.0 ± 5.2	30.0 ± 8.2	22 ± 2	23 ± 2
	Nodes (no.)		Empty Nodes (no.)		Empty Nodes (%)	
Air: CF	11 ± 1	10 ± 1	1 ± 1	1 ± 1	11 ± 12	10 ± 12
Air: NF	11 ± 1	10 ± 1	3 ± 2	4 ± 2	31 ± 15	35 ± 15
Air: O3	11 ± 2	10 ± 2	5 ± 2	5 ± 2	43 ± 16	55 ± 30
Air: AA	10 ± 1	10 ± 2	4 ± 2	4 ± 2	35 ± 14	43 ± 21
	Height (m)					
Air: CF	0.63 ± 0.09	0.63 ± 0.08				
Air: NF	0.63 ± 0.08	0.63 ± 0.10				
Air: O3	0.59 ± 0.10	0.60 ± 0.10				
Air: AA	0.54 ± 0.08	0.54 ± 0.09				

[a]Values are means ± SD of 30 individual plants for weights, 10 in each of three blocked chambers or outside plots; or 90 observations for height and injury (nodes), 3 stems per plant, with 10 plants in each of three blocks.

Table 12. Treatment Means for Yield, Height, and Injury Data from the Second Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air: CF	169 ± 37	153 ± 36	34.6 ± 6.3	29.3 ± 5.9	21 ± 1	18 ± 2
Air: NF	162 ± 29	133 ± 37	32.0 ± 6.1	24.8 ± 6.1	20 ± 1	19 ± 2
Air: O_3	130 ± 20	107 ± 41	26.7 ± 4.5	21.4 ± 8.0	21 ± 1	20 ± 1
Air: AA	160 ± 27	132 ± 39	32.9 ± 4.7	25.8 ± 7.3	20 ± 1	20 ± 2
	Nodes (no.)		Empty Nodes (no.)		Empty Nodes (%)	
Air: CF	10 ± 1	10 ± 1	1 ± 1	1 ± 1	5 ± 9	6 ± 10
Air: NF	11 ± 1	10 ± 1	3 ± 2	4 ± 2	28 ± 18	42 ± 22
Air: O_3	10 ± 1	10 ± 1	5 ± 2	6 ± 3	45 ± 19	57 ± 27
Air: AA	10 ± 1	10 ± 2	2 ± 2	3 ± 2	22 ± 16	35 ± 23
	Height (m)					
Air: CF	0.62 ± 0.08	0.59 ± 0.06				
Air: NF	0.60 ± 0.06	0.58 ± 0.09				
Air: O_3	0.57 ± 0.08	0.53 ± 0.12				
Air: AA	0.54 ± 0.07	0.51 ± 0.09				

[a]Values are means ± SD of 30 individual plants for weights, 10 in each of three blocked chambers or outside plots; or 90 observations for height and injury (nodes), 3 stems per plant, with 10 plants in each of three blocks.

Table 13. Treatment Means for Yield, Height, and Injury Data from the Third Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air: CF	181 ± 103	198 ± 109	41.4 ± 21.3	41.5 ± 20.6	24 ± 2	22 ± 2
Air: NF	138 ± 64	138 ± 57	32.1 ± 15.0	29.1 ± 12.6	23 ± 2	21 ± 2
Air: O_3	88 ± 35	94 ± 42	22.0 ± 9.1	21.9 ± 10.2	25 ± 2	23 ± 3
Air: AA	171 ± 32	138 ± 57	42.3 ± 7.7	34.7 ± 11.8	25 ± 3	22 ± 2
	Nodes (no.)		Empty Nodes (no.)		Empty Nodes (%)	
Air: CF	11 ± 2	12 ± 2	2 ± 2	1 ± 1	17 ± 19	9 ± 12
Air: NF	13 ± 2	13 ± 2	6 ± 3	6 ± 4	45 ± 27	47 ± 23
Air: O_3	12 ± 2	13 ± 2	7 ± 3	8 ± 3	58 ± 23	61 ± 23
Air: AA	12 ± 1	12 ± 2	3 ± 2	4 ± 2	29 ± 14	39 ± 23
	Height (m)					
Air: CF	0.75 ± 0.16	0.74 ± 0.15				
Air: NF	0.70 ± 0.12	0.73 ± 0.12				
Air: O_3	0.61 ± 0.12	0.61 ± 0.15				
Air: AA	0.61 ± 0.10	0.59 ± 0.23				

[a]Values are means ± SD of 30 individual plants for weights, 10 in each of three blocked chambers or outside plots; or 90 observations for height and injury (nodes), 3 stems per plant, with 10 plants in each of three blocks.

Table 14. Predicted and Calculated Reductions in Dry Weight for Alfalfa Exposed to Ozone

Treatments[a]	First Harvest			Second Harvest		
	Pred.[b]	Measured[c]		Pred.	Measured	
		El Dorado	Mesa Sirsa		El Dorado	Mesa Sirsa
NF vs CF	19	3	8	22	8	15
O_3 vs CF	21	11	18	21	23	27
AA vs CF	21	1	10	25	5	12
		Third Harvest			Fourth Harvest	
NF vs CF	20	23	30	24	30	39
O_3 vs CF	21	47	47	21	52	51
AA vs CF	22	(2)[d]	16	22	(2)[d]	17
		First Four Harvests			Fifth Harvest	
NF vs CF	22	23	26	11	39	50
O_3 vs CF	21	36	39	10	45	53
AA vs CF	22	(2)[d]	14	11	4	24

[a]Abbreviations: NF = non-filtered air, CF = charcoal filtered air, O_3 = filtered air plus added ozone, and AA = ambient air in outside plots.

[b]Predicted by an adaptation of the ozone dose-crop loss equation of Temple et al. (1988):
% loss = {1- [(3160 - (10.963 x 12 hr O_3)) / (3160 - (10.963 x CF O_3))]} x 100;
where the ozone concentration is for 0800- 2000 PST in ppb using the numbers in tables 1-3.

[c]Calculated as:
[(mean for CF - mean for NF, O_3, or AA) / mean for CF] x 100;
where the mean is the mean ozone concentration for each growth period.

[d]Higher dry weight vs. CF.

Table 15. Treatment Means for Yield, Height, and Injury Data from the Fourth Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air: CF	270 ± 134	301 ± 145	54.6 ± 23	57.3 ± 24.3	21 ± 3	20 ± 3
Air: NF	189 ± 69	181 ± 77	38.0 ± 14.7	35.2 ± 15.8	20 ± 2	19 ± 1
Air: O_3	125 ± 72	133 ± 88	26.4 ± 16.1	23.3 ± 18.4	21 ± 2	21 ± 2
Air: AA	256 ± 46	227 ± 86	55.7 ± 9.6	47.5 ± 17.5	22 ± 2	21 ± 2
	Nodes (no.)		Empty Nodes (no.)		Empty Nodes (%)	
Air: CF	13 ± 2	12 ± 2	2 ± 2	1 ± 2	12 ± 11	12 ± 12
Air: NF	14 ± 2	14 ± 2	6 ± 3	7 ± 3	43 ± 20	47 ± 20
Air: O_3	13 ± 2	13 ± 3	7 ± 3	8 ± 3	51 ± 24	62 ± 25
Air: AA	13 ± 2	12 ± 3	5 ± 2	6 ± 3	35 ± 16	47 ± 20
	Height (m)					
Air: CF	0.79 ± 0.18	0.77 ± 0.18				
Air: NF	0.73 ± 0.15	0.75 ± 0.19				
Air: O_3	0.59 ± 0.19	0.59 ± 0.18				
Air: AA	0.64 ± 0.11	0.62 ± 0.15				

[a]Values are means ± SD of 30 individual plants for weights, 10 in each of three blocked chambers or outside plots; or 90 observations for height and injury (nodes), 3 stems per plant, with 10 plants in each of three blocks.

Table 16. Treatment Means for Yield, Height, and Injury Data Averaged Across First Four Alfalfa Harvests.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	191 ± 105	197 ± 112	40.9 ± 18.1	40.3 ± 19.5	22 ± 2	21 ± 3
Air:NF	158 ± 54	146 ± 56	33.6 ± 11.4	30.0 ± 11.6	21 ± 2	21 ± 3
Air:O_3	117 ± 45	113 ± 56	26.2 ± 9.9	24.7 ± 11.9	22 ± 3	22 ± 3
Air:AA	184 ± 54	164 ± 70	41.0 ± 11.7	34.5 ± 14.3	22 ± 3	21 ± 2
	Nodes (no.)		Empty Nodes (no.)		Empty Nodes (%)	
Air:CF	12 ± 2	11 ± 2	1 ± 2	1 ± 1	11 ± 14	9 ± 12
Air:NF	12 ± 2	12 ± 3	5 ± 3	5 ± 3	37 ± 22	44 ± 20
Air:O_3	12 ± 2	11 ± 2	6 ± 3	7 ± 3	49 ± 21	59 ± 26
Air:AA	11 ± 2	11 ± 2	4 ± 2	4 ± 3	30 ± 16	41 ± 22
	Height (m)					
Air:CF	0.70 ± 0.15	0.68 ± 0.15				
Air:NF	0.66 ± 0.12	0.67 ± 0.15				
Air:O_3	0.59 ± 0.13	0.58 ± 0.14				
Air:AA	0.58 ± 0.10	0.56 ± 0.12				

[a]Values are means ± SD of 120 individual plants for weights, 10 in each of three blocked chambers or outside plots over four harvests; or 360 observations for height and injury (nodes), 3 stems per plant, with 10 plants in each of three blocks over four harvests.

Table 17. Treatment Means for Yield, Height, and Injury Data from the Fifth Alfalfa Harvest.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air: CF	271 ± 141	323 ± 185	55.8 ± 23	62.2 ± 28	22 ± 4	20 ± 5
Air: NF	162 ± 85	156 ± 82	34.3 ± 18	31.4 ± 20	22 ± 4	19 ± 4
Air: O_3	127 ± 71	135 ± 89	30.5 ± 15	29.4 ± 18	26 ± 8	22 ± 3
Air: AA	228 ± 82	228 ± 101	53.3 ± 15	47.4 ± 20	23 ± 1	20 ± 5
	Nodes (no.)		Empty Nodes (no.)		Empty Nodes (%)	
Air: CF	13 ± 3	14 ± 2	1 ± 2	1 ± 1	9 ± 11	4 ± 1
Air: NF	13 ± 3	12 ± 3	3 ± 2	3 ± 2	19 ± 15	20 ± 18
Air: O_3	12 ± 3	12 ± 2	3 ± 2	2 ± 2	20 ± 12	20 ± 17
Air: AA	13 ± 2	13 ± 2	3 ± 2	3 ± 2	19 ± 13	20 ± 18
	Height (m)					
Air: CF	0.51 ± 0.30	0.85 ± 0.19				
Air: NF	0.54 ± 0.24	0.59 ± 0.24				
Air: O_3	0.44 ± 0.22	0.52 ± 0.16				
Air: AA	0.41 ± 0.24	0.57 ± 0.15				

[a]Values are means ± SD of 30 individual plants for weights, 10 in each of three blocked chambers or outside plots; or 90 observations for height and injury (nodes), 3 stems per plant, with 10 plants in each of three blocks.

Table 18. Results from Statistical Analysis of Yield Data from Pinto Bean Harvest.[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)		Pods
	Plants	Pods	Plants	Pods	Plants	Pods	(no.)
Air: CF vs. NF	*	NS	**	**	NS	NS	NS
Air: NF vs. O_3	NS	NS	**	NS	NS	NS	*
Air: NF vs. AA	NS	NS	NS	NS	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at $p < 0.05$, 0.01, and 0.005, levels, respectively.

Table 19. Treatment Means for Yield Data from the Pinto Bean Harvest[a]

Treatment	Fresh Weight (g)		Dry Weight (g)		Dry/Fresh Wt. (%)		Pods
	Plants	Pods	Plants	Pods	Plants	Pods	(no.)
Air: CF	314 ± 116	162 ± 66	34.1 ± 10.7	27.6 ± 12.2	11 ± 2	19 ± 12	45 ± 15
Air: NF	201 ± 86	112 ± 52	23.8 ± 9.2	13.4 ± 6.2	13 ± 4	13 ± 7	36 ± 13
Air: O_3	109 ± 35	58 ± 21	12.8 ± 5.0	8.7 ± 4.0	12 ± 3	17 ± 11	21 ± 7
Air: AA	179 ± 54	103 ± 37	17.6 ± 8.0	19.2 ± 8.9	10 ± 3	20 ± 10	31 ± 7

[a]Values are means ± SD of 15 individual plants, five in each of three blocked chambers or outside plots.

2. Physiology

There were no general statistically significant differences in water vapor loss from leaves due to added ozone as compared to ambient ozone. Neither stomatal conductance nor transpiration rates showed significant differences between the O_3 and NF treatments during any specific harvest or across four harvests (Tables 20,21). Stomatal conductance for both the O_3 and NF treatments averaged 0.8 to 1.0 cm s^{-1} during the first two harvests (Tables 22,23), and then decreased to 0.4 to 0.7 cm s^{-1} during the third and fourth harvests (Tables 24,25). Transpiration was in the range of 13 to 22 μg cm^{-2} s^{-1} during all four harvests (Tables 22-25).

Table 20. Results from Statistical Analysis of Stomatal Conductance and Transpiration Data for the First Four Harvests[a].

Treatment	First	Second	Third	Fourth
		Conductance (cm s^{-1})		
Air: NF vs CF	NS	**	NS	*
Air: NF vs O_3	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS
Cultivar	NS	NS	NS	NS
Air x Cultivar	NS	NS	NS	NS
		Transpiration (µg cm^{-2} s^{-1})		
Air: NF vs CF	NS	*	NS	NS
Air: NF vs O_3	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS
Cultivar	NS	NS	NS	NS
Air x Cultivar	NS	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with * are statistically significant at $p < 0.05$ level, respectively.

Table 21. Results from Statistical Analysis of Stomatal Conductance and Transpiration Data Across the First Four Harvests[a].

Treatment	Conductance (cm s^{-1})	Transpiration (ug cm^{-2} s^{-1})
Air: NF vs CF	**	**
Air: NF vs O_3	NS	NS
Air: NF vs AA	NS	NS
Cultivar	NS	NS
Air x Cultivar	NS	NS
Harvest	NS	**[b]
Harvest x Air	NS	NS
Harvest x Cultivar	NS	NS
Harvest x Air x Cultivar	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with * and ** are statistically significant at $p < 0.05$, and 0.005 levels, respectively.
[b] Highest value in August, lowest in September.

Table 22. Treatment Means for Stomatal Conductance and Transpiration Data from the First Alfalfa Harvest[a]

Treatment	Conductance (cm s^{-1})		Transpiration (μg cm^{-2} s^{-1})	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	1.20 ± 0.43	1.29 ± 0.62	21.1 ± 6.1	20.5 ± 4.6
Air:O_3	0.93 ± 0.39	1.02 ± 0.37	15.2 ± 5.4	17.6 ± 5.0
Air:NF	0.78 ± 0.32	1.05 ± 0.29	15.0 ± 5.1	18.7 ± 3.9
Air:AA	0.92 ± 0.26	0.99 ± 0.28	16.7 ± 4.5	17.8 ± 3.5

[a]Values are means ± SD of nine single plant replicates, three from each of three chambers or outside plots.

Table 23. Treatment Means for Stomatal Conductance and Transpiration Data from the Second Alfalfa Harvest.

Treatment	Conductance (cm s^{-1})		Transpiration (μg cm^{-2} s^{-1})	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	1.41 ± 0.31	1.51 ± 0.29	25.4 ± 3.8	27.0 ± 3.3
Air:O_3	1.06 ± 0.13	0.98 ± 0.22	21.4 ± 2.1	19.4 ± 3.7
Air:NF	1.08 ± 0.28	1.00 ± 0.30	22.3 ± 1.8	20.1 ± 4.8
Air:AA	1.11 ± 0.36	1.20 ± 0.27	21.7 ± 3.8	23.1 ± 3.4

[a]Values are means ± SD of nine single plant replicates, three from each of three chambers or outside plots.

Table 24. Treatment Means for Stomatal Conductance and Transpiration Data from the Third Alfalfa Harvest.

Treatment	Conductance (cm s^{-1})		Transpiration (μg cm^{-2} s^{-1})	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	1.00 ± 0.32	0.83 ± 0.45	20.2 ± 6.1	17.2 ± 7.6
Air:O_3	0.71 ± 0.23	0.57 ± 0.31	16.3 ± 4.2	13.3 ± 6.0
Air:NF	0.69 ± 0.39	0.65 ± 0.37	18.8 ± 8.4	16.6 ± 5.8
Air:AA	0.62 ± 0.41	0.58 ± 0.29	15.4 ± 8.0	14.6 ± 5.5

[a]Values are means ± SD of nine single plant replicates, three from each of three chambers or outside plots.

Table 25. Treatment Means for Stomatal Conductance and Transpiration Data from the Fourth Alfalfa Harvest.

Treatment	Conductance (cm s^{-1})		Transpiration (μg cm^{-2} s^{-1})	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	1.01 ± 0.28	0.89 ± 0.45	25.3 ± 5.3	23.1 ± 7.7
Air:O_3	0.39 ± 0.16	0.36 ± 0.19	14.6 ± 5.3	13.4 ± 6.3
Air:NF	0.47 ± 0.19	0.61 ± 0.17	15.9 ± 5.7	20.3 ± 4.5
Air:AA	0.61 ± 0.33	0.60 ± 0.20	17.8 ± 7.7	19.7 ± 6.0

[a]Values are means ± SD of nine single plant replicates, three from each of three chambers or outside plots.

Leaf chlorophyll concentrations tended to be lower with added ozone compared to ambient ozone, which was similar to the pattern of greater leaf senescence with added ozone. However, the differences in chlorophyll (a,b and total) between the O_3 and NF treatments were statistically significant primarily when the data were pooled across all four harvests (Tables 26-30). The only significant difference between O_3 and NF treatments at a particular harvest was for the reduction in chlorophyll b with added ozone at the fourth harvest. The lack of significant differences between treatments may be due primarily to the large variability in chlorophyll concentrations for each treatment as shown in Tables 31 to 34. There were no differences in leaf carotenoid concentrations between the O_3 and NF treatments.

C. Non-filtered vs. Filtered Air Effects

1. Growth, Yield, Injury

Ambient-ozone (NF treatment) tended to reduce plant growth, but the effects were not as dramatic as for the O_3 treatment. Weights were significantly different for the NF vs. CF treatment at the fourth (dry) and fifth harvests (fresh and dry) (Tables 8,10). This indicated that the effect of ambient ozone on yield tended to be cumulative, reaching a peak with the 50% reductions in yield at the last two harvests (Table 14). No other growth or yield parameters were statistically significant at any harvest.

Table 26. Results from Statistical Analysis of Pigment Data for the First Harvest (in µg per mg dry weight)[a].

Treatment	Chl. a	Chl. b	Carot.	Total Chl.
Air: NF vs CF	NS	NS	NS	NS
Air: NF vs O_3	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS
Cultivar	***	*	***	NS
Air x Cultivar	NS	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *,**, and *** are statistically significant at $p < 0.05$ and 0.005 levels, respectively.

Table 27. Results from Statistical Analysis of Pigment Data for the Second Harvest (in µg per mg dry weight)[a].

Treatment	Chl. a	Chl. b	Carot.	Total Chl.
Air: NF vs CF	*	NS	NS	**
Air: NF vs O_3	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS
Cultivar	NS	***	NS	***
Air x Cultivar	NS	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at $p < 0.05$, 0.01, and 0.005 levels, respectively.

Table 28. Results from Statistical Analysis of Pigment Data for the Third Harvest (in µg per mg dry weight)[a].

Treatment	Chl. a	Chl. b	Carot.	Total Chl.
Air: NF vs CF	*	*	NS	**
Air: NF vs O_3	NS	NS	NS	NS
Air: NF vs AA	NS	NS	NS	NS
Cultivar	**	***	NS	***
Air x Cultivar	NS	NS	**	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at p <0.05, 0.01, and 0.005 levels, respectively.

Table 29. Results from Statistical Analysis of Pigment Data for the Fourth Harvest (in µg per mg dry weight)[a].

Treatment	Chl. a	Chl. b	Carot.	Total Chl.
Air: NF vs CF	NS	NS	NS	**
Air: NF vs O_3	NS	**	NS	**
Air: NF vs AA	NS	NS	NS	NS
Cultivar	NS	***	***	***
Air x Cultivar	NS	NS	NS	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with *, **, and *** are statistically significant at p <0.05, 0.01, and 0.005 levels, respectively.

Table 30. Results from Statistical Analysis of Pigment Data Across the First Four Harvests (in µg per mg dry weight)[a].

Treatment	Chl. a	Chl. b	Carot.	Total Chl.
Air: NF vs CF	*	**	NS	***
Air: NF vs O_3	**	***	NS	***
Air: NF vs AA	NS	NS	NS	NS
Cultivar	***	***	NS	***
Air x Cultivar	NS	NS	NS	NS
Harvest	NS	*[b]	***	***
Harvest x Air	NS	NS	NS	NS
Harvest x Cultivar	NS	***	***	***
Harvest x Air x Cultivar	NS	NS	*	NS

[a]Results based on analysis of variance with chamber as the experimental unit. Parameters and treatments with * and ** are statistically significant at $p < 0.05$, and 0.005 levels, respectively.
[b]Highest value in August, lowest in September.

Table 31. Treatment Means for Leaf Pigment Data at First Harvest in ug/mg dry wt[a].

Treatment	Chlorophyll a		Chlorophyll b	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	7.32 ± 1.37	5.69 ± 2.52	4.98 ± 2.09	6.10 ± 1.87
Air:O_3	5.78 ± 2.29	4.72 ± 1.96	4.24 ± 2.36	5.04 ± 1.93
Air:NF	6.97 ± 1.23	5.99 ± 1.37	5.14 ± 1.43	5.74 ± 2.08
Air:AA	6.50 ± 1.04	4.85 ± 1.36	4.85 ± 1.32	5.32 ± 1.20
	Carotenoids		Total Chlorophyll	
Air:CF	0.41 ± 0.29	0.20 ± 0.23	12.97 ± 3.41	12.47 ± 4.01
Air:O_3	0.59 ± 0.30	0.30 ± 0.36	10.02 ± 4.48	9.76 ± 3.60
Air:NF	0.57 ± 0.35	0.17 ± 0.17	12.11 ± 2.51	12.40 ± 3.10
Air:AA	0.57 ± 0.46	0.22 ± 0.21	11.34 ± 2.00	10.17 ± 2.43

[a]Values are means ± SD of 15 single plant replicates, five from each of three chambers or outside plots.

Table 32. Treatment Means for Leaf Pigment Data at Second Harvest in µg/mg dry wt[a].

Treatment	Chlorophyll a		Chlorophyll b	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	6.83 ± 2.16	6.84 ± 1.14	5.73 ± 2.10	5.10 ± 0.82
Air:O_3	3.90 ± 1.47	4.15 ± 1.69	3.65 ± 1.27	3.33 ± 1.32
Air:NF	5.84 ± 1.55	4.74 ± 1.34	5.09 ± 1.24	3.69 ± 1.03
Air:AA	5.72 ± 1.23	4.90 ± 1.47	5.09 ± 1.50	3.85 ± 1.19
	Carotenoids		Total Chlorophyll	
Air:CF	0.26 ± 0.39	0.12 ± 0.14	13.90 ± 2.69	11.95 ± 1.92
Air:O_3	0.15 ± 0.15	0.13 ± 0.13	7.55 ± 2.69	7.48 ± 2.97
Air:NF	0.14 ± 0.12	0.14 ± 0.17	10.93 ± 2.73	8.43 ± 2.31
Air:AA	0.20 ± 0.18	0.18 ± 0.17	10.81 ± 2.67	8.75 ± 2.64

[a]Values are means ± SD of 15 single plant replicates, five from each of three chambers or outside plots.

Table 33. Treatment Means for Leaf Pigment Data at Third Harvest in µg/mg dry wt[a].

Treatment	Chlorophyll a		Chlorophyll b	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	6.96 ± 3.47	7.19 ± 2.33	7.12 ± 2.25	5.91 ± 1.54
Air:O_3	4.47 ± 1.60	3.40 ± 1.33	3.76 ± 1.15	2.89 ± 1.34
Air:NF	6.46 ± 1.61	4.19 ± 2.12	5.39 ± 1.23	3.80 ± 2.29
Air:AA	6.27 ± 1.08	5.39 ± 1.45	5.26 ± 0.99	4.20 ± 1.13
	Carotenoids		Total Chlorophyll	
Air:CF	0.07 ± 0.14	0.34 ± 0.51	16.75 ± 2.51	13.81 ± 2.55
Air:O_3	0.37 ± 0.18	0.16 ± 0.18	8.23 ± 2.71	6.29 ± 2.62
Air:NF	0.21 ± 0.19	0.17 ± 0.18	11.85 ± 2.75	7.94 ± 4.42
Air:AA	0.22 ± 0.18	0.32 ± 0.46	11.53 ± 1.96	9.59 ± 2.22

[a]Values are means ± SD of 15 single plant replicates, five from each of three chambers or outside plots.

Table 34. Treatment Means for Leaf Pigment Data at Fourth Harvest in µg/mg dry wt[a].

Treatment	Chlorophyll a		Chlorophyll b	
	El Dorado	Mesa Sirsa	El Dorado	Mesa Sirsa
Air:CF	6.39 ± 3.69	7.21 ± 2.95	5.70 ± 3.92	5.85 ± 2.30
Air:O_3	5.50 ± 2.25	4.06 ± 1.42	5.42 ± 2.17	3.22 ± 1.17
Air:NF	6.38 ± 2.18	5.39 ± 2.64	7.20 ± 1.97	4.62 ± 2.15
Air:AA	6.64 ± 1.53	5.96 ± 1.38	6.52 ± 1.84	4.33 ± 0.94
	Carotenoids		Total Chlorophyll	
Air:CF	0.00 ± 0.00	0.23 ± 0.36	18.86 ± 3.91	15.06 ± 2.95
Air:O_3	0.07 ± 0.10	0.24 ± 0.19	11.61 ± 5.25	7.28 ± 2.59
Air:NF	0.03 ± 0.08	0.30 ± 0.25	14.91 ± 3.06	10.69 ± 4.90
Air:AA	0.05 ± 0.09	0.34 ± 0.24	13.16 ± 3.32	10.29 ± 2.18

[a]Values are means ± SD of 15 single plant replicates, five from each of three chambers or outside plots.

The NF treatment had a highly significant effects on alfalfa leaf injury at all harvests, as measured as # empty nodes and % empty nodes. This increased leaf senescence for alfalfa due to ambient air previously had been demonstrated in many studies conducted in the South Coast Air Basin and Central Valley of California (Olszyk et al. 1986b, 1987; Oshima et al., Temple et al. 1988).

The NF treatment had a significant effect on both vegetative and fruit weights for bean plants (Table 18). Plant fresh and dry weights and pod dry weights were reduced with NF compared to CF air.

2. Physiology

Ambient ozone tended to reduce stomatal conductance and transpiration for all harvests. Stomatal conductance was significantly different between the NF and CF treatments during the first and fourth harvests (Table 20), and across all four harvests (Table 21). Transpiration was significantly different between the NF and CF treatments for the second harvest (Table 20), and across all four harvests (Table 21). The reduction in conductance due to ambient ozone has been reported for many other species (Tingey and Taylor, 1982).

Ambient ozone reduced leaf chlorophyll concentrations at three of the four harvests and across all four harvests. Chlorophyll concentrations were significantly lower for the NF compared to the CF treatment for the following parameters and harvests: chlorophyll a for the second and third harvests, and across all four harvests (Tables 27,28,30); chlorophyll b for third harvest and across all harvests (Tables 28,30); and total chlorophyll for the second, third and fourth harvests, and across all four harvests (Tables 27-30). The reduction in leaf chlorophyll concentration due to ambient ozone was expected as shown by the significant increase in leaf senescence with this treatment compared to filtered air. Leaf carotenoid concentrations were not affected by ambient ozone at any harvest.

D. Chamber Effects

1. Growth, Yield, Injury

Plant weight was not affected by the chambers at any harvest as shown by the lack of significant differences between the NF and AA treatments for both either alfalfa and pinto beans (Tables 5-10, 19). However, there was an increase in height for NF chambers compared to AA plots for one of the five harvests and across the first four harvests (Tables 5-6, 9-11). This was indicative of a chamber effect on etiolation of alfalfa as described in previous air pollution studies (Olszyk et al. 1986b). The chamber also had an effect on leaf injury with a different number of empty nodes for the NF vs. AA treatments at three of the first four harvests and across the four harvests (Table 5). Percentage empty nodes was greater for the NF treatment vs. AA treatments at the first harvest (Table 5). The chambers had no effect on growth of beans (Table 18).

2. Physiology

The chambers had no effect on physiological responses of the alfalfa plants in this study. This was shown by lack of significant differences between the NF and AA treatments for stomatal conductance and transpiration (Tables 20,21), or any leaf pigment parameter (Tables 26-30).

E. Cultivar Effects

1. Growth, Yield, Injury

There were statistically significant differences between the two cultivars in terms of weights and leaf injury responses (Tables 5-10). El Dorado grew better than Mesa Sirsa at most harvests, exhibiting greater weights, heights, and number of nodes (Tables 11-12, 15-17). Mesa Sirsa was more sensitive to ozone than El Dorado, exhibiting greater numbers of empty nodes and percentage of empty nodes and percentage reduction dry weight in O_3 or NF compared to CF air (Table 13).

2. Physiology

Cultivar had no effect on water vapor exchange responses of the alfalfa plants in this study. There were no significant differences between El Dorado and Mesa Sirsa for stomatal conductance and transpiration (Tables 20,21). Evidently, the greater ozone injury to Mesa Sirsa compared to El Dorado was not due to greater stomatal conductance as found in other species.

El Dorado had higher leaf chlorophyll concentrations than Mesa Sirsa for nearly all parameters and at all harvests (Tables 26-30). The only exceptions were chlorophyll a at the second and fourth harvests (Tables 27,28). These chlorophyll results coincided with the general observation that El Dorado leaves were greener that Mesa Sirsa leaves. Mesa Sirsa had a higher leaf carotenoid concentration at the fourth harvest (Table 29), but since this response did not occur at any other harvest its importance was questionable.

F. Interactions

1. Growth, Yield, Injury

The primary air x cultivar interaction was greater leaf senescence for Mesa Sirsa than El Dorado in response to ozone. There was a greater percentage empty nodes for Mesa Sirsa than El Dorado with ozone

exposure for each of the first four harvests and across the first four harvests (Tables 5-9). In addition, the number of empty nodes was greater for Mesa Sirsa than El Dorado for the second and third harvests, and across all four harvests (Tables 6-7,9). The only growth or yield parameter which had a significant air x cultivar interaction was the greater effect of ozone on height for Mesa Sirsa compared to El Dorado at the fifth harvest (Table 10).

There were a number of significant harvest x treatment interactions (Table 9). This was expected as the environmental conditions were slightly different during each growth period as reflected by the response on each harvest. The harvest x cultivar interactions were the most important with the relative ozone response of Mesa Sirsa vs. El Dorado differing with the different harvest day for each response parameter. There also were significant harvest x air, and harvest x air x cultivar interactions for percentage dry weight. This indicated that the water content of the plant material varied with harvest, and the water content affected the relative response of the different cultivars to ozone.

2. Physiology

There were no significant interactions between cultivar and air pollutant treatments except for carotenoids at the third harvest (Table 28). The interaction occurred because El Dorado has the highest carotenoid concentration with added ozone and lowest with filtered air, whereas Mesa Sirsa had the highest concentration with filtered air and lowest with added ozone. This interaction may not be of any general importance since this pattern was not statistically significant for any other harvest.

There were significant day x cultivar interactions for chlorophyll b, carotenoid, and total chlorophyll concentrations across all four harvests (Table 29). This was expected as the growing conditions were slightly different for each harvest, i.e. daylight (0800-2000) air temperature averaged about 25-27°C for the first; 27-35°C for the second, 27-29°C for the third; 26-30°C for the fourth, and 19-27°C for the fifth harvest.

IV. DISCUSSION

A. What Roles do Non-Ozone Pollutants Play in Ambient Oxidant Effects?

The reasons for the increased effects of added the O_3 treatment are open to further research, however, several possibilities exist. The first is that the higher nitrogen dioxide concentrations or other trace pollutants in NF compared to O_3 treatments may have in some way protected plants from ozone injury. The most obvious way for this to happen is for nitrogen dioxide to cause stomatal closure which would prevent entry of ozone into leaf tissues. However, experimental results indicate that nitrogen dioxide has little effect on stomatal closure by itself (Amundson and Weinstein 1981). There is no definitive information on the effects of nitrogen dioxide and ozone on stomata (Amundson and MacLean 1982).

Another possible explanation for the greater effects in the O_3 treatment is that the higher concentrations of nitric oxide may have increased the sensitivity of the plants to ozone compared to the NF treatment. The increased nitric oxide was probably due to remission from the charcoal without subsequent oxidation by ozone to nitrogen dioxide as in the NF treatment. However, nitric oxide is even less toxic to plants than nitrogen dioxide (EPA, 1982). Because of the general lack of concern for nitric oxide effects there have been no detailed studies of the response of stomata to combinations of nitric oxide and ozone.

A third explanation for the increased injury in the O_3 treatment is the presence of trace oxidants such as nitric acid vapor (HNO_3) emitted by the ozone generator when using dry air over the first four harvests. Reasons for this possibility are: (a) the smallest difference in leaf injury and yield responses between the O_3 and NF treatments occurred for the fifth harvest when O_3 was generated from oxygen, (b) scientific literature indicates that nitric acid vapor is the most important trace pollutant generated with ozone from dry air (Harris et al. 1982), and (c) the air quality monitoring did not indicate any increase in nitrogen oxides or peroxyacetyl nitrate in the O_3 treatment compared to NF air. If (3) is confirmed, this study will provide a strong indication that other trace oxidants besides nitrogen dioxide and peroxyacetyl nitrate may add to the toxicity of ozone to vegetation. The NF vs. O_3 treatment comparison was not statistically significant for nearly all growth (height, # nodes), or

yield (fresh weight, dry weight, % dry/fresh weight) parameters at every harvest.

Thus, an unexpected conclusion from this study was that non-ozone pollutants may play a greater role in ambient oxidant effects than previously suspected. Detailed research by Harris et al. (1982) indicated that nitric acid vapor (HNO_3) may be the main pollutant of concern from corona discharge ozonizers as used in this study. These researchers determined the production of various nitrogen trace gases, organic trace gases, and hydrogen peroxide using pure oxygen, dry air, and various other source gases for the ozonator. In general their results showed that the amount of extra nitrogen dioxide, PAN, hydrogen peroxide, formaldehyde, ammonia, and other trace gases were either below the detection limit or the rate of production was less than 1/1,000 of that for ozone. Organic compounds were in general oxidized to carbon dioxide or carbon monoxide (both non-toxic to plants) by the ozonizer. Only nitric acid and dinitrogen pentoxide were present in any substantial amounts. The concentrations of these gases were dependent on water vapor concentrations in the source gas, with little of either gases detected unless dry air was used for the ozonizer. With dry air the nitric acid produced could become quite high, possibly up to 40% of the ozone concentration produced by the ozonator. Since dry air was used for generating the first four harvests of this study, nitric acid vapor is the prime suspect for the possible increased injury in the O_3 treatment.

There have been a few spot measurements of nitric acid vapor in the atmosphere using recently developed monitoring technology (Forrest et al., 1982; Spicer et al., 1982). Much of the research has focused on the South Coast Air Basin of California (Bytnerowicz et al., 1987), where the high inputs of nitrogen oxides from automobiles lead to high concentrations of a variety of nitrogen species. In one study in the San Gabriel Mountains the concentration of nitric averaged about 5 ppb compared to 30 ppb of nitrogen dioxide over a six hour period (Bytnerowicz et al., 1987). However, the Bytnerowicz study could not indicate peak values which may be much higher. Furthermore, nitric acid values have not been well documented for the Riverside area where the reported study was conducted.

There have been no detailed studies of nitric acid toxicity to vegetation. However, based on recent, unpublished pollutant deposition

research, the deposition velocity of nitric acid to leaves is approximately three times that for nitrogen dioxide (G. Taylor, Jr., Oak Ridge National Laboratory.) It is uncertain how much of the nitric acid actually enters leaves compared to nitrogen dioxide because comparatively more nitric acid is absorbed to the leaf surfaces. However, ignoring this point for now, if the toxicity of nitric acid was the same as nitrogen dioxide, than the atmospheric concentration (exposure) of nitric acid necessary to injure plants could be one-third that for nitrogen dioxide.

Metabolic and growth effects from nitrogen dioxide were reported with atmospheric concentrations ranging from approximately 0.5 ppm for one hour to 0.2 ppm for 10 hours (US EPA, 1982). The threshold for necrotic foliar lesions is much higher at >2 ppm for one hour to 1 ppm for 10 hours. Thus, using the potential three fold greater deposition of nitric acid compared to nitrogen dioxide would indicate that from >0.67 ppm nitric acid for one hour to 0.33 ppm for 10 hours would be necessary for foliar injury. While the potential concentrations of nitric acid generated in this study were likely only up to about 0.1 ppm, the lack of any specific nitric acid effects research leaves the question of whether nitric acid did cause the extra injury in the O_3 treatments wide open.

B. Does Added Ozone Exposure Simulate Ambient Ozone Conditions?

In terms of chamber grown plants, the comparison between predicted and actual yield losses suggests that the non-filtered exposure appears to simulate actual ambient ozone conditions better than the added ozone treatment (Table 14). The percentage losses on projected and calculated basis were more similar for the NF vs CF comparison, and not the O_3 vs CF comparison on a whole season basis. In addition, nonfiltered air contained the entire range of ambient pollutants including nitrogen oxides which may have protected plants and added oxidants which may have have increased injury. In contrast, the O_3 treatment did not have the nitrogen oxides and may have had extra added oxidants.

The use of the predicted losses based on the equation from Temple et al. (1988) appears to be reasonable. The research by Temple et al. was part of the National Crop Loss Assessment Program, and as such used a defined protocol to represent actual field exposure conditions in a commercial growing area at Shafter in the San Joaquin Valley. These included

field grown plants, irrigation, normal cultural practices, and use of open-top field chambers. The research described in this report used similar chambers and cultural practices, but the plants were grown in pots under experimental conditions at Riverside, and different cultivars were used than that used by Temple et al.

The study by Temple et al. (1988), used ozone generated from oxygen to produce the higher ozone concentrations for the dose response equation which are similar to the concentrations found in Riverside. Since the ozone was added to nonfiltered air, background concentrations of nitrogen dioxide and other oxidants should have been present in the chambers. Thus, the primary difference between an ozone concentration as represented by the Temple equation as opposed to the same concentration in the reported study, was the lack of the incremental amount of nitrogen dioxide and other oxidants above backgroud in proportion to the added ozone. In other words, in the Temple study when ozone was increased to 2.0 times the nonfiltered concentration, e.g. 0.10 ppm; the concentrations of other oxidants were likely only one-half of what they would have been if the 0.10 ppm ozone would have occurred in ambient air.

It is possible that the lower concentrations of other oxidants in the Temple compared to the reported study may have been responsible for the slightly lower predicted losses (4-7%) with the Temple equation vs. those found with the NF treatment in this study (Table 14). This difference was similar to the difference in estimated alfalfa yield losses for Riverside county found with Temple's equation vs. an equation generated from previous Riverside studies (Olszyk et al. 1986b). The comparison was made as part of the ARB sponsored crop loss assessment project (Thompson and Olszyk, 1988). This comparison indicated a slightly lower whole growing season alfalfa yield loss of 14% with the Temple equation compared 18% with the Thompson-Olszyk equation.

The primary conclusion of the above discussion is that to simulate ambient ozone conditions, it is preferable to use nonfiltered air as compared to charcoal filtered air plus added ozone. If a range of ozone concentrations is desired it would be much preferable to have different degrees of filtration of ambient oxidants (Kats et al. 1985), than charcoal filtered air with different concentrations of ozone added to it. However, this type of protocol requires experimental sites in rela-

tively highly polluted areas such as Riverside, and is not appliable to more pollutant-free areas. For those sites where ozone must be added to generate a dose-response equation it is much more difficult to produce a realistic pollutant exposure representative of the actual mix of oxidants in which ozone would occur.

Generation of ozone from oxygen would result in a defined pollutant exposure, but would not actually represent effects from an ambient ozone exposure due to the lack of the associated other oxidants. Use of ozone from oxygen for long term exposures would require very large amounts of oxygen which can be expensive and time consuming. This is especially critical for forest effects studies where large open-air release systems at remote sites are being considered (Hogsett et al. 1987).

In contrast, use of dry air or compressed air would be a much simpler and less expensive means of generating ozone, especially at remote sites or with open air release systems. Furthermore, generation of ozone from dry or compressed air would result in ozone mixed including other oxidants that are more qualitatively representative of ambient ozone exposures. The main, and potentially greatest, problem is that the added oxidant pollutants besides ozone would likely be at higher concentrations than in ambient air - and based on the reported study these would enhance the ozone effects.

All of the above are very important considerations which must be taken into account when designing new studies or interpreting old studies on the effects of ozone on vegetation.

The calculated losses were similar for the NF and AA treatments for all harvests except the third. This indicated, that, in general there was no obvious chamber effect on plant response to ozone. The yields in the NF chambers were much lower than the AA yields at the third harvest, possibly due to cooler, more humid weather early in the growth period which would have encouraged etiolation and lodging in the chambers.

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