Growth, Physiological, and Biochemical Response of Ponderosa Pine (Pinus ponderosa) to Ozone

Final Report

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Abstract

This report describes results of the 1989/1990 field studies on the effects of ambient and elevated ozone concentrations on growth, development of injury, physiological processes and chemistry of ponderosa pine (Pinus ponderosa Dougl. ex Laws) seedlings. The study was performed at a typical ponderosa pine site at Shirley Meadow in the Greenhorn Range of the southern Sierra Nevada. In the study, the two-year old ponderosa pine seedlings from the Greenhorn Range stock were grown in pots filled with soil native to this location. To test the effects of air pollution, the seedlings were exposed in open-top chambers to four different treatments: 1) clean air; 2) clean air with the addition of ambient ozone concentrations; 3) clean air with the addition of doubled ambient ozone concentrations; and 4) ambient air. In addition, to test the effects of chamber enclosures on plant performance the plants were exposed to ambient air in the outside chamberless plots.

During the study, ambient ozone concentrations at Shirley Meadow were both elevated and typical of other southern Sierra Nevada locations. Ozone was the most important phytotoxic air pollutant in the area; however, concentrations of nitric acid vapor and ammonia were also elevated.

In the first year of the exposures, no significant changes in growth of the seedlings exposed to the ambient ozone concentrations were determined. However, these seedlings had an increase of foliar injury and a tendency toward reduced net photosynthesis. For the seedlings exposed to the doubled ambient ozone concentrations, a significant increase of foliar injury was accompanied by increased senescence of the previous year's needles, reduced rates of net photosynthesis, and a tendency toward reduced chlorophyll fluorescence.

Effects of ozone on ponderosa pine seedlings became more pronounced during the second season of exposures. At ambient concentrations, the effects of ozone were still subtle; however, a significant increase of the previous year's foliar injury was accompanied by their premature senescence, a tendency toward reduced net photosynthesis and chlorophyll fluorescence, and reduced starch accumulation in the foliage. At the doubled ambient ozone concentrations, increased needle injury was

accompanied by decreased stomatal conductance, net photosynthesis, chlorophyll fluorescence, pigment concentrations, and starch accumulation. As a result of these changes, biomass of foliage, stems, and roots of the plants was significantly reduced.

It was also shown that plants grown in the open-top chambers developed needles faster, had modified stomatal conductance, and higher concentrations of foliar starch compared to the plants from the outside, chamberless plots.

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Disclaimer

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Glossary of Terms, Abbreviations and Symbols

Annular denuder	An assembly consists of a cyclone, pyrex glass tubes, and filter system holders connected to vacuum pumps used to collect samples of trace gases and particulate air pollutants.
ARB	California Air Resources Board
Carotenoid(s)	The yellow or orange pigments in the leaves of plants. Lipids that function as accessory pigments to protect chlorophyll molecules from photooxidation and absorb and transfer light energy to chlorophyll.
Chlorophyll(s)	The green pigments of plant leaves; lipids that are the primary light absorbing molecules in the light reactions of photosynthesis.
Chlorophyll fluorescence	Partial re-emission of radiant energy captured by chloroplasts in plant cells.
Chlorotic mottle	Typical symptom of ozone caused injury on needles of conifers.
cm	Centimeter, equal to 1×10^{-2} m.
Conifer	A cone-bearing plant; a plant classified as being a member of the order Coniferales.
Cotyledon mark	A distinctive depression on a seedling stem denoting the location of the seed leaves.
d	Diameter of a seedling stem measured at a cotyledon mark.
d^2h	Measure of stem volume calculated as the diameter of a stem squared, multiplied its height.
$\delta^{13}C$	Measure of stable carbon isotope ratio
EPA	Environmental Protection Agency
EPRI	Electric Power Research Institute
F_m	Maximum chlorophyll fluorescence
F_v	Variable chlorophyll fluorescence
Gas exchange	Processes involved in the movement of carbon dioxide or water vapor into and out of leaves (within the context of this study).

h	Height of seedlings measured from the cotyledon mark.
HPLC	High performance liquid chromatography.
M	Molarity, moles per liter.
mm	Millimeter, equal to 1×10^{-3} m.
Net photosynthesis	Net amount of carbon dioxide molecules incorporated into the plant system, calculated as a difference between gross photosynthesis and respiration.
NH_3	Ammonia
NH_4^+	Ammonium ion
nm	Nanometer, equal to 1×10^{-9} m.
NO	Nitric oxide
NO_2	Nitrogen dioxide
NO_x	Nitrogen oxides
NO_3^-	Nitrate ion
O_3	Ozone
Open-top chamber	A cylindrical structure comprised of a metal frame and PVC panels routinely used in field studies to examine the effects of air pollution on vegetation.
Photosynthesis	The process by which chlorophyll-containing cells in green plants convert incident light to chemical energy and synthesize organic compounds from inorganic compounds, especially carbohydrates.
Ponderosa pine	Pinus ponderosa Dougl. ex Laws; western yellow pine, or blackjack pine
ppb	Parts per billion
ppm	Parts per million
PST	Pacific Standard Time
S	Sulfur
Seedling	A young tree less than three feet high
SO_2	Sulfur dioxide

Stomatal conductance	A measure of stoma openness based on the water vapor conductance gradient between the atmosphere and the interior of the leaf.
Transpiration	The evaporation of water vapor from the leaves of plants especially through stomata.
µg	Microgram, equal to 1×10^{-6} g.
µM	Micromoll, equal to 1×10^{-6} M.

Summary and Conclusions

Forest decline related to air pollution has been observed for many years in vast areas of Central and Eastern Europe (Ashmore et al., 1985; Blank, 1985) and eastern North America (Tomlinson, 1983; McLaughlin, 1985). In most of the affected areas, ozone, sulfur dioxide and acidic precipitation are believed to be the main phytotoxic agents. Negative effects of ozone on some forests in the western United States were determined much earlier. Increased mortality of trees, reduced needle retention, increased foliar injury, changes in physiological processes and reduced growth of some coniferous species due to ozone in the San Bernardino Mountains of southern California were noticed for several decades (Miller et al., 1963; Miller and Millecan, 1971). More recently, reduced needle retention and increased foliar injury of ponderosa and Jeffrey pine were found over large areas of the western slopes of the Sierra Nevada (Duriscoe and Stolte, 1989). Significant reduction of Jeffrey pine radial growth was also determined in the Sierra Nevada (Peterson et al., 1987). Despite these important findings, there are still gaps in the knowledge of the mechanisms by which ozone damages forest trees, and to close those gaps plants need to be exposed in controlled field conditions and results carefully evaluated.

In the last few years a number of mechanistic studies on the effects of ozone and other environmental stresses on coniferous seedlings have been carried out in California. In most of these studies, ponderosa pine (Pinus ponderosa Dougl. ex Laws), an economically and ecologically important tree species in the western United States has been used. In an ARB-sponsored study performed in Riverside, the effects of acidic fog and ambient concentrations of ozone on ponderosa pine and white fir seedlings were studied, but no significant changes in growth, injury development or physiological processes due to one-season of ozone exposures were found (Bytnerowicz et al., 1989d). Nor were significant effects of ambient concentrations of ozone during a single season found in a Southern California Edison-sponsored study on four different species of pine at Tanbark Flat, in the San Gabriel Mountains (Bytnerowicz et al., 1989a; Bytnerowicz and Takemoto, 1989). In the ROPIS (Responses of Plants to Integrated Stresses) study sponsored by the Electric Power Research

Institute (EPRI) at Whitaker Forest, Sequoia National Forest, seedlings of ponderosa pine were exposed to ambient and elevated concentrations of ozone, acidic rain, dry deposition and water stress treatments for three seasons. While exposures of plants for one season did not cause any significant trends in plant growth or physiology, after the second season significant changes in development of injury, physiology and growth of the plants exposed to elevated levels of ozone started to be seen, and became even more pronounced after the third season of the exposures (Temple et al., 1989; 1992).

As reported in this study, the two-year-old ponderosa pine seedlings from the Greenhorn Range stock were grown in pots filled with native soil. The seedlings were exposed to various air pollution treatments in open-top chambers located at Shirley Meadow, Greenhorn Range, southern Sierra Nevada (1950 m elevation) in the summer-fall seasons of 1989 and 1990. The air pollution treatments were as follows: a) clean air (CA); b) clean air with the addition of concentrations of ambient ozone (CA + O_3); c) clean air with addition of doubled concentrations of ambient ozone (CA + 2 x O_3); and d) ambient air (Ambient). Ozone effects on plants were tested by comparing results from the CA, CA + O_3, and CA + 2 x O_3 treatments. Effects of other non-ozone air pollutants were tested by comparing the CA + O_3 and Ambient treatments. Additionally plants were grown in chamberless outside plots (Outside). By comparing the plants from the Ambient and Outside treatments the effects of chamber enclosure on plant behavior were assessed. Growth, injury development, and physiological and biochemical processes were investigated. In addition to plant exposures, monitoring of ozone, nitrogen dioxide and total sulfur compounds, as well as wind speed, wind direction, and air temperature were continuously conducted. Concentrations of nitric acid, nitrous acid, ammonia, sulfur dioxide and ions in atmospheric aerosols were measured during intensive studies.

The objectives for the study were as follows:

1. To determine if exposures of ponderosa pine seedlings to ambient and elevated ozone concentrations would cause changes in growth and injury development.

2. To determine if exposures to ozone would cause changes in gas exchange and starch production of pine seedlings.

3. To evaluate changes in foliar chlorophyll and carotenoid concentrations and ratios caused by ozone exposures.

4. To determine if ozone exposures would affect chlorophyll fluorescence of the seedlings.

5. To determine if non-ozone air pollutants at ambient concentrations would change responses of trees to ozone.

No significant changes in growth of the seedlings exposed to the ambient ozone concentrations were determined during the first season. These seedlings, however, had increased foliar injury and a tendency toward reduced net photosynthesis. For the seedlings exposed to the doubled ambient ozone concentrations, a significant increase of foliar injury was accompanied by increased senescence of the previous year's needles, reduced net photosynthesis, and a trend toward reduced chlorophyll fluorescence of foliage.

During the second season of the exposures, the effects of ozone on the seedlings became more pronounced. At ambient concentrations the effects of ozone were still subtle, however, a significant increase of injury of the previous year's foliage was accompanied by their premature senescence, a tendency toward reduced net photosynthesis and chlorophyll fluorescence, as well as reduced starch accumulation of the foliage. At the doubled ambient ozone concentrations, increase of foliar injury was accompanied by decreased stomatal conductance, net photosynthesis, chlorophyll fluorescence, pigment concentrations, and starch accumulation. As a result of these changes, the biomass of foliage, stems, and roots of the seedlings were significantly reduced.

The following conclusions can be drawn from the study:

1. In the southern Sierra Nevada, more than two seasons of exposures to ambient levels of ozone were needed for reduction of biomass of ponderosa pine seedlings.

2. At the doubled ambient ozone levels, two seasons of the exposures caused significant reduction of physiological processes, increase of foliar injury, reduction of foliar starch reserves and the biomass of foliage, stems and roots.

3. Ozone effects on ponderosa pine seedlings progressed and accumulated with time. Initially the increased visible injury and accelerated senescence of the foliage were observed. Later changes, such

as reduced net photosynthesis, stomatal conductance, chlorophyll fluorescence, starch accumulation, and pigment concentrations led to reduced growth and biomass of the plants.

4. Faster and more pronounced reduction of net photosynthesis than stomatal conductance suggests that ozone injury to mesophyll, carboxylation, or excitation components of the carbon dioxide diffusion pathway were greater than injury to the stomata.

5. Ozone effects on ponderosa pine seedlings did not appear to be modified by nitric acid, ammonia or particulate pollutants at ambient levels. However, elevated ambient concentrations of these nitrogenous compounds might potentially change forest growth in the Sierra Nevada after long periods of time.

6. It was also shown that plants enclosed in the open-top chambers may change physiological processes and growth compared to plants grown in outside plots.

Recommendations

1. Long-term (more than two years) studies on the effects of ozone at ambient concentrations are needed for more complete understanding of mechanisms leading to decline of forest trees in the California mountains.

2. Systems other than open-top chambers should be tested for investigations of air pollutant effects on trees.

3. Continuation of monitoring of trace gases such as nitric acid, ammonia, and particulate pollutants in the Sierra Nevada should be continued and expanded in order to better understand a potential for long-term effects of nitrogenous and other air pollutants on forest ecosystems.

4. Exchange and comparison of results from this study with other in situ studies on ponderosa pine seedlings in which ambient concentrations of air pollutants have been used (ROPIS study, studies sponsored by Southern California Edison, the EPA study at Garner Valley in the San Jacinto Mountains, the Lawrence Livermore National Laboratory study with branch chambers, and others) is needed. Initially, an informal exchange of information is possible, but later a symposium with formal presentation of the results should be organized. This would enable verification of the results from our study with others, and allow better understanding of the potential for air pollutant damage to ponderosa pines in the South Coast Air Basin and southern Sierra Nevada mountain ranges.

I. INTRODUCTION

Ozone is the only air pollutant that has been proven to be phytotoxic at ambient levels in some areas of the western United States. One of the best known examples of this is decreased growth, reduced foliar retention and visible injury in ponderosa and Jeffrey pines (Pinus ponderosa and P. Jeffreyi) on the north western slopes of the San Bernardino Mountains (Miller et al., 1963, 1969). Elevated ozone concentrations have also been correlated with reduced radial growth of Jeffrey pines (Peterson et al., 1987), and foliar injury in ponderosa and Jeffrey pines on the western slopes of the Sierra Nevada (Duriscoe and Stolte, 1989). Elevated concentrations of ozone have also been recorded in some mountain locations near Seattle but with no accompanying injury of coniferous trees (Basabe et al., 1989).

In aqueous solutions, O_3 is a highly reactive compound that decomposes to form molecular and singlet oxygen (Heath, 1980). Ozone reacts with a variety of biological compounds, including unsaturated fatty acids, proteins, sulfhydryls, and ring-containing compounds (Mudd et al., 1984). While the direct oxidation of biomolecules by O_3 can disrupt plant cell electrochemistry, the production of oxygen free radicals and hydrogen peroxide (Heath, 1980), which are phytotoxic at low concentrations. At the cellular level of organization, O_3 is known to alter membrane permeability (Heath, 1975).

Growth responses of a variety of forest tree species have been reported to be inhibited by exposure to ambient or close to ambient concentrations of O_3. For example, eastern deciduous tree species such as Liriodendron tulipifera (Jensen, 1985); Populus deltoides x trichocarpa (Reich et al., 1984); and Populus tremuloides (Wang et al., 1986) have been found to exhibit reduced growth responses following O_3 treatment. Additionally, reduced stem diameter, plant height, and dry weight responses were observed in two species of Pinus elliottii following 112 days of O_3 fumigation (Hogsett et al., 1985); however, growth of Pinus strobus was not significantly affected by O_3 after three months of exposure (Reich et al., 1986). The results of these studies indicate that exposure to O_3 can have a negative impact on tree seedling growth responses, but the duration of exposure and inherent O_3-susceptibility of

a tree species appear to be important considerations with respect to O_3 effects on growth.

Central to most hypotheses on the physiological basis of tree decline is decreased plant vitality. Reduced foliar nutrient and pigment levels resulting in decreased photosynthesis is often cited as being fundamental to the disruption of normal physiological functions by air pollutants (Heath, 1975; Taylor, 1978). Rates of photosynthesis in a variety of deciduous tree species (Carlson, 1979; Reich and Amundson, 1985), white pine (Reich et al., 1987), and ponderosa pine (Coyne and Bingham, 1981) have been reported to be reduced by O_3 stress. Moreover, in the study on ponderosa pine (Coyne and Bingham, 1981), the rate of photosynthetic decline due to needle aging was accelerated in accordance with the amount of visible injury to needles. Thus, the rate of change in the levels of photosynthetic pigments (i.e., chlorophylls and carotenoids) may be important with respect to characterizing incipient metabolic alterations of photosynthetic decline and plant senescence.

Coniferous trees exhibit inherently low physiological process rates, but the year-round retention of needles provides a compensatory measure which allows for generating adequate amounts of photoassimilates for growth, homeostasis, and storage (Larcher, 1975; Kramer and Kozlowski, 1979). However, low physiological activity (i.e., stomatal conductance of water vapor) may be a beneficial adaptive measure for avoiding excessive pollutant uptake (Taylor, 1978), particularly for pine species that grow in areas where acute O_3 episodes are frequent. Nevertheless, the stress imposed by O_3 on above-ground plant structures has been shown to cause increased carbon retention in white pine (McLaughlin et al., 1982) and soybean (McLaughlin and McConathy, 1983). In this regard, alterations in foliar starch storage responses may result if chronic O_3 stresses are applied to sensitive pine species (e.g., ponderosa pine or Jeffrey pine). It is likely that increased needle starch levels may be observed in response to moderate O_3 stress, but decreased levels may occur if needle health is severely reduced. While the net effect of altered needle starch levels must be considered with respect to the adequacy of carbon allocations to other sink tissues (i.e., roots, stems, and buds), the changes may provide an indication of growth responses in the following year.

In recent years several studies on the effects of ozone on western conifers were undertaken by the researchers of the Statewide Air Pollution Research Center of the University of California and the USDA Forest Service PSW Research Station in Riverside. In the ARB-sponsored study, responses of ponderosa pine and white fir (Abies concolor) seedlings to ozone and acidic fog were studied in the field in Riverside for a single season. The Southern California Edison sponsored study investigated for two seasons responses of ponderosa pine and bigcone Douglas fir seedlings (Pseudotsuga macrocarpa) to ambient and sub-ambient levels of photochemical smog and reduced supplies of nutrients in the San Gabriel Mountains of Southern California. In the ROPIS (Responses of Plants to Integrated Stresses) study, sponsored by the Electric Power Research Institute, ponderosa pine seedlings were exposed for three seasons to ambient and elevated concentrations of ozone, and different levels of acidic precipitation, drought stress, and dry atmospheric deposition. In all those mentioned studies, effects on growth, development of injury, physiological processes and chemistry and biochemistry of the seedlings were investigated to determine the extent to which ozone and other pollutants stress plants.

II. PROJECT OBJECTIVES

The objectives for the study were as follows:

A. To determine if exposures to ambient and elevated ozone concentrations at Shirley Meadow (a typical site for this species in the southern Sierra Nevada) could induce changes in growth or cause injury development in ponderosa pine seedlings.

B. To determine if exposures to ozone affect gas exchange and starch production of pine seedlings.

C. To evaluate changes in pigment concentrations caused by ozone exposures.

D. To determine if ozone exposures affect chlorophyll fluorescence in foliage.

E. To determine if non-ozone air pollutants at ambient concentrations modify responses of plants to ozone.

The following hypotheses were tested during the study:

A. Exposure to ambient and elevated ozone concentrations supress growth and increase injury to plant foliage.

B. Exposure to ozone will reduce stomatal conductance and net photosynthesis of plants, resulting in decreased plant starch reserves.

C. Ozone exposure can lead to changes of foliar pigment concentrations.

D. Exposure of conifer seedlings to ozone will reduce the ratio of variable chlorophyll fluorescence (F_v) to maximal chlorophyll fluorescence (F_m).

E. At ambient concentrations responses of conifer seedlings to ozone alone are different from responses of such seedlings exposed to ozone together with other gaseous and particulate air pollutants.

III. MATERIALS AND METHODS

A. Experimental Design

The experimental design was a completely randomized block, with two replications. Each block consisted of five ozone exposure regimes: four in the open-top chambers and one on the outside chamberless plots. Ozone concentration was the main treatment effect, with the principal aim being to determine if ozone, at ambient and elevated concentrations typical of the chosen ponderosa pine forest stand, would alter growth, physiology and biochemistry of the seedlings compared with seedlings grown in clean air. Second, comparison of plant responses in ambient air chambers with plant responses in the clean air chambers to which ambient concentrations of ozone were added to provide information on the way ozone effects may be modified by the presence of other (gaseous and particulate) air pollutants typical of the area. Third, comparison of plant responses in outside plots to those grown in ambient air chambers to provide a measure of the effects of enclosure on plants in open-top chambers.

B. Site Location

The study was performed at Shirley Meadow, which is located on the eastern slope of the Greenhorn Range of the Sequoia National Forest, southern Sierra Nevada. The site is located 6500 feet (1950 m) above sea level at 35°42'N and 118°33'W, about 50 km NE of Bakersfield, California (Figure 1). The site was constructed on a clearing surrounded by a white fir-mixed conifer community of the lower montane forest, where it is typical for ponderosa pine to be one of the dominant tree species (Rundel et al., 1977). Ozone monitoring in several mountain locations of the southern Sierra Nevada showed that at the Greenhorn Summit (location near Shirley Meadow) concentrations were the highest with hourly peaks reaching 0.017 ppm (Vogler, 1982). Elevated ozone concentrations contained in urban plumes are transported up the western slopes of the Sierra Nevada by diurnal up-valley winds (Miller et al., 1972).

C. Plant Material

Ponderosa pine is an ecologically and economically important tree species in the western United States (Peattie, 1980; Little, 1981), and a dominant coniferous tree species in the lower montane forests in the Sierra Nevada and other mountain ranges in California (Rundel et al., 1977). It grows from sea level to approximately 9,000 ft (2,800 m) in elevation, but the best growth is attained on plateaus from 4,200 to 8,600 ft (1,300-2,600 m) in elevation. Ponderosa pine is tolerant of a variety of soils, but reaches it greatest size in deep, well-drained soils. It frequently exists in pure stands, although it is often found growing with other coniferous species. The trees mature quickly and may live from 300 to 600 years. The wood of this important tree is light and varies from soft to hard, and from fine to coarse-grained (Elias, 1987).

On February 16, 1989 approximately 700 two-year-old ponderosa pine seedlings from the Greenhorn Range stock were received from the Chico USDA Forest Service Nursery. The USDA Forest Service code for the seedlings, 122-540.60-13-54-4-0516=85, identifies them as propagated from seeds obtained during a general collection in 1985 in the Greenhorn Range District, from an elevation of about 5,400 feet (1,650 m), from 16 trees scattered over five different stands. The trees from which the seeds were collected were of an average good but not superior condition. The best looking 600 seedlings were planted during the second part of February 1989 in 6 L pulp pots filled with native soil collected in the Greenhorn Range from a typical ponderosa pine stand. From the time they were planted, the seedlings were watered with deionized water as needed (typically once a week).

The trees were put in the UC Riverside lathhouse on March 1, 1989 and were kept there until March 27, 1989. Because relatively high concentrations of ozone occurred in Riverside in the last half of March (reaching 0.13 ppm) the plants were moved to Garner Valley, where they were kept until they were moved to the Shirley Meadow site. Garner Valley is a typical site for ponderosa pine in the San Jacinto Mountains at an elevation of 4,000 feet (1,200 m). On April 25-28, 1989 the air temperatures at Garner Valley dropped at night to between -1 and -5°C, causing frost injury to the seedlings. All of their 1988 needles were damaged, but 1989 needles were affected only on about 20% of the trees.

After moving the trees to the Shirley Meadow site the best 400 which did not have freeze injury to the 1989 needles were selected for the experiment. Because of frost damage, the 1988 needles were excluded from injury or physiological determinations.

D. Air Pollution Exposures and Monitoring

Pine seedlings were exposed to air pollution in open-top chambers and in the outside plots. Air pollution treatments in open-top chambers were conducted as follows:

- Clean air (CA) - air filtered through activated charcoal and RIGA-FLO 200 air filters. This combination of filters in other studies removed approximately 90-95% of particles <2 micrometers in diameter, and excluded approximately 80% of ozone concentrations and approximately 50% of nitrogen dioxide concentrations (Bytnerowicz et al., 1989b).
- Clean air containing ambient concentrations of ozone (CA + O_3).
- Clean air containing 2 x ambient concentrations of ozone (CA + 2 x O_3).
- Ambient air (Ambient) - air filtered through Type DP2 60 Media air filters, which removed approximately 50% of particles <2 micrometers in diameter but did not significantly reduce concentrations of gaseous pollutants.

Plants were grown in open-top chambers typically designed for plant studies (Heagle et al., 1973) and, additionally, in outside chamberless plots (Outside) where concentrations of gases and particles were at the ambient level. Since mid-day air temperatures inside open-top chambers may exceed ambient temperatures by about 2-4°C (Musselman et al., 1986; Olszyk et al., 1989), a shade cloth was installed on the tops and sides of the chambers and over outside chamberless plots to reduce light intensity to about 55%. Eight open-top chambers and two outside plots allowed air pollution regimes to be duplicated. Ozone was produced with a Griffin ozonizer by exposing oxygen to UV light, and was then delivered to the open-top chambers through Teflon tubing. One designated Dasibi ozone instrument was used to monitor ambient concentrations of ozone. The readings were sent to the ISAAC-Apple II+ data acquisition system, which translated ozone ambient concentration values into a voltage signal. Changes in the voltage signal controlled production of ozone by the

Griffin ozonizer. Samples of air were taken by a scanivalve system in all the experimental plots and on the roof of the instrument trailer and were sent to a second Dasibi ozone monitor, as well as to the $NO-NO_2$ Monitor Lab analyzer and the total S Meloy analyzer. Collection time at each sampling point took five minutes, allowing pollutant concentrations to be monitored at each point once an hour.

Total atmospheric nitrate, ammonium and sulfate ions in the chambers of different air pollution regimes were collected with low volume sample trains as described by John et al. (1984b). The sample trains consisted of aluminum cyclone (John et al. 1984a), Sierra Instruments Constant Flow Air Samplers Model No. 113, and nylon filters. The cyclones were those developed by the Air and Industrial Hygiene Laboratory in Berkeley, California (John and Reischl, 1980), with the addition of a Teflon coating in the internal surfaces to minimize adsorption of gaseous nitric acid. Air was pulled through the systems at 22L/min, providing a 50% cutoff of the 2.5 μm diameter particles. Nitric acid vapor, as well as particles <2.5 μm, were sampled and absorbed on Nylasorb 47 mm diameter filters with 1 μm pore size placed in Nucleopore polycarbonate filter holders. Particles >2.5 μm were deposited inside the cyclones. Samples of air were taken from the chambers of various air pollution regimes (CA, CA + 2 x O_3, Ambient) and from the chamberless outside plots.

In addition, ambient concentrations of HNO_3, HNO_2, NH_3, SO_2 and ions in particles <2.2 μm were collected with the annular denuder systems (Possanzini et al., 1983), modified by Peake and Legge (1987). HNO_3, HNO_2, and SO_2 were deposited on two glass denuder tubes coated with $NaHCO_3$. NH_3 was deposited on a third annular denuder tube coated with citric acid. Particulate matter was collected on 47 mm diameter Teflon filters backed by 47 mm diameter Nylasorb nylon filters. Preparation of denuder tubes and assembly of the systems was done in a room with clean air. After air pollution collections, the annular systems were disconnected, sealed and transported to the laboratory. In the laboratory they were disassembled within 24 hrs. Glass tubes were placed in plastic bags and stored at -18°C prior to the extractions. Annular denuder tubes used for collection of acidic gases were extracted with weak carbonate buffer, and tubes used for collection of NH_3 were extracted with ultrapure water. Teflon and Nylon filters were extracted with carbonate buffer.

Extract solutions were immediately analyzed for NH_4^+, and within 24 hrs for NO_3^-, NO_2^-, and SO_4^{2-}.

E. Meteorological Monitoring

A Sierra/Misco Inc. Model 1036HM Wind Sensor was used to monitor wind speed and direction. This sensor was installed 4 m above ground level on the roof of the instrument trailer. The air temperature was monitored with an AD590 sensor, and relative humidity with a HMD30U sensor. The amount of rain precipitation was measured with a rain gauge Model 2501. Meteorological data was stored on floppy disks and later reported as hourly values.

F. Measurements of Plant Growth

During the first season, the measurements of plant growth were made on all 400 seedlings (40 seedlings per chamber/plot). Because half of the plants were harvested after the first season, during the second season the measurements were made on the remaining 200 seedlings (20 seedlings per chamber/plot). Growth of the plants was measured before the experiment started and every two months during the exposures. Height of the plants was measured in centimeters from the cotyledon mark to the end of the main shoot, and diameter was measured in millimeters at the cotyledon mark. Because a cross-section of a tree is not an ideal circle, two measurements of stem diameter were taken, and their averages were used for further calculations. Length of growth buds on a main stem were measured in millimeters. During the harvests the upper parts of the seedlings were separated from the roots at the cotyledon mark, and were divided into stems, needles, and buds of different age classes. The plant samples were dried at about 40°C for two weeks, and then at 70°C for 12 hours before weighing. The weight of roots, stems, needles and buds were recorded in grams.

In the beginning of the second season of the exposures, development of needles was evaluated. Percentages of needles on each tree assigned to each of the four different categories (stages of development) was determined. The categories were assigned as follows:

Stage 1 - needles not developed

Stage 2 - developing needles <2 mm long

Stage 3 - developing needles >2 mm and <10 mm

Stage 4 - developing needles >10 mm

Statistical analysis of needle development data was done after an arcsin transformation was calculated for percentages of occurrences of various stages (Steel and Torrie, 1980).

G. Injury Evaluation

Injury development of the 1989 foliage was determined every two months during the experiment. Tip burn and chlorotic mottling were the main symptoms of needle damage. Percentages of foliage area affected by these two symptoms were estimated and presented both as the percentage of foliage area affected by chlorotic mottle (considered as a typical symptom of ozone injury) and as a total area of foliage affected by the two symptoms. Statistical analysis of injury data was done after an arcsin transformation was calculated for percentages of needle area affected both by the chlorotic mottle and by the sum of chlorotic mottle and tip burn.

In addition to the assessment of visual injury symptoms, the scanning electron microscopy (SEM) technique was used to determine the needle surface damage. Prior to the final harvest in the second year of the study, needles were collected, air dried and shipped to Ms. Minna Turunen of the Department of Botany, University of Oulu, Finland, for the SEM evaluation of surface changes. The needles were covered with gold-palladium (45nm) with a Polaron E 5100 sputter device, and micrographed with an SEM, Jeol JSM-35 at 15 kV and exposure of 90 s.

H. Physiological Measurements

Measurements of carbon dioxide and water vapor exchange were made with a portable photosynthesis system (LI-COR 6200, Lambda Instruments, Lincoln, NE). The LI-COR 6200 system simultaneously measured irradiance, relative humidity, carbon dioxide concentration, leaf temperature, and air

temperature (LI-COR 6200 Primer, 1989). These data were used to calculate net photosynthesis and stomatal conductance of water vapor. Transpiration rate depends upon two conductances of the flow, from the sub-stomatal aperture and from the conductance of a thin boundary layer of air that envelopes the leaf. Since the boundary layer conductance for a leaf in the leaf chamber is usually different from the same leaf out of the chamber, the measured transpiration rate is usually artificial. Therefore, no transpiration rates are presented in this report. The 0.25 L leaf cuvette was used in this study, with a total sampling time per seedlings of 60 s. For each seedling mean values of net photosynthesis and stomatal conductance of water vapor represent the average of ten 6 s measurements. After gas exchange measurements were taken, the number of needles enclosed in the cuvette was counted. Equations were generated to estimate total needle surface area from measures of needle number and needle length and width, in order to quantify needle surface area non-destructively. All values of plant gas exchange were expressed on a total needle surface area basis. The gas exchange measurements were performed four times a month. On each sampling day, measurements were made on branches from four different seedlings per plot. Plot means were used for statistical analysis of the data.

In addition, field measurements of chlorophyll fluorescence were made during the course of the experiment. According to Oquist and Wass (1988) the F_v/F_m ratios (variable fluorescence/maximal fluorescence) are sensitive indicators of environmental stresses. Chlorophyll fluorescence of the foliage was measured with the portable Plant Stress Meter (Polartech, Umea, Sweden) on loan from Dr. Bjorn Martin, NPI, Salt Lake City, Utah. The readings were made in the evening hours after sunset. Foliage used for measurements was allowed to adapt to the dark for about 20 minutes, after which its F_v/F_m values were recorded. The measurements were performed once a month on four seedlings in each of the experimental plots.

I. Biochemical Assays

In the initial phase of the study, a high performance liquid chromatography (HPLC) method was used for determination of chlorophyll and carotenoid pigments in plant extracts according to the procedure described

by Goodwin and Britton (1988) and Wolfenden et al. (1988). This method offered a possibility of determining concentrations of chlorophyll a, chlorophyll b, beta carotene, violaxanthin, antheraxanthin, neoxanthin, lutein and other pigments. Concentrations of individual pigments and ratios between some of the pigments (such as violaxanthin/antheraxanthin), can serve as sensitive indicators of oxidant air pollutants effects on plants (Wolfenden et al., 1988). Unfortunately, after several months of laboratory tests, we found that the method was not applicable for the analysis of ponderosa pine needle material. This was mainly due to a lack of stability of the extracted samples and clogging of precolumns and columns of the chromatograph.

We decided to substitute the procedure with the method well tested in our laboratory, which had been developed by Hiscox and Israelstam (1979). Needles stored at -18°C were used for the pigment assay. Three needles were cut into 1 cm long pieces and placed in a scintillation vial containing 10 mL dimethyl sulfoxide (DMSO). The needles were incubated for 7 hours at 65°C and cooled to room temperature. Absorptions of the extracts were determined at 470, 646 and 664 nm with the Hewlett-Packard UV-VIS diode array spectrophotometer Model 8452A. Concentrations of chlorophyll a, chlorophyll b, and carotenoids were calculated according to formulas described by Lichtenthaler and Wellburn (1983).

Starch concentrations in needles were determined according to the following procedure.

Dried needles, obtained from the destructive harvest were ground with a Wiley mill to pass through a 40 mesh screen. The needles' starch content was determined by assaying glucose content of foliage samples subjected to chemical and enzymatic digestion (Huber and Israel, 1982). Sub-samples of needle preparations (50 to 100 mg) were extracted four times with 80% ethanol (v/v) in an 80°C water bath until the samples were pigment-free. After the supernatant ethanol was removed, 1 mL of 0.2 M KOH was added to the needle brei, and the mixture was incubated at 100°C for 30 min to facilitate the chemical digestion process. Samples were allowed to cool for 10 min, after which the mixture was neutralized by adding 0.2 mL of 1 M acetic acid. The mixture was stirred and placed in a 55°C water bath for 5 min before adding 1 mL of amyloglucosidase from Rhizopus sp. (400 units mL^{-1}, Sigma Chemical Co., St. Louis, MO) in 50 mM

sodium acetate buffer (pH 4.5). Enzymatic digestion was allowed to continue for 90 min, after which the samples were placed in a 100°C water bath for 1 min to stop the enzyme digestion process. The samples were cooled to room temperature and centrifuged. The supernatant was adjusted to 10 mL with distilled water, and kept frozen until it was assayed. Aliquots (0.2 to 0.5 mL) were analyzed for glucose content by measuring the production of oxidized O-dianisidine (Glucose Diagnostic Kit, Sigma Chemical Co., St. Louis, MO) at 450 nm with a spectrophotometer. Starch content (expressed as glucose equivalents) was calculated on a dry weight basis.

J. Chemical Analysis of Plant Material

Samples of needles were collected during the 1989 and 1990 harvests. The samples were dried overnight at 70°C, ground with a Wiley mill, and stored for the analysis in plastic vials. The ground plant material was digested in a Kjeldahl mixture of H_2SO_4, H_2O_2, selenium powder and $LiSO_4$ H_2O (Parkinson and Allen, 1975). Concentrations of N and P were determined colorimetrically with a Technicon TRAACS 800 Analyzer. Concentrations of Ca, Mg, and K were determined with an atomic absorption spectrophotometer (Perkin-Elmer 5000). All these assays were performed at the laboratory of the USDA Forest Service PSW Research Station in Riverside.

Dried and ground needle samples were sent to the laboratory of Dr. Ehleringer, Department of Biology of the University of Utah for determinations of stable carbon isotope ratio. The fractionation of carbon isotopes during photosynthesis involves several distinct biochemical and physical processes which control CO_2 uptake. Such processes include stomatal conductance, which can be readily affected by air pollutants, and photosynthetic fixation of CO_2 by the primary carboxylating enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (Martin et al., 1988; Berry, 1989). The results were presented as $\delta^{13}C$.

K. Chemical Analysis of Soil Samples

Analysis of soil extracts was performed in the chemical laboratory of the USDA Forest Service PSW Research Station in Riverside. Samples of soils from randomly selected pots in each of the experimental plots, as

well as the original soil sample, were extracted in distilled water and strontium chloride solutions. Concentrations of chloride, nitrate, phosphate and sulfate were determined with ion chromatography (Dionex 4000i system) and colorimetrically (Technicon TRAACS 800 Analyzer). Concentrations of metals were determined with an atomic absorption technique (Perkin Elmer Atomic Absorption Spectrophotometer Model 5000).

L. Statistical Analysis of Data

Statistical analysis of data was performed for a completely randomized design using analysis of variance. Plot means were used for the analysis of data. Significance of air pollution treatments as well as significance of contrasts between the individual treatments were determined. The results are presented as means and standard deviations for plot means (measure of variability between the plots for each treatment), as well as p values for all air pollution treatments and contrasts between the individual treatments (Snedecor and Cochran, 1978).

M. Brief Chronological Description of Activities

1988

During 1988 most of the equipment and instrumentation needed were ordered and purchased. During that time negotiations on obtaining permits needed to perform research in the area of the Sequoia National Forest started. Ponderosa pine seedlings needed for the study were reserved; instrument trailer was purchased and remodeled; native soil for tree planting was excavated, prepared, and stored; and wooden stands for holding tree pots started to be made.

1989

At the beginning of 1989 purchase of all equipment and instrumentation was completed. All of the pot stands were made. The seedlings were received and planted into pots. Plants were initially maintained on the UC Riverside Campus and later at Garner Valley of the San Bernardino Mountains. Remodeling of the instrument trailer was finished and air pollution and meteorological instruments were tested at the UC Riverside Campus. The research site was developed in May 1989. Activities during that time included fencing the research area, setting up open-top chambers and outside plots for plant exposures, connecting electric lines,

installing and calibrating instruments, and testing the ozone dispensing system. The plants were moved to the Shirley Meadow site in the beginning of June 1989, and immediately after that the air pollution exposures begun. During the exposures (June through October), growth, injury and physiological processes of the plants were measured, and samples for pigment analysis were collected and stored. The exposures ended in the middle of October, at which time the fencing, chambers, instrument trailer, and all the instrumentation were removed from the research site and stored for the winter. Half of the seedlings were moved to the vicinity of the research site where they were kept during winter. The remaining plants were moved to Riverside where destructive harvest took place at the end of October. Measurements of plant growth (height, diameter, weights of needles, stems, and roots) were made during harvest. The plant samples were dried, ground and preserved for analysis of starch concentrations, stable carbon composition and mineral composition.

1990

In winter the growth measurements of the harvested seedlings continued as well as the analysis of plant pigments with the HPLC technique. In February the status of the experimental seedlings stored at Shirley Meadow for winter was checked. All the air pollution and meteorological monitoring instrumentation was thoroughly checked and repaired if needed. Preparation of the field site for the second season of exposures started in April and was finished on May 9, 1990. On that day the seedlings were moved into the chambers, and two days later air pollution exposures started. During the 1990 growing season, measurements of plant growth, injury development, and physiological processes continued according to the research plan. Plant material was collected every two months for the pigment analysis. Ambient concentrations of air pollutants were monitored during the period of the exposure. Trace air pollutants were analyzed inside the chambers that received different air pollutant exposure regimes. The exposures lasted until mid October 1990. The plants were moved to Riverside, where the last set of measurements of height, development of growth buds of the seedlings was performed. Destructive harvest of the seedlings took place immediately after the measurements. During harvest the last set of samples were collected for

pigment determinations. Samples were dried and determinations of biomass of the seedlings began. Dried needles were ground with a Wiley mill and samples were used for analysis of starch concentrations, stable carbon isotope composition and mineral composition.

1991

During 1991 the remaining determinations of seedlings biomass and plant chemical assays were completed. Chemical analyses of soil samples collected at the end of the air pollution exposures were done. All of the remaining results of air pollution and meteorological monitoring, measurements of growth and injury development, chemical determinations, and physiological measurements were stored in computer memory and tabulated. Statistical analysis of the results using the ANOVA was performed, and a draft final report for the entire study prepared.

IV. RESULTS

A. Air Quality

Concentrations of ozone, nitrogen dioxide, nitric oxide and total sulfur compounds measured during the 1989 and 1990 seasons in open-top chambers with different filtration regimes and in the outside chamberless plots are presented in Table 1 through 4. During most of the study, concentrations of ozone in the CA + O_3 and Ambient chambers were similar to the levels measured in the Outside plots. In the CA + 2 x O_3 chambers, concentrations of ozone were about two-fold larger than those in the CA + O_3, Ambient and Outside air pollution treatments. Low monthly 24-hr average O_3 concentration occurred at the beginning of the first year of exposures (June 1989) because of a malfunction of the O_3 generating system. The monthly 24-hour average concentrations of O_3 in the CA treatment did no exceed 0.024 ppm during the entire study, nor did the highest 1-hour peak values exceed 0.055 ppm. No large differences in ozone concentrations between the individual months and years occurred during two years of the study. Diurnal concentrations of ozone for all the individual months during the study, are shown in Figures A1 through A11. These profiles were similar for most months. The lowest concentrations of ozone occurred during morning hours and the highest during afternoons. During mid-day ozone concentrations remained stable: no decrease of ozone concentration at night was detected in any of the months.

Concentration of nitrogen dioxide, nitric oxide and sulfur compounds were very low during the entire study, and no significant differences between the air pollution treatments were observed (Tables 2 through 4). Detailed air pollutant monitoring data for every air pollutant treatment are contained in a diskette attached to this report.

Determinations of trace gases and atmospheric ion concentrations in ambient air determined with annular denuder systems (KAPS) are presented in Table 5. Concentrations of gaseous pollutants fell within the following ranges: nitric acid 0.16 - 4.82 $\mu g\ m^{-3}$, nitrous acid 0 - 1.94 $\mu g\ m^{-3}$, ammonia 0.31 - 5.47 $\mu g\ m^{-3}$, and sulfur dioxide 0.13 - 2.55 $\mu g\ m^{-3}$. Concentration of ions in the fine particulate fraction (<2.2 μm) were in the following ranges: nitrate 0.03 - 2.20 $\mu g\ m^{-3}$, ammonium 0 - 0.53 μg

m^{-3}, and sulfate 0 - 2.21 μg m^{-3}. Concentrations of nitric acid were higher during the day than at night, but day and night concentrations of other pollutants were similar.

On two dates (August 15 and September 25, 1989) air samples were collected with the annular denuder (KAPS) system from the chambers (CA and Ambient), as well as from the outside plots. Concentrations of nitric acid in the Outside plots were several times higher than in the Ambient chambers (equipped with the low-efficiency particulate filters). Concentrations of nitric acid in the CA chambers (equipped with high-efficiency particulate filters and charcoal filters), were about 60-70% of those determined in the Ambient chambers. No significant differences in nitrous acid or ammonia concentrations were determined for the studied plots. During the first period of the comparisons, concentrations of sulfur dioxide were the highest in Outside plots, and lowest in the CA chambers. However, during the second period of the measurements such differences were not seen. No clear differences in concentration of ions in fine particulate fraction occurred between the plots (Table 6).

Additional determinations of ion concentrations in plots of different filtration regimes were made with cyclone trains. These comparisons were performed in the end of July 1990. Concentrations of nitrate, ammonium and sulfate in the fine particulate and gaseous fraction were highest in Outside plots and lowest in the CA and CA + 2 x O_3 treatments. For the coarse particulate fraction, highest ionic concentrations were found for the Outside plots, and no clear differences between the chamber treatments were determined (Table 7).

B. Meteorology

Air temperature measurements for the two seasons of the study are summarized in Table 8. Maximum and minimum air temperatures varied considerably during all months, which is typical for mountainous locations. Average monthly 24-hour air temperatures for the entire period of exposure ranged from 7.5 to 20.2°C. Detailed air temperature data are contained on a diskette included with the report.

Results of measurements of wind direction and speed for individual months during the study are presented in Figures B1 through B18. In September and October malfunctioning equipment prevented wind speed and

wind direction data from being collected. During most of the time winds came from the WSW and SW directions and had an average speed of 1.5-2.5 miles/hour.

C. Growth Measurements

Height of the seedlings was not significantly affected by the air pollution treatments. However, in the second year of the study a trend toward the least increases of height of the seedlings exposed to the CA + 2 x O_3 treatment started to appear, although it was not significant. During the second season of the exposures, seedlings from the Ambient treatment became significantly taller than the trees from the Outside plots (Figure 2 and Table 9).

Similarly, diameter of the seedlings were not affected by air pollution treatments during the first season of the exposures. During the second year, however, a trend toward decrease of seedling diameter caused by the ozone exposures started to be evident. At the end of the study a difference between the CA and CA + 2 x O_3 treatments was significant at p=0.071. A tendency toward better growth of the seedlings in the Ambient chambers compared with seedlings from the Outside Plots became more visible and on September 4, 1990 the difference was significant at p=0.084 level (Figure 3 and Table 10).

The volume of the stems, expressed as d^2h, was not affected during the first season of the study. The effect of ozone on stem volume started to be evident in the second season, and a difference between the CA and CA + 2 x O_3 treatments on September 4, 1990 was significant at p=0.052. Also the difference in stem volume growth between the Ambient and Outside treatments became significant (p=0.042) - (Figure 4 and Table 11).

Observations of new needle development in the spring of 1990 (following one season of ozone exposures), indicated that new needles were emerging sooner on the seedlings preexposed to ozone. The higher the ozone concentrations in the preceding season, the higher the portion of more developed needles (stages 1 and 2). A significant effect of chamber enclosure on bud development was also indicated by a significantly higher portion of the more developed buds in the Ambient treatment compared with seedlings from the Outside treatment (p=0.018 for the stage 2 needles) - (Figure 5 and Table 12).

Results of measurements of height, diameter, and volume of the stems were confirmed by the results of biomass determinations made during the harvest following the first and second seasons of air pollutant exposures. After the first season of exposure, no significant changes in biomass of stems (both total biomass and 1989 portion) were seen. Similarly, no difference in biomass of 1989 needles and roots, buds or bud length were determined after the first season of exposure. However, seedlings from the CA + 2 x O_3 had significantly reduced 1988 needle biomass compared to seedlings from the CA as well as CA + O_3 treatments ($p=0.041$ and 0.024, respectively). There was also a tendency for seedlings from the Outside plots to have lower 1988 needle biomass than the seedlings from Ambient chambers ($p=0.073$) - (Figure 6 and Table 13). After the second season, effects of air pollution on biomass of the seedlings became more evident. Seedlings exposed to the CA + 2 x O_3 treatment had the lowest biomass of the entire stems, and 1990 and 1989 portions of stems (p = 0.090, 0.167, and 0.067, respectively). The effect of ozone exposure on biomass of the 1989 needles was most pronounced. The difference between the CA and CA + O_3 treatments was significant at $p=0.017$, while the difference between the CA and CA + 2 x O_3 treatments was significant at $p=0.000$ level. Decrease of biomass of the roots for the CA + 2 x O_3 treatment compared with the CA treatment was significant at $p=0.061$ level, while exposures at the CA + O_3 chambers did not cause reduction of the root biomass. After the second season of exposure, the differences between Ambient chambers and Outside plots also became significant. The biomass of entire stems, as well as their 1990 and 1989 portions was lower for the Outside plots compared with the Ambient chambers ($p=0.026$, 0.016, and 0.093, respectively). Decrease of the biomass of the 1990 needles, 1988 needles and roots of the seedlings from the Outside plots compared with the Ambient chambers was also evident ($p=0.027$, 0.079, and 0.039, respectively) - (Figure 7 and Table 14).

D. Injury Development

Significant increase of the 1989 needle injury (expressed both as chlorotic mottle and total injury) caused by the ozone exposure occurred in the middle of the first season of the study. At the beginning of the second year of the exposures these differences were less evident, but

later in the season they became significant again. Increase in foliar injury was evident in the ambient ozone treatments (CA + O_3 treatment) but was most pronounced in the CA + 2 x O_3 treatment. At the beginning of the study, needles in the Outside plots were more injured than the needles in the Ambient chambers, however, at the end of the second season this trend was reversed (Figures 8 and 9 and Tables 15 and 16).

Scanning electron microscopy (SEM) examination of needles collected in the end of the second year of the exposures revealed melting of wax fibrils in stomatal cavities of the needles from the highest ozone treatments. Most of the stomates in this treatment had this appearance (Figure 10).

E. Gas Exchange

On July 6, 1989 diurnal measurements of gas exchange of the plants were performed on four seedlings in an Outside plot. Negative net photosynthesis values were determined in the early morning (before 0715 PST); positive values of photosynthesis occurred at 0815 PST; and maximum photosynthetic rates were reached at about 1015 PST until 1445 PST. Photosynthetic activity started to decline at 1615 PST, and at 1915 PST was close to zero (Figure 11). Based on the results of these measurements we decided that for the entire study the gas exchange data would be collected only in the middle of the day, between 1100 and 1400 PST.

During the first season of exposure, the air pollution treatments did not have a significant effect on stomatal conductance of 1989 needles (Figure 12 and Table 17). However, during the entire season there was a trend toward reduced net photosynthesis at elevated ozone concentrations, and this trend was significant on a few occasions (Figure 13 and Table 18). During the second season of exposure there was a trend toward reduced stomatal conductance in 1989 needles caused by ozone exposures. For the CA and CA + 2 x O_3 treatments, significant differences occurred on several occasions, and for the CA and CA + O_3 treatments during the last set of measurements (Figure 14 and Table 19). Ozone exposures caused even more pronounced changes in net photosynthesis of the 1989 foliage (Figure 15 and Table 20). Similar to the 1989 foliage during the first season of the study, in the second season no clear effects of ozone exposures on the

stomatal conductance and a tendency toward reduced net photosynthesis of the 1990 foliage were seen (Figures 16 and 17, Tables 21 and 22).

F. Chlorophyll Fluorescence

During the 1989 season the plants in the CA + 2 x O_3 treatment had reduced F_v/F_m ratio compared with the CA treatment. During the same season, a tendency toward lower F_v/F_m values in the CA + O_3 compared with the CA treatment was seen, but was not statistically significant (Figure 18 and Table 23). During most of the second season of exposures, the higher the ozone concentration, the lower the F_v/F_m values of the 1989 needles. Significant differences between the plants from the CA + 2 x O_3 and CA treatments continued throughout the season and became greater with time. In the second part of the season, when the 1990 needles were measured, no clear effects of air pollution treatments on chlorophyll fluorescence were observed (Figure 19 and Table 24).

G. Starch Content

No clear effects of air pollution exposures on needle starch concentrations were determined after one season of exposures. After the second season, however, a trend (p=0.168) toward reduced starch concentrations was seen in 1989 needles at the CA + O_3 treatment compared to the CA plants. A strong effect of chamber enclosure was determined for 1989 needles at the 1989 harvest; needles from the Ambient treatment had more starch than needles from the Outside plots (p=0.000). Reduction of starch concentrations caused by the ozone exposures was determined for 1990 needles during the 1990 harvest. Significantly less starch was in the CA + O_3 needles compared with the CA needles (p=0.033), and in the CA + 2 x O_3 needles compared with the CA needles (p=0.008). A trend toward reduced starch concentrations in needles from the Outside plots compared with the Ambient plots continued (p=0.253) - (Figure 20 and Table 25).

H. Stable C Isotope Composition

No effects of air pollution exposures on the composition of carbon isotopes in the needles were determined. The only significant effect was a lower negative value of the $\delta^{13}C$ in the Outside treatment compared with the Ambient treatment (Table 26).

I. Pigment Concentrations

Unfortunately, due to a failure of the HPLC method for analysis of plant pigments, no results for the needles collected during the 1989 season are presented. The results of the DMSO method for the second season of the exposures indicate that pigment levels in the 1989 needles were lowered by ozone beginning in June 1990. There was a trend ($p=0.075$) toward reduced concentrations of chlorophyll a, and a significant ($p=0.027$) reduction of chlorophyll b concentration in the CA + 2 x O_3 treatment compared with the CA treatment. Destruction of pigments at the highest ozone treatment was more pronounced after an additional two months of the exposures. In the samples collected in August, a highly significant reduction of the concentrations of chlorophyll a, chlorophyll b, and carotenoids was determined for the CA + 2 x O_3 treatment compared with the CA and CA + O_3 treatments. No reduction in pigment concentrations was determined for the plants of the CA + O_3 treatment (Figure 21 and Table 27). Analysis of the 1990 needles collected at the final harvest (October 1990) did not indicate any significant effects of air pollution exposures on pigment concentrations (Table 28).

J. Mineral Composition of Needles

No clear effects of air pollution exposures on the mineral composition of plant foliage was seen. After the first season the only effect seen was a decrease in the concentration of P in the foliage from CA + 2 x O_3 treatment compared with the CA foliage (Table 29). In the 1989 foliage after the second season of the exposure, the only significant effect was a lower K concentration in the needles from the CA treatment compared with the CA + 2 x O_3 treatment (Table 30). Similarly, no significant effects of air pollution exposure on the mineral composition of the 1990 needles were determined (Table 31). In general, the 1989 needles in the first season of the study had much higher concentrations of N and P than both the 1989 and 1990 needles in the second season of the study.

K. Mineral Composition of Soil

Comparison of the results of the mineral analysis of soil samples collected in the beginning of the study and after two seasons of the exposures indicated no differences for most of the analyzed ions. The only exception, was a sharp reduction of the SO_4^{2-} and Na^+ ions in soil for all the air pollution treatments compared with the original soil sample (Table 32).

V. DISCUSSION

A. Air Quality and Meteorological Conditions

Ozone

Air pollution monitoring revealed that ozone was the most abundant and important air pollutant at the Shirley Meadow research site. During the period of plant exposures, ambient concentrations of this gas were relatively stable, not exceeding 0.118 ppm for 1 hour maximum averages, and remaining between 0.049 and 0.073 ppm for monthly 24-hour averages. These results are similar to those found at the same location by Taylor et al. (1986) in the summer of 1982. However, the measured concentrations of ozone were lower than the ozone monitoring data collected at the adjacent Greenhorn Summit between the years 1978 and 1981. During that period, ozone concentrations reached 0.170 ppm; and in the summer of 1978 there were 68 hours in which 1-hour average concentrations exceeded the Federal ozone standard of 0.120 ppm (Vogler, 1982). In addition, ozone concentrations at Shirley Meadow in the summer of 1989 were similar to, but slightly higher than, the concentrations of this pollutant at the Whitaker Forest study site during the 1988-1989 seasons (Temple et al., 1989), and fell within the range of values determined during the summers of 1974 through 1976 at locations in the middle portion of the San Bernardino Mountains, such as Barton Flats, Rock Camp and Running Springs (Miller et al., 1986). The ozone levels at Shirley Meadow were much higher than in a remote mountainous site in the Canadian Rockies (Legge and Krupa, 1989), and can be considered as moderately high if compared with other locations in the western United States (Bohm, 1989). If the ozone concentrations at the Shirley Meadow site are evaluated with regard to possible damage to forest trees, they may be considered as potentially hazardous to the forest (see discussion in Chapter V.B and V.D). Concentrations of ozone in the CA chambers were reduced to very low, non-toxic, levels, while in the CA + O_3 and Ambient chambers the concentrations were similar to the Outside plots during most of the study. However, during several days in June ozone was not added to the CA + O_3 and CA + 2 x O_3 chambers due to technical problems. Therefore, the average 24-hour concentrations of ozone for June in these two chamber treatments were lower than in the Ambient and Outside treatments.

Nitrogen and Sulfur Oxides

Ambient concentrations of nitrogen dioxide were very low and can be considered as non-toxic to the plants (Amundson and Maclean, 1982). The recorded ambient concentrations were much lower than concentrations occurring in the South Coast Air Basin or the San Bernardino Mountains (Bytnerowicz et al., 1987). Concentrations of nitric oxide monitored continuously during the study were also very low and had no potential to affect plants. Concentrations of nitrogen dioxide in the CA, CA + O_3, and CA + 2 x O_3 chambers were lower than in the Ambient chambers and the Outside treatment because charcoal filters absorbed the pollutant. Concentrations inside those chambers were reduced by about 50% compared to the ambient concentrations, which is typical of air filtered through charcoal (Bytnerowicz et al., 1989b).

Concentrations of sulfur dioxide determined with the annular denuder samplers (KAPS) confirmed the results obtained by continuously monitoring sulfur dioxide. The sulfur dioxide concentrations were very low, only slightly exceeding values determined at the Canadian Rockies remote site (Legge and Krupa, 1989), and were in the range of values determined at Shirley Meadow during the 1982 season (Taylor et al., 1986). Those concentrations are not toxic to plants (Mudd, 1975). Air filtration had no clear effect on concentrations of sulfur dioxide inside open-top chambers.

Nitric acid, nitrous acid, ammonia

Ambient concentrations of gaseous nitric acid rose to their highest level in the middle of the summer (July through August), when they exceeded 4.8 $\mu g/m^3$. The levels of nitric acid concentrations at Shirley Meadow are compared in Table 33 with the levels of this pollutant in other locations. Compared with some remote locations in North America, the concentrations of nitric acid at Shirley Meadow should be considered as elevated. Nitric acid concentrations were in the range of concentrations determined at the Whitaker Forest, southern Sierra Nevada, during the summers of 1989 and 1990. However, mean concentrations of nitric acid for both the day and night periods were higher at Shirley Meadow than at Whitaker Forest. The concentrations at Shirley Meadow were much lower than levels found at Tanbark Flat in the San Gabriel Mountains of the South Coast Air Basin. It should be remembered, however, that the Tanbark

Flat site is well known for its extremely high concentrations of nitrogenous pollutants (Bytnerowicz et al., 1987). Filtration of air into the chambers through particulate filters alone and through particulate and charcoal filters significantly reduced nitric acid concentrations.

Concentrations of nitrous acid fell within a range typical of ambient levels for this gas; however, they were higher than the values determined for the remote mountainous site in the Canadian Rockies (Legge and Krupa, 1989). In the open-top chambers, concentrations of nitrous acid fell below detection limits.

Concentrations of ammonia were in the range of 0.34 - 5.47 μg m^{-3}, and were the highest in summer. No clear differences between day and night values were determined. These values were several times higher than the values found at the remote mountainous site of the Canadian Rockies. They were also higher than concentrations at other mountainous sites in North America, but lower than the values in rural areas of the Netherlands and England (Table 34). Air filtration did not reduce ammonia concentrations in the chambers. Elevated ambient concentrations of nitric acid and ammonia at the Shirley Meadow site probably resulted from long-range transport of these pollutants from the automobiles, stationary combustion sources and agricultural emissions in the San Joaquin Valley.

Ions in Atmospheric Particles

Concentrations of ions in the aerosol fraction <2.2 μm fell within a range typical of ambient values (Legge and Krupa, 1989). The ionic concentrations were slightly higher than the values determined for the remote mountainous site in the Canadian Rockies (Legge and Krupa, 1989), but were much lower than the concentrations found at Tanbark Flat in the San Gabriel Mountains (Bytnerowicz et al., 1987).

Meteorological Conditions

Meteorological data for Shirley Meadow indicated that most of the winds come from the WSW and SW directions, bringing heavily polluted air masses from the Bakersfield-Oildale area in the San Joaquin Valley. These data confirm that the dispersion of air pollution from the Bakersfield area during the summer is heavily influenced by the general up-valley flow of air masses coming from the NW. Transport of the polluted air from the NW as well as local production of air pollution from the numerous stationary and mobile sources in the heavily populated Bakersfield-Oildale

area cause this portion of the Valley to be considered as the most polluted (Unger, 1978; Carroll and Baskett, 1979). As a result of such transport, forests in the southern portion of the Sierra Nevada have been exposed to elevated concentrations of air pollutants (mostly ozone), which could pose serious problems for trees (Peterson et al., 1987).

B. Growth Measurements

A single season of the air pollution exposures did not significantly affect height, diameter and stem volume (d^2h) of the pine seedlings. Despite a lack of significant changes in growth of the seedlings during the first season of the exposures, ozone significantly affected a potential of pine seedlings to produce new growth. It was reflected by the stimulation of new needle growth by ozone in the spring following the first season of the exposure. During the second season of the exposures, however, other changes in seedlings development were seen. The seedlings exposed to doubled ambient ozone concentrations had reduced height, diameter and stem volume compared to the seedlings grown in the clean air. The observed changes in growth development were accompanied by significant reduction of biomass of stems, needles and roots at this ozone treatment. In other studies on coniferous seedlings, ozone exposures caused a decrease in radial growth in Jeffrey pine (Peterson et al., 1987), and reduced stem diameter, plant height, top and root dry weight, needle number and needle length of the two varieties of slash pine (Hogsett et al., 1985). However, such exposures did not have significant effects on growth of eastern white pine (Reich et al., 1987), or Scots pine (Skeffington and Roberts, 1985).

Reduction of biomass of the previous year's foliage was the most sensitive indicator of ozone effects on the plants. Even a single season exposure to ambient ozone concentrations caused a significant reduction of the biomass of the previous year foliage. The reason for that was stimulation of premature senescence. Ozone is known for stimulating premature senescence (Heath, 1975), and this phenomenon has been reported for many forest tree species, including ponderosa pine (Miller and Van Doren, 1981; Temple et al., 1991).

Our results as well as the other studies indicate that ambient or slightly elevated concentrations of ozone very subtly affect changes in growth of trees. Therefore, to be able to determine with certainty that such changes actually take place, some basic conditions have to be met. These include long-term (multi-year) exposures, large number of the experimental trees and sensitive methods of the measurements.

Seedlings grew much better in open-top chambers than the chamberless plots. Better growth in the Ambient chambers than in the Outside plots started to be seen as a trend already in the first season, and became strongly significant in the second season of the experiment. Modification of plant growth in the chambers was probably caused by changes in temperature, relative humidity, light intensity and air movement observed in open-top chambers (Musselman et al., 1986; Olszyk et al., 1986).

C. Injury of Foliage

A statistically significant increase in foliar injury (either expressed as chlorotic mottle or total injury) was observed both for the CA + O_3 and CA + 2 x O_3 treatments by the beginning of the second year of exposures. The difference between chlorotic mottle-type injury and the total injury was higher for the CA + 2 x O_3 treatment than for any of the other treatments, indicating that high concentrations of ozone may increase tip burn injury of pine foliage as well. During the 1988 study at Tanbark Flat, ponderosa pine seedlings grown in the clean air treatment showed lower foliar injury than seedlings grown in the ambient air treatments. That difference, however, was not statistically significant (Bytnerowicz et al., 1989a). Similarly, ponderosa pine seedlings exposed to ambient air in Riverside did not develop significantly higher amount of injury than seedlings grown in clean air (Bytnerowicz et al., 1989c). However, at Tanbark Flat and Riverside, environmental conditions are different from sites where ponderosa pine grows naturally, and may have increased the resistance of the seedlings to ozone. At the ROPIS at Whitaker Forest, increased injury of foliage was seen during the second season of seedling exposures to the 1.5 x ambient ozone concentrations and at the ambient levels in the third season of the exposures (Temple et al., 1991).

Ozone concentrations in the CA + 2 x O_3 treatment caused significant increase in foliar injury similar to that found in highly polluted sites in the San Bernardino mountains, where ponderosa pine trees show severe symptoms of ozone damage (Miller and Millecan, 1971; Miller et al., 1986). An increase in chlorotic mottle and total injury in seedlings exposed to ambient concentrations of ozone provides evidence that development of foliar injury is a sensitive indicator of ozone stress.

D. Physiology and Biochemistry of Plants

A single season of the exposures to ambient and elevated ozone concentrations did not cause significant changes in stomatal conductance of the current year needles. Ozone had a greater effect on net photosynthesis, where needles in the doubled ambient ozone treatment exhibited a trend toward reduced net photosynthesis in mid-July 1989, which continued until the end of the year. Effects of the highest ozone concentrations on gas exchange by current year foliage in the second season of exposures were similar to the effects seen during the first season - reduction of net photosynthesis was not accompanied by similar reduction of stomatal conductance. This would suggest that for the young (current year growth) needles, losses in photosynthetic capacity were not controlled by changes in stomatal conductance but rather by injury to the mesophyll, carboxylation or excitation components of the carbon dioxide diffusion pathway (Coyne and Bingham, 1981). In the second season, ozone effects on gas exchange of the previous year's foliage were very pronounced. A significant reduction of stomatal conductance also took place, which caused an even greater reduction of net photosynthesis than in the first season. Reduction of photosynthetic capacity of the plants was reflected by the diminished reserves of carbohydrates in the second season of the exposures - both the previous year's and current year's needles of the plants exposed to ozone showed reduced concentrations of starch.

Despite clear effects of ozone exposures on net photosynthesis of the seedlings, no differences in stable carbon isotope composition expressed as $\delta^{13}C$ were seen. Stable carbon isotope composition is associated with the primary photosynthetic carboxylation reaction (catalyzed by ribulose-1,5-bis-phosphate carboxylase) and diffusion rate differences between ^{13}C

and ^{12}C (Ehleringer et al., 1986). Increases of $\delta^{13}C$ due to ozone fumigations were found in the leaves and roots of radish and soybean plants (Greitner and Winner, 1988), as well as in the leaves and wood of the trees exposed to air pollutants (Martin et al., 1988).

Chlorophyll fluorescence is a sensitive indicator of photosynthetic energy conversion (Papageorgiu, 1975). The specific site of the ozone damage is believed to be on the photosystem II (PS II) donor site (H_2O-splitting enzyme system) prior to any decrease in energy transfer efficiency within the pigment system. With increasing exposure to ozone, the electron transport from PS II to PS I also becomes inhibited (Schreiber et al., 1978). In this study, during the second year of exposures, significant reductions in the ratio of variable fluorescence to maximal fluorescence (F_v/F_m) was determined. It may be an indication of changes in the efficiency of PS II and transport of energy between PS I and PS II (Oquist and Wass, 1988).

Ozone exposure had a very strong effect on concentrations of pigments in the previous year's needles during the second season of the exposures. These effects began to appear in June 1990, and became very pronounced two months later. Concentrations of chlorophyll a, chlorophyll b, carotenoids and also a ratio chlorophyll a/chlorophyll b were significantly reduced due to exposures to the doubled ambient ozone concentrations. It should be emphasized that even two years of the exposures at the ambient ozone levels did not cause significant changes in plant pigments. Little is known about the mechanism of pigment destruction by ozone. However, a decline of chlorophyll concentration has been proposed as an indicator of ozone phytotoxicity (Knudson et al., 1977). In a short experiment with elevated ozone concentrations, significant reductions in chlorophyll content of the Rocky Mountains provenances of ponderosa pine seedlings were found (Aitken et al., 1984).

E. Evaluation of the Responses of Ponderosa Pine Seedlings to Ambient and Elevated Ozone Concentrations

Results presented here indicate a wide spectrum of changes in physiology, biochemistry and biomass production of ponderosa pine seedlings exposed to the doubled ambient ozone concentrations. During the first season of the exposure no changes in stomatal conductance were

observed. Without restriction of gas uptake, the seedlings absorbed high doses of ozone which caused damage to their photosynthetic apparatus, a phenomenon reflected by reduced rates of photosynthesis. Due to the accelerated senescence of the previous year's (1988) needles, the total photosynthetic capacity of those seedlings was even more depleted. Continuation of ozone exposures during the second season reduced net photosynthesis of the previous year's needles even further. During that season a reduction of photosynthetic capacity of those needles was more pronounced due to reduced stomatal conductance, reduced concentrations of pigments and lowered efficiency of PS II. Since the middle of the second season, net photosynthesis of the current year's (1990) foliage was also significantly reduced. Consequently, carbon assimilation of the seedlings became so severely reduced that in the end of the second season the needle starch accumulation as well as biomass of foliage, stems, and roots were drastically diminished.

The first season of the exposures to the ambient ozone concentrations did not cause significant changes in physiology of the seedlings, however, increase of visible injury of the previous year's foliage occurred. During the second season of the exposures an increased foliar injury, reduced chlorophyll fluorescence, reduced starch reserves of the foliage, and increased senescence of the previous year's foliage became significant. This indicates that photosynthetic effects of ozone on physiology and metabolism of ponderosa pine seedlings at ambient ozone concentrations are low and cumulative. It seems obvious that only long-term exposures to ambient levels of ozone can to produce significant reduction of seedlings biomass.

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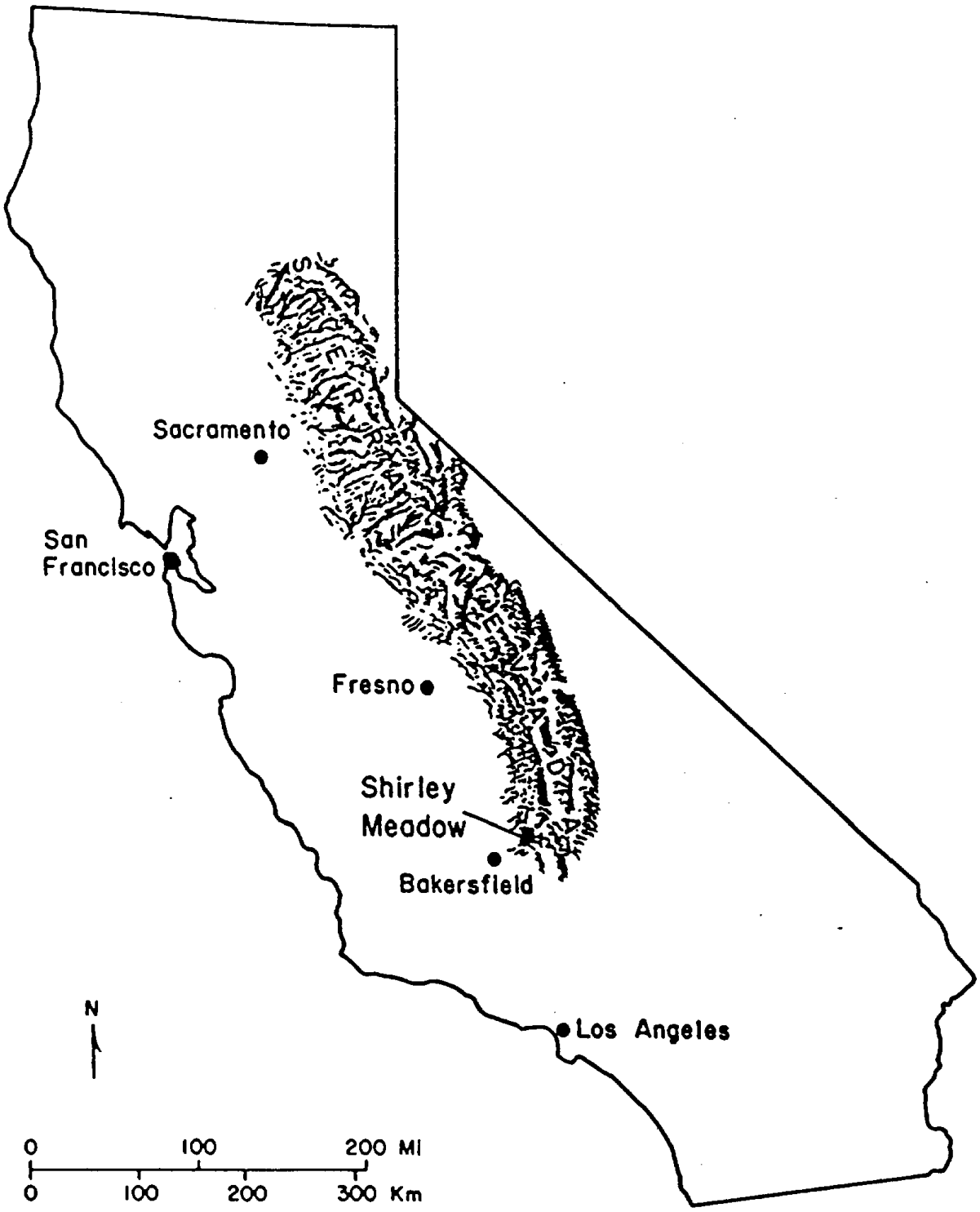

Figure 1. Location of Shirley Meadow.

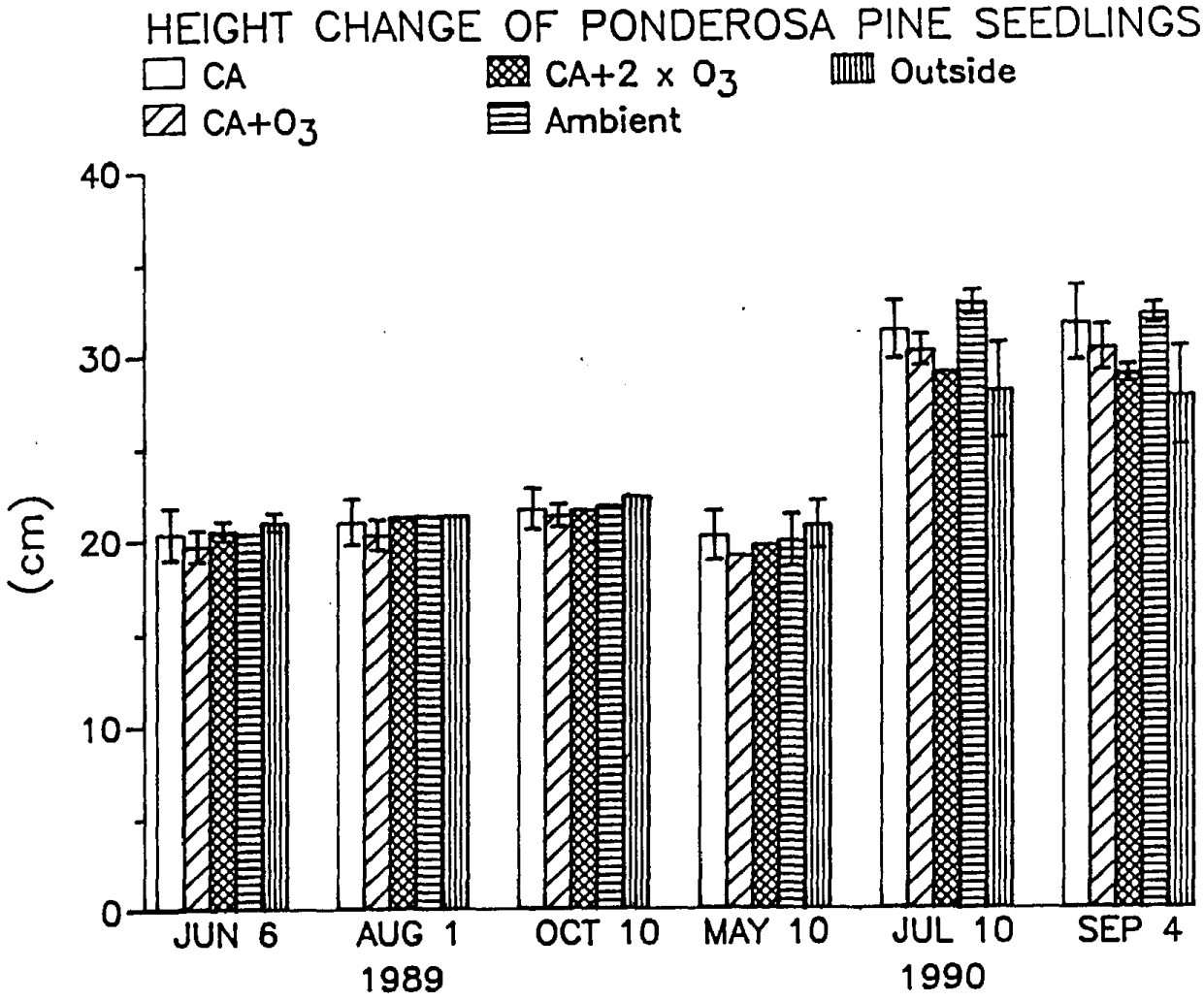

Figure 2. Height changes of ponderosa pine seedlings expressed as treatment means and standard deviations for plot means. For each plot mean n = 40 in the 1989 season, and n = 20 in the 1990 season.

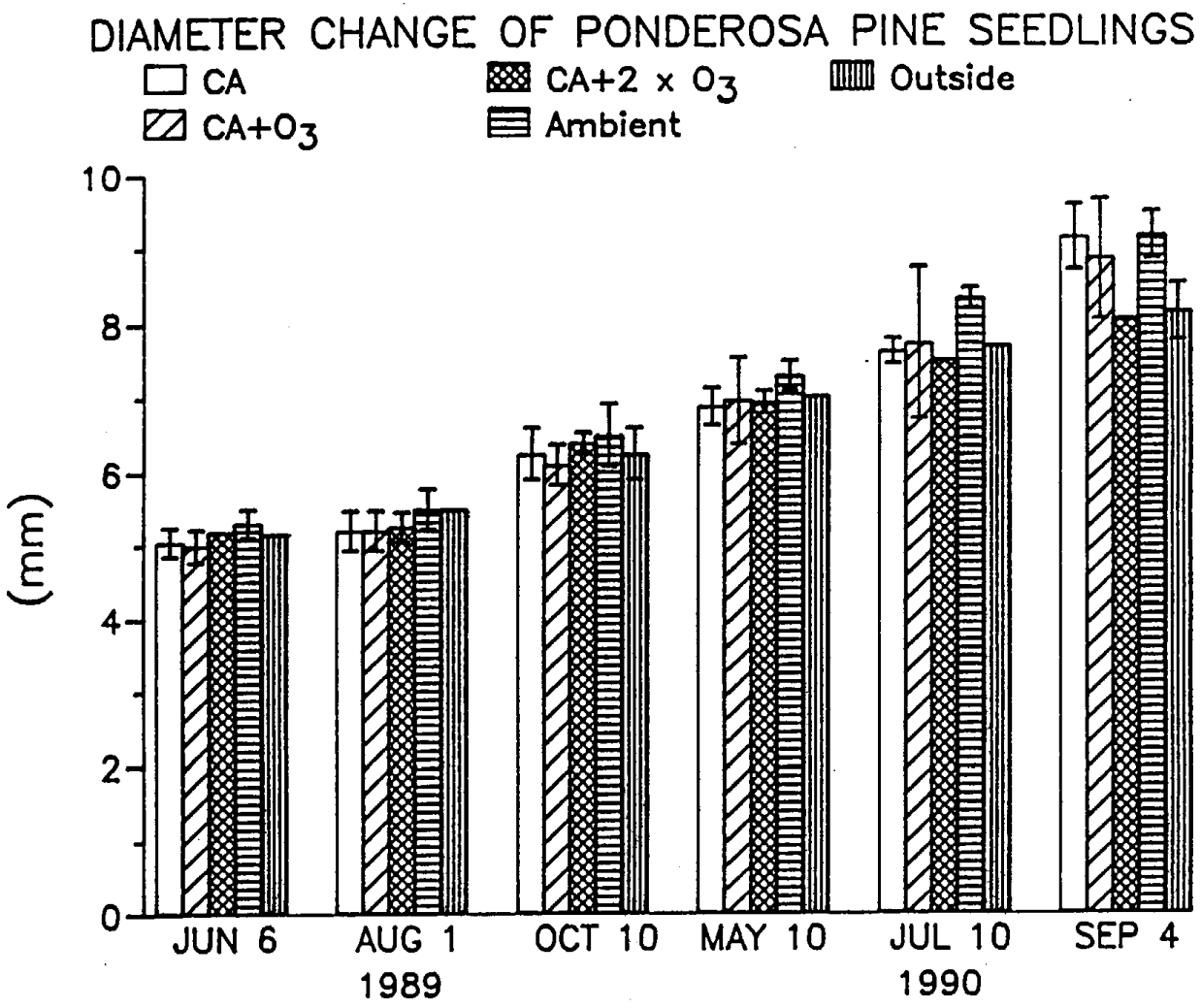

Figure 3. Diameter changes of ponderosa pine seedlings expressed as treatment means and standard deviations for plot means. For each plot mean n = 40 in the 1989 season, and n = 20 in the 1990 season.

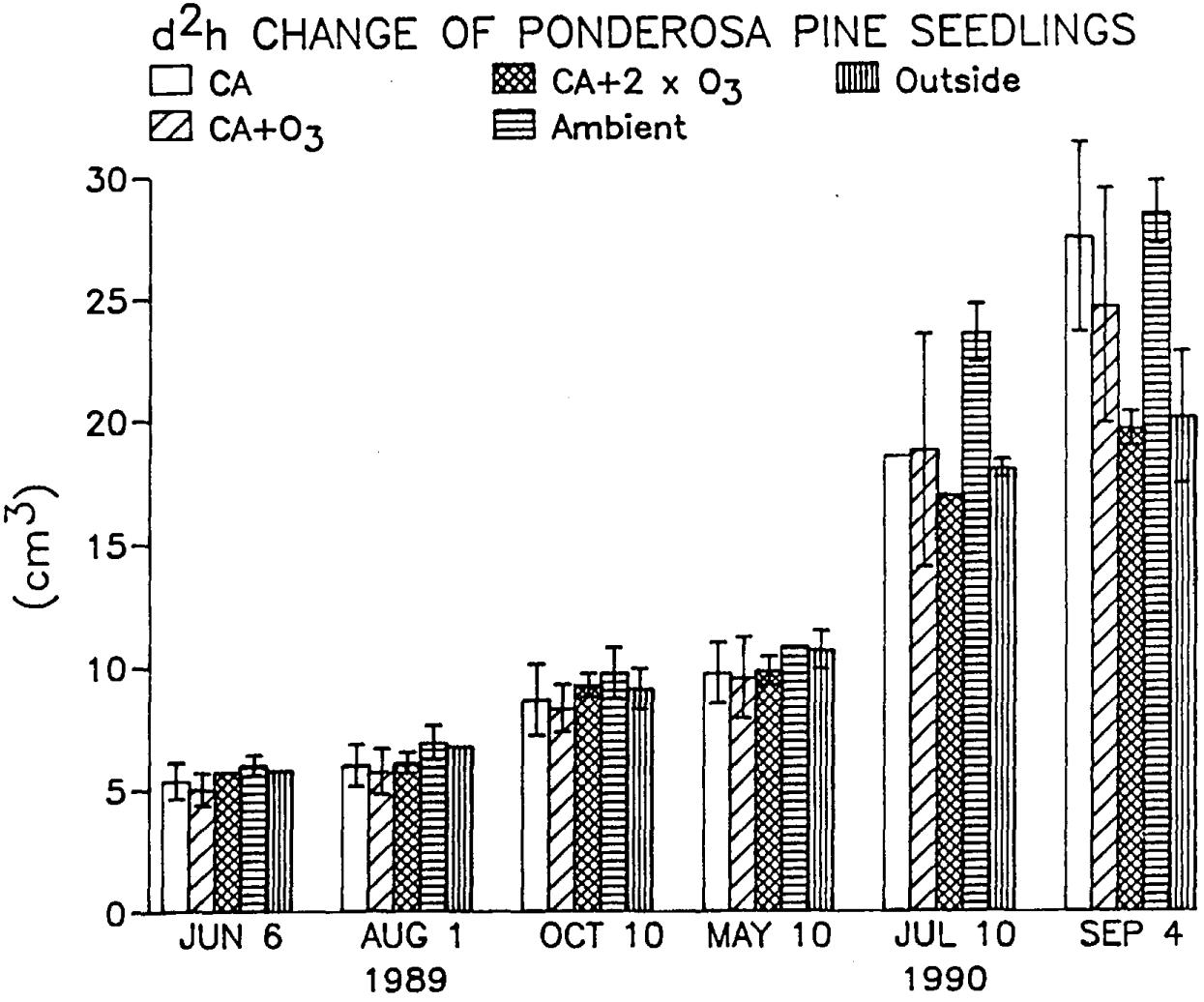

Figure 4. Stem volume (d^2h) changes of ponderosa pine seedlings expressed as treatment means and standard deviations for plot means. For each plot mean n = 40 in the 1989 season, and n = 20 in the 1990 season.

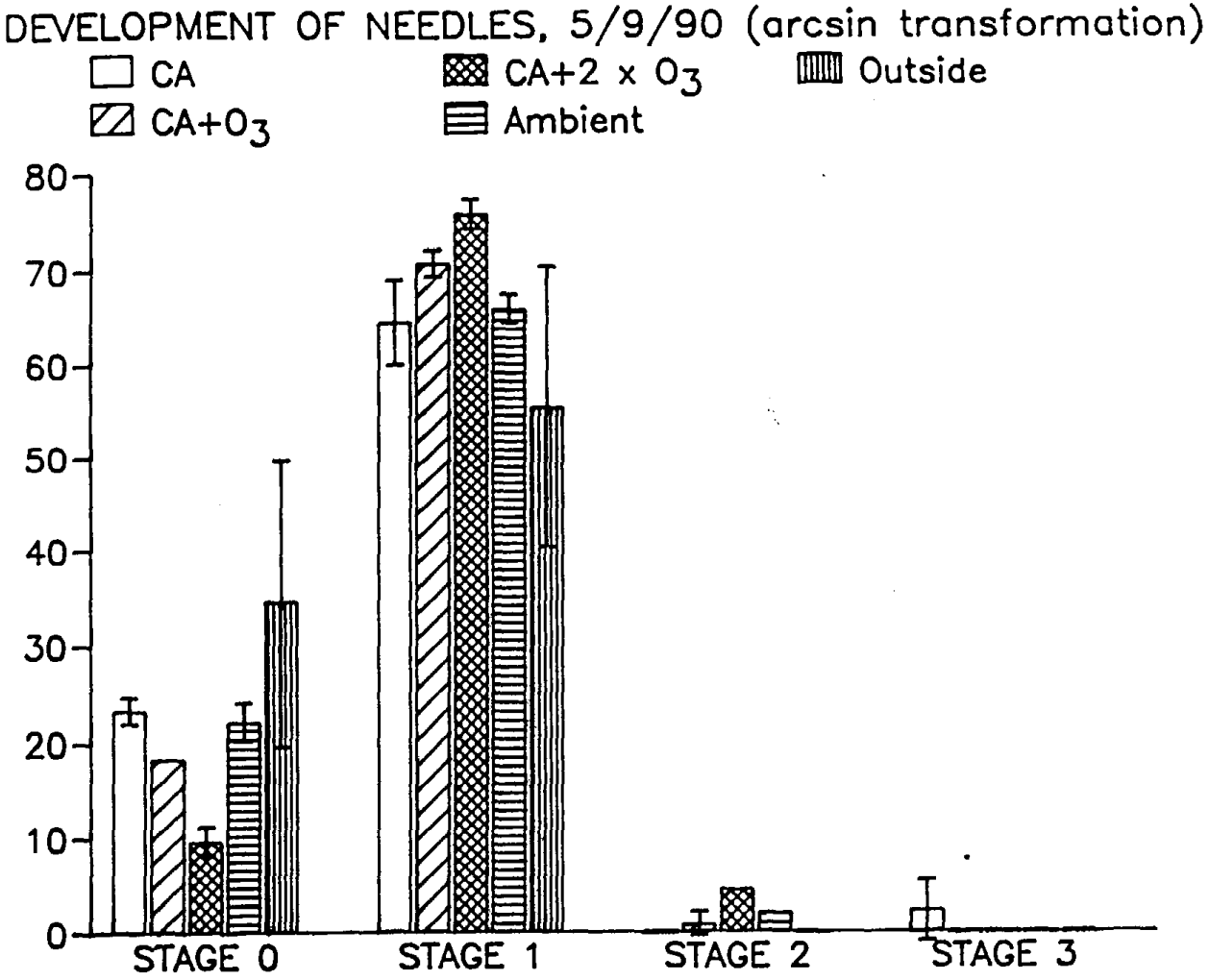

Figure 5. Development of needles (description of the stage of development in the text) expressed as treatment means and standard deviations for plot means. For each plot mean 20 measurements on individual trees were made.

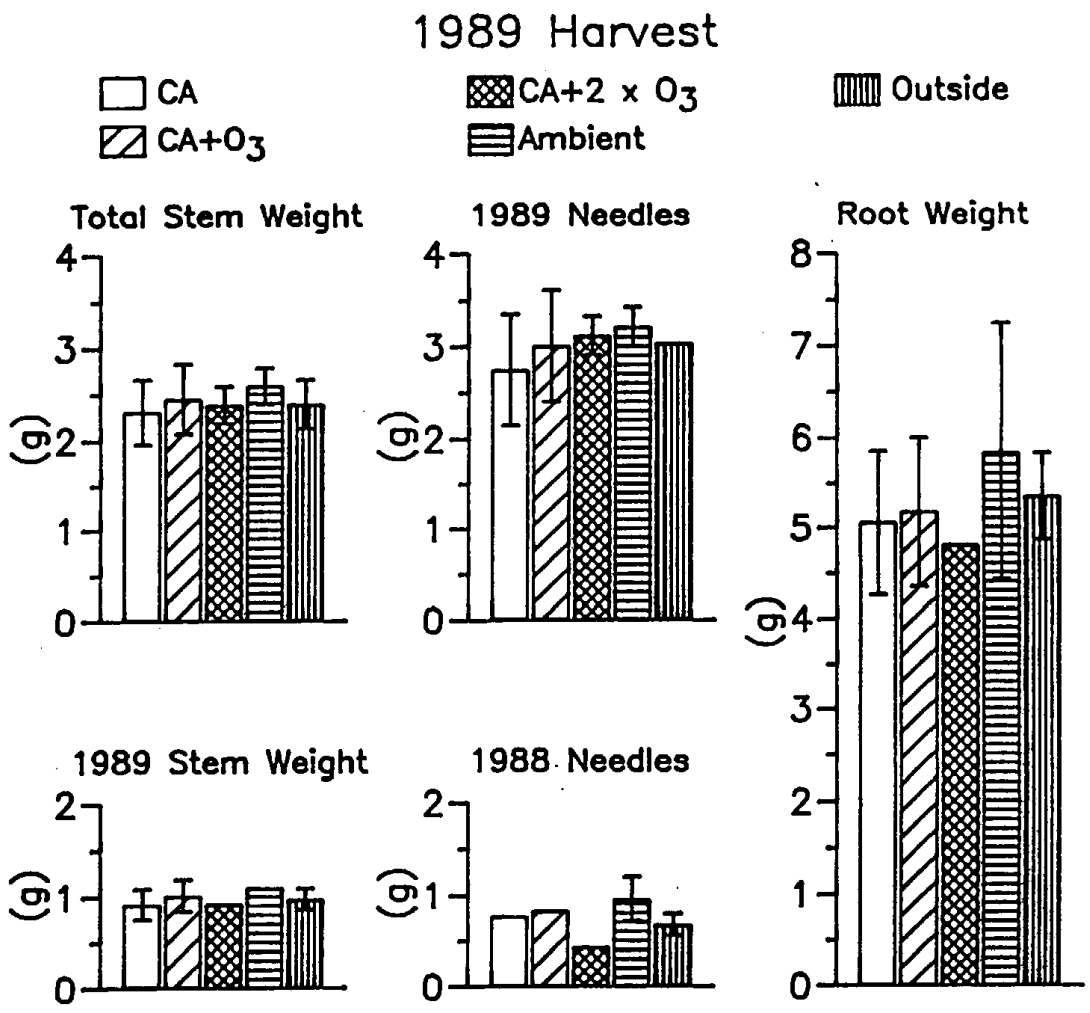

Figure 6. Biomass of ponderosa pine seedlings exposed to air pollution treatments for one season expressed as treatment means and standard deviations for plot means. For each plot 20 plants were harvested.

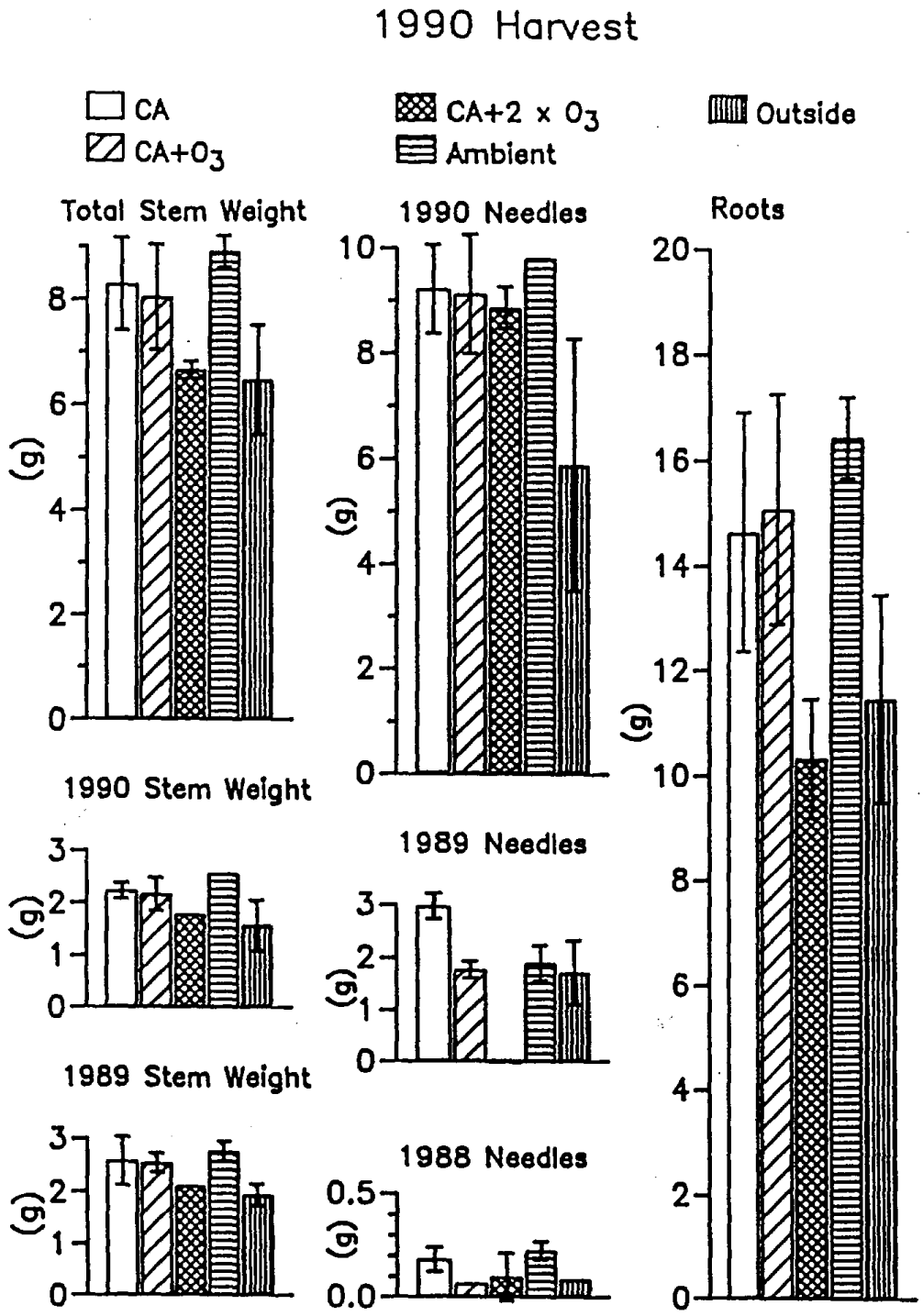

Figure 7. Biomass of ponderosa pine seedlings exposed to air pollution for two seasons expressed as treatment means and standard deviations for plot means. For each plot 20 plants were harvested.

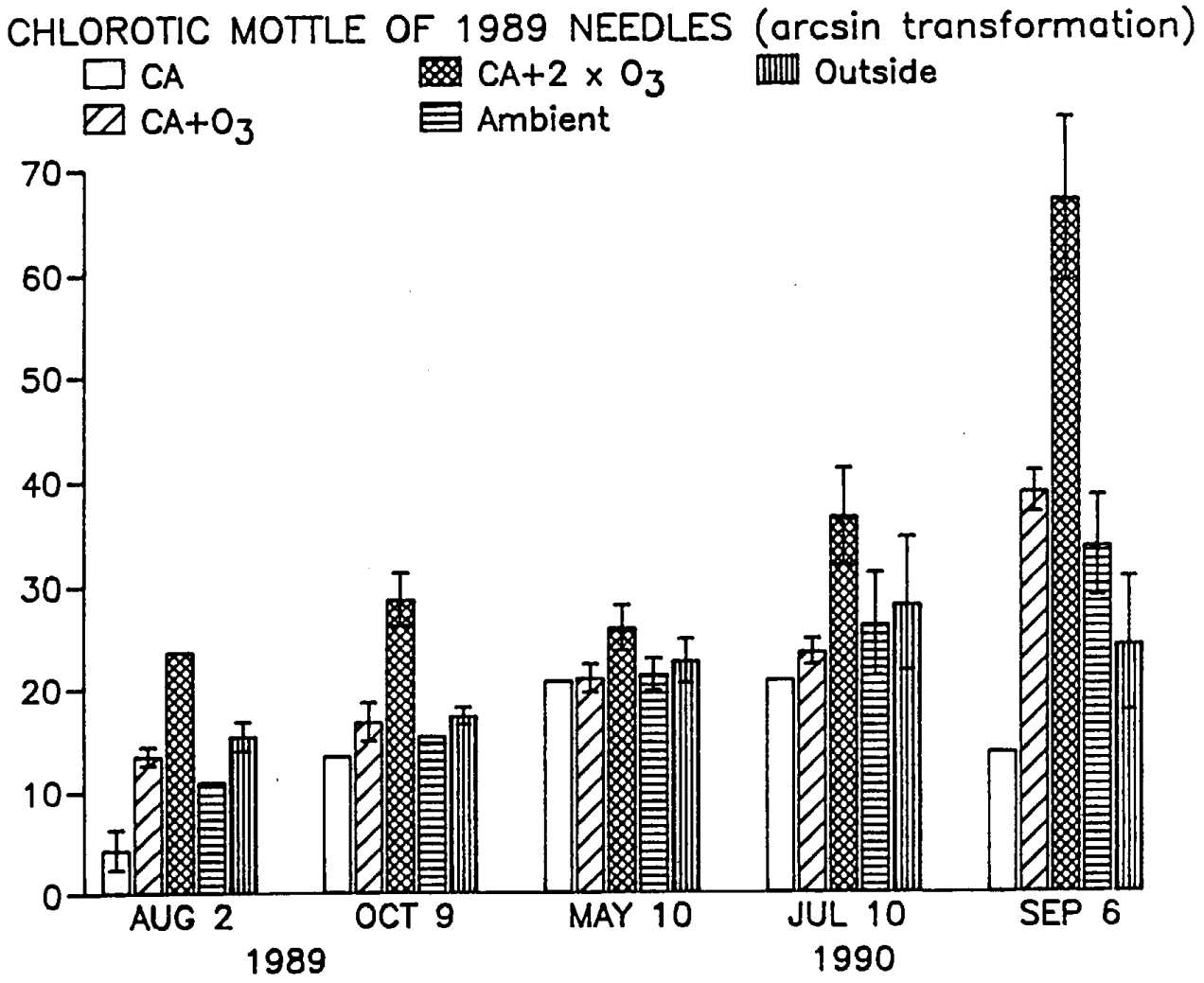

Figure 8. Chlorotic mottle of needles expressed as treatment means and standard deviations for plot means (after the arcsin transformation). For each plot mean n = 40 in the 1989 season and n = 20 season in the 1990 season.

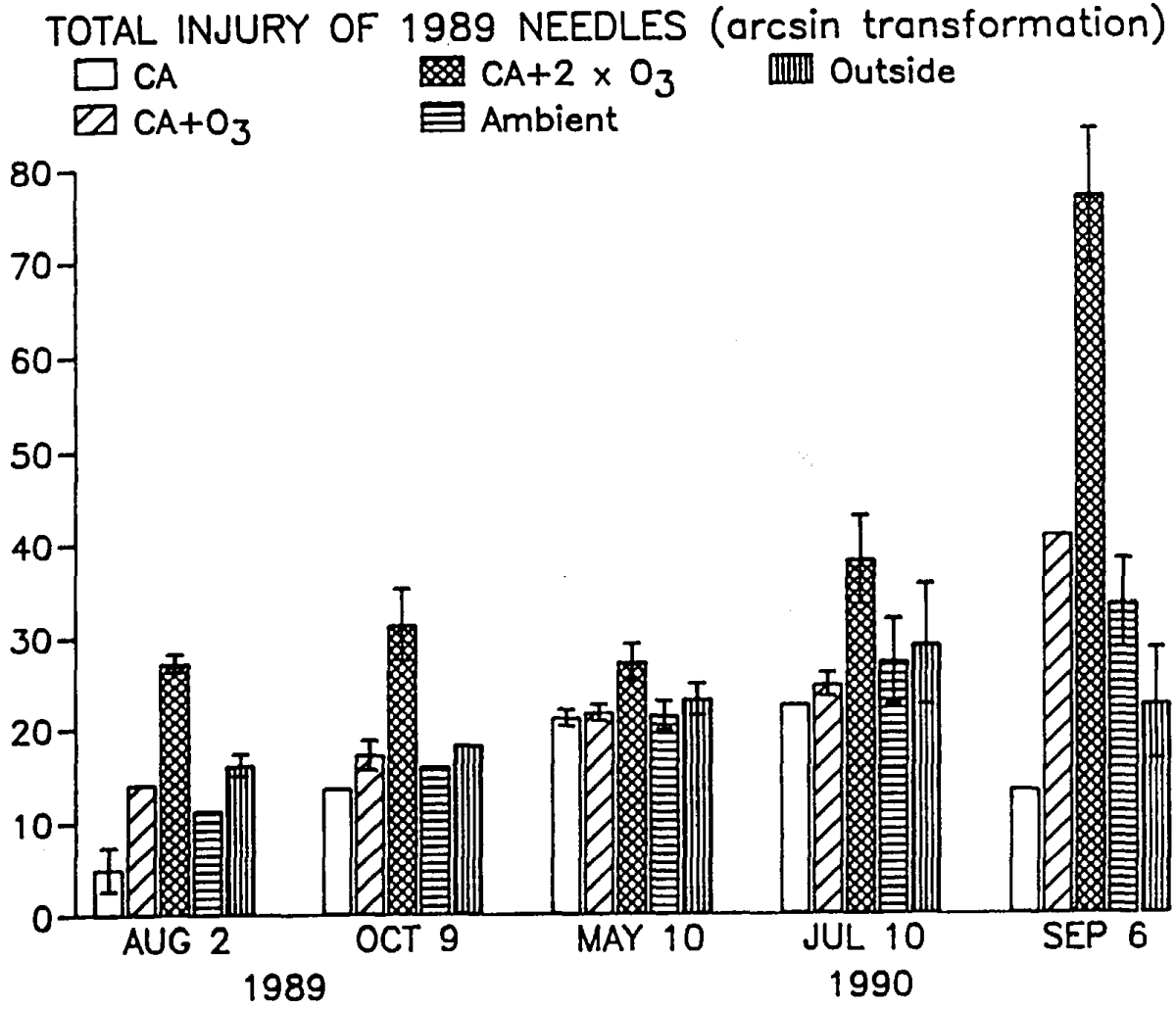

Figure 9. Total injury of needles expressed as treatment means and standard deviations for plot means (after the arcsin transformation). For each plot mean n = 40 in the 1989 season and n = 20 in the 1990 season.

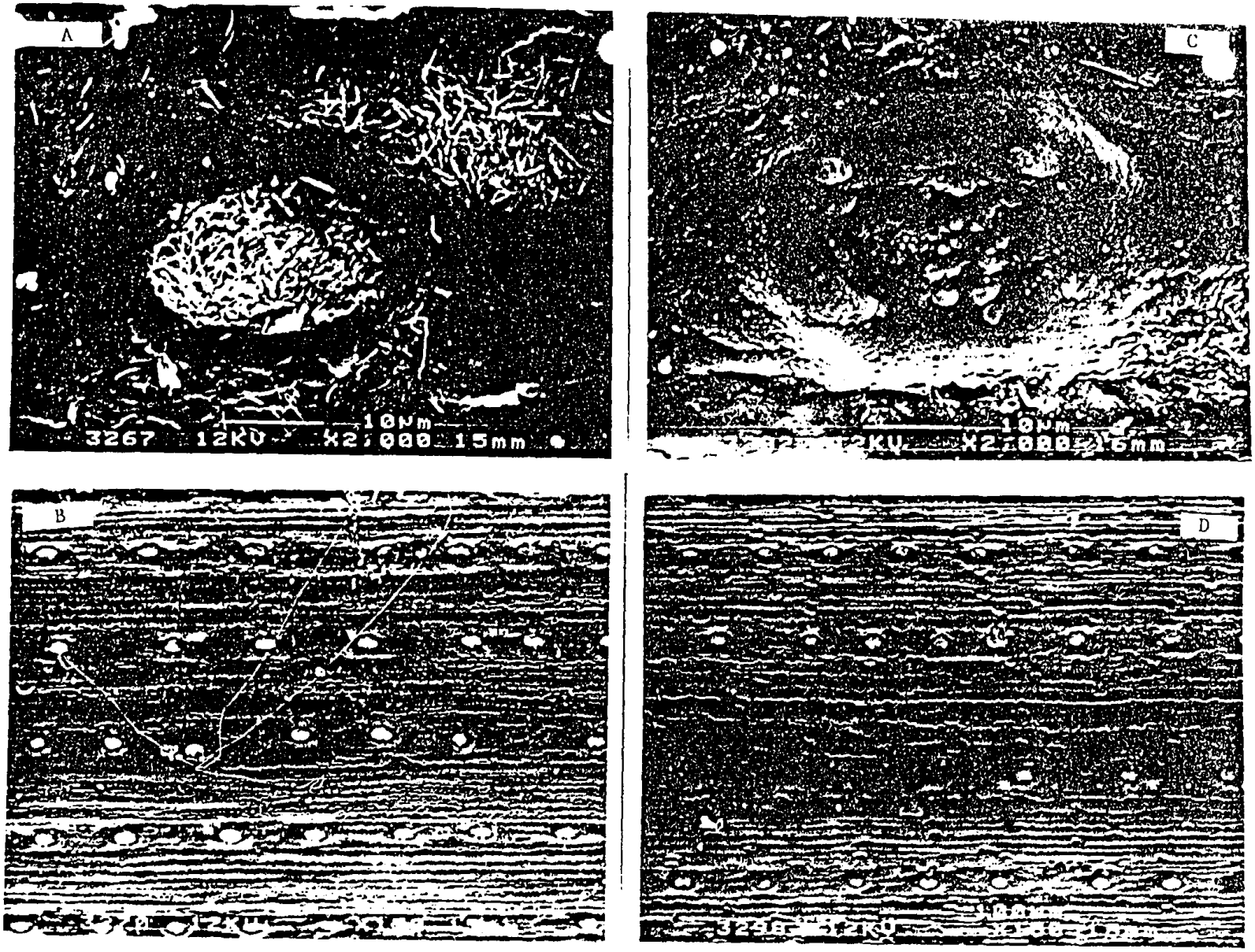

Figure 10. Scanning electron micrographs of pine needles exposed to ozone. A. Typical appearance of a stomata from the CA treatment - epistomatal wax tubes are well developed and preserved. B. Wax tubes are concentrated inside stomatal cavities, however, single wax tubes can also be found in the interstomatal area. C. Heavily eroded epistomatal tubes completely occluding the epistomatal chamber at the CA + 2 x O_3 treatment. D. At the CA + 2 x O_3 treatment higher percentage of stoma are completely occluded by the eroded epistomatal tubes.

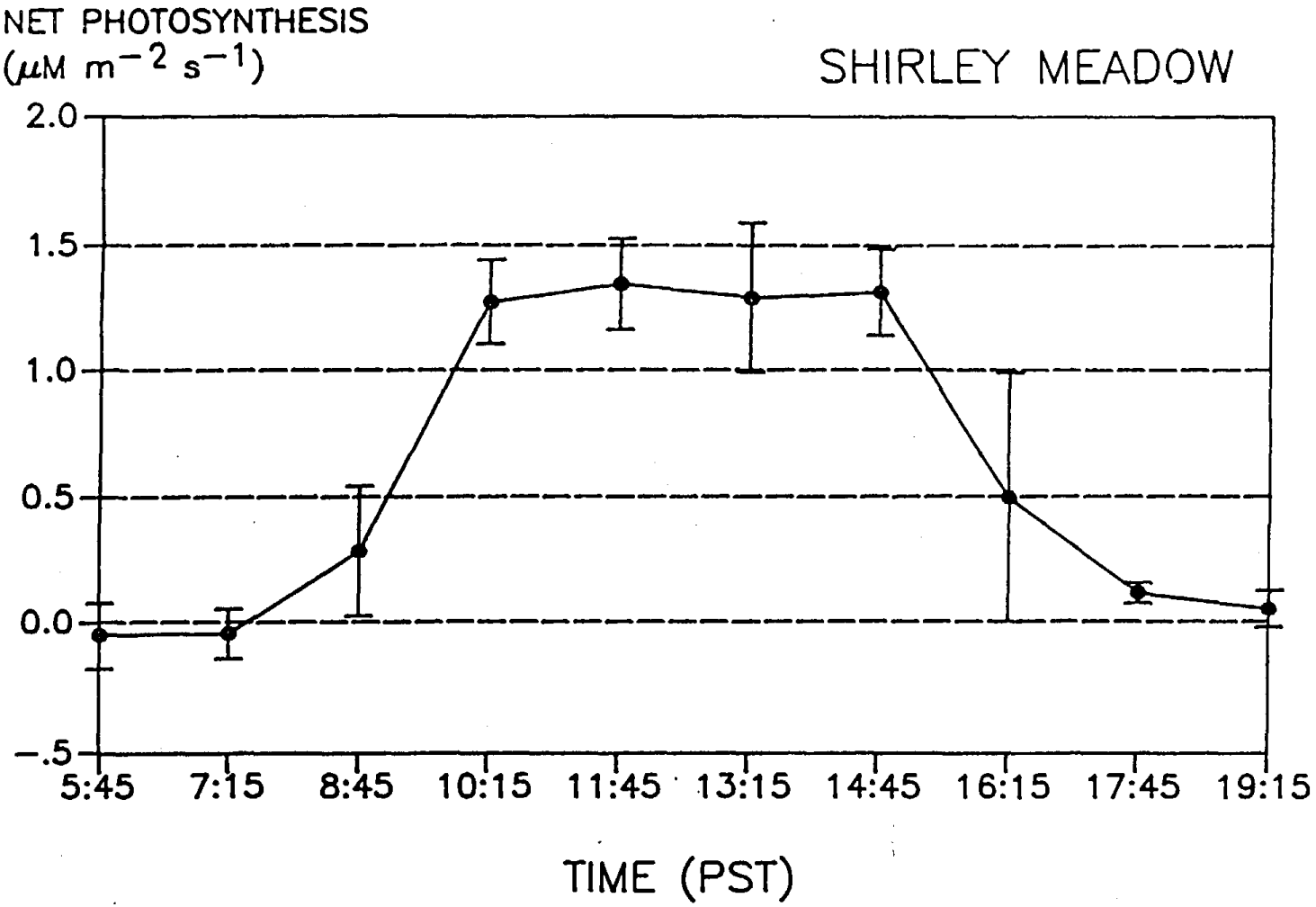

Figure 11. Diurnal profile of net photosynthesis of pine seedlings measured on July 6, 1989. Each point represents a mean of four measurements and their standard deviation.

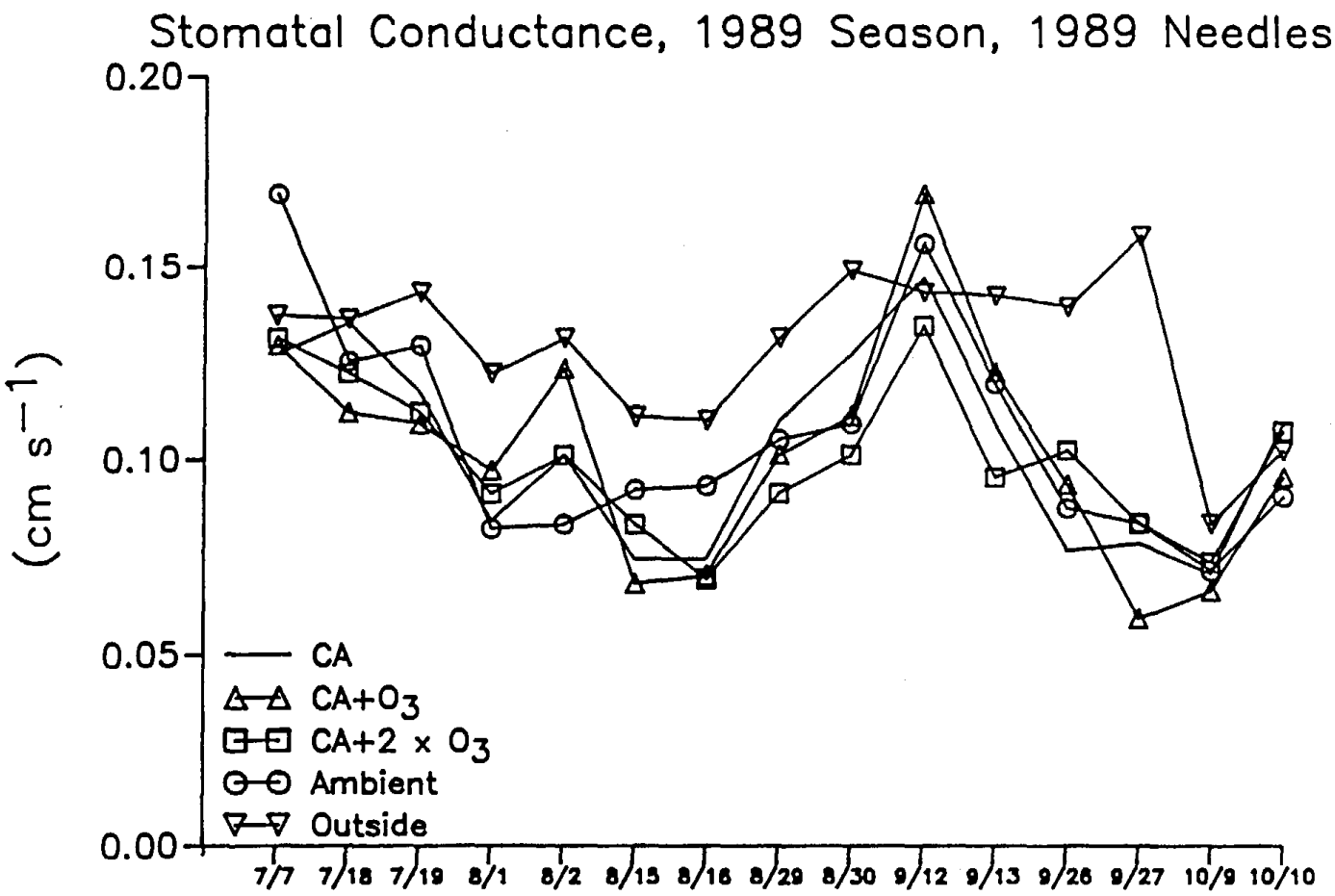

Figure 12. Stomatal conductance of 1989 needles during the first season of the exposures. Each point represents a mean of four measurements.

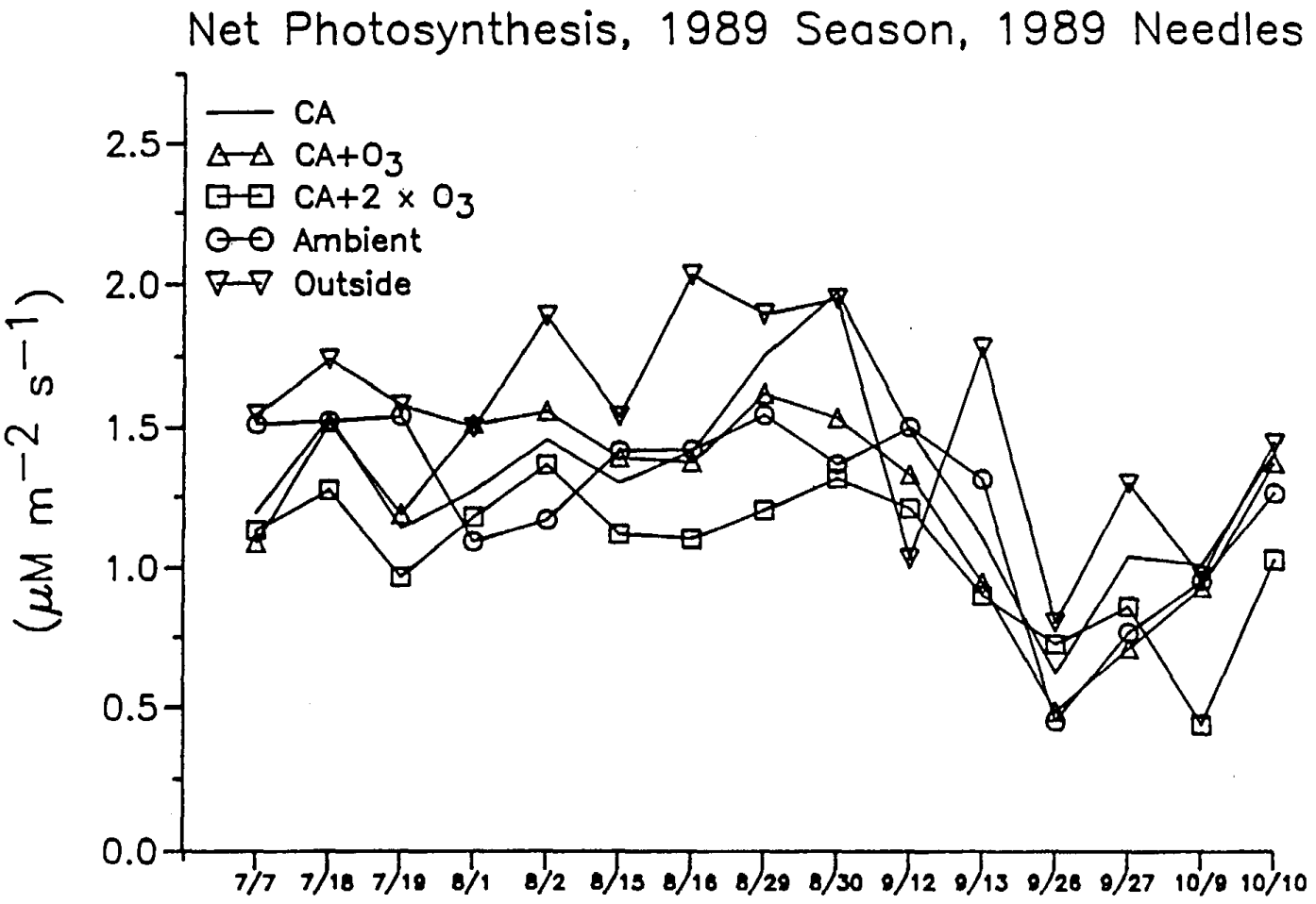

Figure 13. Net photosynthesis of 1989 needles during the first season of the exposures. Each point represents a mean of four measurements.

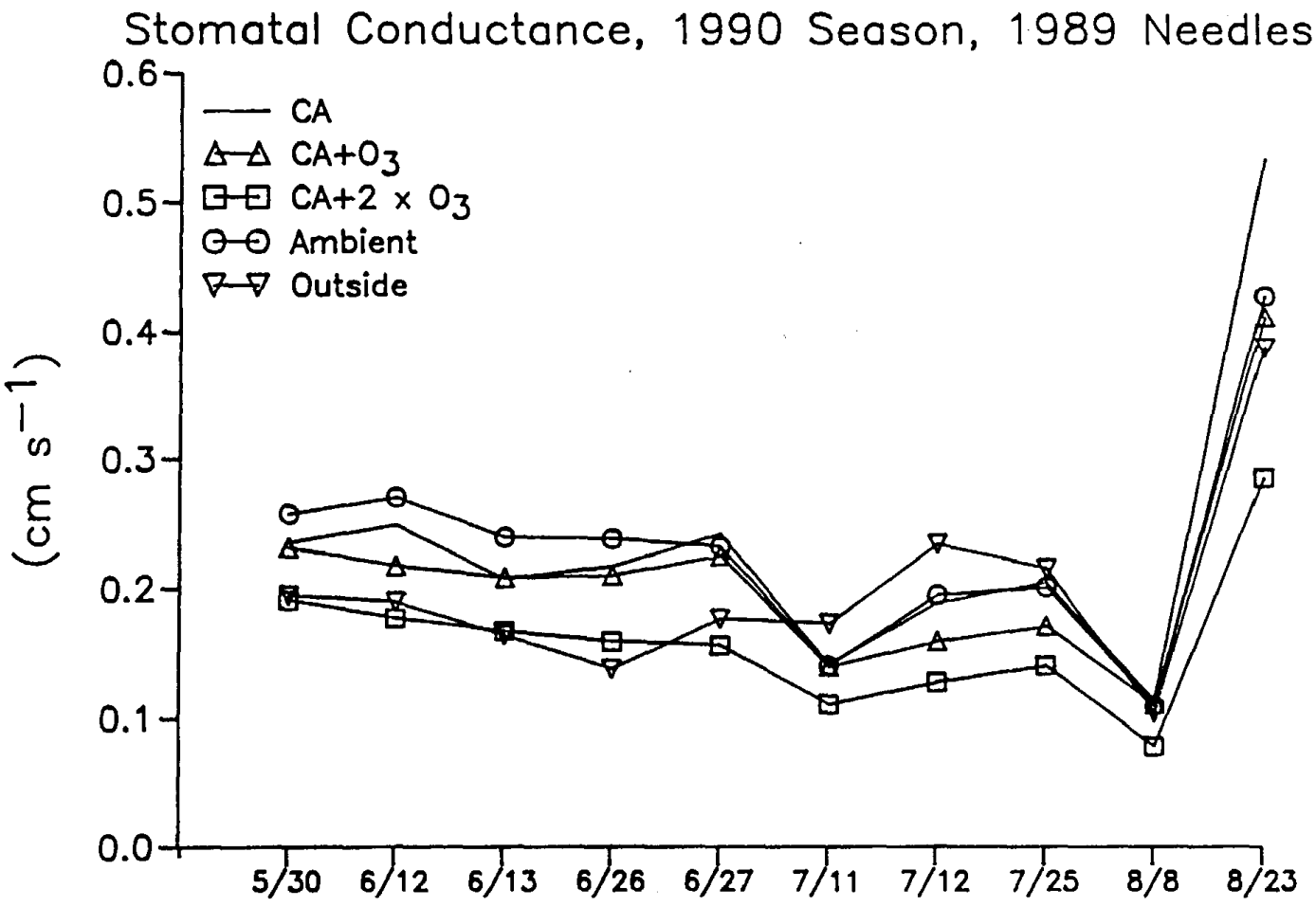

Figure 14. Stomatal conductance of 1989 needles during the second season of the exposures. Each point represents a mean of four measurements.

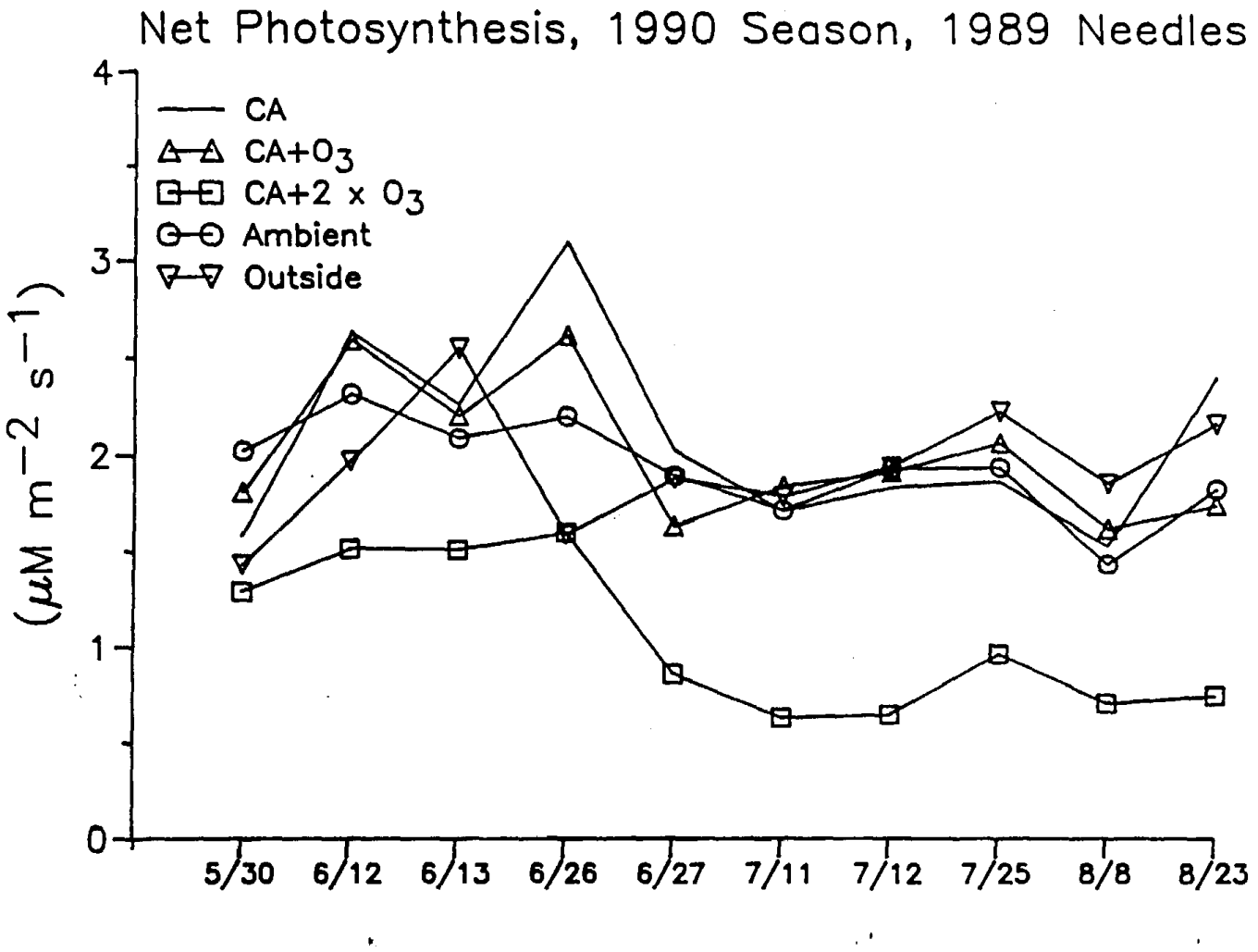

Figure 15. Net photosynthesis of 1989 needles during the second season of the exposures. Each point represents a mean of four measurements.

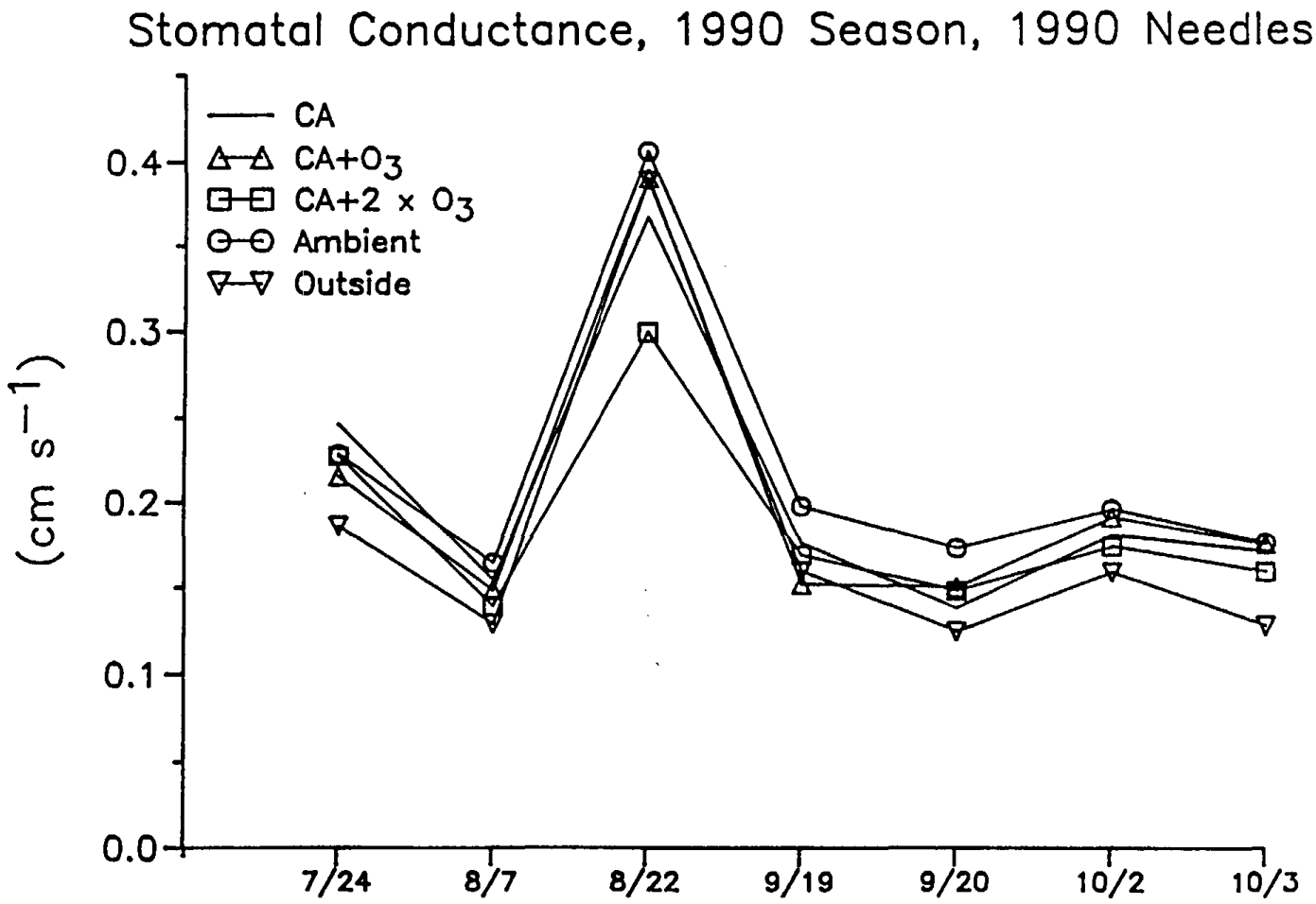

Figure 16. Stomatal conductance of 1990 needles during the second season of the exposures. Each point represents a mean of four measurements.

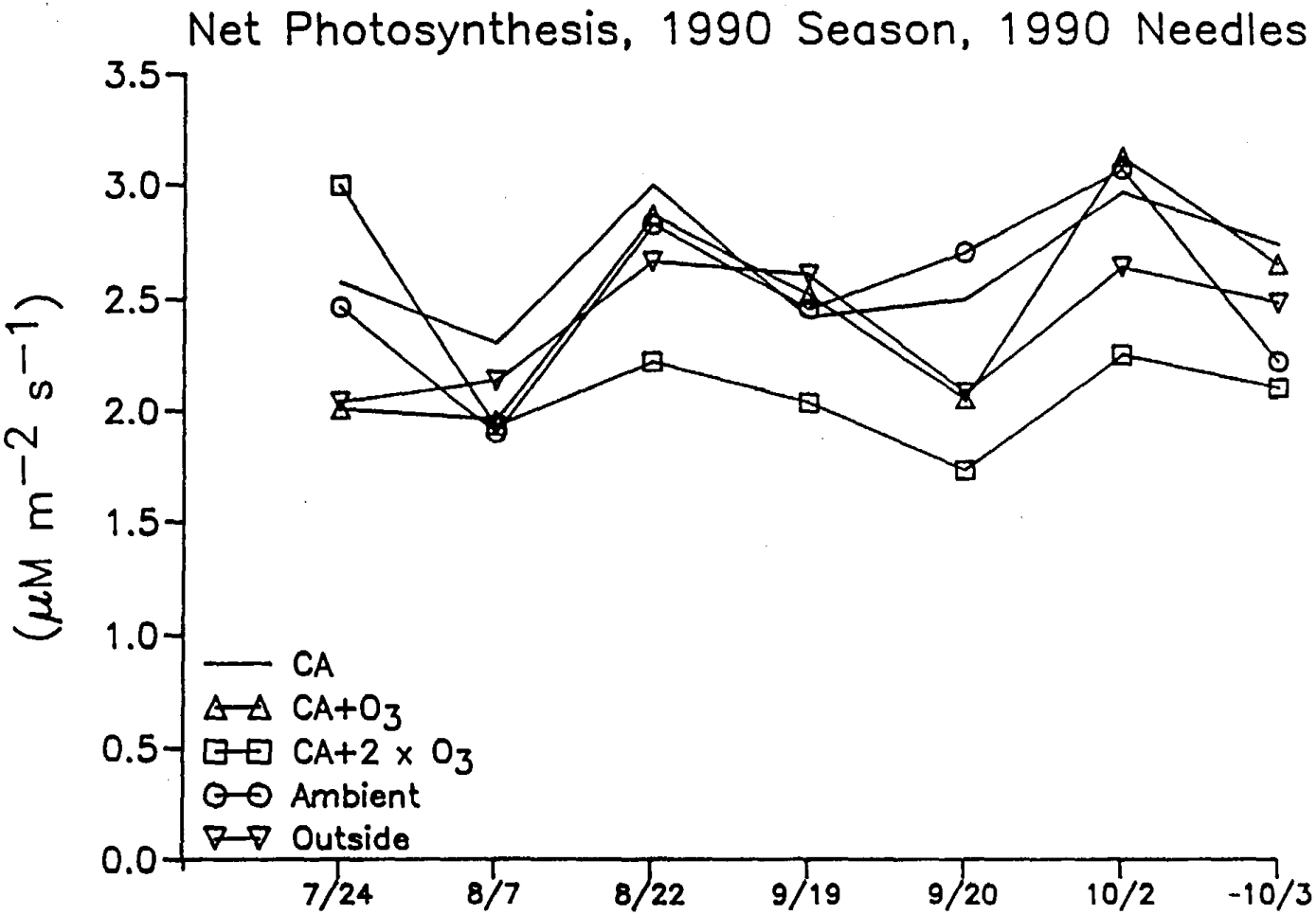

Figure 17. Net photosynthesis of 1990 needles during the second season of the exposures. Each point represents a mean of four measurements.

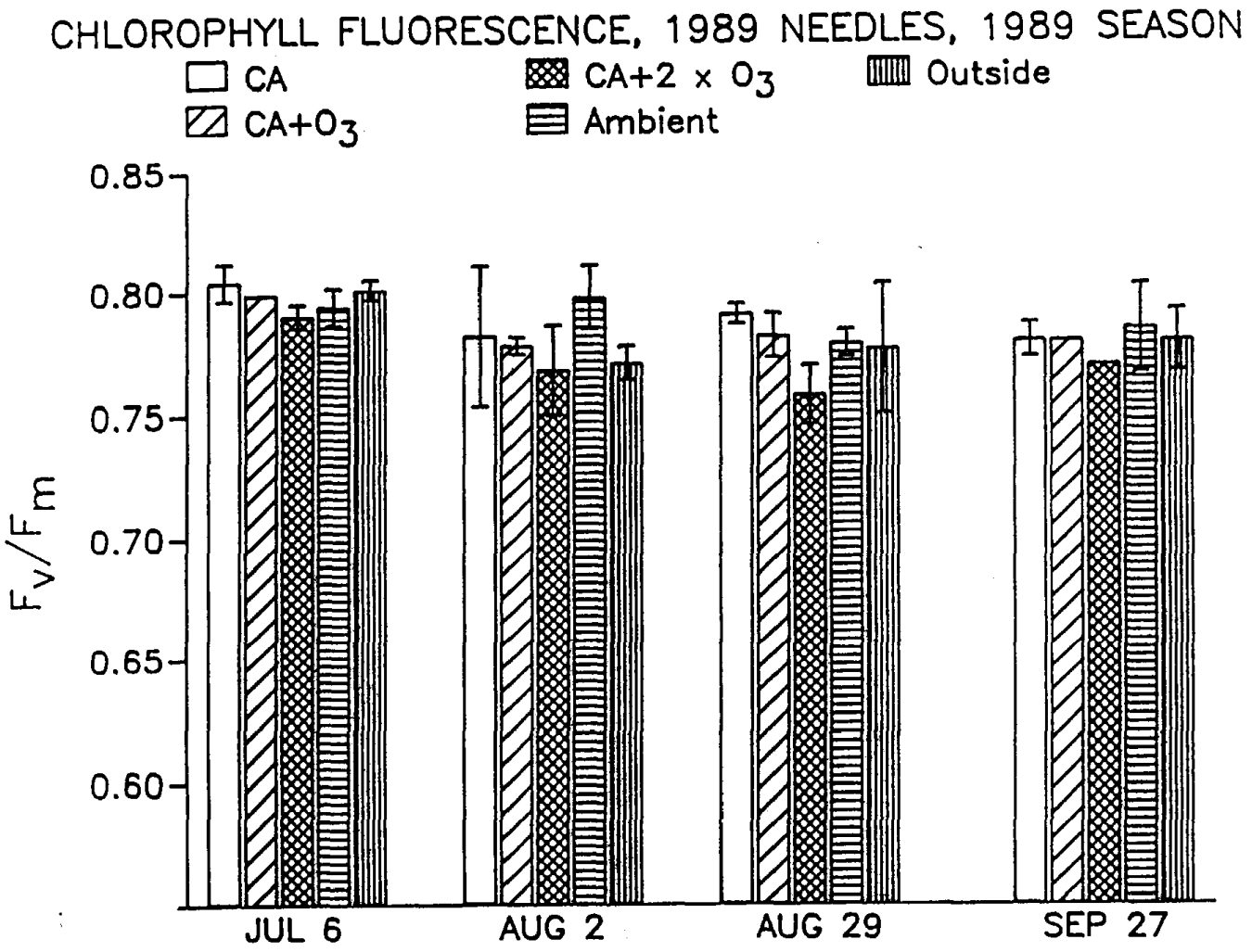

Figure 18. Chlorophyll fluorescence (F_v/F_m) changes in 1989 needles during the first season of the exposures expressed as treatment means and standard deviations for plot means. For each plot mean four measurements were made.

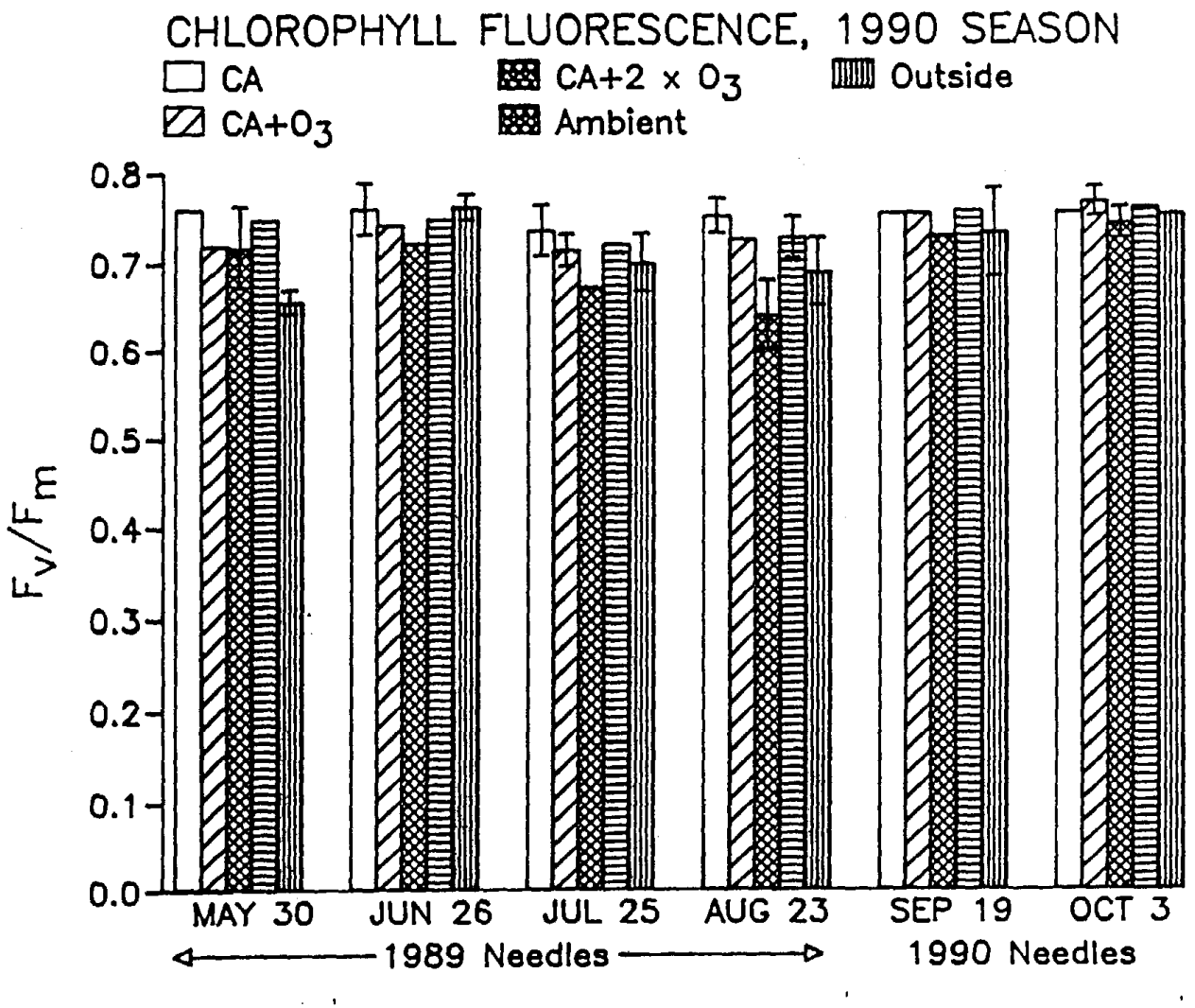

Figure 19. Chlorophyll fluorescence (F_v/F_m) changes in 1989 and 1990 needles during the second season of the exposures expressed as treatment means and standard deviations for plot means. For each plot mean four measurements were made.

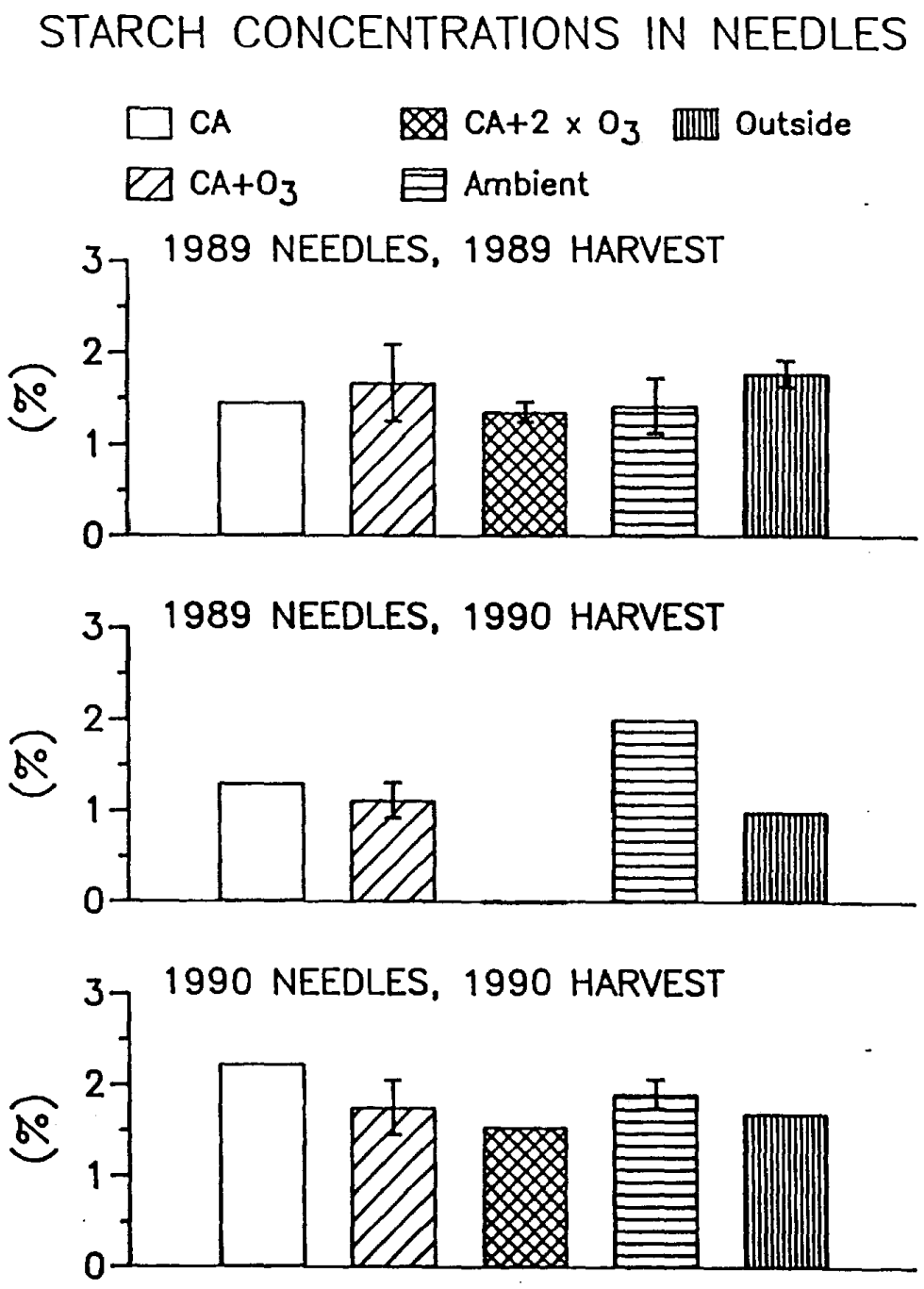

Figure 20. Starch accumulation in needles collected after one and two seasons of the exposures expressed as treatment means and standard deviations for plot means. For each plot mean five composite samples, each of them consisting of four randomly selected individual plant samples were analyzed.

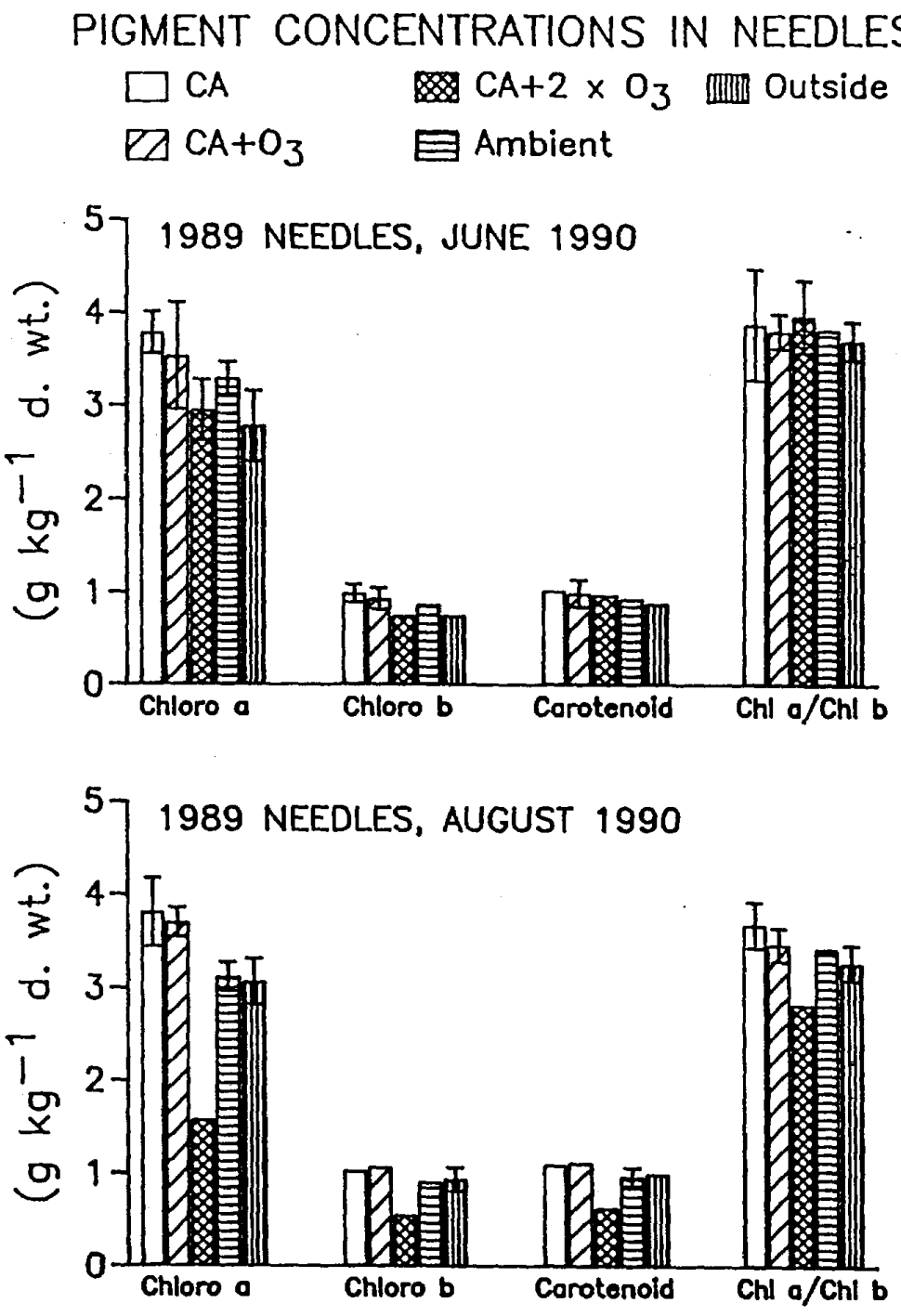

Figure 21. Pigment concentrations in 1989 needles collected during the second season of the exposures expressed as treatment means and standard deviations for plot means. For each plot mean determinations on six randomly selected individual plants were made.

Table 1. Comparison of O_3 Concentrations [Monthly 24-h Averages and 1-h Peak Values (in parentheses)] Between Different Air Pollution Regimes (ppm)

Month	Air Pollution Regime				
	CA	CA + O_3	CA + 2 x O_3	Ambient	Outside
			1989		
June	0.022 (0.044)	0.036 (0.109)	0.055 (0.219)	0.064 (0.110)	0.069 (0.110)
July	0.024 (0.033)	0.069 (0.124)	0.130 (0.255)	0.067 (0.103)	0.073 (0.118)
August	0.022 (0.035)	0.063 (0.105)	0.117 (0.206)	0.060 (0.086)	0.065 (0.093)
September	0.021 (0.031)	0.053 (0.107)	0.092 (0.200)	0.053 (0.092)	0.055 (0.101)
October	0.020 (0.043)	0.050 (0.095)	0.087 (0.187)	0.051 (0.084)	0.053 (0.094)
			1990		
May	0.018 (0.055)	0.051 (0.079)	0.103 (0.163)	0.048 (0.077)	0.049 (0.082)
June	0.020 (0.031)	0.053 (0.086)	0.122 (0.193)	0.059 (0.096)	0.061 (0.103)
July	0.021 (0.033)	0.061 (0.090)	0.142 (0.203)	0.068 (0.108)	0.068 (0.106)
August	0.019 (0.030)	0.056 (0.084)	0.128 (0.197)	0.062 (0.105)	0.061 (0.102)
September	0.020 (0.031)	0.055 (0.096)	0.129 (0.221)	0.061 (0.106)	0.060 (0.103)
October	0.020 (0.039)	0.069 (0.122)	0.154 (0.267)	0.064 (0.106)	0.064 (0.103)

Table 2. Comparison of NO_2 Concentrations [Monthly 24-h Averages and 1-h Peak Values (in parentheses)] Between Different Air Pollution Regimes (ppm)

Month	Air Pollution Regime				
	CA	CA + O_3	CA + 2 x O_3	Ambient	Outside
			1989		
June	0.003 (0.007)	0.003 (0.007)	0.003 (0.006)	0.005 (0.008)	0.005 (0.009)
July	0.003 (0.005)	0.003 (0.006)	0.003 (0.006)	0.004 (0.010)	0.004 (0.013)
August	0.003 (0.006)	0.004 (0.007)	0.004 (0.006)	0.005 (0.010)	0.005 (0.006)
September	0.001 (0.005)	0.001 (0.005)	0.001 (0.005)	0.002 (0.007)	0.002 (0.008)
October	0.000 (0.002)	0.000 (0.002)	0.000 (0.003)	0.001 (0.006)	0.001 (0.006)
			1990		
May	0.000 (0.003)	0.000 (0.003)	0.000 (0.003)	0.001 (0.002)	0.001 (0.004)
June	0.000 (0.002)	0.000 (0.002)	0.001 (0.004)	0.001 (0.004)	0.001 (0.004)
July	0.000 (0.001)	0.000 (0.002)	0.001 (0.004)	0.001 (0.004)	0.001 (0.005)
August	0.000 (0.012)	0.000 (0.015)	0.001 (0.015)	0.001 (0.040)	0.002 (0.048)
September	0.000 (0.001)	0.000 (0.001)	0.000 (0.002)	0.001 (0.003)	0.001 (0.006)
October	0.000 (0.001)	0.000 (0.002)	0.001 (0.003)	0.001 (0.004)	0.001 (0.004)

Table 3. Comparison of NO Concentrations [Monthly 24-h Averages and 1-h Peak Values (in parentheses)] Between Different Air Pollution Regimes (ppm)

Month	Air Pollution Regime				
	CA	CA + O_3	CA + 2 x O_3	Ambient	Outside
			1989		
June	0.001 (0.006)	0.001 (0.005)	0.001 (0.004)	0.001 (0.002)	0.001 (0.002)
July	0.001 (0.003)	0.000 (0.002)	0.000 (0.002)	0.000 (0.001)	0.000 (0.001)
August	0.000 (0.002)	0.000 (0.002)	0.000 (0.001)	0.000 (0.001)	0.000 (0.001)
September	0.001 (0.004)	0.001 (0.004)	0.001 (0.004)	0.001 (0.003)	0.001 (0.003)
October	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)
			1990		
May	0.000 (0.002)	0.000 (0.003)	0.000 (0.001)	0.000 (0.001)	0.000 (0.000)
June	0.000 (0.003)	0.000 (0.002)	0.000 (0.002)	0.000 (0.002)	0.000 (0.002)
July	0.001 (0.004)	0.001 (0.004)	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)
August	0.001 (0.006)	0.001 (0.007)	0.001 (0.002)	0.001 (0.009)	0.001 (0.012)
September	0.001 (0.004)	0.001 (0.004)	0.001 (0.002)	0.001 (0.002)	0.001 (0.003)
October	0.001 (0.003)	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)	0.001 (0.002)

Table 4. Comparison of Total S Concentrations [Monthly 24-h Averages and 1-h Peak Values (in parentheses)] Between Different Air Pollution Regimes (ppm)

Month	Air Pollution Regime				
	CA	CA + O_3	CA + 2 x O_3	Ambient	Outside
			1989		
June	0.000 (0.004)	0.000 (0.002)	0.000 (0.004)	0.000 (0.007)	0.000 (0.018)
July	0.000 (0.005)	0.000 (0.005)	0.000 (0.004)	0.000 (0.002)	0.000 (0.002)
August	0.000 (0.004)	0.000 (0.000)	0.000 (0.001)	0.000 (0.002)	0.000 (0.000)
September	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.002)
October	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
			1990		
May	--	--	--	--	--
June	--	--	--	--	--
July	0.000 (0.001)	0.000 (0.002)	0.000 (0.002)	0.000 (0.005)	0.000 (0.006)
August	0.000 (0.002)	0.000 (0.002)	0.000 (0.002)	0.000 (0.004)	0.000 (0.001)
September	0.000 (0.001)	0.000 (0.001)	0.000 (0.001)	0.000 (0.001)	0.000 (0.001)
October	0.000 (0.000)	0.000 (0.000)	0.000 (0.001)	0.000 (0.001)	0.000 (0.000)

Table 5. Ambient Concentrations of Gaseous and Particulate Pollutants Measured with Annular Denuder (KAPS) Systems

Date	Time (PST)	Pollutant ($\mu g\ m^{-3}$) Gases HNO_3	HNO_2	NH_3	SO_2	Particles NO_3^-	NH_4^+	SO_4^{2-}
		1989 Season						
July 5-6	18-6	1.01	0.36	2.87	2.55	1.90	0.41	1.57
July 6	6-18	2.79	0.33	2.64	2.40	0.32	0.04	0.54
July 6-7	18-6	2.19	0.23	2.65	2.29	0.78	0.34	1.35
July 7	6-18	2.33	1.22	2.65	1.57	0.03	n.d.[1]	n.d.
August 15	6-18	2.67	n.d.	2.12	1.14	0.47	0.12	0.03
August 15-16	18-6	1.18	0.06	1.17	1.13	0.10	0.10	n.d.
September 25	6-18	1.34	n.d.	0.82	0.60	0.94	0.12	0.67
September 25-26	18-6	0.59	0.02	0.34	0.14	0.18	0.32	1.34
		1990 Season						
May 30	6-18	0.28	n.d.	1.03	0.16	0.84	0.14	0.55
May 30-31	18-6	0.16	0.10	0.31	0.19	0.18	n.d.	0.13
May 31	6-18	0.37	0.17	0.39	0.20	0.20	n.d.	0.18
May 31-June 1	18-6	0.18	0.06	0.42	0.13	0.13	0.03	0.09
June 25-26	18-6	0.66	0.09	2.87	1.94	2.20	0.53	1.41
June 26	6-18	1.25	0.04	1.69	2.14	0.19	0.07	0.46
June 26-27	18-6	0.76	0.08	1.77	1.72	1.46	0.30	1.28
June 27	6-18	1.80	0.04	1.45	1.64	0.53	0.26	0.54
July 10-11	18-6	1.14	0.08	2.38	1.15	0.67	n.d.	2.21
July 11	6-18	3.37	0.04	2.61	1.33	0.65	n.d.	2.09
July 11-12	18-6	1.75	0.08	3.02	1.27	0.33	n.d.	1.46
July 12	6-18	4.82	0.15	3.88	1.88	0.27	n.d.	1.03
August 6	6-18	1.06	1.94	5.47	0.76	0.31	n.d.	1.16
August 6-7	18-6	2.15	0.53	4.64	0.45	0.32	n.d.	0.82
August 7	6-18	0.95	0.96	2.81	0.59	0.52	n.d.	1.04
August 7-8	18-6	2.46	0.64	4.65	0.93	0.22	n.d.	1.27
September 4-5	18-6	0.59	0.08	0.93	0.50	0.31	0.04	0.56
September 5	6-18	2.19	0.13	2.02	1.41	0.78	0.07	1.05
September 6	6-18	1.93	0.25	1.63	0.85	0.21	0.16	0.94
October 1-2	18-6	0.52	0.21	1.32	1.16	0.56	n.d.	0.13
October 2	6-18	1.43	0.22	1.93	1.13	0.91	0.19	0.81
October 2-3	18-6	0.70	0.19	1.45	0.91	0.69	0.19	0.73
October 3	6-18	0.56	0.13	1.82	0.20	0.07	0.01	0.19

[1]Not detected, below detection limits.

Table 6. Comparison of Trace Pollutant Concentrations in Plots of Different Filtration Regimes Performed with Annular Denuder Systems (KAPS) ($\mu g\ m^{-3}$)

Type of Filtration	Gases				Fine Particles		
	HNO_3	HNO_2	NH_3	SO_2	NO_3^-	SO_4^{2-}	NH_4^+
			August 15, 1989, 6-18 PST				
Outside	2.672	n.d.[1]	2.119	1.137	0.167	0.028	0.120
Ambient	0.426	n.d.	1.174	0.275	0.214	n.d.	0.128
CA	0.263	0.396	1.338	0.085	0.471	0.007	0.111
			September 25, 1989, 6-18 PST				
Outside	1.339	n.d.	0.817	0.595	0.935	0.670	0.115
Ambient	0.416	n.d.	1.031	0.438	0.681	0.811	0.113
CA	0.282	n.d.	1.138	0.439	0.420	0.722	0.052

[1]Not detected, below detection limits.

Table 7. Comparison of Concentrations of Atmospheric Ions in Plots of Different Filtration Regimes Performed with Cyclone Trains ($\mu g\ m^{-3}$)

Type of Filtration	Fine Particles (<2.5 μg m) and Gases			Coarse Particles (>2.5 μg m)		
	NO_3^-	NH_4^+	SO_4^{2-}	NO_3^-	NH_4^+	SO_4^{2-}
			July 23-24, 1990			
Outside	1.389	0.334	5.505	0.438	0.054	0.067
Ambient	0.248	0.129	0.811	0.510	0.023	0.003
CA	0.122	0.027	0.389	0.598	0.044	0.051
CA + 2 x O_3	0.083	0.076	0.947	0.119	0.003	0.013
			July 24, 1990			
Outside	3.800	0.338	9.716	0.690	0.075	0.051
Ambient	0.536	0.116	0.717	0.297	0.014	n.d.[1]
CA	0.178	0.048	0.492	0.324	0.020	0.010
CA + 2 x O_3	0.236	0.085	0.591	0.167	0.034	n.d.
			July 24-25, 1990			
Outside	0.366	0.117	0.705	0.754	0.068	0.126
Ambient	0.088	0.052	0.344	0.279	0.013	0.042
CA	n.d.	0.006	0.074	0.039	0.016	0.152
CA + 2 x O_3	n.d.	0.042	0.256	0.094	n.d.	n.d.
			July 25, 1990			
Outside	3.322	0.449	6.023	1.036	0.083	0.142
Ambient	1.846	0.015	8.162	0.625	0.024	0.088
CA	1.153	0.034	5.596	0.181	n.d.	0.034
CA + 2 x O_3	0.191	0.034	0.132	0.137	n.d.	0.029

[1]Not detected, below detection limits.

Table 8. Ambient Air Temperatures at Shirley Meadow During the Ozone Exposures (°C)

Month	24-Hour Average	Hourly Minimum	Hourly Maximum
	1989		
June	15.0	5.6	28.3
July	20.1	7.8	33.3
August	18.0	8.3	31.0
September	15.5	1.7	30.0
October	13.2	0.6	27.8
	1990		
May	7.5	1.7	18.3
June	15.9	2.8	30.0
July	20.2	7.8	33.3
August	18.3	5.0	36.1
September	16.6	6.7	33.9
October	14.4	5.0	28.9

Table 9. Results of the ANOVA for Height Measurements of Ponderosa Pine Seedlings During the 1989 and 1990 Seasons (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
June 6, 1989	0.648	0.451	0.851	0.357	0.434	0.488
August 1, 1989	0.549	0.356	0.681	0.206	0.188	1.000
October 10, 1989	0.539	0.578	0.936	0.632	0.393	0.434
May 10, 1990	0.654	0.363	0.661	0.616	0.494	0.461
July 10, 1990	0.110	0.482	0.176	0.452	0.134	0.022
September 4, 1990	0.168	0.443	0.156	0.440	0.302	0.043

Table 10. Results of the ANOVA for Diameter Measurements of Ponderosa Pine Seedlings During the 1989 and 1990 Seasons (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
June 6, 1989	0.493	0.767	0.451	0.310	0.136	0.454
August 1, 1989	0.546	1.000	0.842	0.842	0.264	1.000
October 10, 1989	0.772	0.664	0.664	0.399	0.274	0.477
May 10, 1990	0.704	0.805	0.853	0.951	0.325	0.419
July 10, 1990	0.503	0.831	0.816	0.658	0.249	0.230
September 4, 1990	0.164	0.570	0.071	0.153	0.538	0.084

Table 11. Results of the ANOVA for d^2h Measurements of Ponderosa Pine Seedlings During the 1989 and 1990 Seasons (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
June 6, 1989	0.444	0.531	0.508	0.225	0.122	0.747
August 1, 1989	0.446	0.725	0.887	0.624	0.147	0.832
October 10, 1989	0.685	0.747	0.584	0.397	0.217	0.555
May 10, 1990	0.654	0.845	0.935	0.782	0.266	0.891
July 10, 1990	0.154	0.923	0.502	0.447	0.082	0.054
September 4, 1990	0.101	0.405	0.052	0.165	0.270	0.042

Table 12. Results of the ANOVA for Needle Development on May 9, 1990 (Arcsin Transformation)

Parameter	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
Stage 0	0.102	0.506	0.103	0.259	0.608	0.128
Stage 1	0.193	0.419	0.171	0.506	0.525	0.201
Stage 2	0.002	0.189	0.000	0.001	0.108	0.018
Stage 3	0.486	0.175	0.175	1.000	1.000	1.000

Table 13. Results of the ANOVA for Biomass of Ponderosa Pine Seedlings at the Harvest of 1989

Parameter	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
Total Stem	0.893	0.651	0.807	0.832	0.651	0.523
1989 Stem	0.660	0.503	0.971	0.525	0.547	0.386
1989 Needles	0.826	0.552	0.406	0.798	0.637	0.686
1988 Needles	0.054	0.658	0.041	0.024	0.357	0.073
Roots	0.793	0.892	0.783	0.683	0.469	0.586
Buds	0.867	0.594	0.656	0.927	0.394	0.493
# Buds	0.530	0.441	0.553	0.192	0.485	0.400

Table 14. Results of the ANOVA for Biomass of Ponderosa Pine Seedlings at Final Harvest of 1990

Parameter	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
Total Stem	0.090	0.769	0.090	0.136	0.314	0.026
1990 Stem	0.080	0.829	0.167	0.224	0.209	0.016
1989 Stem	0.159	0.654	0.067	0.123	0.449	0.093
1990 Needles	0.130	0.945	0.790	0.843	0.617	0.027
1989 Needles	0.003	0.017	0.000	0.004	0.731	0.620
1988 Needles	0.182	0.120	0.229	0.634	0.053	0.079
Roots	0.075	0.815	0.061	0.045	0.478	0.039

Table 15. Results of the ANOVA for Chlorotic Mottle Injury of the 1989 Needles (Arcsin Transformation) - p values

	Source of Variation					
		Contrasts				
Date	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
August 2, 1989	0.000	0.000	0.000	0.000	0.092	0.015
October 9, 1989	0.000	0.082	0.000	0.000	0.389	0.250
May 10, 1990	0.127	0.898	0.031	0.036	0.836	0.485
July 10, 1990	0.083	0.553	0.014	0.029	0.565	0.664
September 6, 1990	0.001	0.004	0.000	0.003	0.359	0.123

Table 16. Results of the ANOVA for Total Injury of the 1989 Needles (Arcsin Transformation) - p values

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
August 2, 1989	0.000	0.001	0.000	0.000	0.099	0.017
October 9, 1989	0.001	0.123	0.000	0.000	0.567	0.305
May 10, 1990	0.046	0.724	0.011	0.015	0.853	0.301
July 10, 1990	0.078	0.634	0.014	0.024	0.580	0.688
September 6, 1990	0.000	0.002	0.000	0.000	0.178	0.074

Table 17. Results of the ANOVA for Stomatal Conductance of 1989 Needles During the 1989 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
July 7	0.046	0.879	0.713	0.829	0.013	0.028
July 18	0.859	0.410	0.641	0.703	0.640	0.658
July 19	0.738	0.797	0.860	0.935	0.508	0.644
August 1	0.241	0.465	0.699	0.719	0.392	0.057
August 2	0.481	0.451	0.996	0.454	0.199	0.136
August 15	0.011	0.430	0.287	0.096	0.022	0.040
August 16	0.131	0.821	0.786	0.963	0.179	0.293
August 29	0.031	0.324	0.072	0.289	0.643	0.023
August 30	0.179	0.389	0.195	0.605	0.937	0.069
September 12	0.011	0.013	0.063	0.002	0.083	0.061
September 13	0.170	0.418	0.409	0.135	0.818	0.196
September 26	0.175	0.448	0.273	0.699	0.787	0.063
September 27	0.138	0.574	0.886	0.486	0.479	0.065
October 9	0.368	0.616	0.735	0.413	0.565	0.174
October 10	0.420	0.234	0.839	0.307	0.651	0.313

Table 18. Results of ANOVA for Net Photosynthesis of 1989 Needles During the 1989 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
July 7	0.058	0.484	0.676	0.768	0.030	0.809
July 18	0.330	0.911	0.225	0.261	0.974	0.307
July 19	0.502	0.903	0.676	0.592	0.402	0.920
August 1	0.526	0.432	0.756	0.290	0.197	0.206
August 2	0.351	0.762	0.786	0.571	0.276	0.072
August 15	0.252	0.601	0.314	0.155	0.881	0.472
August 16	0.132	0.887	0.316	0.379	0.875	0.080
August 29	0.076	0.490	0.029	0.071	0.698	0.109
August 30	0.171	0.179	0.068	0.480	0.582	0.093
September 12	0.315	0.501	0.265	0.619	0.472	0.090
September 13	0.159	0.634	0.546	0.893	0.296	0.192
September 26	0.941	0.801	0.845	0.656	0.951	0.519
September 27	0.318	0.276	0.533	0.604	0.841	0.108
October 9	0.727	0.873	0.276	0.340	0.961	0.970
October 10	0.151	0.454	0.032	0.085	0.532	0.306

Table 19. Results of the ANOVA for Stomatal Conductance of 1989 Needles During the 1990 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
May 30	0.005	0.702	0.008	0.012	0.052	0.002
June 12	0.039	0.217	0.025	0.138	0.067	0.017
June 13	0.268	0.977	0.290	0.280	0.388	0.073
June 26	0.226	0.879	0.226	0.276	0.523	0.060
June 27	0.255	0.686	0.080	0.138	0.854	0.214
July 11	0.420	0.938	0.333	0.368	0.963	0.309
July 12	0.625	0.682	0.403	0.652	0.621	0.596
July 25	0.293	0.357	0.113	0.406	0.408	0.692
August 8	0.779	0.935	0.303	0.337	0.952	0.878
August 23	0.012	0.025	0.001	0.023	0.674	0.333

Table 20. Results of the ANOVA for Net Photosynthesis of 1989 Needles During the 1990 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
May 30	0.366	0.553	0.449	0.205	0.571	0.157
June 12	0.132	0.920	0.033	0.038	0.514	0.408
June 13	0.116	0.851	0.054	0.069	0.717	0.191
June 26	0.023	0.204	0.006	0.029	0.274	0.126
June 27	0.424	0.552	0.116	0.263	0.688	0.985
July 11	0.125	0.768	0.047	0.032	0.774	0.867
July 12	0.065	0.843	0.025	0.020	0.964	0.974
July 25	0.052	0.545	0.034	0.016	0.698	0.390
August 8	0.230	0.842	0.115	0.088	0.687	0.372
August 23	0.007	0.043	0.001	0.010	0.746	0.219

Table 21. Results of the ANOVA for Stomatal Conductance of 1990 Needles During the 1990 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
July 24	0.191	0.199	0.405	0.592	0.567	0.096
August 7	0.725	0.831	0.589	0.739	0.578	0.243
August 22	0.765	0.807	0.472	0.348	0.865	0.858
September 19	0.598	0.433	0.802	0.581	0.170	0.246
September 20	0.426	0.632	0.700	0.923	0.365	0.093
October 2	0.409	0.624	0.700	0.395	0.809	0.107
October 3	0.654	0.922	0.739	0.668	0.976	0.233

Table 22. Results of the ANOVA for Net Photosynthesis of 1990 Needles During the 1990 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
July 24	0.016	0.031	0.078	0.003	0.061	0.078
August 7	0.387	0.167	0.141	0.901	0.790	0.314
August 22	0.317	0.711	0.072	0.118	0.914	0.660
September 19	0.591	0.788	0.331	0.232	0.865	0.686
September 20	0.338	0.372	0.149	0.502	0.210	0.229
October 2	0.077	0.561	0.038	0.019	0.837	0.153
October 3	0.426	0.819	0.135	0.184	0.283	0.501

Table 23. Results of the ANOVA for Chlorophyll Fluorescence (F_v/F_m) of 1989 Needles During the 1989 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
July 6	0.224	0.383	0.048	0.160	0.470	0.310
August 2	0.507	0.822	0.429	0.561	0.311	0.177
August 29	0.348	0.574	0.070	0.151	0.814	0.893
September 27	0.688	1.000	0.364	0.364	0.654	0.654

Table 24. Results of the ANOVA for Chlorophyll Fluorescence (F_v/F_m) of Needles During the 1990 Season (p values)

Date	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Ambient	Ambient vs. Outside
			1989 Needles			
May 30	0.027	0.120	0.106	0.929	0.254	0.008
June 26	0.170	0.277	0.046	0.214	0.701	0.387
July 25	0.168	0.354	0.031	0.109	0.770	0.365
August 23	0.061	0.396	0.011	0.030	0.920	0.235
			1990 Needles			
September 19	0.614	1.000	0.307	0.307	0.899	0.333
October 3	0.421	0.365	0.346	0.097	0.654	0.519

Table 25. Results of the ANOVA for starch Concentrations in Ponderosa Pine Needles (p values)

Age of Needles at Time of Harvest	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Amb.	Amb. vs. Out.
1989 needles, 1989 harvest	0.461	0.426	0.690	0.254	0.351	0.206
1989 needles, 1990 harvest	0.003	0.168	--	--	0.001	0.000
1990 needles, 1990 harvest	0.050	0.033	0.008	0.244	0.399	0.253

Table 26. Stable Carbon Isotope Composition ($\delta^{13}C$) of Ponderosa Pine Needles Exposed to Different Air Pollution Treatments[1]

Treatment	1989 Harvest	1990 Harvest	
	1989 Needles	1989 Needles	1990 Needles
CA	-28.683 ± 0.485	-28.842 ± 0.000	-28.049 ± 0.050
CA + O_3	-28.335 ± 0.250	-28.992 ± 0.323	-28.099 ± 0.180
CA + 2 x O_3	-28.443 ± 0.014	--	-28.184 ± 0.054
Ambient	-28.608 ± 0.322	-28.725 ± 0.144	-28.194 ± 0.314
Outside	-28.808 ± 0.187	-28.816 ± 0.358	-27.505 ± 0.381
p values			
All treatments	0.567	0.770	0.135
CA vs. CA + O_3	0.292	0.585	0.840
CA vs. CA + 2 x O_3	0.454	--	0.593
CA + O_3 vs. CA + 2 x O_3	0.730	--	0.735
CA + O_3 vs. Ambient	0.398	0.350	0.705
Ambient vs. Outside	0.529	0.735	0.034

[1]Results expressed as treatment means and standard deviations for plot means. For each plot mean five composite samples, each of them consisting of four randomly selected individual plant samples were analyzed.

Table 27. Results of the ANOVA for Pigment Concentrations in 1989 Pine Needles During the Second Season of Exposures

Pigment	Source of Variation					
		Contrasts				
	All Air Pollution Treatments	CA vs. CA + O_3	CA vs. CA + 2 x O_3	CA + O_3 vs. CA + 2 x O_3	CA + O_3 vs. Amb.	Amb vs. Out.
			June 1990			
Chlorophyll a	0.174	0.523	0.075	0.180	0.543	0.225
Chlorophyll b	0.082	0.470	0.027	0.068	0.440	0.179
Carotenoids	0.530	0.730	0.566	0.813	0.490	0.571
Chl a/Chl b	0.957	0.834	0.819	0.663	0.954	0.755
			August 1990			
Chlorophyl a	0.000	0.645	0.000	0.000	0.051	0.802
Chlorophyll b	0.003	0.646	0.000	0.000	0.058	0.699
Carotenoids	0.004	0.698	0.000	0.000	0.064	0.728
Chl a/Chl b	0.025	0.263	0.000	0.012	0.833	0.403

Table 28. Concentration of Plant Pigments and Results of the ANOVA for the 1990 Needles Collected During the Final Harvest on October 17, 1990 (g kg^{-1} dry wt)[1]

	Chlorophyll a	Chlorophyll b	Carotenoids	Chlorophyll a / Chlorophyll b
CA	1.53 ± 0.49	0.37 ± 0.11	0.42 ± 0.13	4.20 ± 0.08
CA + O_3	1.69 ± 0.14	0.37 ± 0.04	0.46 ± 0.04	4.65 ± 0.13
CA + 2 x O_3	1.46 ± 0.07	0.34 ± 0.06	0.41 ± 0.02	4.39 ± 0.16
Ambient	1.60 ± 0.25	0.39 ± 0.06	0.43 ± 0.08	4.24 ± 0.01
Outside	1.61 ± 0.35	0.40 ± 0.07	0.50 ± 0.15	4.33 ± 0.36
p Values				
All Treatments	0.941	0.906	0.887	0.259
CA vs. CA + O_3	0.608	1.000	0.698	0.060
CA vs. CA + 2 x O_3	0.818	0.711	0.907	0.356
CA + O_3 vs. CA + 2 x O_3	0.465	0.711	0.616	0.217
CA + O_3 vs. Ambient	0.759	0.772	0.782	0.076
Ambient vs. Outside	0.971	0.845	0.532	0.627

[1]Results expressed as treatment means and standard deviations for plot means. For each plot mean determinations on six randomly selected individual plants were made.

Table 29. Chemical Analysis of the 1989 Ponderosa Pine Needles and Results of the ANOVA after One Season of Air Pollution Exposures (1989 Harvest) - % dry wt.[1]

Treatment	Element				
	N	P	Ca	Mg	K
CA	1.863 ± 0.004	0.294 ± 0.017	0.270 ± 0.031	0.183 ± 0.009	1.075 ± 0.127
CA + O_3	1.766 ± 0.116	0.266 ± 0.020	0.262 ± 0.003	0.176 ± 0.012	1.004 ± 0.062
CA + 2 x O_3	1.687 ± 0.089	0.247 ± 0.001	0.256 ± 0.020	0.204 ± 0.017	0.929 ± 0.081
Ambient	1.655 ± 0.087	0.275 ± 0.013	0.289 ± 0.033	0.197 ± 0.026	1.069 ± 0.076
Outside	1.655 ± 0.030	0.272 ± 0.020	0.264 ± 0.000	0.170 ± 0.020	1.046 ± 0.011
p Values					
All Treatments	0.156	0.193	0.641	0.403	0.438
CA vs. CA + O_3	0.267	0.137	0.734	0.711	0.414
CA vs. CA + 2 x O_3	0.072	0.031	0.557	0.303	0.128
CA + O_3 vs. CA + 2 x O_3	0.353	0.284	0.798	0.184	0.393
CA + O_3 vs. Ambient	0.212	0.575	0.271	0.313	0.458
Ambient vs. Outside	0.995	0.834	0.303	0.198	0.791

[1]Results expressed as treatment means and standard deviations for plot means. For each plot mean five composite samples, each of them consisting of four randomly selected individual plant samples were analyzed.

Table 30. Chemical Analysis of the 1989 Ponderosa Pine Needles and Results of the ANOVA after Two Seasons of Air Pollution Exposures (1990 Harvest) - % dry wt.[1]

Treatment	Element				
	N	P	Ca	Mg	K
CA	1.125 ± 0.001	0.175 ± 0.007	0.348 ± 0.006	0.153 ± 0.007	0.595 ± 0.007
CA + O_3	1.100 ± 0.057	0.181 ± 0.012	0.364 ± 0.047	0.170 ± 0.028	0.666 ± 0.038
Ambient	1.063 ± 0.042	0.159 ± 0.016	0.325 ± 0.007	0.145 ± 0.003	0.623 ± 0.023
Outside	1.047 ± 0.028	0.197 ± 0.005	0.360 ± 0.014	0.176 ± 0.019	0.640 ± 0.000
p Values					
All Treatments	0.300	0.103	0.493	0.366	0.124
CA vs. CA + O_3	0.547	0.586	0.571	0.386	0.033
CA + O_3 vs. Ambient	0.386	0.105	0.200	0.226	0.127
Ambient vs. Outside	0.696	0.026	0.236	0.146	0.501

[1]Results expressed as treatment means and standard deviations for plot means. For each plot mean five composite samples, each of them consisting of four randomly selected individual plant samples were analyzed.

Table 31. Chemical Analysis of the 1990 Ponderosa Pine Needles and Results of the ANOVA after One Season of Air Pollution Exposures (1990 Harvest) - % dry wt.[1]

Treatment	Element				
	N	P	Ca	Mg	K
CA	1.050 ± 0.057	0.164 ± 0.008	0.142 ± 0.011	0.110 ± 0.006	0.912 ± 0.008
CA + O_3	1.165 ± 0.010	0.181 ± 0.001	0.159 ± 0.001	0.120 ± 0.008	0.961 ± 0.013
CA + 2 x O_3	1.191 ± 0.030	0.165 ± 0.007	0.163 ± 0.041	0.131 ± 0.035	0.848 ± 0.099
Ambient	1.050 ± 0.023	1.180 ± 0.003	0.155 ± 0.001	0.119 ± 0.001	0.981 ± 0.033
Outside	0.987 ± 0.112	0.172 ± 0.003	0.171 ± 0.016	0.116 ± 0.002	0.932 ± 0.031
p Values					
All Treatments	0.071	0.063	0.698	0.778	0.206
CA vs. CA + O_3	0.108	0.024	0.442	0.571	0.364
CA vs. CA + 2 x O_3	0.062	0.858	0.350	0.259	0.249
CA + O_3 vs. CA + 2 x O_3	0.677	0.029	0.852	0.534	0.070
CA + O_3 vs. Ambient	0.108	0.858	0.852	0.954	0.701
Ambient vs. Outside	0.330	0.191	0.455	0.840	0.364

[1]Results expressed as treatment means and standard deviations for plot means. For each plot mean five composite samples, each of them consisting of four randomly selected individual plant samples were analyzed.

Table 32. Results of Soil Analysis for Various Treatments at Shirley Meadow Study (mg/kg)[1]

Treatment	Ion Measured								
	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	NH_4^+
CA - 1	4.81	174	26.5	4.29	1257	114	4.32	80.4	1.55
CA - 2	4.13	131	19.1	2.98	1258	100	5.26	68.4	1.03
CA + O_3 - 1	4.36	124	22.4	2.57	979	104	7.07	77.5	1.60
CA + O_3 - 2	4.36	266	34.2	6.42	1533	141	2.86	74.7	1.99
CA + 2 x O_3 - 1	3.01	110	29.0	4.17	1447	129	4.23	109.8	2.00
CA + 2 x O_3 - 2	7.83	150	27.1	4.37	1121	99	2.65	87.8	1.83
Ambient - 1	5.60	126	25.5	2.75	1334	130	5.62	71.9	1.72
Ambient - 2	6.74	137	29.3	4.08	1446	151	3.31	109.4	3.26
Outside - 1	5.24	233	39.0	7.02	1638	156	3.59	87.1	1.25
Outside - 2	4.87	124	31.2	5.10	951	107	5.19	81.9	0.92
Control (original soil sample)	6.83	131	22.7	52.73	1214	111	18.06	116.3	1.58

[1]Samples collected at the end of the study from a single randomly collected pot from each plot.

Table 33. HNO_3 Concentrations at Various Locations ($\mu g\ m^{-3}$)

Location	Type of Area	Period of Measurement	Time of Day	Range	Mean	Reference
Fortress Mt., Alberta	Remote, mountains	3 years	24 h	0.0005-3.50	0.31	Legge & Krupa, 1989
Niwot Ridge, CO	Remote, mountains	October November		0.03-0.48	0.14	Roberts et al., 1988
Point Arena, CA	Remote, coastal	May	24 h	0.11-0.80	0.25	Roberts et al., 1988
Eastern Brook Lake, CA		Winter Summer	24 h		0.15 0.37	D. F. Miller, personal communication
Smoky Mountains, TN	Mountains	August September	Day Night	0.09-1.00 0.04-0.20	0.54 0.12	Cadle & Mulawa, 1988
Whitaker Forest, CA	Mountains	Summer	Day Night	0.90-3.80 0.10-1.10	1.83 0.59	UCF-FS
Shirley Meadow, CA	Mountains	Summer	Day Night	2.30-2.80 1.00-2.20	2.60 1.46	UCR-FS
Tanbark Flat, CA	Mountains	Summer	Day Night	15.40-30.20 0.90-4.50	24.60 2.89	UCR-FS
Claremont, CA	Urban	Summer	Various	0.20-35.0		Appel et al., 1988
Warren, MI	Urban	Summer Fall Winter Spring	24 h 24 h 24 h 24 h	0.6-4.0 0.5-2.0 0.2-4.5 0.4-2.0		Cadle, 1985

Table 34. NH_3 Concentrations at Various Locations ($\mu g\ m^{-3}$)

Location	Type of Area	Period of Measurement	Time of day	Range	Mean	Reference
Fortress Mt., Alberta	Remote, mountains	3 years	24 h	0.04-1.30	0.26	Legge & Krupa, 1989
Smoky Mountain, TN	Mountains	August September	Day Night	0.11-2.20 0.11-3.70	0.75 1.06	Cadle & Mulawa, 1988
Whitaker Forest, CA	Mountains	May through October	Day Night	0.23-3.75 0.51-2.28	2.23 1.28	UCR-FS
Shirley Meadow, CA	Mountains	May through October	Day Night	0.39-5.47 0.31-4.65		UCR-FS
Tanbark Flat, CA	Mountains	Summer	Day Night	1.20-2.60 0.20-1.60	1.82 0.63	UCR-FS
Claremont, CA	Urban	Summer	Various	1-10		Sickles et al., 1988 Appel et al., 1988
Warren, MI	Urban	Summer Fall Winter Spring	24 h	0.4-1.5 0.1-0.4 0.0-0.3 0.0-0.6		Cadle, 1985
Petten, Holland	Rural	March-August	Various	0.5-15		Keuken et al., 1988
Eastern England	Rural Livestock Farm	1.5 years 1.5 years	24 h 24 h	0.5-5.0 3.0-70		Allen et al., 1988

APPENDICES

Appendix A

Diurnal Profiles of Ozone Concentrations for Different Air Pollution Treatments During the Two Seasons of Exposures

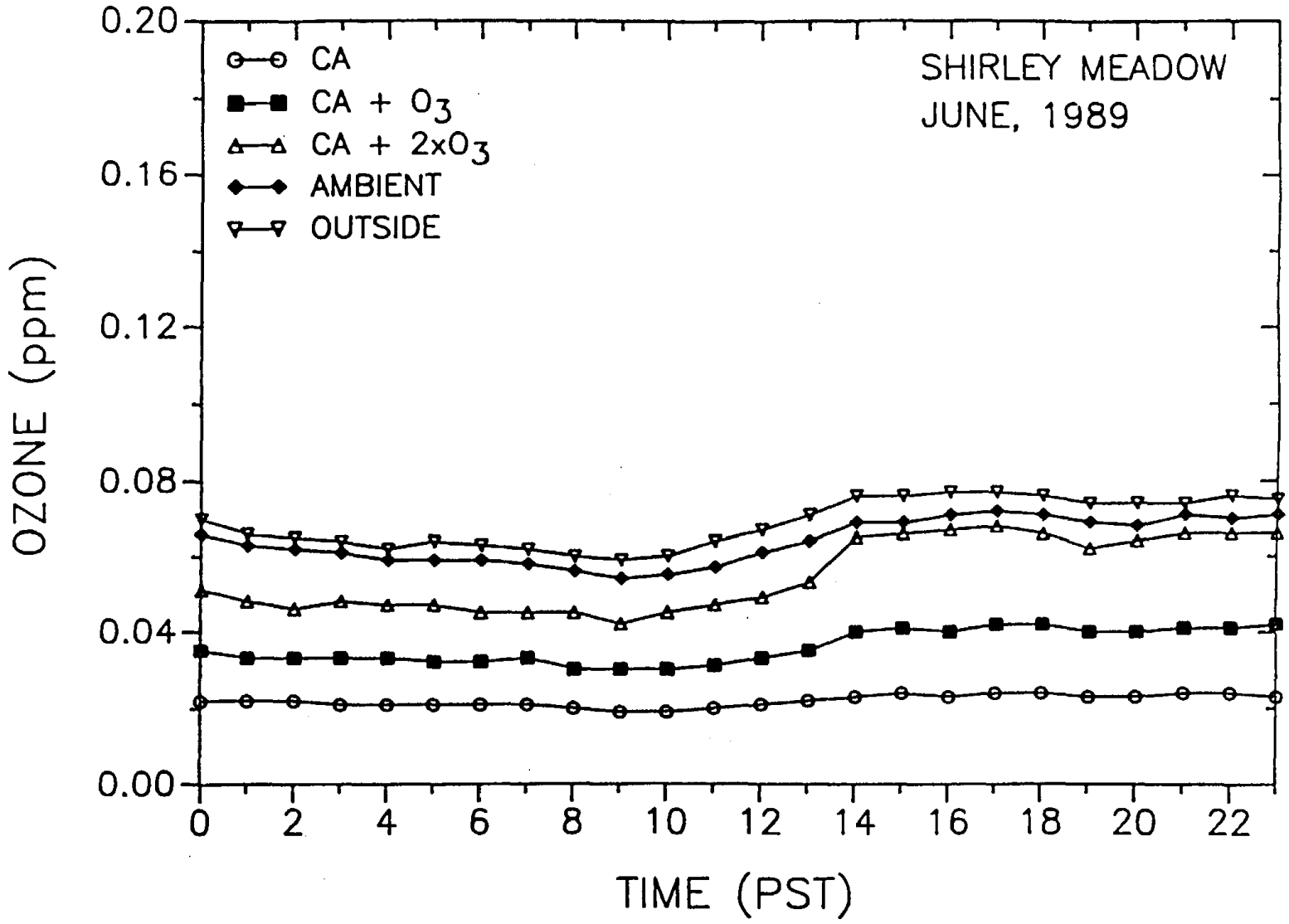

Figure A-1. Diurnal profiles of ozone concentrations for different air pollution treatments - June 1989.

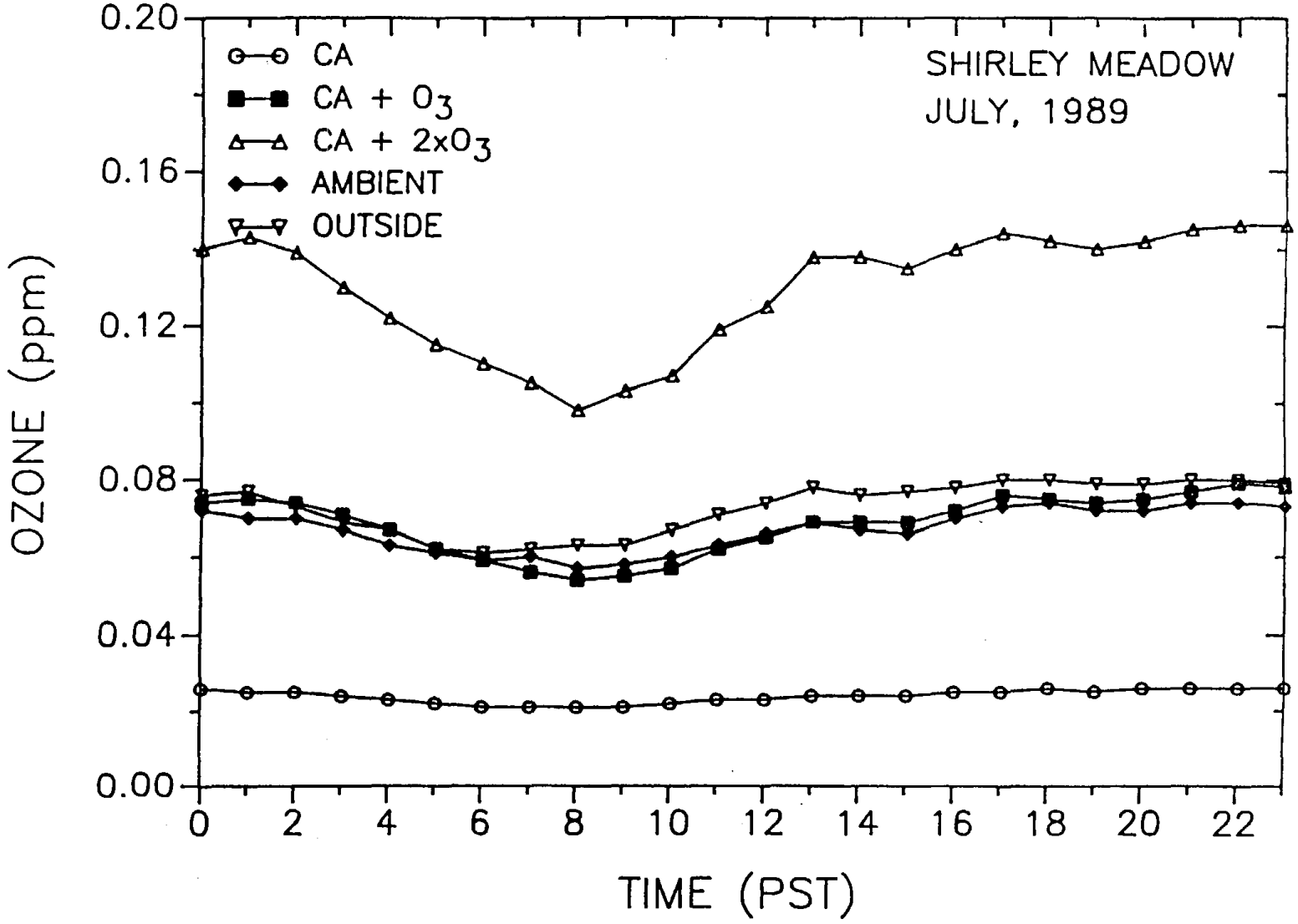

Figure A-2. Diurnal profiles of ozone concentrations for different air pollution treatments - July 1989.

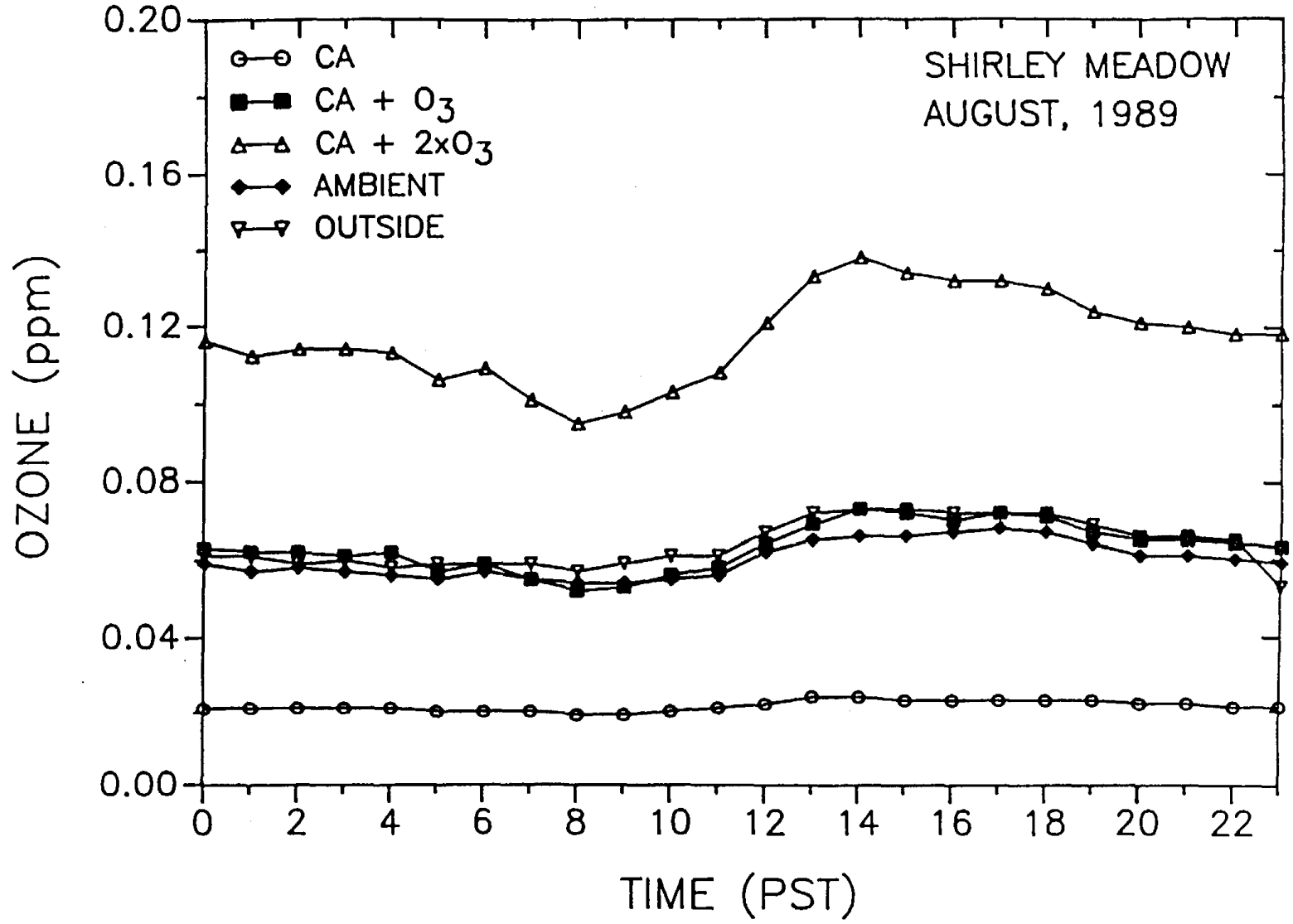

Figure A-3. Diurnal profiles of ozone concentrations for different air pollution treatments - August 1989.

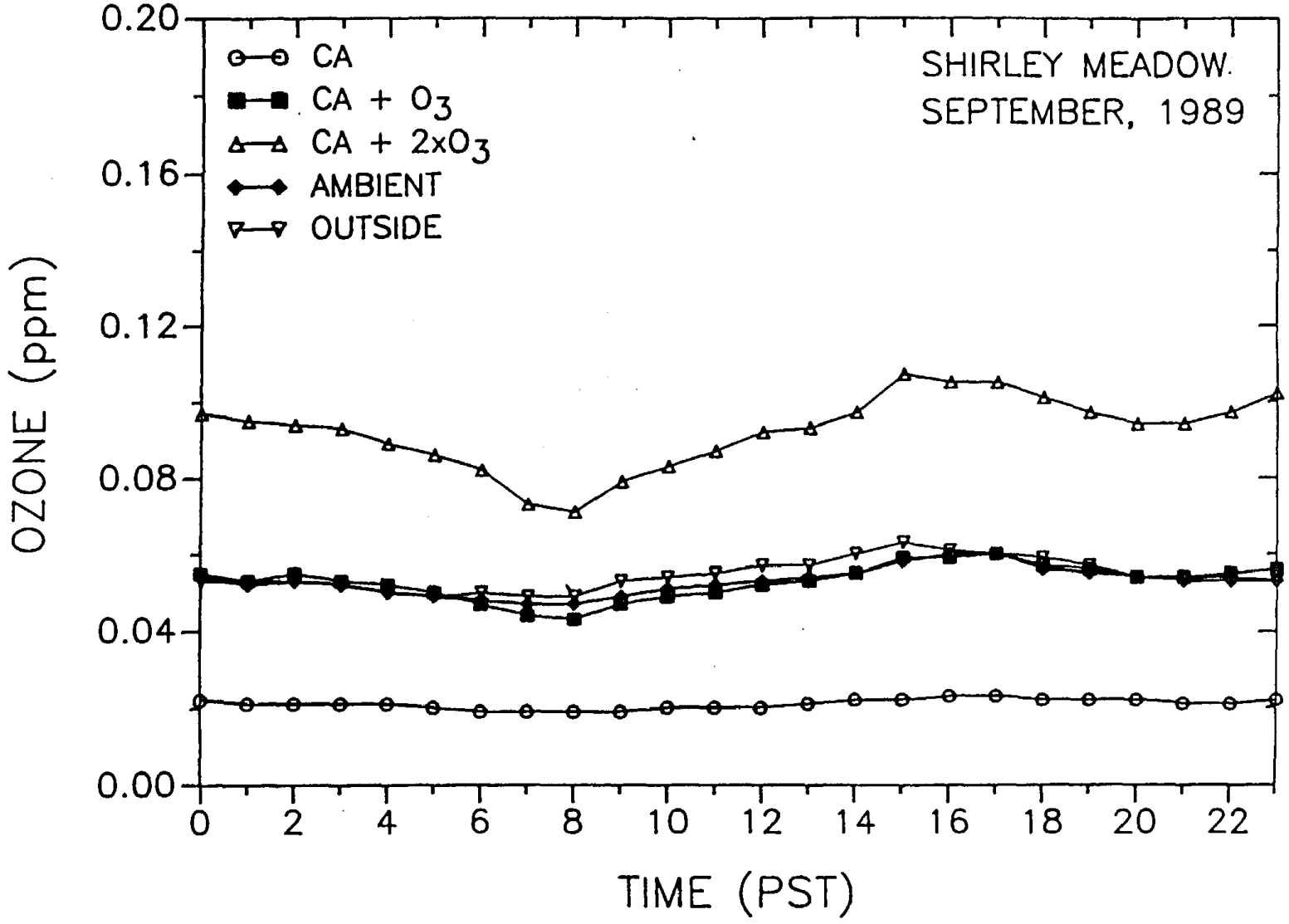

Figure A-4. Diurnal profiles of ozone concentrations for different air pollution treatments - September 1989.

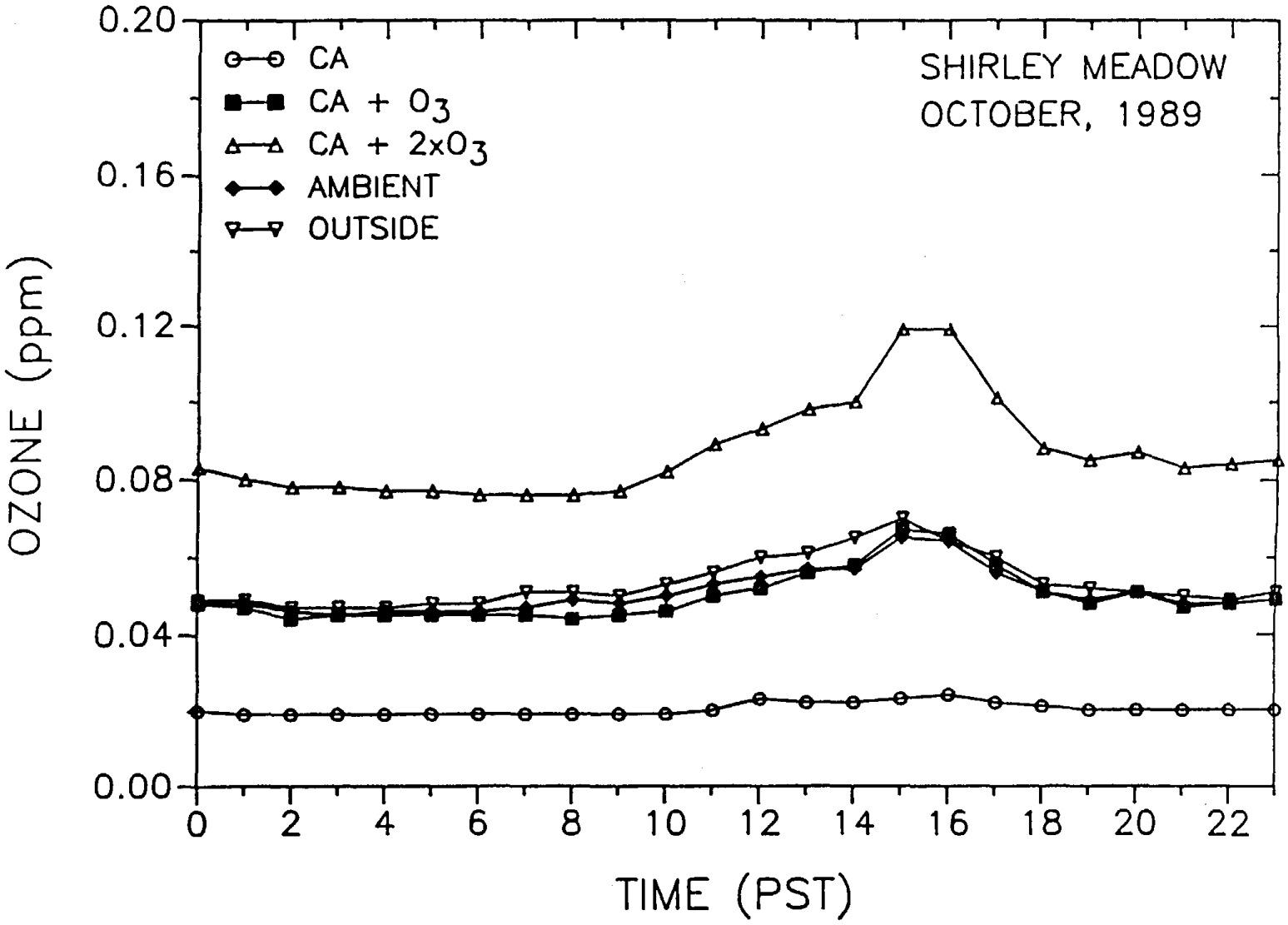

Figure A-5. Diurnal profiles of ozone concentrations for different air pollution treatments - October 1989.

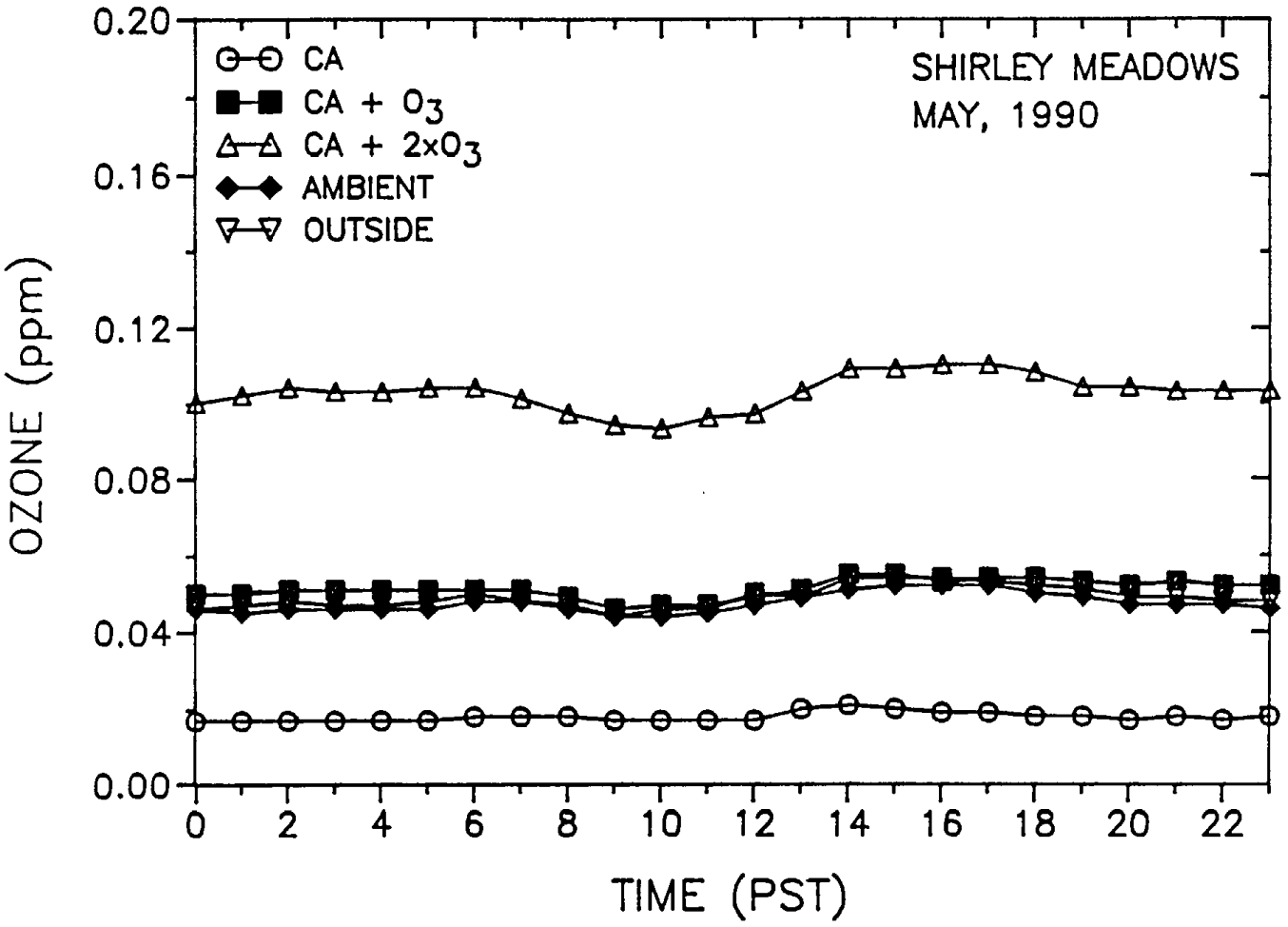

Figure A-6. Diurnal profiles of ozone concentrations for different air pollution treatments - May 1990.

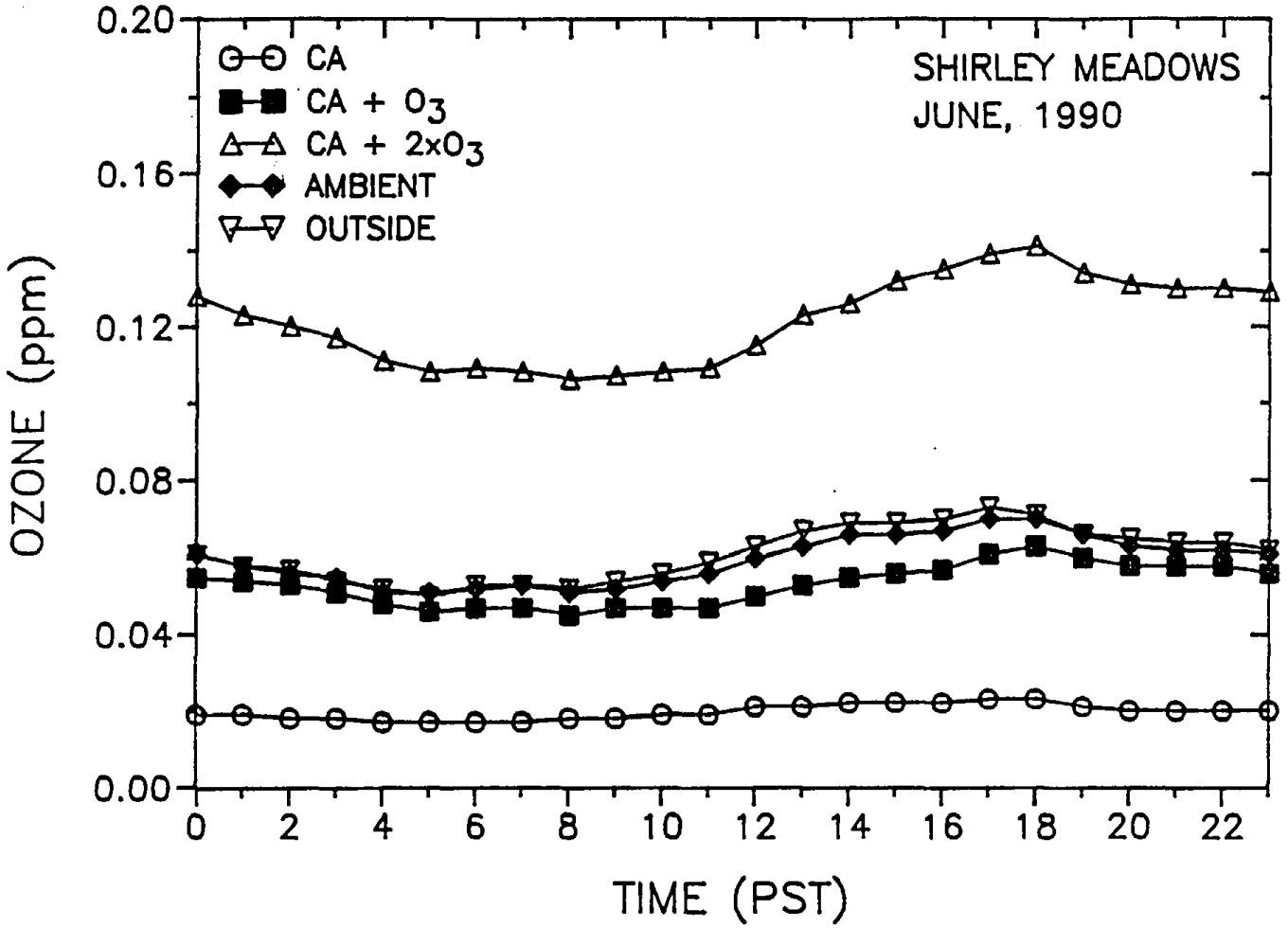

Figure A-7. Diurnal profiles of ozone concentrations for different air pollution treatments - June 1990.

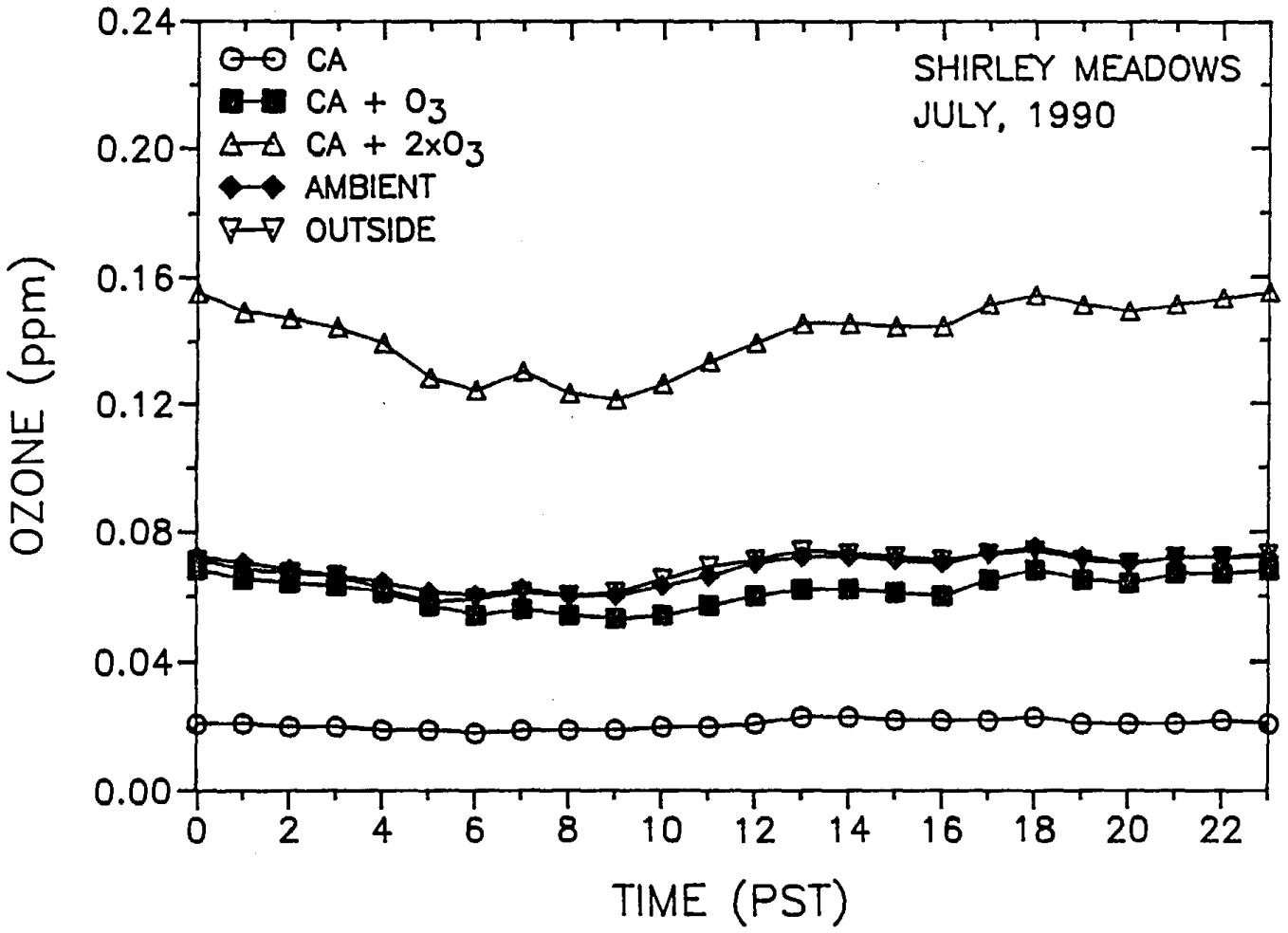

Figure A-8. Diurnal profiles of ozone concentrations for different air pollution treatments - July 1990.

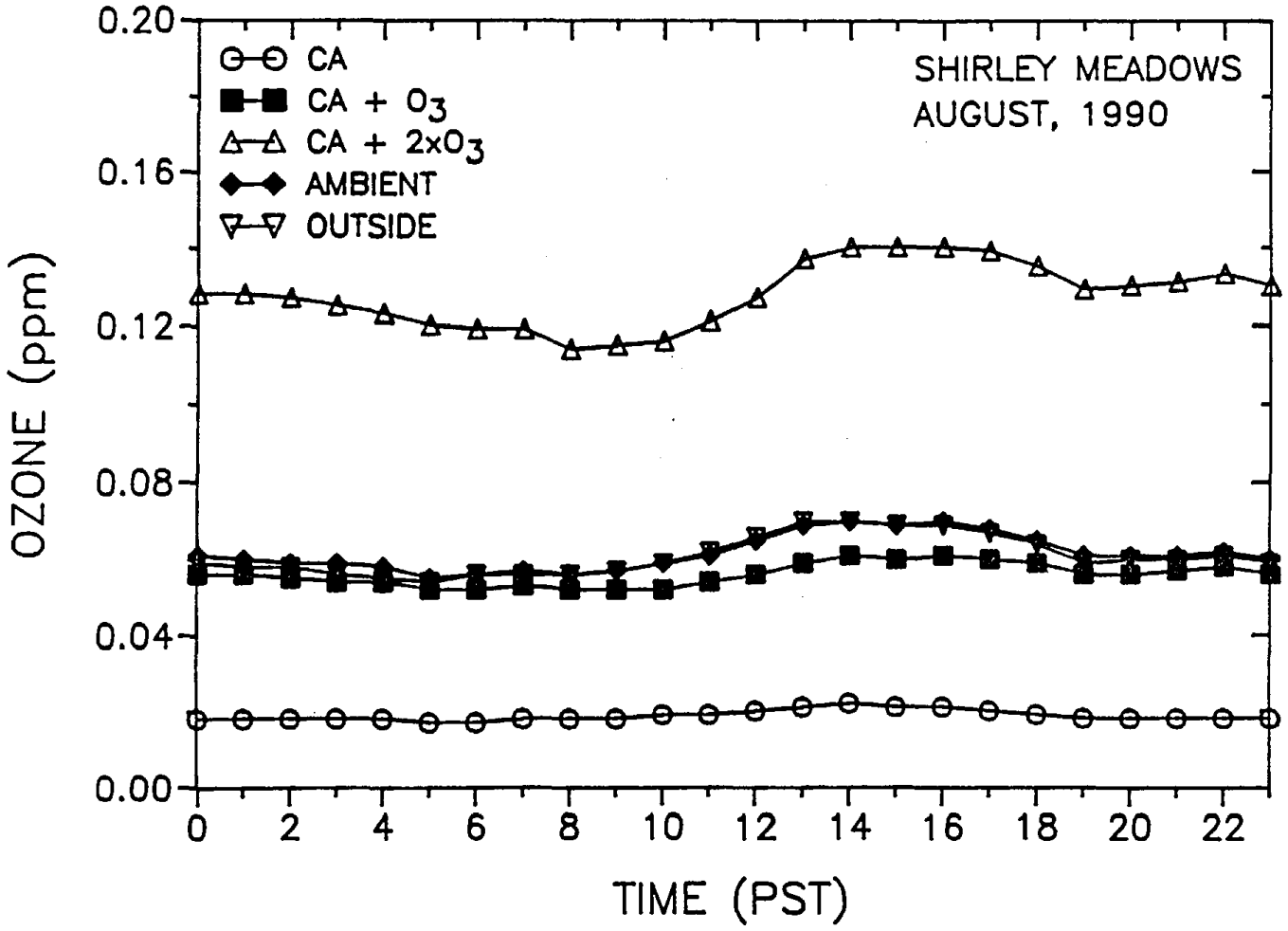

Figure A-9. Diurnal profiles of ozone concentrations for different air pollution treatments - August 1990.

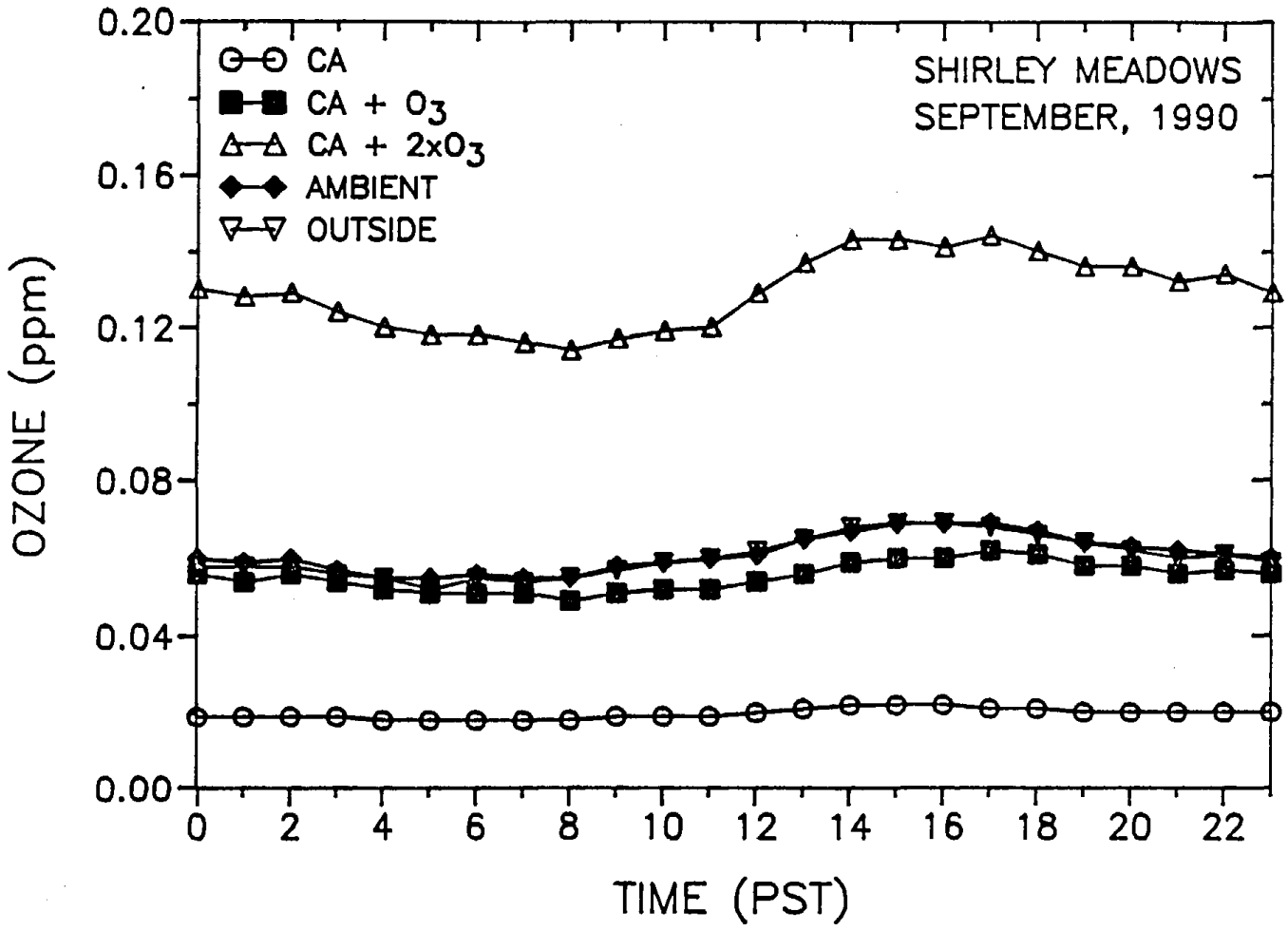

Figure A-10. Diurnal profiles of ozone concentrations for different air pollution treatments - September 1990.

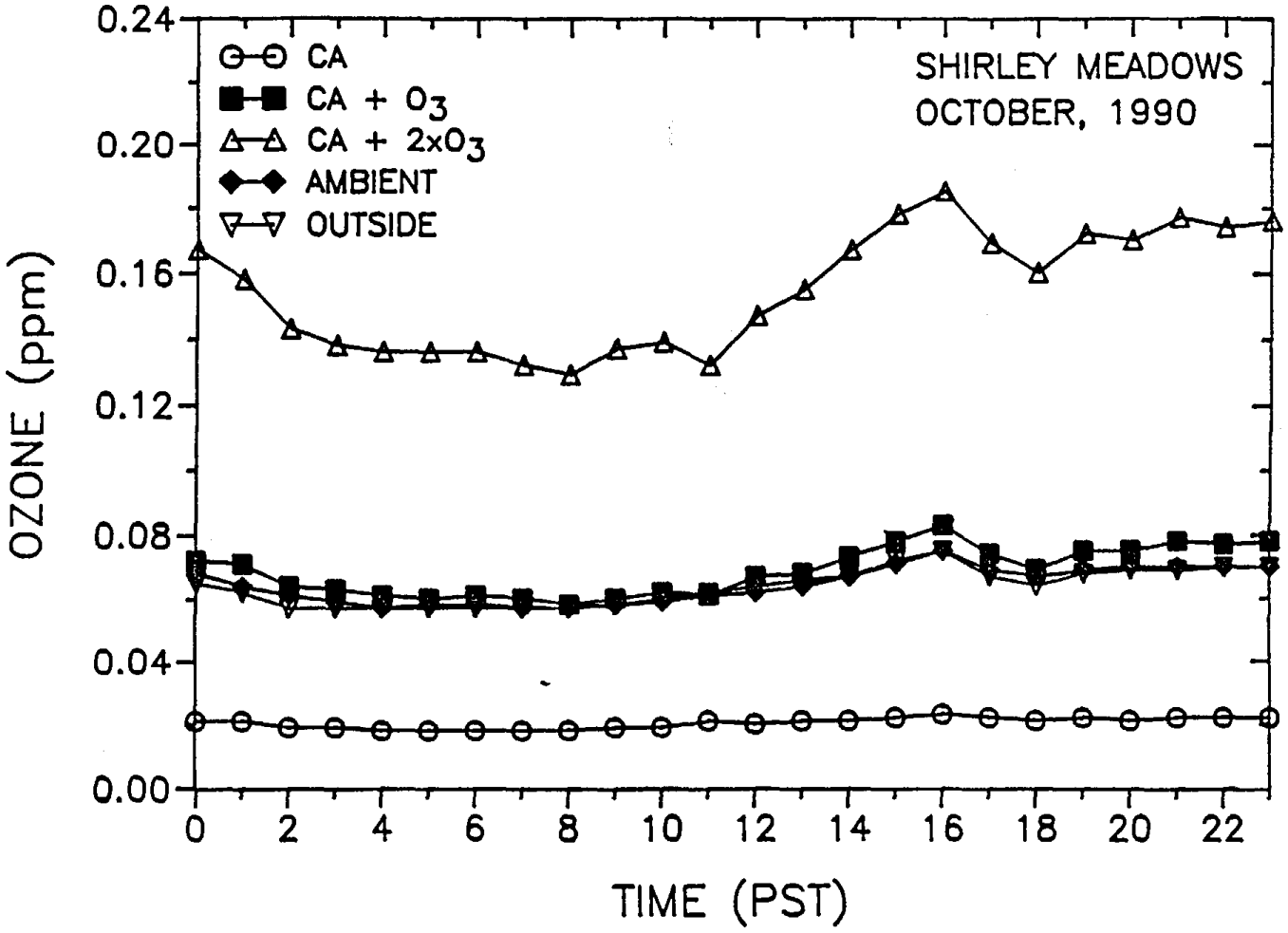

Figure A-11. Diurnal profiles of ozone concentrations for different air pollution treatments - October 1990.

Appendix B

Distribution and Average Wind Speeds at Shirley Meadow During the Course of the Study

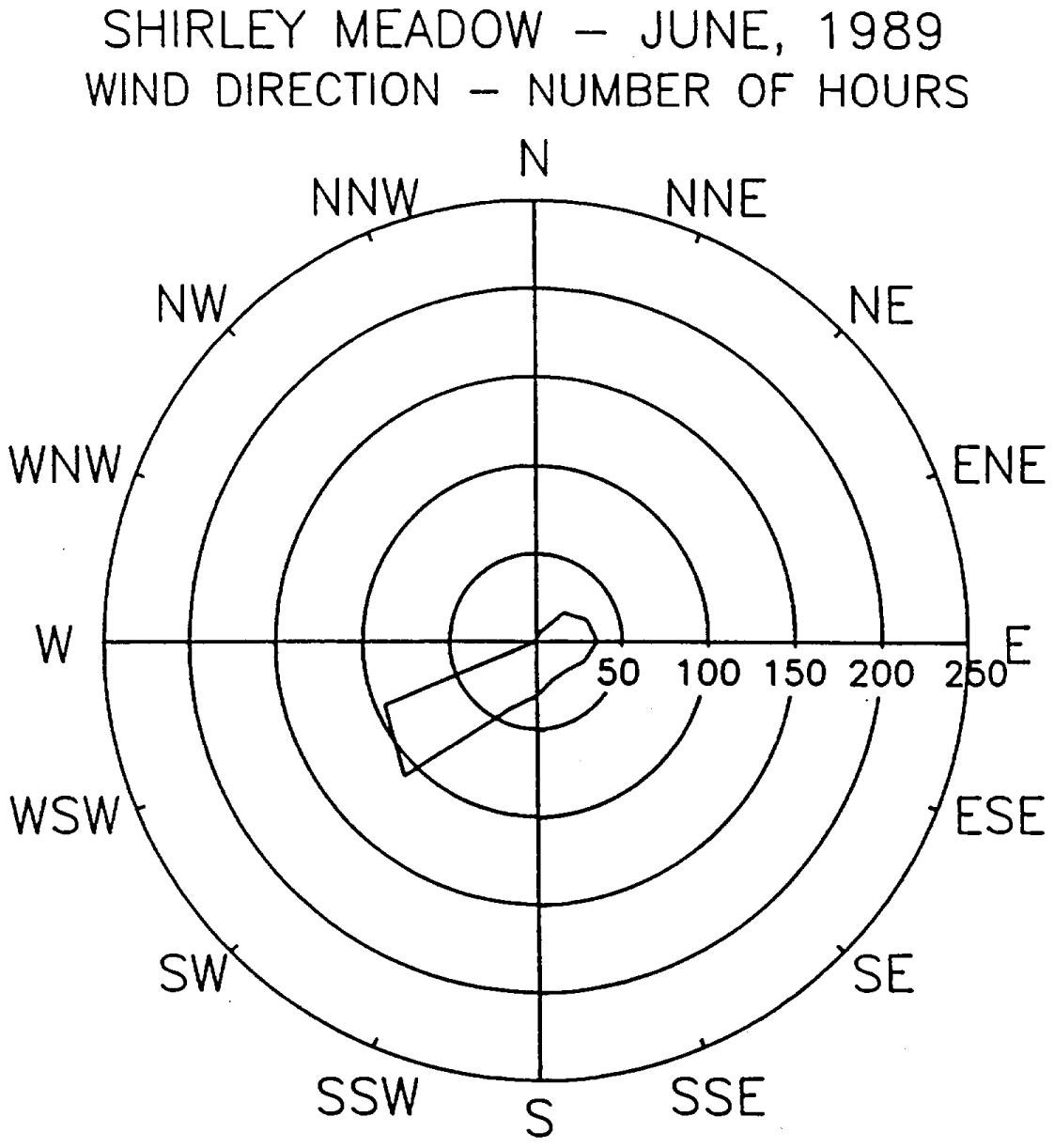

Figure B-1. Distribution of winds at Shirley Meadow in June 1989 expressed as the number of hours winds come from a given direction.

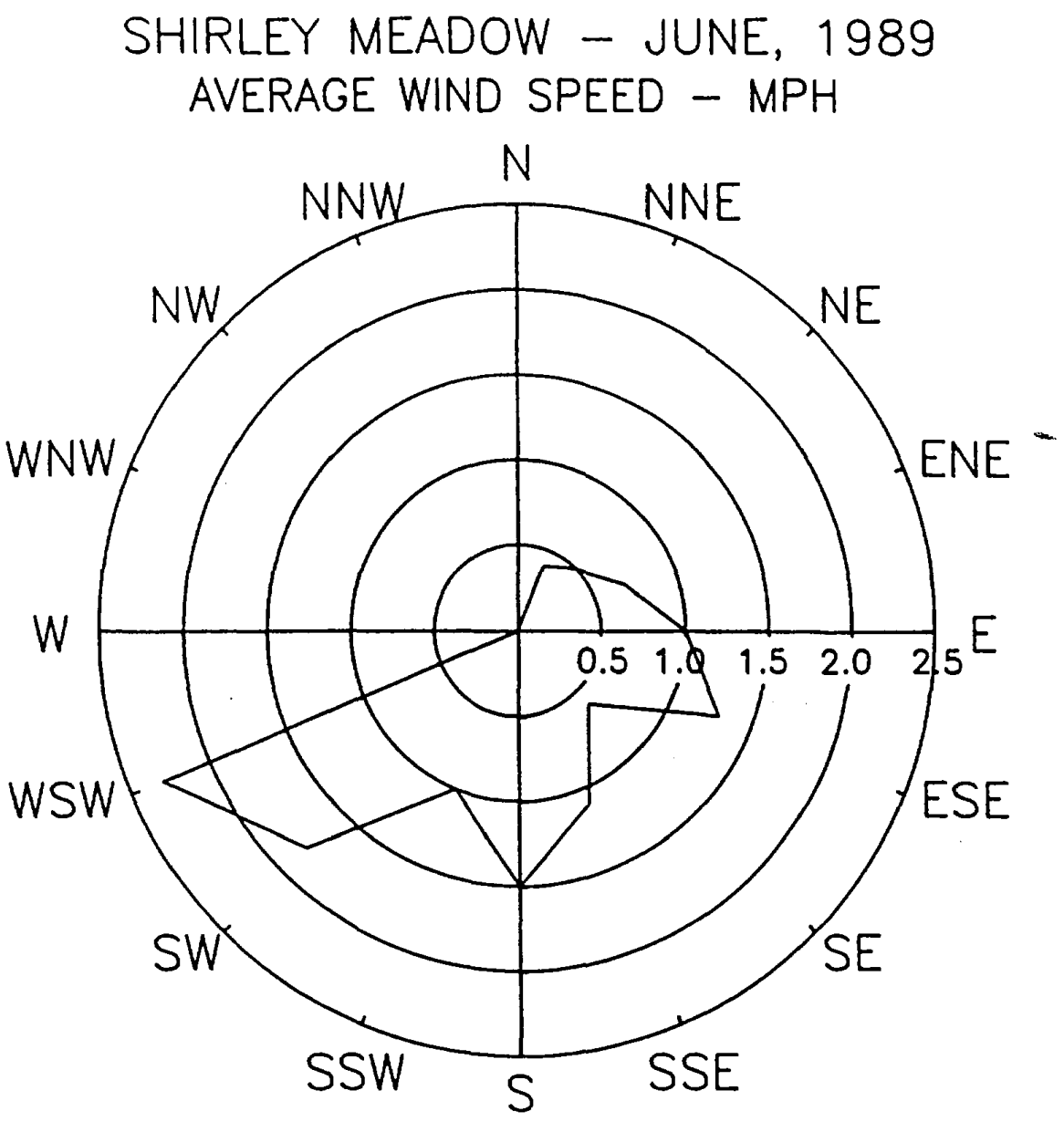

Figure B-2. Average wind speeds at Shirley Meadow in June 1989.

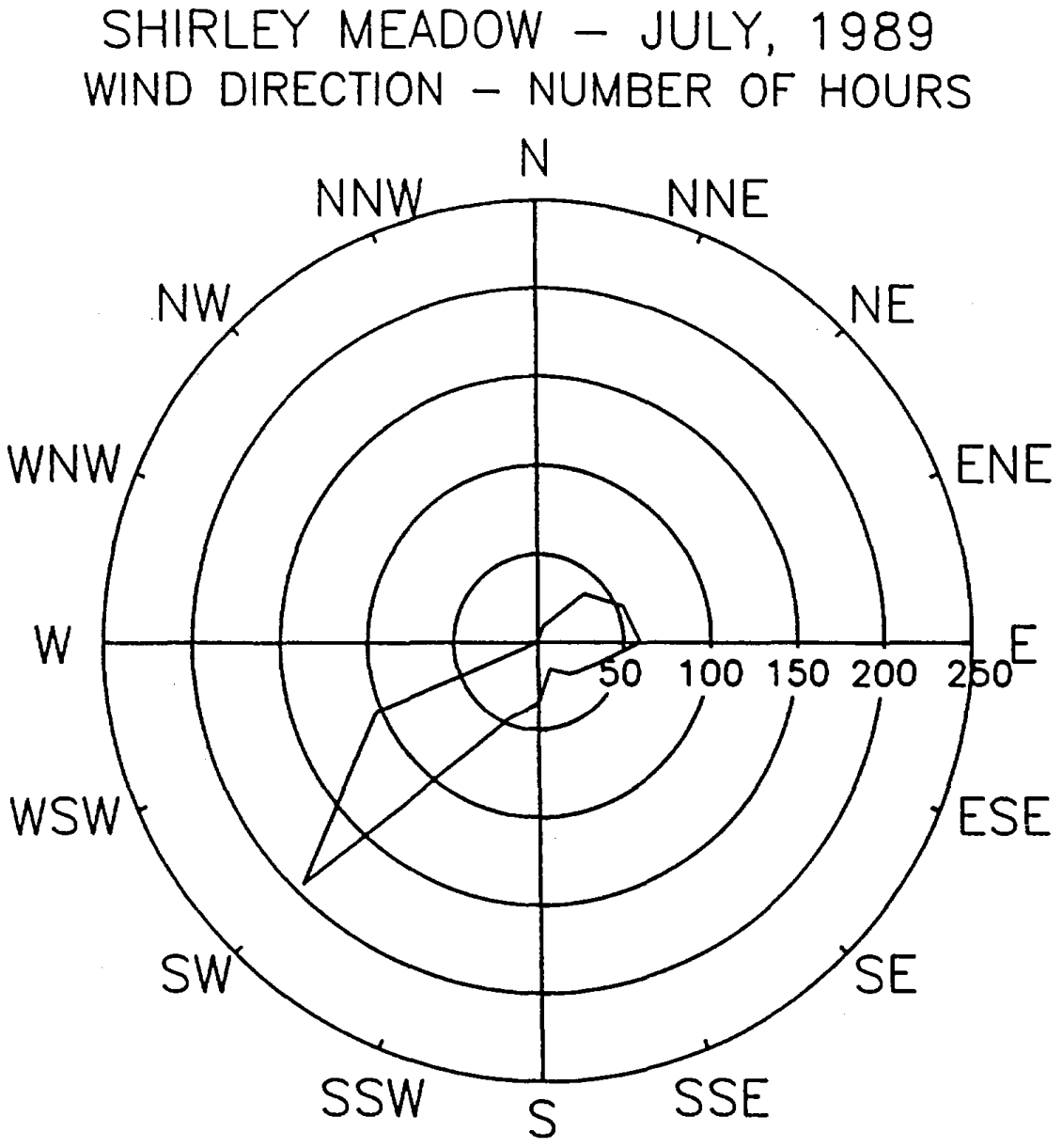

Figure B-3. Distribution of winds at Shirley Meadow in July 1989 expressed as the number of hours winds come from a given direction.

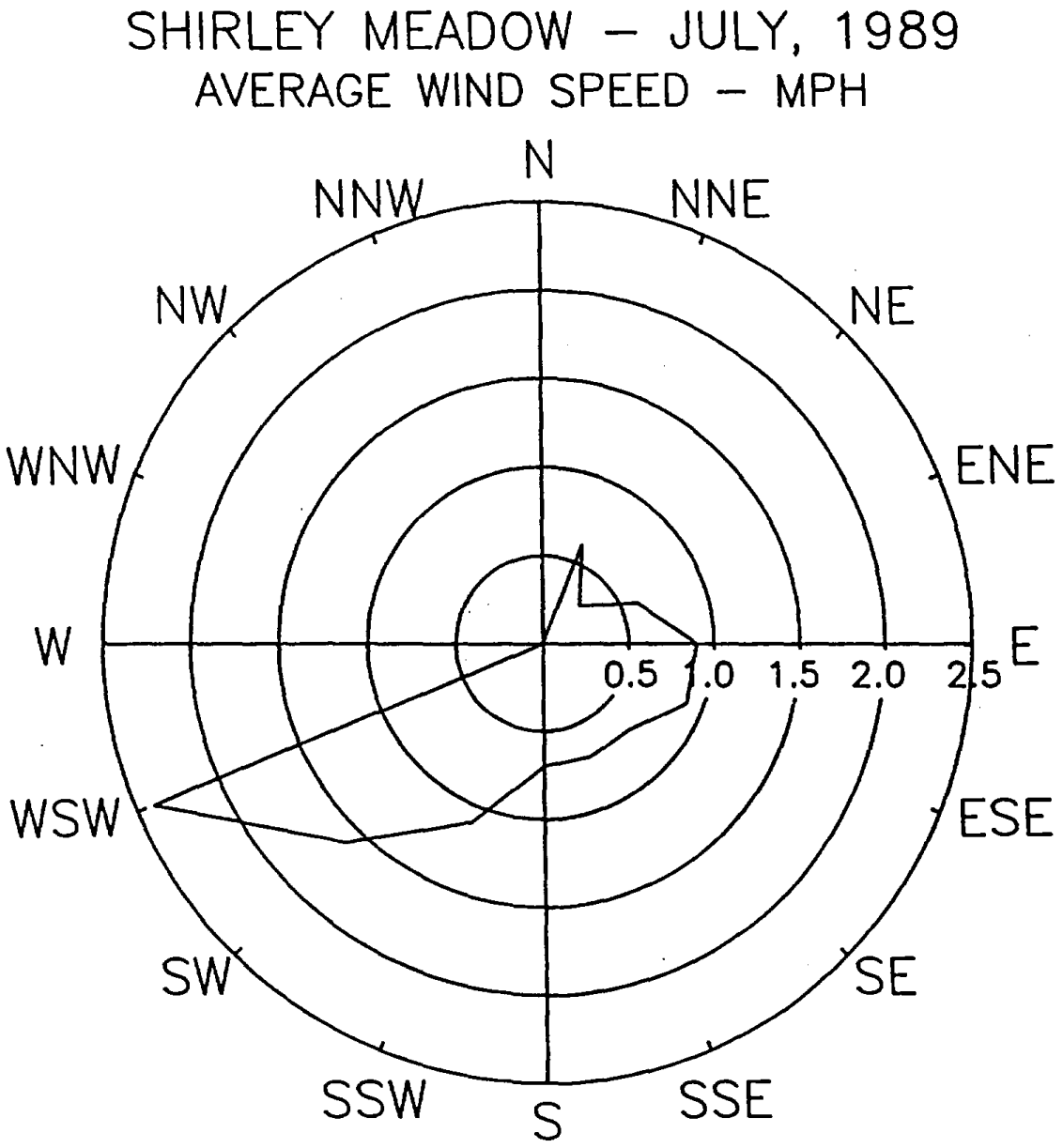

Figure B-4. Average wind speeds at Shirley Meadow in July 1989.

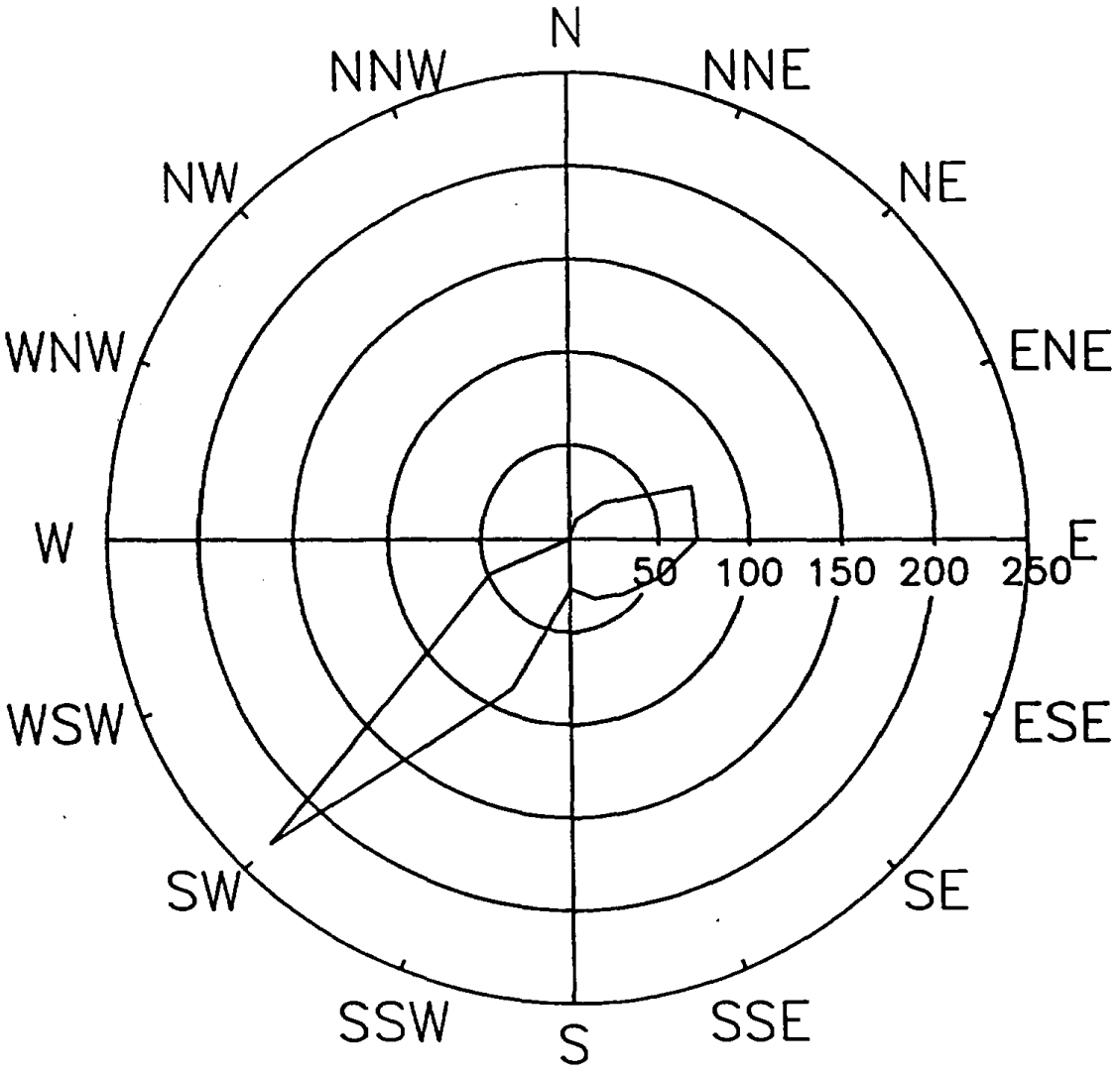

Figure B-5. Distribution of winds at Shirley Meadow in August 1989 expressed as the number of hours winds come from a given direction.

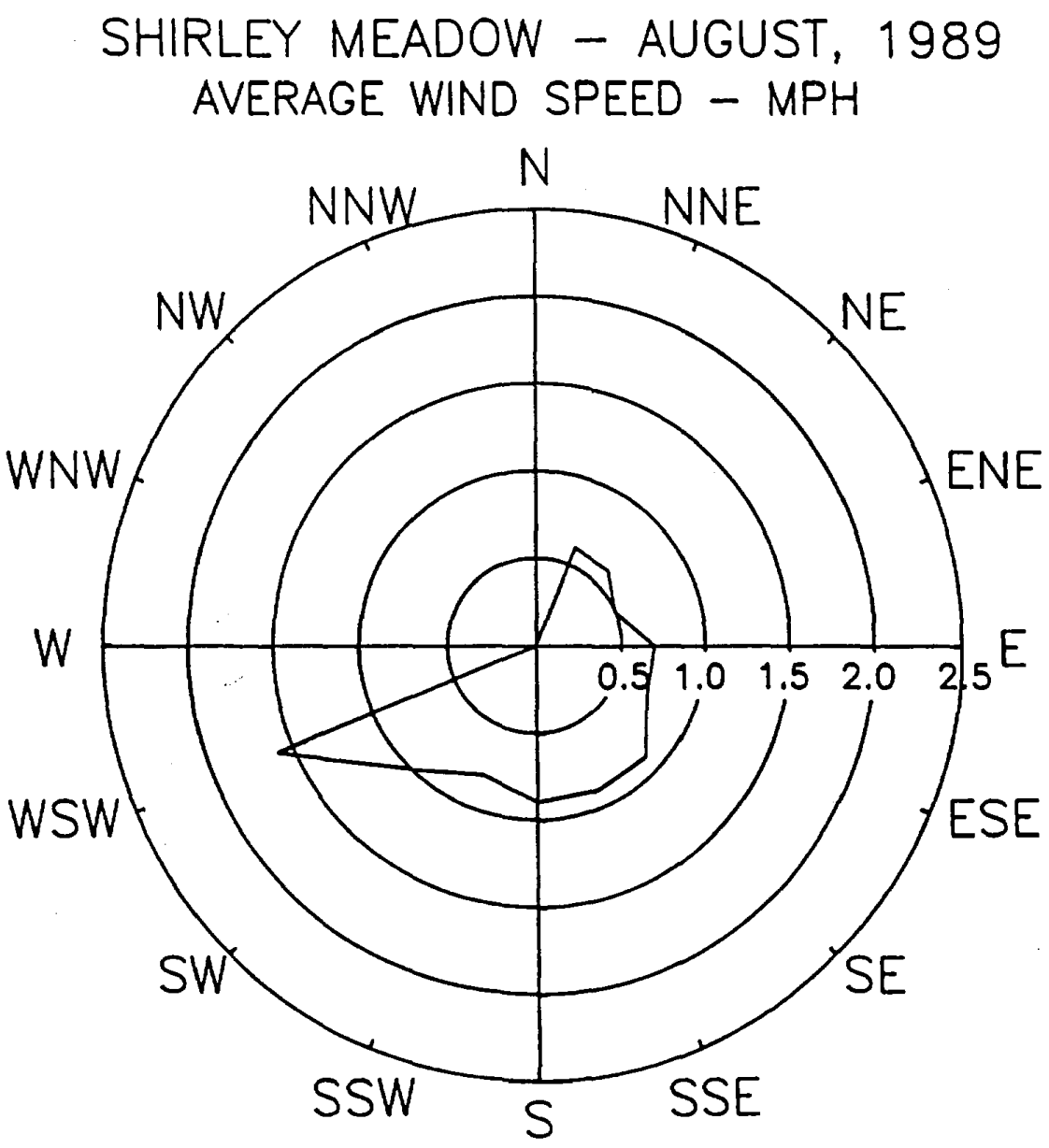

Figure B-6. Average wind speeds at Shirley Meadow in August 1989.

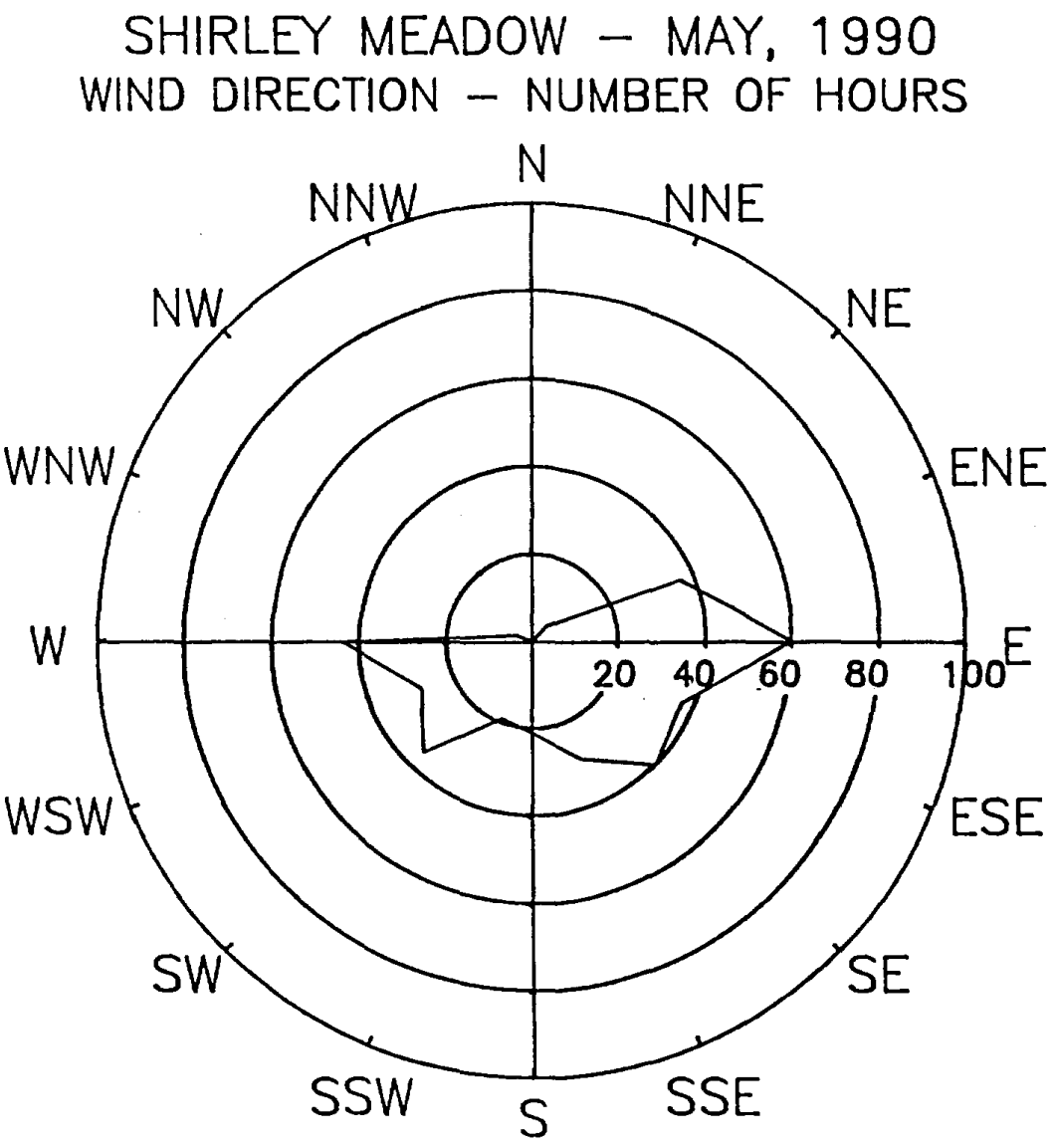

Figure B-7. Distribution of winds at Shirley Meadow in May 1990 expressed as the number of hours winds come from a given direction.

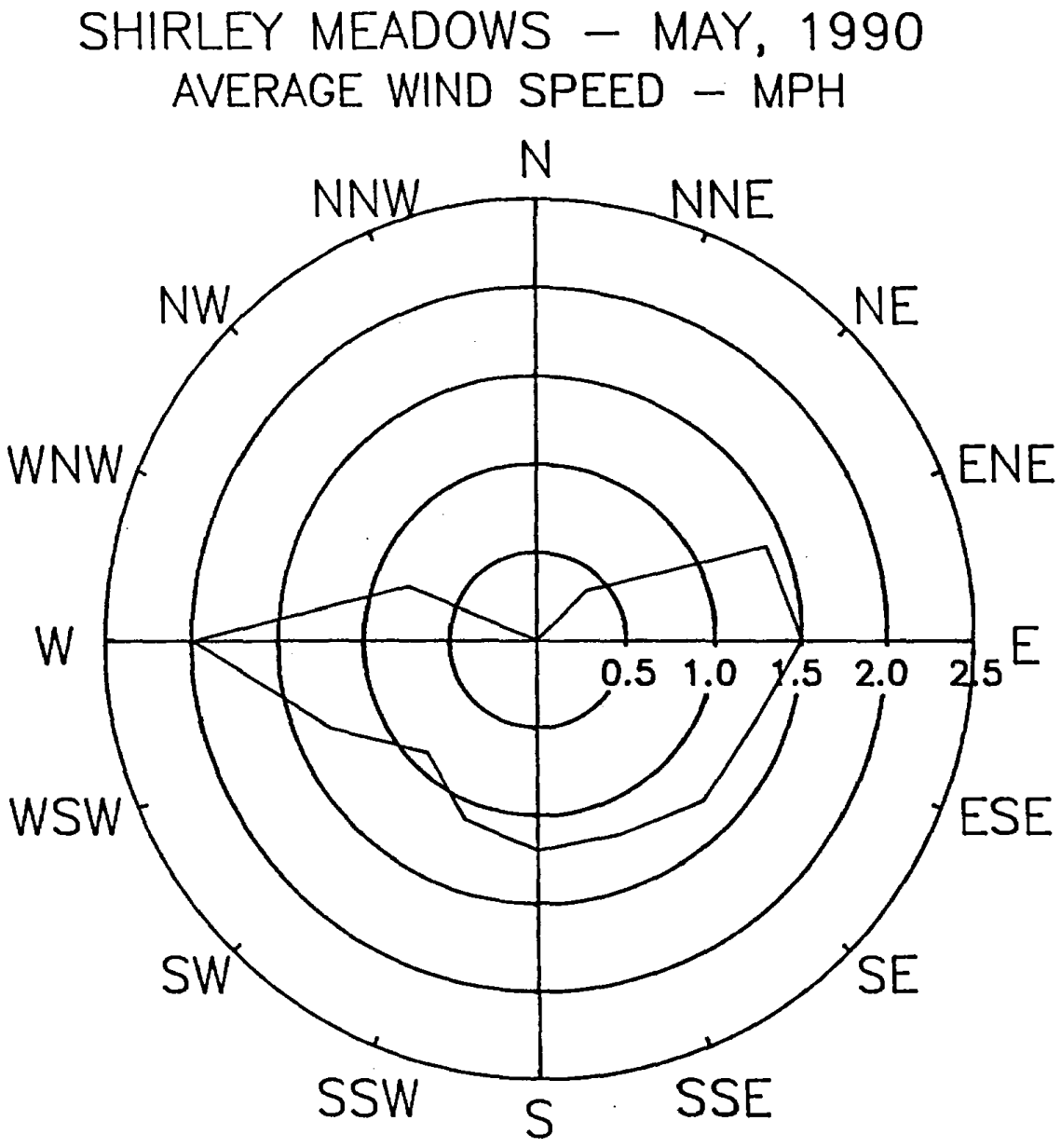

Figure B-8. Average wind speeds at Shirley Meadow in May 1990.

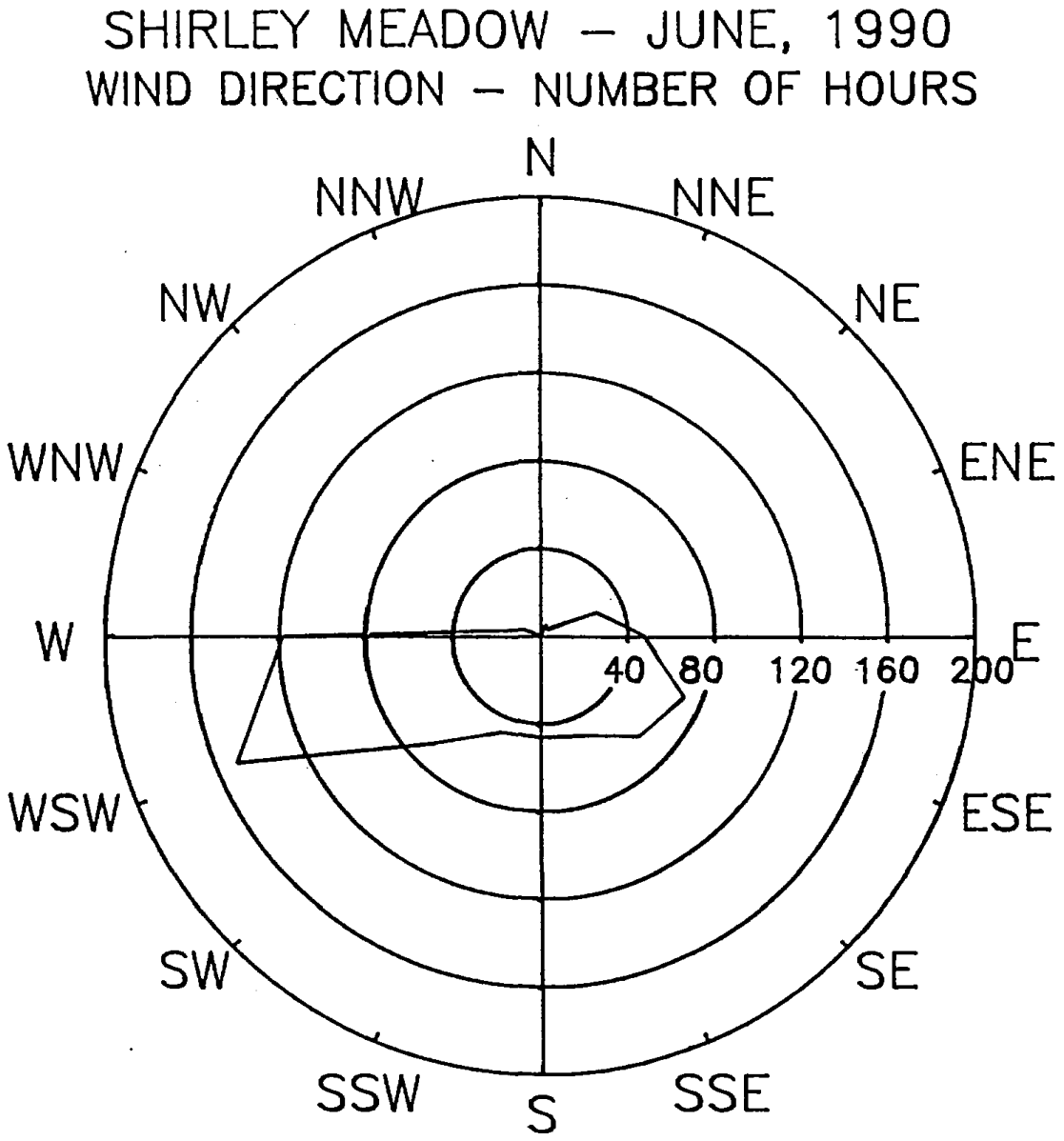

Figure B-9. Distribution of winds at Shirley Meadow in June 1990 expressed as the number of hours winds come from a given direction.

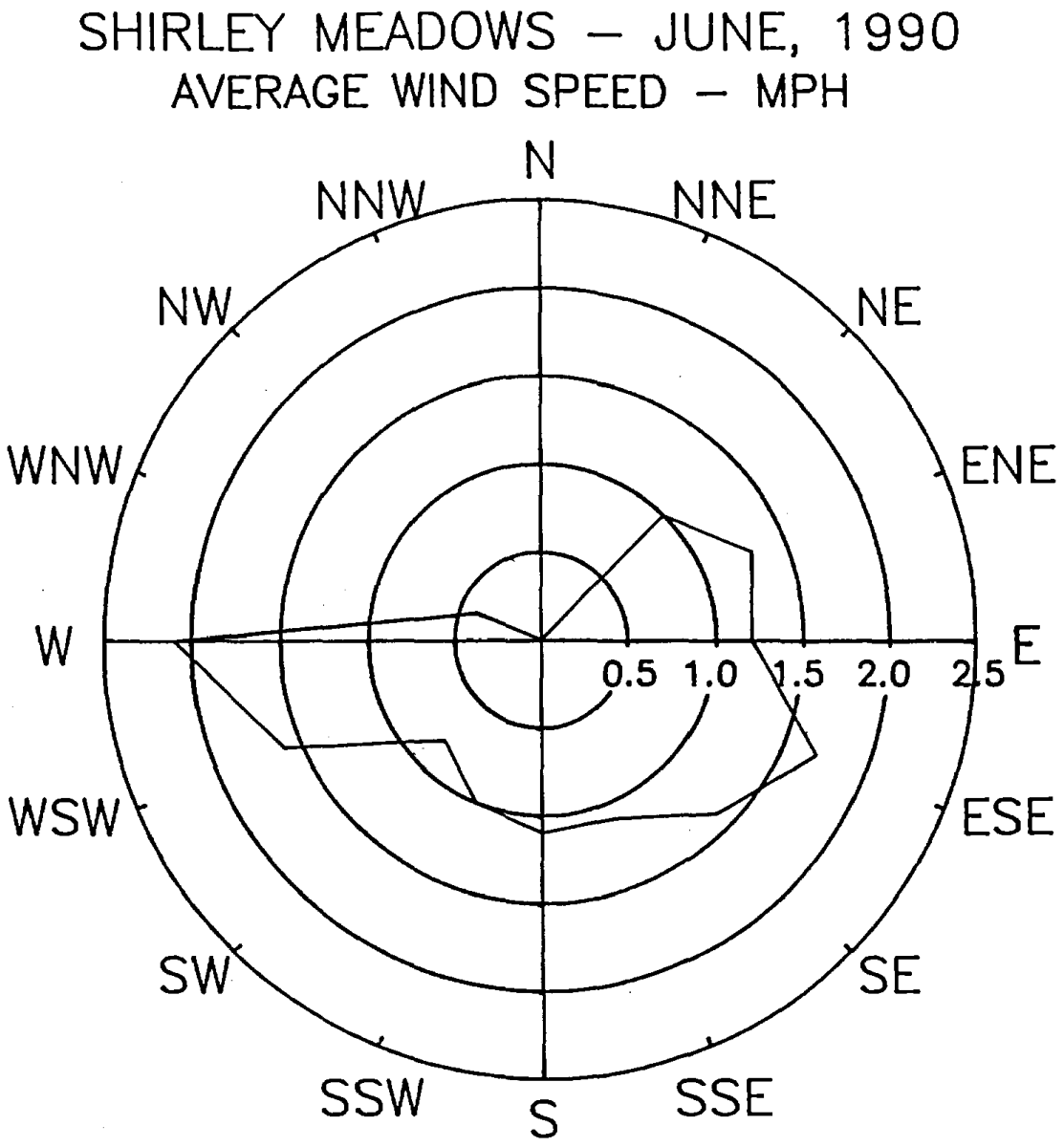

Figure B-10. Average wind speeds at Shirley Meadow in June 1990.

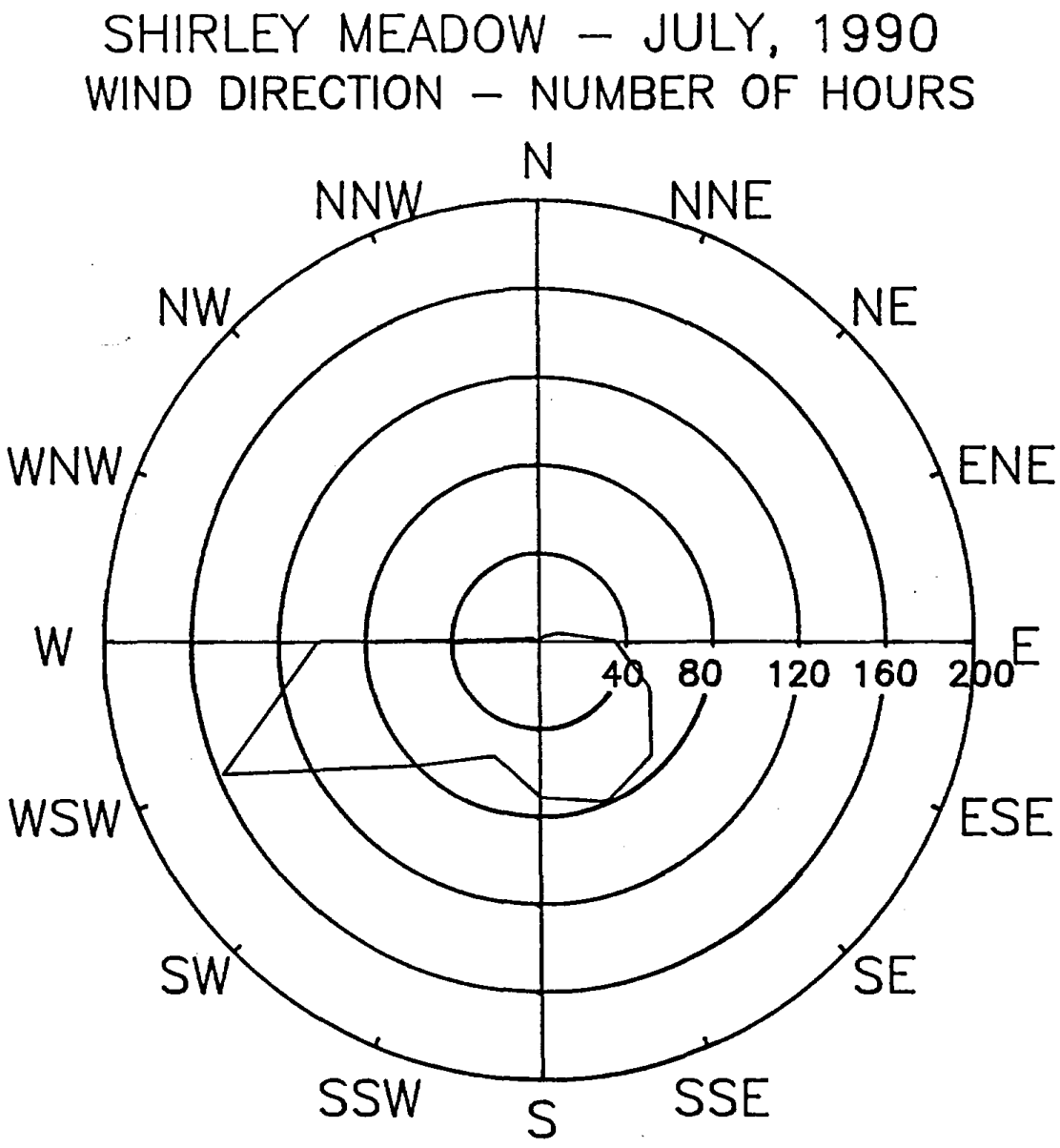

Figure B-11. Distribution of winds at Shirley Meadow in July 1990 expressed as the number of hours winds come from a given direction.

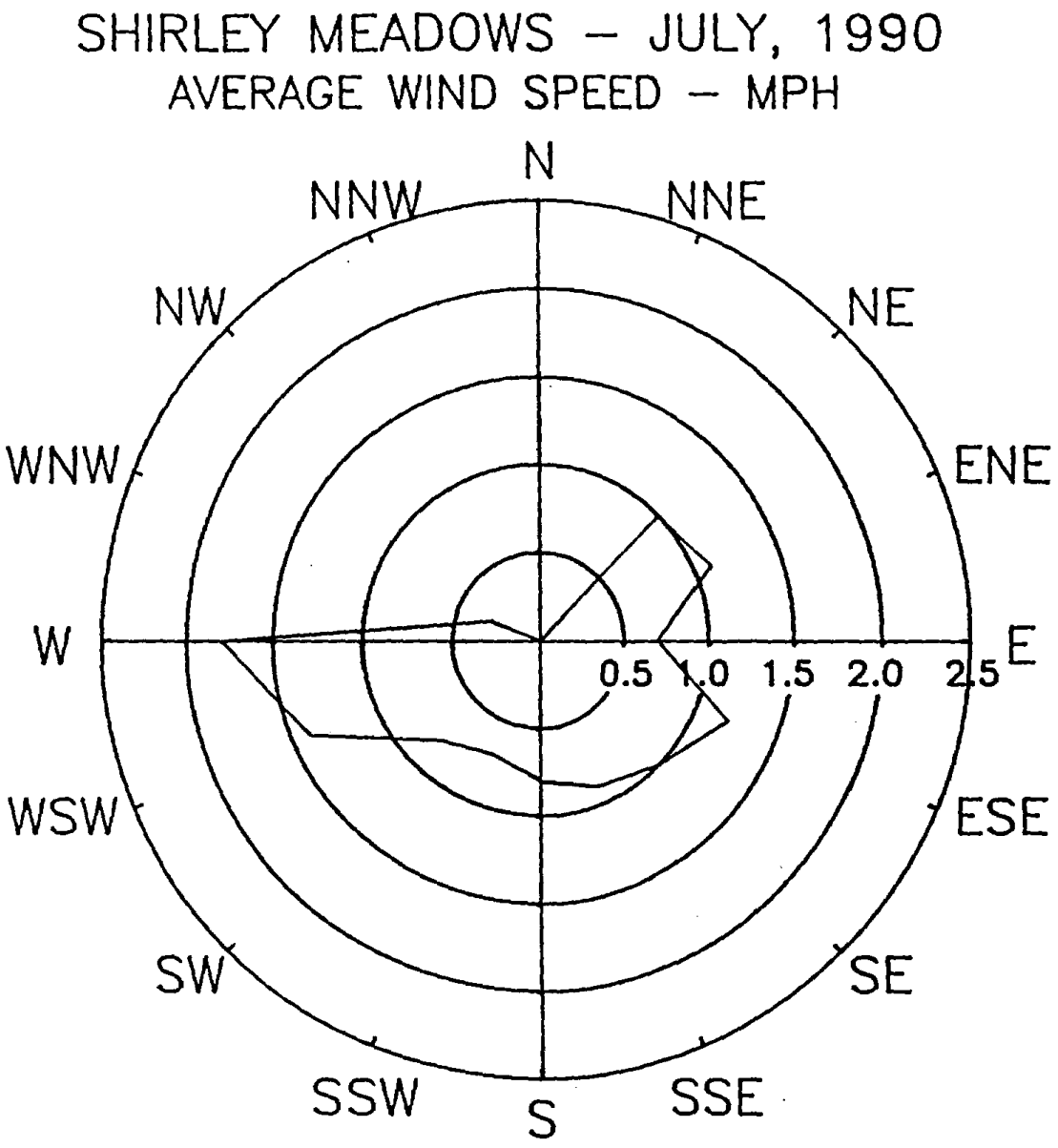

Figure B-12. Average wind speeds at Shirley Meadow in July 1990.

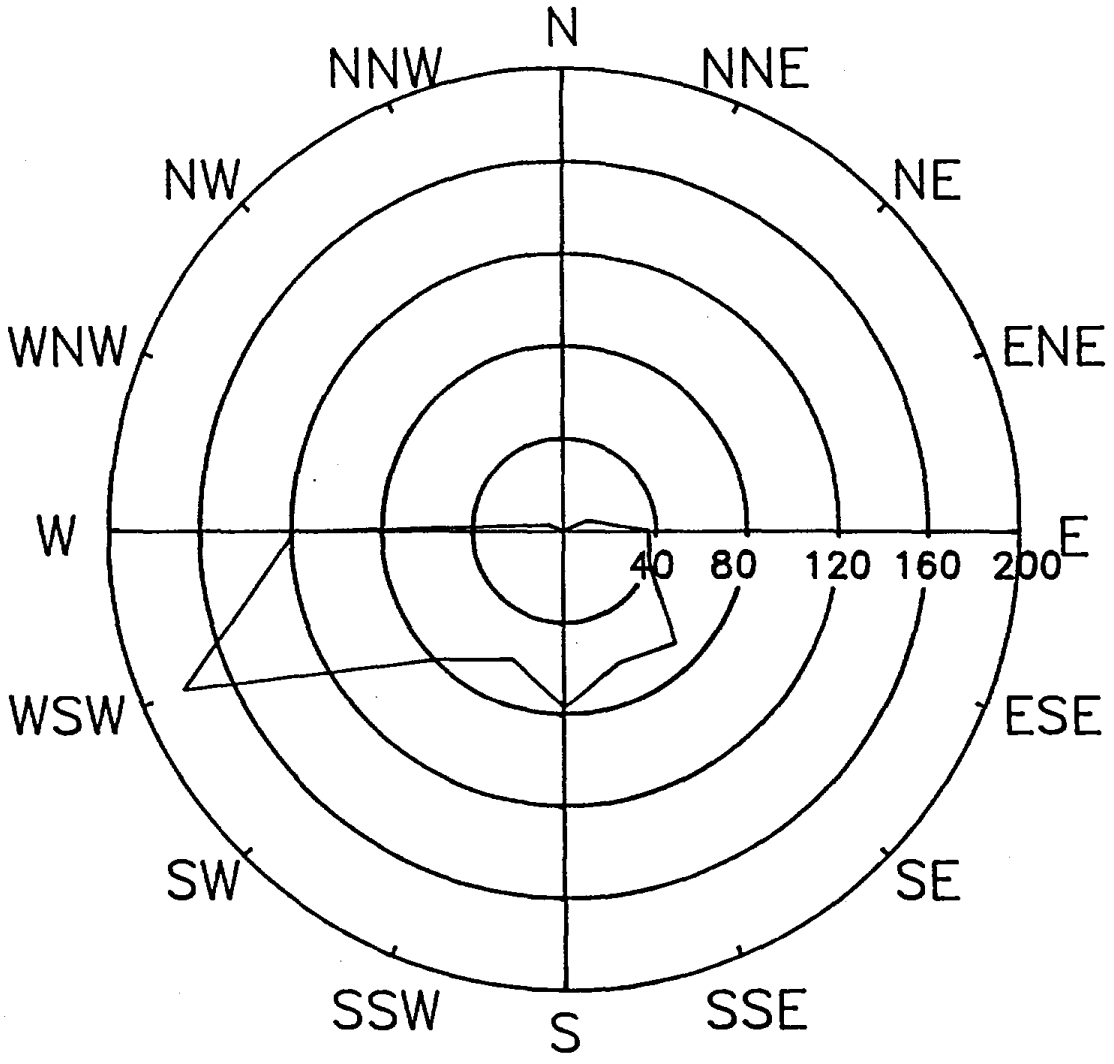

Figure B-13. Distribution of winds at Shirley Meadow in August 1990 expressed as the number of hours winds come from a given direction.

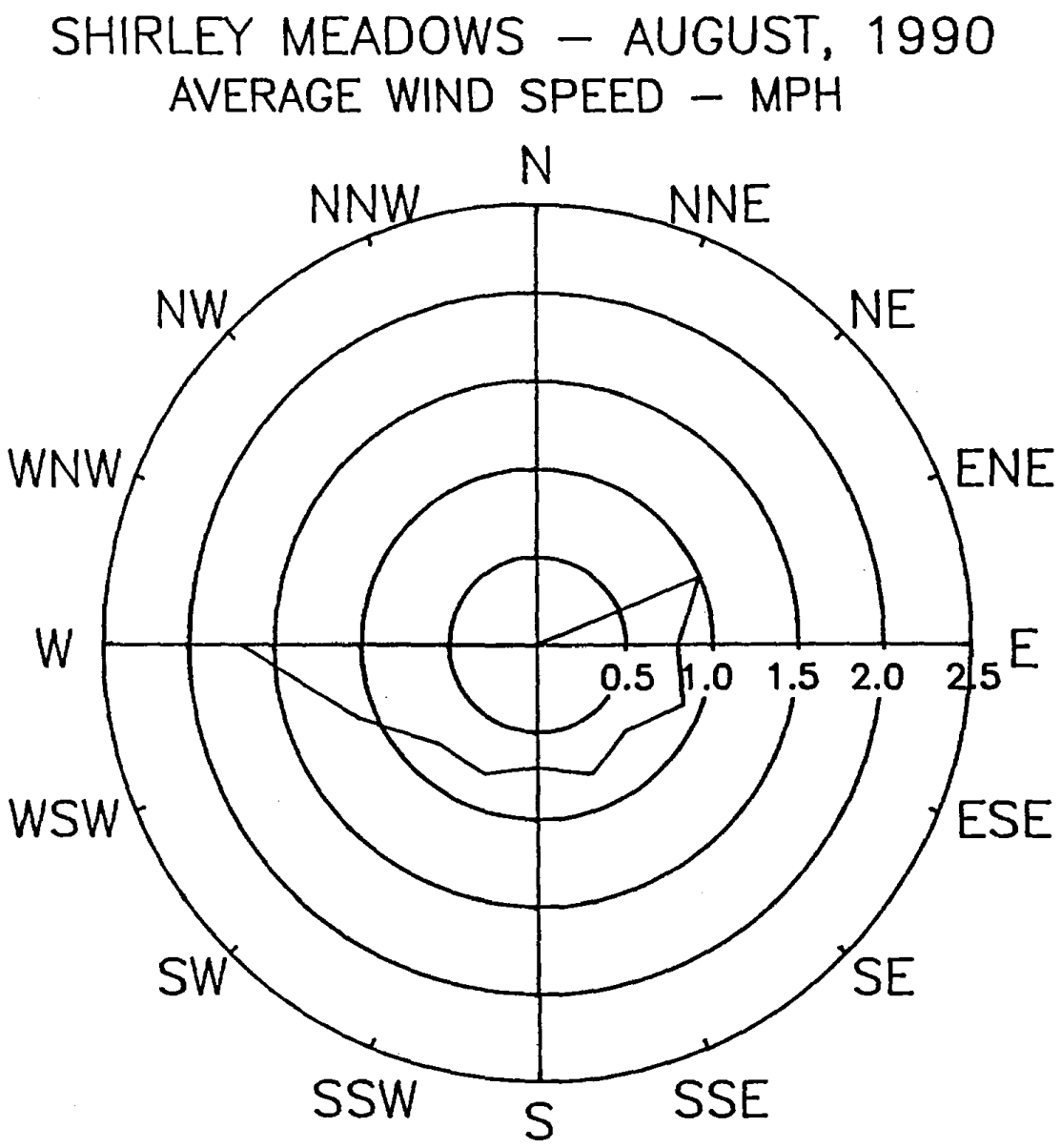

Figure B-14. Average wind speeds at Shirley Meadow in August 1990.

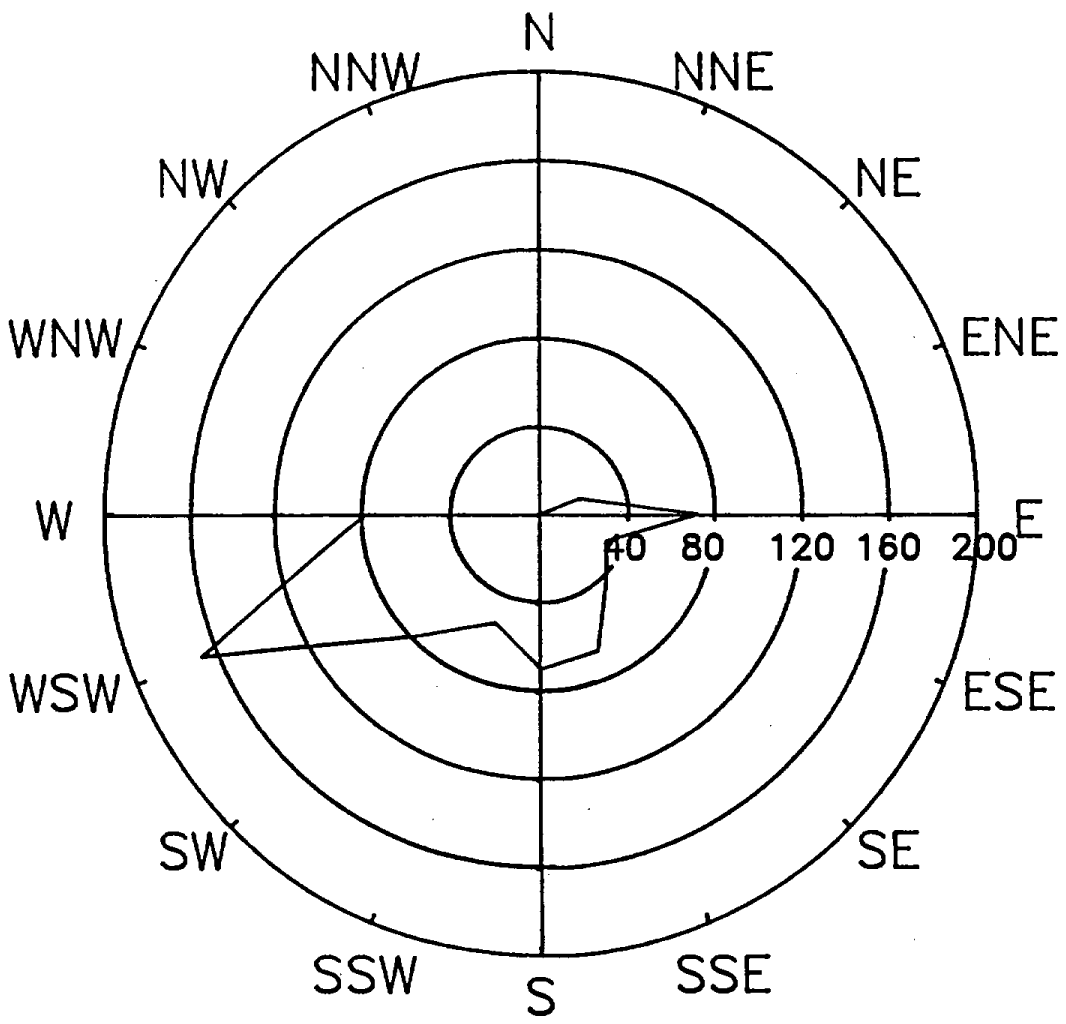

Figure B-15. Distribution of winds at Shirley Meadow in September 1990 expressed as the number of hours winds come from a given direction.

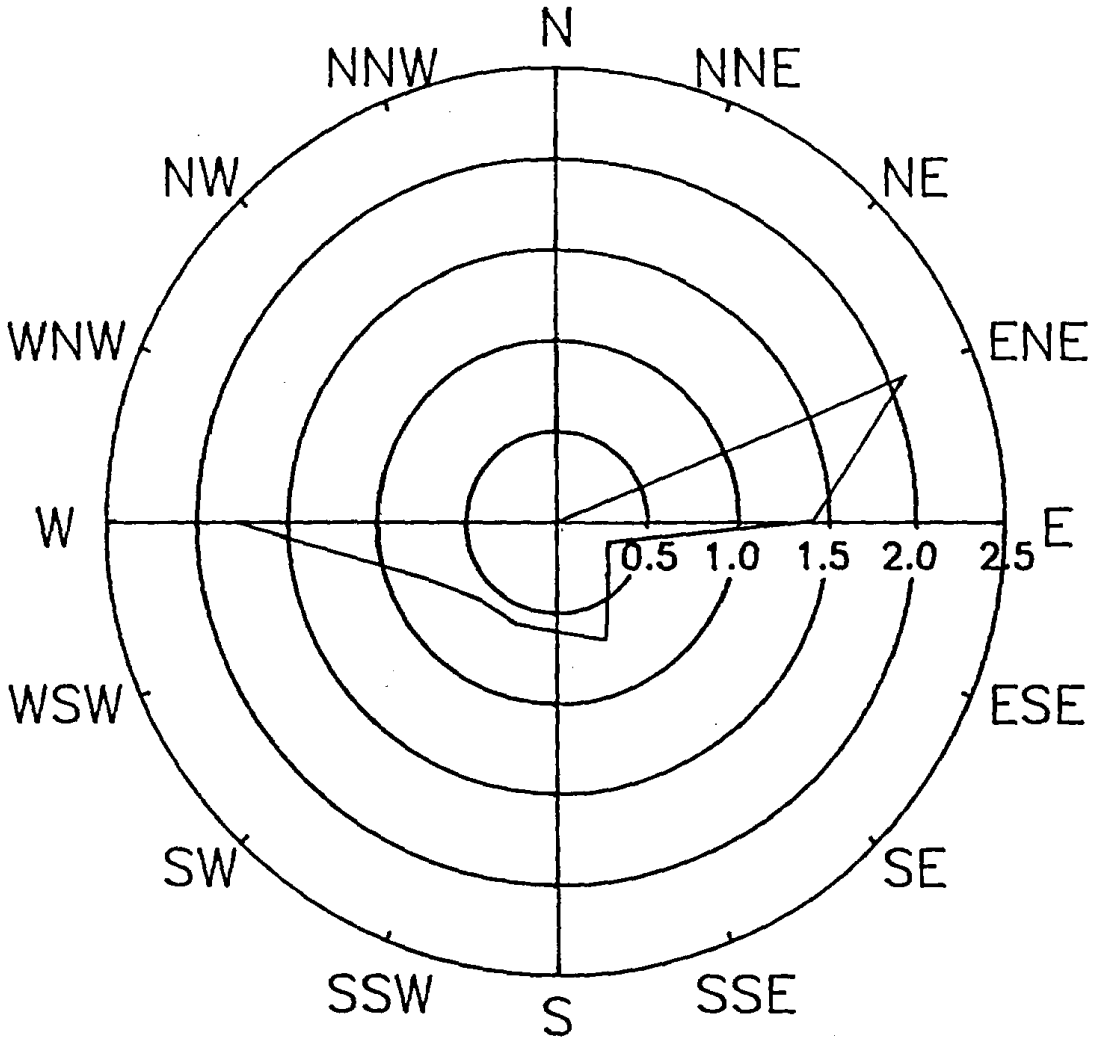

Figure B-16. Average wind speeds at Shirley Meadow in September 1990.

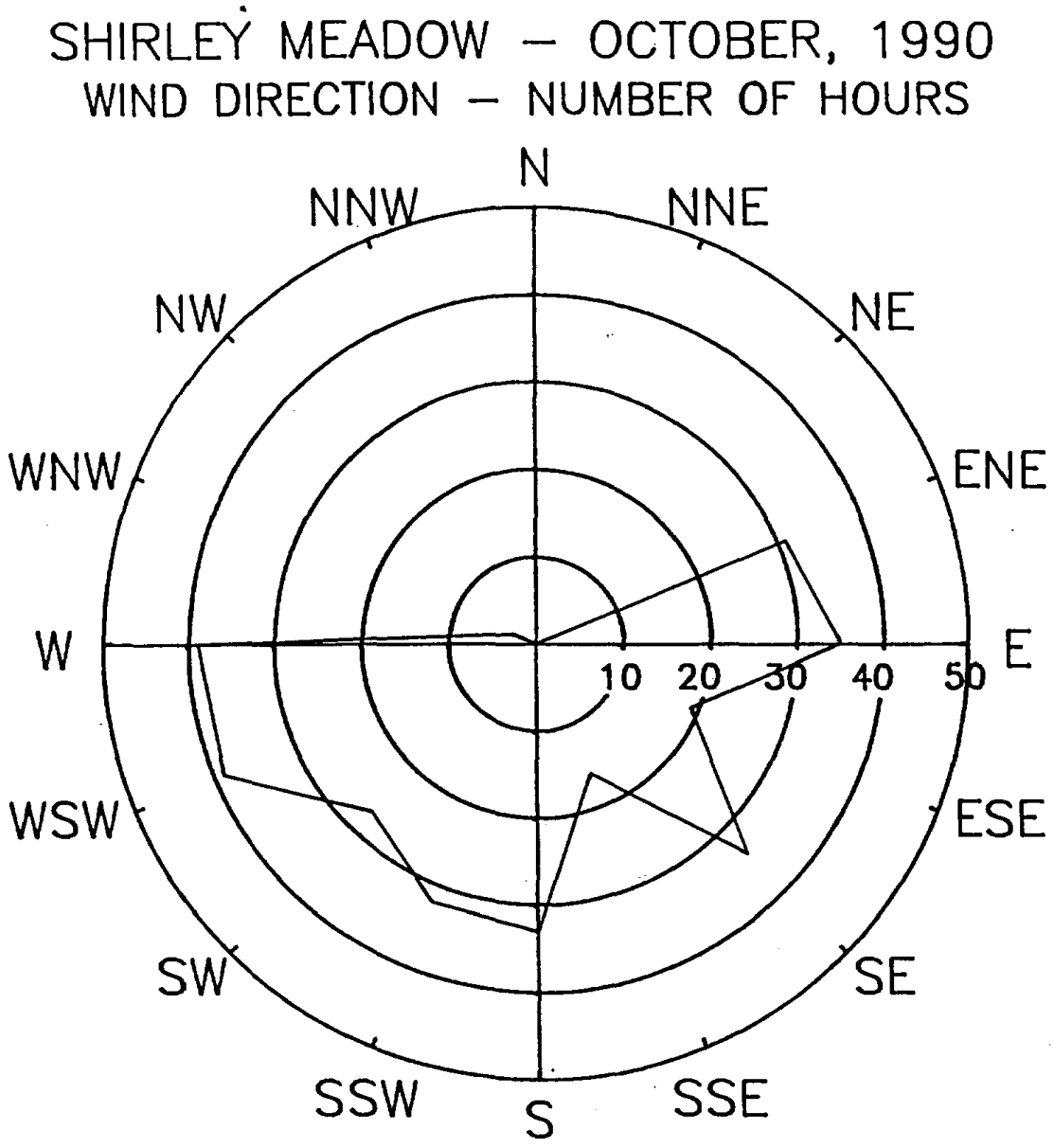

Figure B-17. Distribution of winds at Shirley Meadow in October 1990 expressed as the number of hours winds come from a given direction.

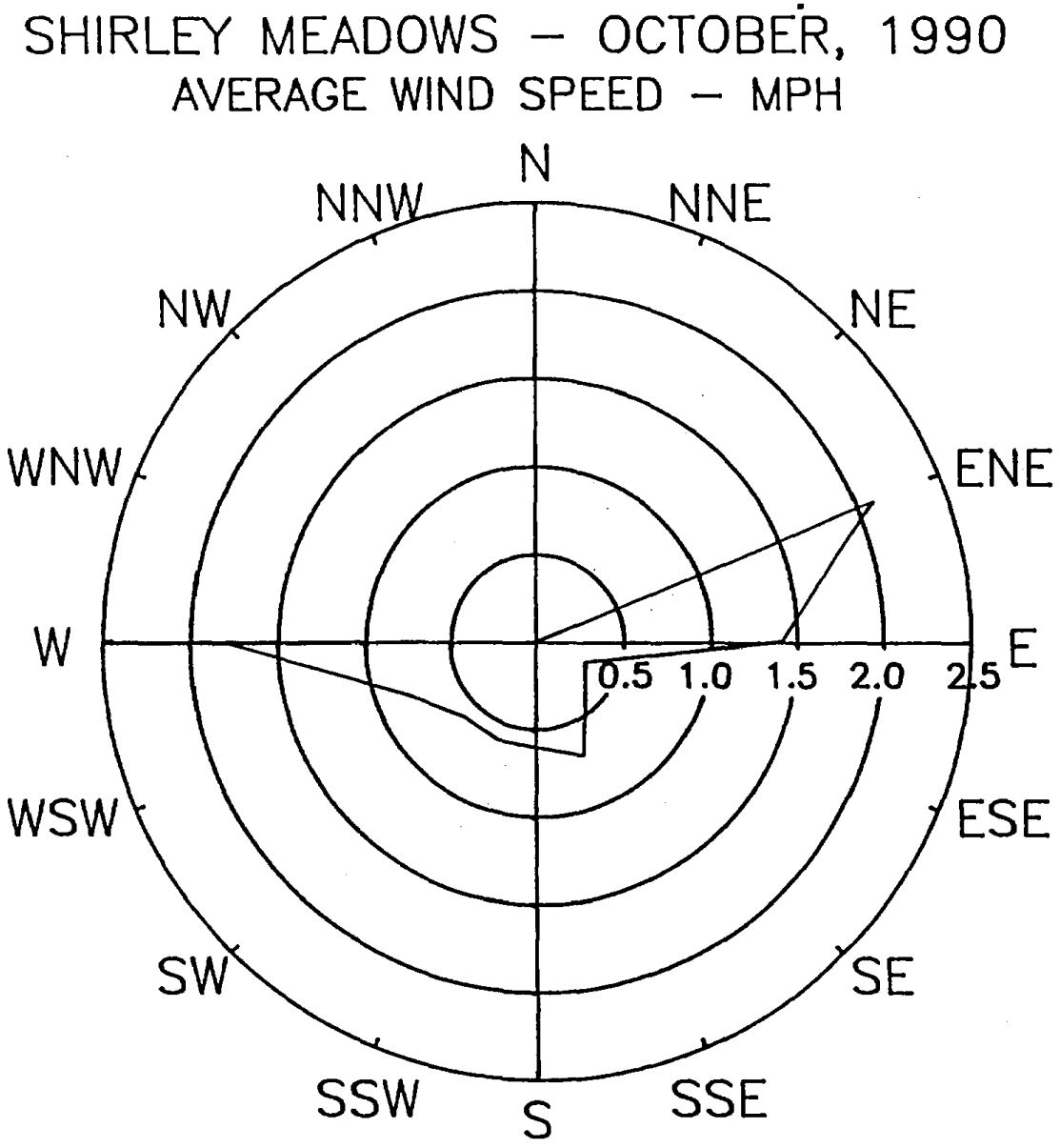

Figure B-18. Average wind speeds at Shirley Meadow in October 1990.