AN ABSTRACT OF THE THESIS OF

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Title: Ozone Flux in Helianthus annuus (L.)

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David T. Tingey

Sunflower (Helianthus annuus L., cv. NK894) seedlings, grown hydroponically, were exposed to ozone concentrations of 0.0, 0.1, 0.2, and 0.3 ppm at 60% RH, or 0.0 and 0.3 ppm at 30% and 90% RH, for 6 h/d, for four consecutive days, at ages 15 to 18 days from seeding. Plant response was detected through measurement of leaf growth rate, and plant dry weight, as well as visible foliar injury. Ozone flux was estimated by a model based on Fick's Law of diffusion, with the assumption that ozone and water vapor experience equal diffusion resistances, and follow the same diffusion pathways.

Visible foliar injury, reductions in leaf growth rate, and reductions in dry weight were highly correlated with predicted ozone flux. Low ozone flux induced a signi-

ficant change in plant biomass partitioning -- an increase in the proportion of total plant dry weight comprised by the leaves, and a reduction in that comprised by the roots -- accompanied by a significant reduction in root, but not total plant dry weight. Visible foliar injury and significant reductions in total plant dry weight were not induced until ozone flux was slightly higher. Significant reductions in leaf growth rate (except at 30% RH), and stem and leaf (except at 30% RH) dry weight occurred only at the highest ozone fluxes, although growth rate was strongly correlated with low, as well as high ozone fluxes.

Ozone flux and plant response to ozone were greater at high, than at low relative humidity. Similar rates of growth and biomass reduction between high (90%) and low (30%) relative humidities suggested that the amount of reduction per unit ozone flux was unaffected by the level of relative humidity. This would support previous evidence that increased plant response to ozone at increasing relative humidity is due to decreased stomatal, rather than internal leaf resistance.

Ozone Flux in *Helianthus annuus* (L.)

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I dedicate this work to John Ruben, without whom it would have been much more difficult to do.

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OZONE FLUX IN *HELIANTHUS ANNUUS* (L.)

INTRODUCTION

I. Ozone -- a component of photochemical smog.

Air pollution is comprised of several classes of pollutants (National Research Council, 1977). Primary air pollutants are those that are emitted directly from sources, such as motor vehicles, industrial plants, and electric power plants, and include sulfur dioxide, nitrogen oxides (NOx) and gaseous hydrocarbons. Certain primary pollutants react together in the presence of sunlight to form secondary pollutants. Ozone, peroxyacetylnitrate (PAN), and hydrogen peroxide are some of the secondary pollutants classified as photochemical oxidants.

Primary pollutants reach peak concentrations when source emissions are greatest. Photochemical oxidants reach peak concentrations subsequent to primary pollutants, because primary pollutants are their precursors. The rates at which photochemical oxidants are formed are dependent upon concentrations of primary pollutants, and meteorological conditions, such as solar radiation, temperature, humidity, and dispersion.

Ozone is the major photochemical oxidant, and it may reach peak concentrations in excess of 0.30 ppm (vol/vol) in urban areas (U.S. Environmental Protection Agency, 1978). Although concentrations are generally lower in rural areas, they may still exceed the 1-h National Ambient Air

Quality Standard of 0.12 ppm.

Ozone exists naturally, and is formed in the stratosphere through the absorption of solar shortwave radiation by molecular oxygen. A proposed mechanism for this reaction is:

$$O_2 + h\nu \text{ -------} \rightarrow O + O \quad (1)$$

$$O + O_2 + M \text{ ---} \rightarrow O_3 + M \quad (2)$$

Decomposition is spontaneous, and probably occurs as:

$$O_3 + h\nu \text{ ------} \rightarrow O + O_2 \quad (3)$$

$$O + O_3 \text{ -------} \rightarrow 2\ O_2 \quad (4)$$

where M is a third body that absorbs the energy of the reaction (Chapman, 1930). A maximum ozone concentration of 0.20-0.50 ppm occurs at an elevation of approximately 22 km (Whitten and Prasad, 1985). This stratospheric ozone layer shields the earth from ultraviolet radiation, which is harmful to life, and plays an important role in the temperature regulation of the troposphere. The concentration of ozone decreases with decreasing elevation, and natural concentrations at ground level average 0.02 to 0.05 ppm, a result of air interchange between the stratosphere and the troposphere.

In the troposphere, ozone is formed through the reaction of nitrogen oxides and sunlight:

$$NO_2 + h\nu \text{ ------} \rightarrow O + NO \quad (5)$$

$$O + O_2 + M \text{ ----} \rightarrow O_3 + M. \quad (6)$$

Decomposition occurs by the reaction:

$$NO + O_3 \text{ -------} \rightarrow NO_2 + O_2. \quad (7)$$

Reactions (5), (6), and (7) are rapid and cyclic, and result in a small steady-state concentration of ozone, as well as the degradation of absorbed sunlight into thermal energy, and do not account for the diurnal increases in ozone concentration that occur near urban centers (National Research Council, 1977).

The key factor involved in increasing ozone concentrations is the gaseous hydrocarbon component of air pollution. Gaseous hydrocarbons and nitrogen oxides reach peak concentrations simultaneously, because they are generally emitted from the same sources. Active gaseous species, such as O, O_3, and various radicals, initiate oxidation of the gaseous hydrocarbons, forming radicals which oxidize nitric oxide. Nitrogen dioxide is formed, without destroying ozone, and ozone accumulates, reaching high concentrations (Fig. 1).

II. Plant response to ozone.

Tingey and Taylor (1982) summarized ozone injury to plants as a four phase process. The first phase is ozone entry into the leaf, which is regulated by leaf conductance. This is followed by perturbation, which consists of primary ozone-induced changes in cell structure and/or function. Homeostasis is the recovery process, during which plants repair or compensate for cell disturbance. Injury, the final response, is the net result of the preceding phases.

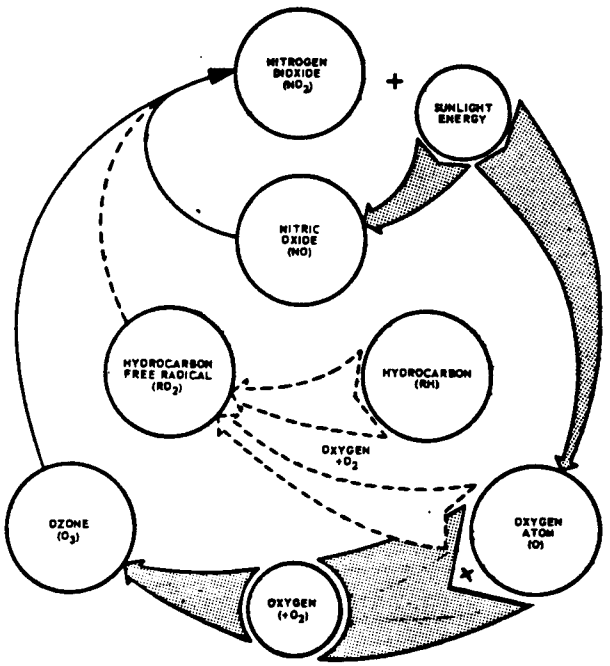

FIG. 1. The interaction of gaseous hydrocarbons with nitrogen oxides and sunlight resulting in increasing ozone concentrations (National Air Pollution Control Administration, 1970).

Ozone enters leaves by passive diffusion through the stomates. Once inside the substomatal cavity, ozone, which is soluble in water to 0.26 cm^3/cm^3 H_2O at 20 degrees C (Hill, 1971), dissolves on the wet surfaces of substomatal cells (mesophyll, guard, and subsidiary cells) (Tingey and Taylor, 1982). In the liquid phase, ozone or its reaction products, which may include other strong oxidants and free

radicals, may react with cell membranes, or cross cell membranes and react with organic molecules inside the cell (Mudd, 1982).

In aqueous solution, ozone spontaneously decomposes to form molecular oxygen, various radicals, hydroxyl ions and hydrogen peroxide (Weiss, 1935). Reactions of ozone or its derivatives with organic compounds include: oxidation of amino acids containing aromatic rings or sulfhydryl groups (Mudd et al, 1969); oxidization of sulfhydryl groups (Mudd, 1973); oxidization of the nicotinamide ring of NAD(P)H (Mudd et al, 1974).

Cellular changes resulting from ozone exposure include: reductions in cellular ATP levels (Tomlinson and Rich, 1968); changes in the levels of some enzymes (Tingey et al, 1976); alterations in the organization of membrane proteins (Swanson et al, 1982); changes in membrane permeability (Sutton and Ting, 1977), resulting in an efflux of potassium ions from cells (Chimlikis and Heath, 1975).

Whole-plant physiological changes include: reduced plant water potential (Evans and Ting, 1974); stomatal closure (Hill and Littlefield, 1969); reduced transpiration (Koritz and Went, 1953); reduced photosynthesis (Todd, 1958); increased respiration (Todd, 1958); reductions in growth and biomass accumulation (Koritz and Went, 1953).

Foliar lesions, including chlorosis, bleaching, bifacial necrosis and pigmented lesions (Hill et al, 1970) are the first visible symptom of ozone injury. Chlorosis, a

yellowing of leaves associated with chlorophyll loss in living cells, may culminate in premature senescence and abscission of leaves. Bleaching appears as small unpigmented necrotic spots on either leaf surface, and may be accompanied by collapse of epidermal and palisade cells. Bifacial necrosis involves death of all tissue layers, and may result in leaf surfaces being drawn together to form a thin, papery lesion. Pigmented lesions appear as localized thickening and pigmentation of adaxial palisade cell walls.

III. Factors affecting plant response to ozone.

Overall, plant response to ozone becomes more severe as the ozone concentration and the duration of the exposure increase (Todd and Probst, 1963). The frequency of exposure also affects plant injury, however, the complex relationship between frequency, concentration, and duration is not yet well understood (Hogsett et al, 1985). Ozone itself may induce stomatal closure (Hill and Littlefield, 1969), and thereby reduce the amount of ozone taken up by leaves.

Environmental factors modify plant response to ozone, apparently through their influence on stomatal aperture, which affects the amount of ozone that enters, and subsequently injures, leaves. Light intensity, which is positively correlated with stomatal opening (Wilson, 1948), is positively correlated with plant response both prior to (Dunning and Heck, 1977), and during (Heck et al, 1965) exposure to ozone. Decreased stomatal resistance is posi-

tively correlated with light-induced plant sensitivity to ozone (Dugger et al, 1963).

Air temperature is also positively correlated with stomatal aperture (Wilson, 1948). The air temperature at which plants are grown prior to (Adedipe and Ormrod, 1974), during (Macdowall, 1965), and subsequent to (Adedipe and Ormrod, 1974) exposure to ozone, affect their sensitivity. However, both direct (Heck et al, 1965; Dunning and Heck, 1977) and inverse (Macdowall, 1965; Cantwell, 1968; Adedipe and Ormrod, 1974) relationships between temperature and ozone-sensitivity have been reported.

Relative humidity is positively correlated with stomatal aperture (Wilson, 1948). Relative humidity is also positively correlated with plant sensitivity to ozone, during exposure (Leone and Brennan, 1969; Otto and Daines, 1969; Rich and Turner, 1972; Dunning and Heck, 1977; McLaughlin and Taylor, 1980), and, to a lesser extent, during the growth period prior to ozone exposure (Dunning and Heck, 1977). Stomatal aperture, indicated by leaf permeability, is positively correlated with injury as relative humidity varies (Otto and Daines, 1969). Stomatal resistance increases during exposure to ozone at low, but not high relative humidity (Rich and Turner, 1972; McLaughlin and Taylor, 1980).

Water stress induces stomatal closure in plants (Larcher, 1980). Water stress reduces plant sensitivity to ozone (Macdowall, 1965; Rich and Turner, 1972; Adedipe et

al, 1973; Tingey et al, 1982; Tingey and Hogsett, 1985). This decrease in sensitivity occurs due to stomatal closure which is induced by water stress (Adedipe et al, 1973; Tingey and Hogsett, 1985). Stomates in water-stressed plants close faster and to a greater extent than in well-watered plants exposed to ozone (Rich and Turner, 1972). Reduced ozone sensitivity during water stress is associated with reductions in leaf water potential (Ting and Dugger, 1971; Adedipe et al, 1973; Tingey et al, 1982). This is complicated by the fact that leaf water content is also lowered by exposure to ozone (Evans and Ting, 1974), but only in ozone-sensitive cultivars (Elkiey and Ormrod, 1979).

Plant sensitivity to ozone is reduced by low light intensity, low relative humidity, water stress, and reduced plant water potential, apparently through reduced stomatal aperture. However, differences in stomatal resistance do not fully explain differences in ozone sensitivity among some plant cultivars (Yingjajaval, 1976; Elkiey et al, 1979; Tingey and Taylor, 1982). Differences in leaf morphology have been examined in an attempt to explain some cultivar differences, but anatomical characteristics such as stomatal density, trichome density, and guard cell size are not always correlated with ozone-sensitivity (Elkiey et al, 1979; Taylor et al, 1982).

Plant sensitivity to ozone is influenced by foliage age. Ozone sensitivity is greatest when rates of leaf

expansion are greatest, just prior to maximum leaf area, in four tobacco varieties (Menser et al, 1963), and in two soybean cultivars (Tingey, et al, 1973), and at intermediate leaf ages in bean (Evans and Ting, 1974) and tobacco (Craker and Starbuck, 1973) plants. The period of maximum sensitivity in soybean leaves coincides with increased intercellular space during this stage of development, resulting in decreased mesophyll resistance, which would facilitate gas flow into tissues (Evans and Ting, 1974).

IV. Ozone flux and the present study.

Because the amount of ozone taken up by plants is affected by environmental and developmental factors, the concentration of ozone plants are exposed to is not, alone, the best indicator of subsequent plant response. Therefore, methods have been established for measuring and estimating ozone flux, the rate at which ozone enters leaves, which can then be correlated to the plant response.

In controlled experiments, ozone flux into plants is commonly measured by monitoring the ozone concentration entering the exposure chamber, and subtracting from it the concentration leaving the chamber plus that adsorbed by chamber surfaces. Estimation of ozone flux into plants involves measurement of the ambient concentration of ozone, as well as several other environmental and plant parameters, and is used in both controlled environment and field experiments. Both techniques involve the analogy of plant ozone flux to an electrical circuit, a concept first

employed by Gaastra (1959) to calculate plant photosynthetic rates. This analogue incorporates leaf resistance, which indicates the degree of stomatal aperture, and therefore accounts for effects of environmental variables. Measured or estimated ozone flux is then usually correlated to visible foliar injury. Correlations of plant response to measured or estimated ozone flux can facilitate the prediction of injury to crops when the concentrations of ozone they are exposed to are known.

In this study, ozone flux is estimated by a model, and correlated with the response of sunflower plants to ozone. Because visible foliar injury is not always a good indicator of other plant responses to ozone, such as reduced photosynthesis (Todd, 1958), the present study assesses leaf growth rate and biomass accumulation, as well as visible foliar injury, for ozone-induced changes.

MATERIALS AND METHODS

Plant Culture

In preliminary experiments, designed to test the relative sensitivities of six sunflower cultivars adapted to the Willamette Valley, and to determine exposure concentrations and durations causing injury, seeds of six cultivars (NK894, NK241, S372A, D070Y, Sunbred 265, and Sunbred 254, obtained from Dr. G. D. Jolliff, Crop Science Department, Oregon State University) were planted in 10-cm plastic pots containing Pro-Mix BX (equal volumes of sphagnum peat moss, vermiculite, and perlite). Seeds were sown three per pot, four pots per cultivar (per treatment), and seedlings were thinned to one per pot eight days after sowing. Seedlings were watered daily with a complete nutrient solution (Appendix A).

In subsequent experiments, designed to correlate ozone flux with its effects, seeds of cultivar NK894 were sown in trays of vermiculite. Eight days after sowing, 16 seedlings of uniform size were selected, adhering vermiculite was rinsed from roots, and each seedling placed in an individual hydroponic unit with its stem supported by modeling clay through a hole in the lid of the unit.

All seedlings were grown in a Conviron Model E15 growth chamber under a 16:8 h light:dark photoperiod (light period 0400-2000 h) at 30/22 degrees C light/dark air temperatures. Relative humidity (RH) was maintained

constant at 60%, except in preliminary experiments, when it was not controlled. Light was provided by a mixture of 16 165-watt cool-white fluorescent, four 35-watt deluxe cool-white fluorescent, and 12 67-watt clear incandescent lamps. Photosynthetically active radiation (PAR) averaged 350 $\mu mol s^{-1} m^{-2}$ (400-700 nm) at canopy height. PAR was measured with a Licor Model 4-1776 solar monitor at evenly spaced intervals throughout the chamber.

Each hydroponic unit, a modification of an apparatus designed for imposing water stress on plants (Snow and Tingey, 1985), consisted of a 2-liter opaque plastic plant reservoir connected by Norprene tubing to a 1-liter PVC nutrient solution reservoir (Fig. 2). Nutrient solution in each plant reservoir was maintained at a level 5 cm below the lid by a float-valve located in a float chamber at the outlet of each nutrient solution reservoir, and was aerated through a 1-mm line by a diaphragm pump. Nutrient reservoirs were refilled when necessary, the level being indicated by a graduated plastic tube buoyed on a styrofoam cylinder. Each nutrient reservoir and float valve chamber was covered with an opaque PVC lid to prevent evaporation and algal growth.

Low oxygen availability to roots can lower growth rates (Letey *et al*, 1962), therefore, the dissolved oxygen content of aerated nutrient solution was analyzed to insure sufficient aeration. Dissolved oxygen concentration in aerated nutrient solution, measured with an oxygen

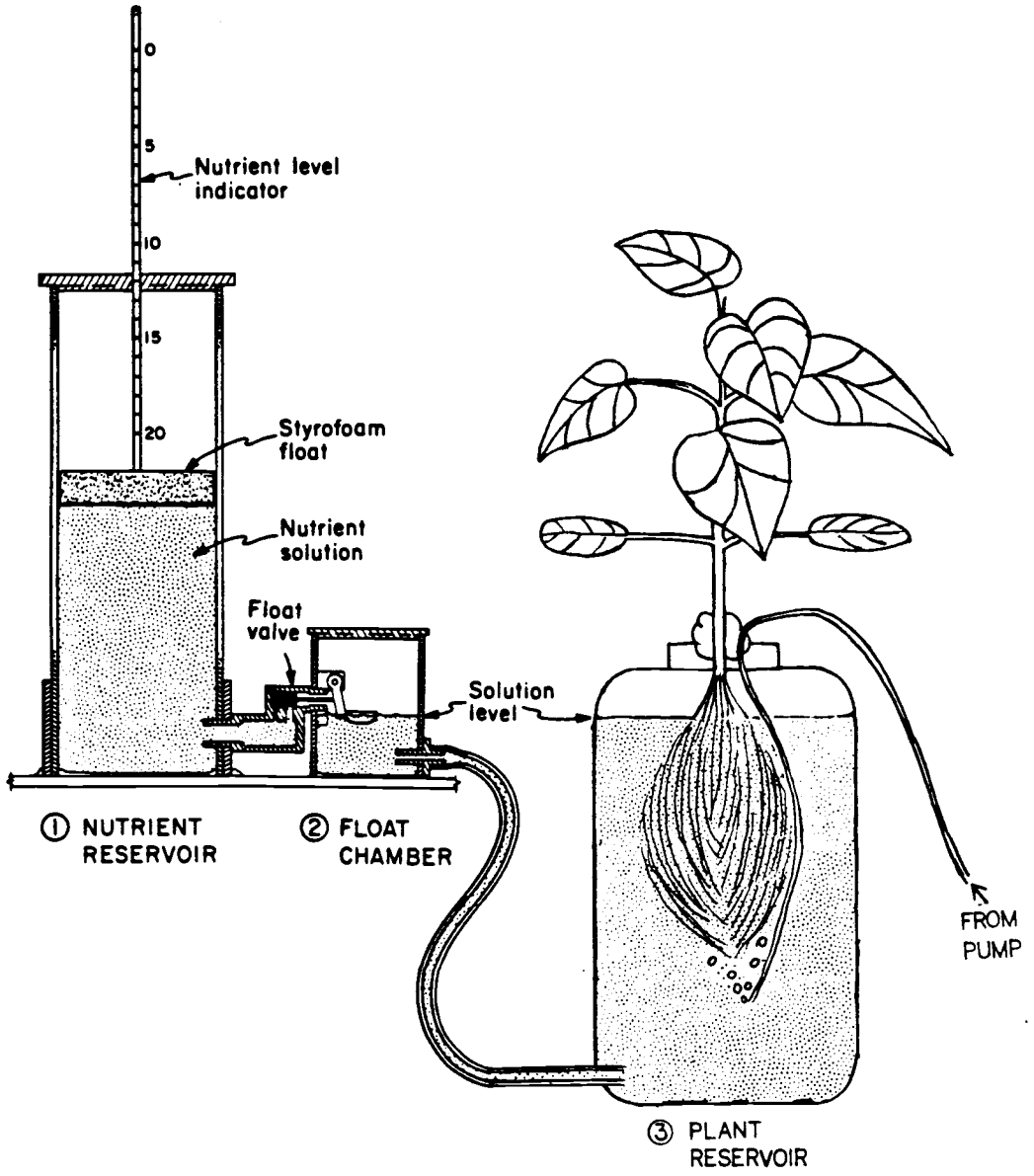

FIG. 2. Hydroponic unit (modified from Snow and Tingey, 1985).

electrode, averaged 7.86 mg O_2/l H_2O (n=3), 100% of saturation at the temperature of the nutrient solution.

Pollutant Exposure Chambers

The pollutant exposure chambers were modified from the design of Heck et al (1967): a perforated upper barrier had been installed in each chamber for increased uniformity of pollutant gas distribution in the chamber, and the volume of the chambers increased to 0.36 m^3 (Olszyk and Tingey, 1984) (Fig. 3). The four pollutant exposure chambers were housed in two Sherer Model CEL 37-14 growth chambers.

The photoperiod and day/night temperatures in the exposure chambers were identical to those in the growth chambers: 16:8 h light:dark and 30/22 degrees C, respectively. Environmental conditions differing from those in the growth chambers included: relative humidity was maintained constant at 30%, 60%, or 90% RH, except in preliminary experiments, when it averaged 65% to 85%; light was provided by a mixture of 18 165-watt cool-white fluorescent and 10 67-watt clear incandescent lamps; PAR averaged 250 $\mu mol\ s^{-1}m^{-2}$ at canopy height; air flow averaged 18.3 m^3h^{-1} (equivalent to an air turnover rate of once per 1.2 min).

Ozone was generated in each exposure chamber by an ultraviolet lamp located beneath the perforated chamber floor. Ozone concentration was controlled by varying UV irradiation time and was sampled continuously in each exposure chamber by a Monitor Labs Model 8410 chemilumines-

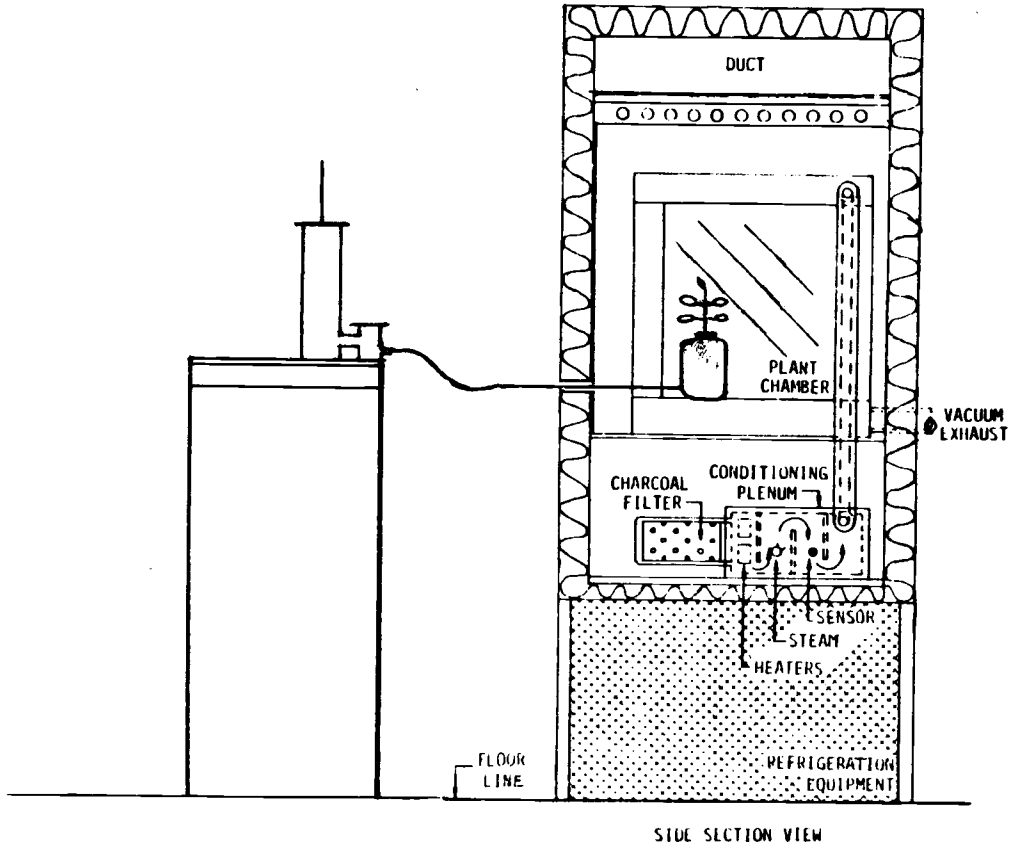

FIG. 3. Exposure chamber (modified from Heck et al, 1967). The exposure chamber is housed within a growth chamber, and the placement of one hydroponic unit is shown.

cent ozone analyzer. A sampling line of clean Teflon tubing, wrapped with insulating tape to prevent condensation, was positioned at canopy height in the center of each exposure chamber. Ozone concentration, averaged over 60-s intervals, was recorded continuously on a Leeds and Northrop multipoint recorder.

The Monitor Labs ozone analyzers, and a Monitor Labs Model 8500 ozone calibrator (used for zero and span checks), were calibrated prior to any experiments with a Dasibi Model 1008PC ozone analyzer (see Appendix B for calibration procedure). The internal operation of the electrical and optical systems and ethylene flow were tested and zero and span checks made weekly. The precision of ozone concentration measurement was $\pm$ 10%.

Relative humidity was increased above ambient by adding steam from a boiler to the air entering the exposure chambers. Relative humidity in each exposure chamber was maintained by adjusting the rate of steam-flow, and measured by a Vaisala 6061 HM thin film capacitance humidity sensing element. Sensing elements were calibrated with a Vaisala HMK 11 humidity meter calibrator, using LiCl and NaCl for continuous measurement of 30% and 60% RH, and LiCl and K_2SO_4 for continuous measurement of 90% RH (as per Vaisala Humicap humidity meter Instruction Manual). Each sensor was connected to a Vaisala Model HMI 14 relative humidity indicator for constant display, and relative humidity was recorded continuously on a Leeds and Northrop

multipoint recorder. The precision of relative humidity measurement was ± 5%.

Treatments

Four treatments were performed simultaneously, one treatment per exposure chamber, and each treatment was repeated four times, once in each of the four exposure chambers.

To find injury-causing ozone exposures, treatments in preliminary experiments consisted of a range of exposure durations - 1, 2, or 4 h - at a constant ozone concentration - 0.4 ppm, and a range of ozone concentrations - 0.2, 0.3, 0.4, and 0.6 ppm - at a constant duration - 8 h.

In subsequent experiments, to correlate ozone flux with its effects, the exposure duration was constant -- 6 h per day, and exposures were performed on four consecutive days. The effects of varying ozone concentration were tested with treatments consisting of four ozone concentrations -- 0.0, 0.1, 0.2, and 0.3 ppm -- at one relative humidity -- 60% RH. The effects of ozone at differing relative humidities were tested with treatments consisting of a control and a high ozone concentration -- 0.0 and 0.3 ppm -- and two relative humidities -- 30% and 90% RH.

Exposure Schedule

Fourteen days after sowing, seedlings were transferred from the Conviron growth chamber to the four pollutant

exposure chambers. Plants were allowed a 24-h acclimation period prior to ozone exposure. In the preliminary experiments, 24 seedlings (four per cultivar) were placed in the four exposure chambers, six seedlings per chamber. In subsequent experiments, 16 seedlings (cv NK894) were placed in the exposure chambers, four per chamber. Leaves were labeled with small numbered tags.

Prescribed treatments were begun on the fifteenth day after sowing (0900 h). Following the four days ofozone exposures, plants were allowed to grow for three days to permit full development of injury symptoms, then harvested.

Measurements

At harvest, the percentage of the adaxial area showing visible injury (necrosis and chlorosis) on each leaf was estimated in 5% increments (Gumpertz et al, 1982). In the ozone flux studies, plant water use was measured during and between the 6-h ozone exposures, by noting fluid-level changes in plant and nutrient solution reservoirs before (0900 h) and after (1500 h) each exposure; length and width of leaves 3.0 cm or greater in length were measured (1500-1600 h) on the day prior to the exposure, on each exposure day, and at harvest; projected leaf area (i.e., the area of one side of the leaf) was measured by a Licor Model 3100 leaf area meter at harvest; roots, stems (including petioles), and leaves were bagged separately and placed in a drying oven at 75 degrees C for one week, then weighed.

Calculated Parameters in Ozone Flux Studies

(1) Leaf Area:

Daily leaf area was estimated from the daily leaf length and leaf width measurements, on days 14 through 18, as follows. The natural logarithm of leaf area (at harvest) versus the natural logarithm of the product of leaf length and width (at harvest) were plotted. The slope and intercept of the line were obtained through linear regression:

$$LN(LEAF\ AREA) = B_0 + B_1\ [LN\ (LFLN \times LFWD)], \qquad (8)$$

(B_0=intercept and B_1=slope).

The data from the variable ozone and variable humidity studies were analyzed and applied separately. The regression equations obtained were, respectively:

ln [estimated leaf area] =

(-0.317466) + 0.994991 (ln [leaf length x leaf width])

(r^2= 0.9971).

ln [estimated leaf area] =

(-0.304700) + 0.991759 (ln [leaf length x leaf width])

(r^2= 0.9954).

Excellent fits of the predicted lines to the data were obtained, as expressed by the high r^2 values. The average residual, the difference between the leaf area predicted by the above equations and the leaf area measured at harvest, was 4% of the measured leaf area.

To test whether one equation could be applied to all leaves, a regression was performed using leaves measured

at harvest from both studies, with the following results:

ln[estimated leaf area] =

(-0.312141) + 0.993652 (ln [leaf length x leaf width])

(r^2= 0.9957).

Residuals averaged 4%, therefore, a single equation could have been used for all leaves. This indicates consistency in leaf shape for the duration of the studies.

(2) Relative Leaf Growth Rate:

Average relative leaf growth rate (RGR) was estimated from the average slope of the natural logarithm of total leaf area over time curve for each treatment, for the 4-day exposure period and the 7-day period when the leaves were measured (see Data Analysis section for details).

(3) Transpiration Rate:

Transpiration rate was calculated from daily water use divided by estimated daily projected leaf area, which was multiplied by a constant to convert to effective (transpiring) leaf area, because sunflowers are amphistomatous. Leaf resistance (r) was used to convert projected leaf area to effective leaf area, because it is inversely proportional to effective leaf area (Nobel, 1983):

$$\text{total effective leaf area} \propto \frac{1}{r(\text{total})}, \text{ and} \qquad (9)$$

$$\text{effective abaxial leaf area} \propto \frac{1}{r(\text{abaxial})}. \qquad (10)$$

Therefore,

$$\frac{\text{total effective leaf area}}{\text{effective abaxial leaf area}} \propto \frac{r(\text{abaxial})}{r(\text{total})} . \qquad (11)$$

Total effective leaf area is proportional to:

$$\frac{r(\text{abaxial})}{r(\text{total})} \times \text{effective abaxial leaf area.} \qquad (12)$$

The diffusion of water vapor through the adaxial leaf surface is in parallel with that through the abaxial leaf surface. Consequently, total leaf resistance is equal to:

$$r(\text{total}) = \frac{r(\text{adaxial}) \times r(\text{abaxial})}{r(\text{adaxial}) + r(\text{abaxial})} \qquad (13)$$

(Gates, 1980). Leaf resistances, obtained from a similar study on cultivar NK894 seedlings at the Corvallis EPA Environmental Research Laboratory (Tom Moser, Northrop Services, unpubl. data, 1985), averaged 1.00 s/cm on the abaxial surface, and 1.27 s/cm on the adaxial surface. Substituting these values into Equation (12) gives:

$$r(\text{total}) = \frac{(1.27 \text{ s/cm}) \times (1.00 \text{ s/cm})}{(1.27 \text{ s/cm}) + (1.00 \text{ s/cm})} = 0.56 \text{ s/cm},$$

and $$\frac{r(\text{abaxial})}{r(\text{total})} = \frac{1.00 \text{ s/cm}}{0.56 \text{ s/cm}} = 1.79.$$

Effective leaf area was therefore calculated as projected leaf area x 1.79, and transpiration (TR) rate as:

$$TR = \frac{\text{daily water use}}{\text{effective leaf area}} . \qquad (14)$$

(4) Leaf Temperature:

Leaf temperature, necessary for calculating the water vapor concentration in the leaf, was calculated by an equation derived to estimate leaf temperatures in growth chambers during ozone exposure (Omasa et al, 1980):

$$\text{leaf temp} = -6.4 \times 10^5 \times TR + \text{air temp} + 2.2. \quad (15)$$

(5) Ozone flux:

Ozone flux was estimated by a model based on Fick's First Law of Diffusion, which states that the rate of diffusion (flux) of species i (J_i, units = mass/area/time), along a pathway of length x, is related to the concentration gradient of species i over the distance x (dc_i/dx), and the diffusion coefficient of species i (D_i):

$$J_i = -D_i dc_i/dx \quad (16)$$

(the negative value indicates that flux is in the direction of decreasing concentration). Fick's Law has previously been used to describe photosynthetic CO_2 flux (Gaastra, 1959), transpiration (Meidner and Mansfield, 1968), and ozone flux (Bennett et al, 1973), and is used in this study to describe transpiration and ozone flux under the following assumptions:

(a) The length and area of, and the resistances experienced in, diffusion pathways between the atmosphere and leaf air spaces, are the same for ozone and water vapor;

(b) The concentration gradients of ozone and water vapor are linear between the atmosphere and leaf air

spaces;

(c) Diffusion processes in leaves are passive for both gases;

(d) The relative humidity in leaf air spaces is 100%;

(e) The concentration of ozone at the surfaces of substomatal cells is zero.

Under these assumptions, ozone and water vapor fluxes are related by a constant, k:

$$k = J(O_3)/J(H_2O). \quad (17)$$

Substituting flux from Equation (16) (Fick's Law) into Equation (17) gives:

$$k = \frac{D(O_3)\ dc(O_3)/dx(O_3)}{D(H_2O)\ dc(H_2O)/dx(H_2O)} . \quad (18)$$

By assumption, $dx(O_3) = dx(H_2O)$, therefore:

$$k = \frac{D(O_3)\ dc(O_3)}{D(H_2O)\ dc(H_2O)} . \quad (19)$$

The ratio of the diffusion coefficients of ozone and water vapor were calculated according to Graham's Law, which states that the diffusion coefficients of two molecular species diffusing through the same medium vary inversely with the square roots of their molecular weights (Devlin and Witham, 1983):

$$\frac{D(O_3)}{D(H_2O)} = \frac{MW(H_2O)^{1/2}}{MW(O_3)^{1/2}} = \frac{(18.015\ g/mol)^{1/2}}{(47.998\ g/mol)^{1/2}} = 0.6126. \quad (20)$$

Substituting the value of the ratio of the diffusion coefficients from Equation (20) into Equation (19) gives:

$$k = (0.6126) \frac{dc(O_3)}{dc(H_2O)} . \quad (21)$$

Substituting the equivalence of k from Equation (21) into Equation (17), and rearranging, gives:

$$J(O_3) = (0.6126) \frac{dc(O_3)}{dc(H_2O)} J(H_2O), \quad (22)$$

where $J(H_2O)$ = transpiration rate.

Ozone flux was predicted by Equation (22), using measured transpiration rate, air temperature, chamber water vapor and ozone concentrations, calculated leaf temperature, and assumed leaf water vapor and ozone concentrations.

(6) Adjusted Dry Weight:

To compensate for initial differences in plant size, dry weight was adjusted, using plant leaf area at plant age 14 days as a covariate (Ormrod et al, 1983), as follows. A positive correlation between dry weight and plant leaf area at age 14 days was confirmed. Simple linear regression was performed separately for root, stem, leaf and plant dry weight on plant leaf area at age 14 days. The slopes (B_1) of the curves were used to calculate adjusted (ADJ) root (RT), stem (ST), leaf (LF), and plant (PL) dry weight from the formulae:

ADJ RTWT = RTWT - (RTWT B_1 x area at age 14),

ADJ STWT = STWT - (STWT B_1 x area at age 14),

ADJ LFWT = LFWT - (LFWT B_1 x area at age 14),

ADJ PLWT = PLWT - (PLWT B_1 x area at age 14).

(7) Dry Weight Ratios:

Root weight ratio (RWR), stem weight ratio (SWR), and leaf weight ratio (LWR) were calculated according to the following formulae (Evans, 1972):

RWR = root dry weight/plant dry weight,

SWR = stem dry weight/plant dry weight,

LWR = leaf dry weight/plant dry weight.

Data Analysis

In the cultivar sensitivity studies, the six cultivars were ranked on the basis of the mean percentage of total plant leaf area showing visible foliar injury.

In the ozone flux studies, treatments were performed in a Latin Squares design to reduce experimental error and improve separation of variation between treatments (Sokal and Rohlf, 1969). Each treatment was performed once in each chamber to compensate for possible differences between chambers. The four treatments were performed simultaneously to compensate for day-to-day variation in plant growth and response. Exposures were performed at the same time of day to compensate for diurnal variation in plant growth.

Data were computer-analyzed by SAS procedures (SAS

User's Guide: Statistics, 1982). An alpha level of 0.05 was used for all analyses. Treatments that were not performed simultaneously were not co-analyzed.

Leaf area estimation equations were calculated as described previously, using the SAS procedure, PROC REG, to perform simple linear regression.

Average relative growth rates were analyzed for differences between treatments by multivariate analysis of variance, using the SAS procedure, PROC MATRIX. This procedure uses matrix algebra to calculate the slopes of the changein ln(area) over time curves, and the characteristic roots of the hypothesis matrix divided by the error matrix. The design employed, curve fitting for repeated measures, is specific for growth curve analysis, and takes into account the high correlation between measurements made over time on the same plant (Morrison, 1976). The Pillai greatest characteristic root was used to calculate the test statistic, which was compared to the appropriate F-table critical value.

Water use, transpiration rate, and ozone flux were tested for differences between treatments by analysis of variance, using the SAS procedure, PROC ANOVA.

Adjusted root, stem, leaf, and plant dry weight were tested for differences between treatments by analysis of variance using a covariate, plant leaf area at age 14 days, using the SAS procedure, PROC GLM (General Linear Models).

RESULTS

Cultivar Sensitivity

Preliminary studies were designed to determine the ozone sensitivity of, and the ozone concentrations and exposure durations causing injury in, six Helianthus annuus cultivars adapted to the Willamette Valley. Cultivar NK894 was found to be the most ozone-sensitive of the six cultivars tested (Table 1). When the cultivars were ranked, based on the percentage of leaf area showing visible foliar injury, cultivar NK894 ranked highest in the majority of the seven treatments. Cultivar NK241 was found to be the most ozone-resistant, showing nearly half the visible foliar injury seen in cultivar NK894. Cultivars Sunbred 265, Sunbred 254, D070Y, and S372A were intermediate, and similar, in their sensitivity to ozone.

The ozone-sensitive cultivar, NK894, was used in subsequent studies. Injury was induced in this cultivar by an ozone concentration of 0.2 to 0.3 ppm, and an exposure duration of 4 to 8 h. In subsequent experiments, ozone concentrations of up to 0.3 ppm, an exposure duration of 6 h, and an exposure period of four consecutive days (to allow changes in plant biomass), were used.

Variable Ozone / Constant Humidity

To correlate ozone flux at different ozone concentrations with its effects on sunflower, seedlings of cultivar NK894 were exposed to a range of ozone concentrations (0.0,

Table 1. Percent Leaf Area Showing Visible Foliar Injury in Six Helianthus annuus Cultivars

Treatment+		Cultivar					
O3 (ppm)	Dur. (h)	NK894	NK241	SNB265	SNB254	DO70Y	S372A
0.2	8	2 (3)*	3 (1)	2 (3)	1 (5.5)	2 (3)	1 (5.5)
0.3	8	6 (1)	2 (5.5)	5 (2.5)	5 (2.5)	2 (5.5)	3 (4)
0.4	8	29 (4)	22 (6)	31 (1)	28 (5)	30 (2.5)	30 (2.5)
0.6	8	32 (1)	8 (6)	30 (2)	21 (5)	25 (4)	29 (3)
0.4	1	12 (2)	12 (2)	12 (2)	11 (4)	8 (6)	9 (5)
0.4	2	10 (1)	2 (6)	5 (4)	5 (4)	5 (4)	6 (2)
0.4	4	14 (1)	6 (6)	7 (5)	9 (3)	8 (4)	12 (2)
Overall % VFI		15	8	13	11	11	13
Overall Rank		(1)	(6)	(2.5)	(4.5)	(4.5)	(2.5)

+ Each treatment was performed on a different day (n = 16 plants per treatment).
* Cultivars were ranked (1-6) on the basis of highest to lowest percentage leaf area showing visible foliar injury (VFI).

0.1, 0.2, and 0.3 ppm) at a constant relative humidity (60%). The increase in plant leaf area, over the seven days when the leaves were measured, was nearly exponential in each treatment, as indicated by the nearly straight ln(area) vs. age curves (Fig. 4).

Average relative growth rates, determined by the increase in ln(plant leaf area) over time, were lower during the 7-day period when the leaves were measured than during the 4-day exposure period (Table 2a). This was due to a gradual decline in relative growth rate (RGR), which may have been a result of the plants being transferred to the exposure chambers, which had a lower light intensity than the growth chambers. Ozone at 0.1 and 0.2 ppm had no significant effect on either the 4-day or 7-day RGRs, based on multivariate component analyses. However, at 0.3 ppm, ozone significantly reduced the average 4-day RGR (13%). The 7-day RGR at 0.3 ppm was similar to the control, but was significantly lower than the 0.1 and 0.2 ppm treatments (8-9%).

Average daily water use increased at a rate of 20% per day during the 4-day exposure period, due to increasing plant size. Average water use during the exposure period, which was typically 62% of daily water use, was significantly reduced by ozone at 0.2 ppm (24%) and 0.3 ppm (20%) (Table 2a).

Average daily transpiration rate decreased at a rate of 10% per day during the 4-day exposure period, possibly

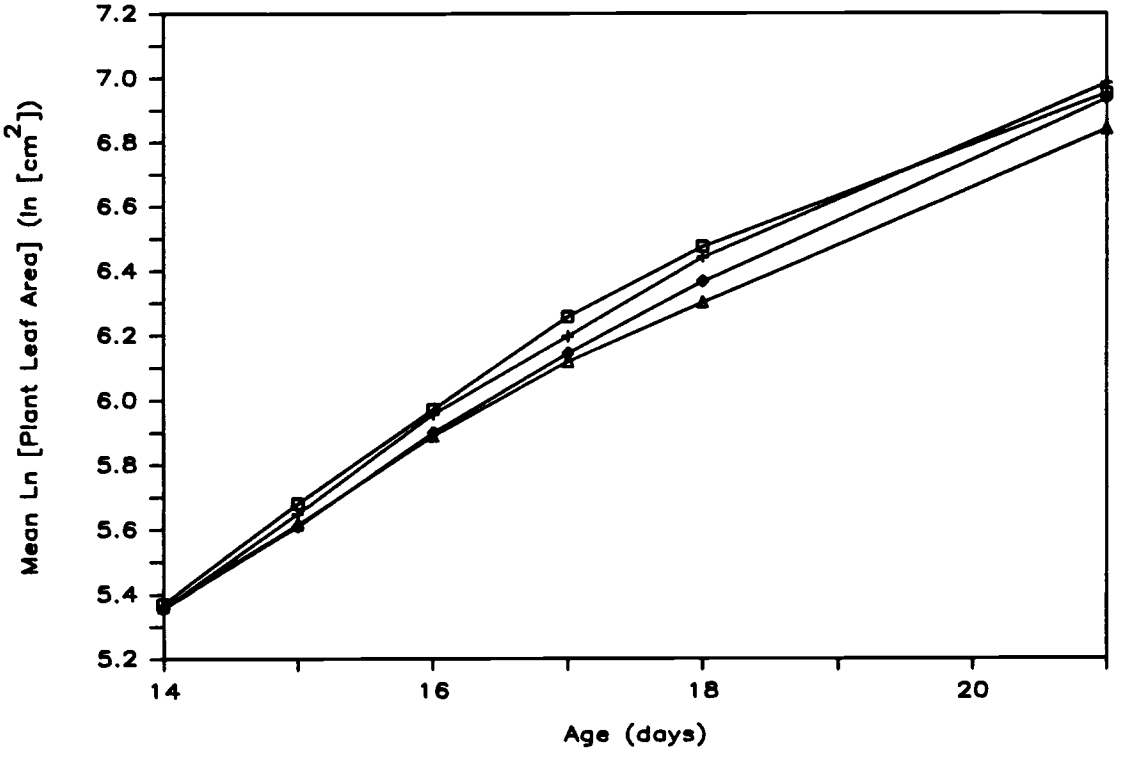

FIG. 4. Effects of increasing ozone concentration on the increase in sunflower plant leaf area over time. Treatments consisted of exposure to 0.0 (□), 0.1 (+), 0.2 (◇), or 0.3 (△) ppm ozone, at 60% RH, 6 h/d, days 15 through 18 (n = 16 plants per treatment).

Table 2. Plant Parameters Measured During Ozone Exposure+

	Treatment		RGR++		Water Use*	Trans-piration*	Ozone Flux*	Leaf Temp*
	O3 (ppm)	RH (%)	4-day ($cm^2/cm^2/d$)	7-day ($cm^2/cm^2/d$)	(mol/h)	($mol/m^2/h$)	($\mu mol/m^2/h$)	(°C)
(a)	0.0	60	0.288 a	0.227 ab	0.722 a	8.92 a	2 d	29.3
	0.1	60	0.281 a	0.239 a	0.648 ab	8.15 ab	33 c	29.6
	0.2	60	0.269 ab	0.237 a	0.552 b	7.02 b	56 b	30.0
	0.3	60	0.251 b	0.218 b	0.580 b	8.10 ab	98 a	29.6
(b)	0.0	30	0.229 b	0.226 a	0.616 a	17.48 a	3 c	26.6
	0.3	30	0.193 bc	0.199 ab	0.508 b	15.44 a	140 b	27.3
	0.0	90	0.273 a	0.242 a	0.250 c	5.70 b	7 c	30.4
	0.3	90	0.180 c	0.166 b	0.185 c	5.82 b	409 a	30.3

+ Means with the same letter are not significantly different at 0.05 alpha level (n = 16 plants per treatment).

++ RGRs were determined over the 4-day exposure period, and over the 7-day period when the leaves were measured. Means were tested by the F test using the Pillai test statistic, in multivariate analysis curve fitting for repeated measures (Morrison, 1976).

* Means of water use, transpiration, ozone flux, and leaf temp., over 4 6-h exposures performed on consecutive days, were tested by the Tukey and Scheffe multiple comparisons tests (SAS User's Guide: Statistics, 1982).

due to the transfer of plants to a lower lightintensity. Average transpiration rate during the exposure period was significantly reduced by ozone at 0.2 ppm (21%), but not at 0.1 or 0.3 ppm (Table 2a).

Ozone caused significant reductions in adjusted dry weight (Fig. 5). Adjusted root dry weight was significantly reduced by ozone at 0.1 ppm (24%), 0.2 ppm (42%), and 0.3 ppm (75%) (Table 3a). Ozone at 0.3 ppm significantly reduced adjusted stem (65%) and leaf (43%) dry weights. Adjusted plant dry weight was significantly reduced by ozone at 0.2 ppm (57%) and 0.3 ppm (30%).

Ozone caused significant changes in dry weight partitioning (Table 3a). All concentrations of ozone significantly reduced the root weight ratio (RWR), with the greatest reduction at 0.3 ppm. The stem weight ratio (SWR) was similar between all treatments. The leaf weight ratio (LWR) was significantly increased at all ozone concentrations, with the greatest increase at 0.3 ppm.

At harvest, the percentage of plant leaf area showing visible foliar injury (necrosis or chlorosis) increased as ozone concentration increased, but was absent at 0.1 ppm (Table 3a).

Average predicted ozone flux significantly increased with each increase in ozone concentration, at a rate proportional to the increase in ozone concentration (Table 2a). Although the only significant reduction in the average RGR during the 4-day exposure period occurred at 0.3 ppm,

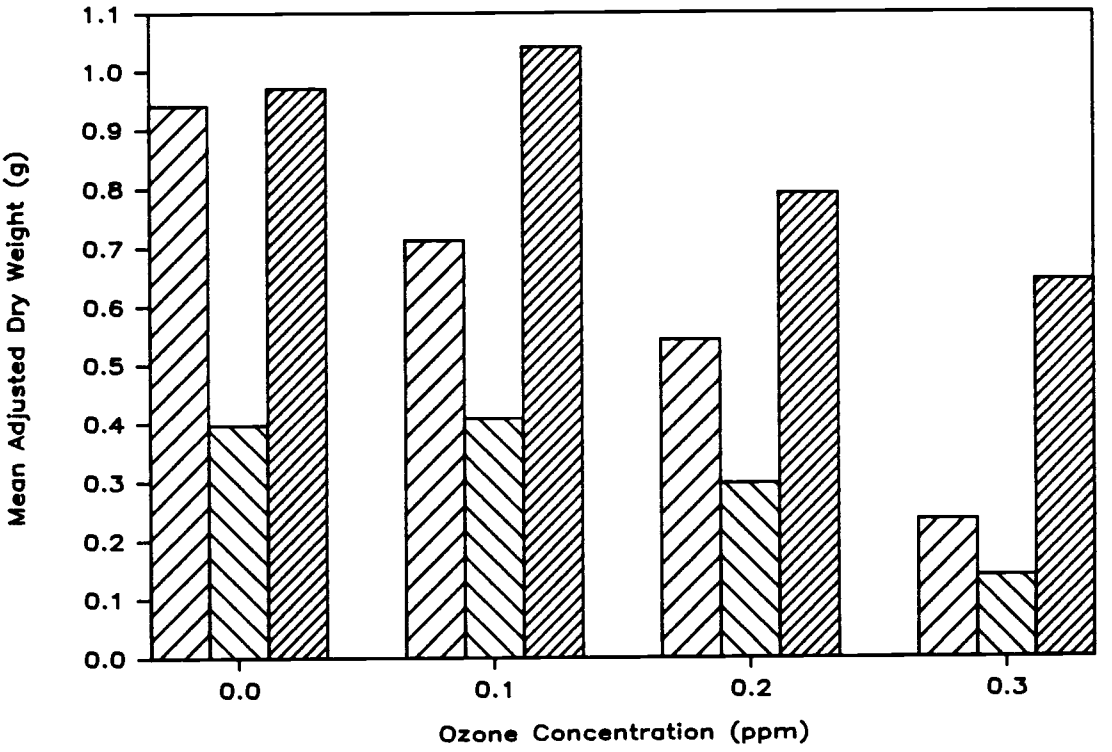

FIG. 5. Effects of increasing ozone concentration on adjusted root (▨), stem (▧), and leaf (▩) dry weight in sunflower. Treatments consisted of exposure to 0.0, 0.1, 0.2, or 0.3 ppm ozone, at 60% RH, 6 h/d, for 4 d (n = 16 plants per treatment). Plants were harvested three days after the final exposure, and dried for one week. Dry weights are adjusted by a covariate, plant leaf area.

Table 3. Plant Parameters Measured at Harvest+

	Treatment		Adjusted Dry Weight				Dry Weight Partit.			VFI
	O3 (ppm)	RH (%)	Root (mg)	Stem (mg)	Leaf (mg)	Plant (g)	RWR	SWR	LWR	(%)
(a)	0.0	60	942 a	398 a	972 a	2.3 a	29 a	24 a	46 c	0
	0.1	60	713 b	409 a	1042 a	2.2 ab	27 b	25 a	48 b	0
	0.2	60	543 b	298 ab	793 ab	1.6 bc	26 b	25 a	49 b	5
	0.3	60	236 c	140 b	645 b	1.0 c	24 c	25 a	51 a	15
(b)	0.0	30	449 a	290 b	683 b	1.4 b	27 a	25 a	48 bc	0
	0.3	30	165 b	120 c	430 b	0.7 c	24 b	25 a	51 b	30
	0.0	90	526 a	467 a	937 a	1.9 a	26 ab	26 a	48 c	0
	0.3	90	-16 c	7 d	280 c	0.3 d	21 c	24 a	54 a	40

+ Means with the same letter are not significantly different at 0.05 alpha level, when tested by the Tukey and Scheffe multiple comparisons tests (SAS User's Guide: Statistics, 1982) (n = 16 plants per treatment). Dry weights were adjusted with a covariate, plant leaf area. RWR = root weight ratio; SWR = stem weight ratio; LWR = leaf weight ratio; VFI = visible foliar injury.

RGR decreased as ozone flux increased, showing a strong negative correlation with ozone flux (r^2=0.99) (Fig. 6). A negative correlation between RGR and ozone flux was not as apparent during the 7-day period when the leaves were measured (r^2=0.46). Recovery from injury is suggested by relative increases in RGR during the 3-day post-exposure period, at all ozone concentrations (6-9%) (i.e., the decreases in RGR between the 4-day and 7-d periods was less in the ozone treatments than in the control) (Table 2a). Adjusted dry weights of roots, stems, and leaves showed strong negative correlations with total predicted ozone flux (r^2=1.00, 0.93, 0.88, respectively).

Variable Humidity / Constant Ozone

To correlate ozone flux at different relative humidities with its effects on sunflower, plants were exposed to no, or high (0.3 ppm) ozone, at low (30%) or high (90%) relative humidity. Plant leaf area increased exponentially during the 7-day period when the leaves were measured (Fig. 7), as indicated by the straight ln(area) vs. age curves, in each treatment except the high relative humidity, ozone treatment, where average plant leaf area decreased from day 17 to day 18, due to curling and abscission of severely injured leaves.

The average RGRs observed during the 7-day period when the leaves were measured were similar to, or lower than those observed during the 4-day exposure period (Table 2b),

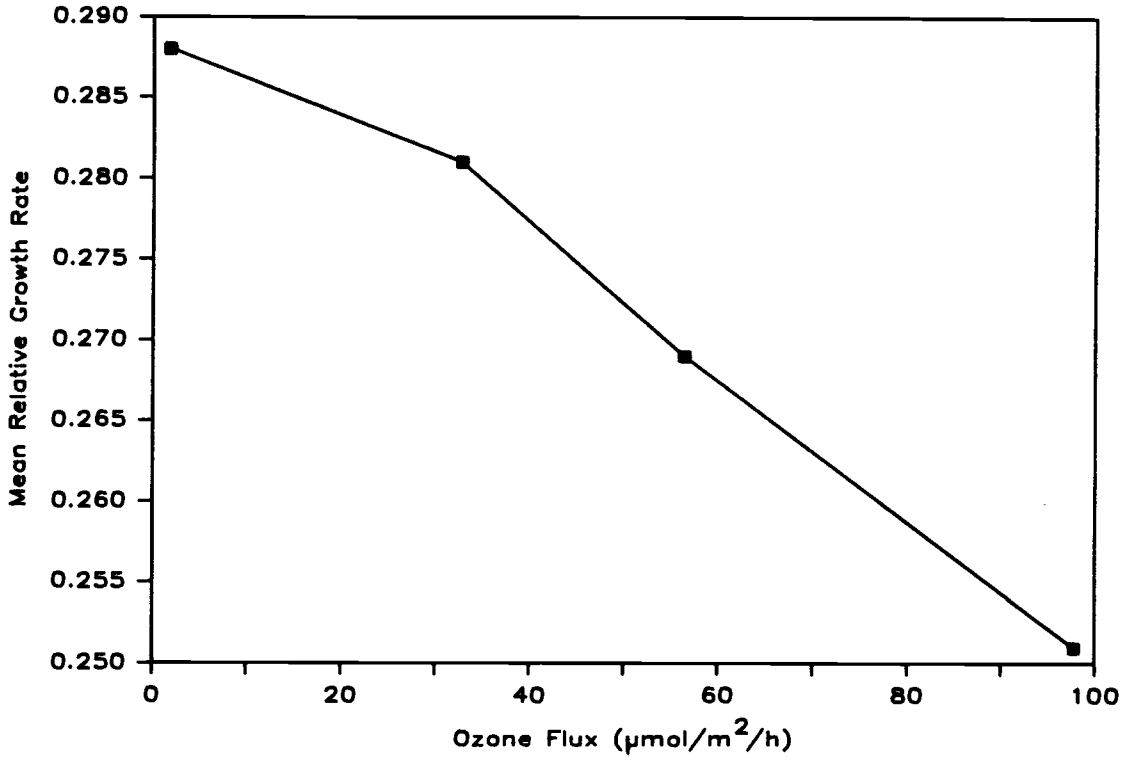

FIG. 6. Mean relative growth rate of sunflower leaves vs. predicted ozone flux. Treatments consisted of exposure to 0.0, 0.1, 0.2, or 0.3 ppm ozone, at 60% RH, 6 h/d, for 4 d (n = 16 plants per treatment). Growth rates ($cm^2/cm^2/d$) are over the 4-day exposure period.

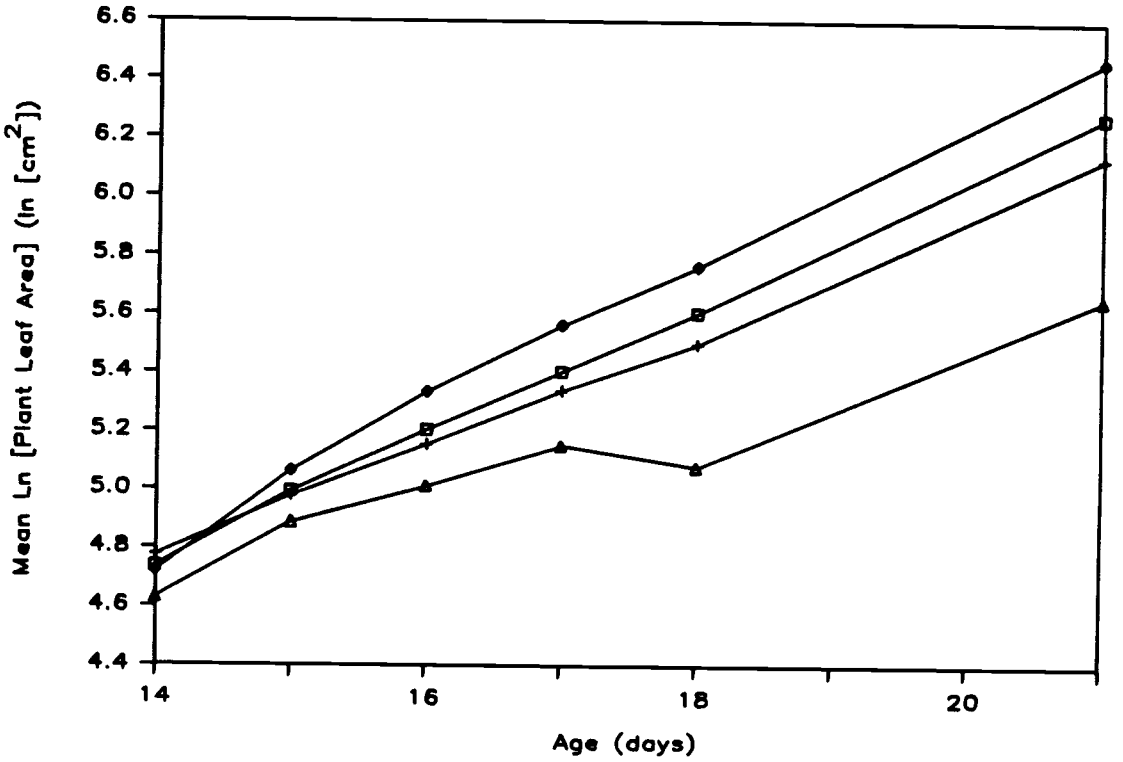

FIG. 7. Effects of ozone and relative humidity on the increase in sunflower plant leaf area over time. Treatments consisted of exposure to 0.0 ppm/ 30% RH (□), 0.3 ppm/ 30% RH (+), 0.0 ppm/ 90% RH (◇), or 0.3 ppm/ 90% RH (△) ozone/ relative humidity, 6 h/d, days 15 through 18 (n = 16 plants per treatment).

possibly due to the transfer of plants to a lower light intensity. Ozone significantly reduced the 4-day RGR (34%) at high, but not low relative humidity. The 4-day RGRs were significantly higher (19%) at 90% RH compared to 30% RH in controls, but significantly lower (7%) at 90% RH compared to 30% RH in ozone treatments. Ozone significantly reduced the 7-day RGR (31%) at high, but not low relative humidity. The 7-day RGRs were similar between relative humidities in controls, but lower (17%) at 90% RH compared to 30% RH in ozone treatments.

Average daily water use increased at a rate of 12% per day during the 4-day exposure period, due to increasing plant size. Average water use was typically 68% and 53% of daily water use at 30% and 90% RH, respectively. Average water use during the exposure period was higher in both control (246%) and ozone (275%) treatments at low, compared to high relative humidity (Table 2b). Ozone significantly decreased average water use (18%) during the exposure period at low, but not high relative humidity.

Daily transpiration rate decreased at a rate of 10% per day during the 4-day exposure period, possibly due to the transfer of plants to a lower light intensity. The average transpiration rate during the exposure period was higher in both control (307%) and ozone (265%) treatments at 30% RH compared to 90% RH (Table 2b). The average transpiration rate during the exposure period was not significantly altered by ozone at either relative humidity.

Ozone caused reductions in adjusted dry weight at both relative humidities, although all reductions were significantly greater at 90% than at 30% RH (Fig. 8). Average adjusted root dry weight was significantly reduced by ozone at high (103%) and low (63%) relative humidity (Table 3b). Average adjusted stem dry weight was reduced by ozone at high (99%) and low (59%) relative humidity. Average adjusted leaf dry weight was reduced by ozone at high (70%), but not low relative humidity. Average adjusted plant dry weight was reduced by ozone at high (84%) and low (50%) relative humidity.

Dry weight partitioning in controls was similar between relative humidities (Table 3b). The RWR was significantly reduced by ozone at both high (19%) and low (11%) relative humidity. The SWR was not affected by ozone. The LWR was significantly increased by ozone at high (13%), but not low relative humidity.

At harvest, the percentage of plant leaf area showing visible foliar injury was higher at 90% than at 30% RH (Table 3b).

Average predicted ozone flux was negatively correlated with average RGR during both the 4-day exposure period (Fig. 9), and the 7-day period when the leaves were measured. At 90% RH, ozone flux was significantly higher than at 30% RH, and caused a significant reduction in RGR (4-day) (Table 2b). The rate of reduction appears to be similar between 30% and 90% RH, because the slopes of the

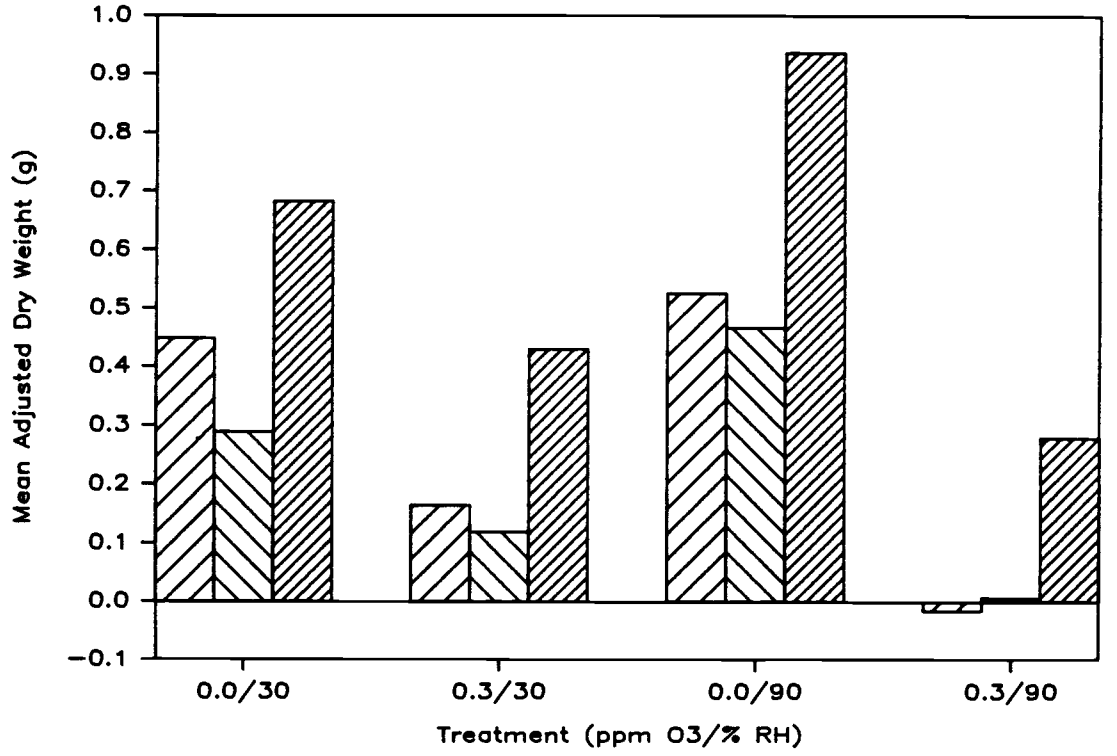

FIG. 8. Effects of ozone and relative humidity on adjusted root (▨), stem (▧), and leaf (▨) dry weight in sunflower. Treatments consisted of exposure to 0.0 or 0.3 ppm ozone, at 30% or 90% RH, 6 h/d, for 4 d (n = 16 plants per treatment). Plants were harvested three days after the final exposure, and dried for one week. Dry weights are adjusted by a covariate, plant leaf area.

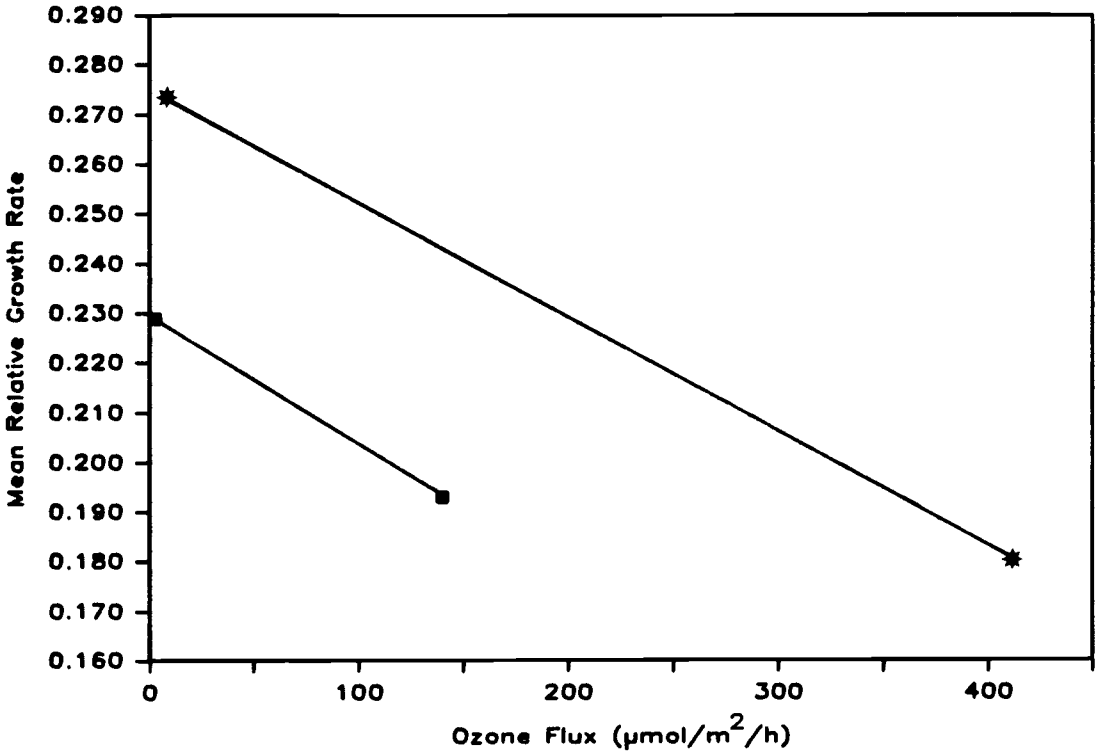

FIG. 9. Mean relative growth rate of sunflower leaves vs. predicted ozone flux. Treatments consisted of exposure to 0.0 or 0.3 ppm ozone at 30% RH (■) or 90% RH (✸), 6 h/d, for 4 d (n = 16 plants per treatment). Growth rates ($cm^2/cm^2/d$) are over the 4-day exposure period.

RGR (4-day) vs. ozone flux lines are similar between the two relative humidities (Fig. 9). Relative increases in RGR during the 3-day post-exposure period suggest that recovery occurred at both relative humidities (3-4%).

Adjusted dry weights of roots, stems, and leaves showed strong negative correlations with total predicted ozone flux. The rate of reduction in dry weight also appears to be similar between 30% and 90% RH, because the slopes of dry weight vs. ozone flux lines for roots, stems, and leaves (not shown) are similar between the two relative humidities.

DISCUSSION

I. Correlations between ozone flux and plant response.

Factors such as light intensity, relative humidity, water stress, and ozone concentration, affect the amount of plant injury induced by ozone, apparently through their influence on stomatal aperture. Because stomatal aperture affects the amount of ozone that enters leaves, plant response to ozone is more closely associated with ozone flux, the rate of ozone diffusion into leaves, than the ambient ozone concentration (Amiro et al, 1984). In the present study, plant response to ozone is related to ozone flux estimated by a model.

According to EPA criteria based on visible foliar injury, sunflower cultivar NK894 is ozone-resistant compared to other crops. The ozone dose required to induce visible injury in this cultivar (0.2 ppm for 6 h) was associated with an ozone flux of 56 μmol $m^{-2}h^{-1}$. Above this level, visible foliar injury was positively correlated with ozone flux, which increased as ozone concentration and relative humidity increased. This observation is consistent with the results of previous studies. Visible foliar injury was correlated with ozone flux in bean plants (Amiro et al, 1984). In begonia varieties, visible foliar injury increased as ozone concentration and relative humidity increased, but was absent at low ozone concentrations (0.1 ppm, at 60% RH) (Leone and Brennan, 1969). In tobacco (Bel

W3) and bean (Pinto) plants, visible foliar injury increased as relative humidity increased from 45% to 75% RH at 0.4 ppm ozone (1 h) (Dunning and Heck, 1977), and similar amounts of visible foliar injury were induced at 0.1 ppm ozone at 95% RH, and 0.3 ppm at 26% RH (90 min) (Otto and Daines, 1969).

As Todd (1958) noted, visible foliar injury is not always a good indicator of ozone-induced metabolic changes, such as reduced photosynthesis. To assess plant response to ozone in the present study, leaf growth rate and biomass accumulation, as well as visible foliar injury, were observed for ozone-induced changes.

A significant reduction in root dry weight was induced by 0.1 ppm ozone (ozone flux = 33 μmol $m^{-2}h^{-1}$), in the absence of a significant change in total plant dry weight. Changes in dry weight partitioning -- a decrease in the root weight ratio (RWR) and an increase in the leaf weight ratio (LWR) -- accompanied the reduction in root dry weight, suggesting that plant biomass was repartitioned, but not reduced, by low ozone flux. Repartitioning of biomass has previously been observed in sunflower (cv. Russian Mammoth) -- a decrease in root dry weight accompanied by an increase in the LWR -- when exposed to low levels of ozone (Shimizu et al, 1984a).

Significant reductions in plant dry weight did not occur until the concentration of ozone reached 0.2 ppm (ozone flux = 56 μmol $m^{-2}h^{-1}$). This level also induced

visible foliar, in addition to significant reductions in root dry weight and RWR, and a significant increase in the LWR. Similar changes -- reductions in root dry weight and RWR, and an increase in the LWR -- were observed in sunflower seedlings (cv. Russian Mammoth) exposed to 0.2 ppm (6 h/d, 12 d) (Shimizu et al, 1984b). The requirement of an ozone concentration of 0.2 ppm to induce visible in sunflower cultivar NK894, is consistent with the observation that visible injury is induced only after a minimum amount of ozone is taken up by bean plants (Amiro et al, 1984).

Significant reductions in relative leaf growth rate were induced by 0.3 ppm ozone (ozone flux = or > 98 μmol $m^{-2}h^{-1}$, except at 30% RH). This level of ozone also caused significant reductions in stem and leaf (except at 30% RH) dry weight, as well as significant reductions in root and plant dry weight and RWR, a significant increase in the LWR (except at 30% RH), and visible foliar injury. Reductions in growth rate were also induced at 0.1 ppm (2%) and 0.2 ppm (7%), but were not significant when tested by analysis of variance. However, these reductions did show a strong negative correlation to ozone flux at 60% RH (r^2=0.99) (Fig. 6), and, according to regression analysis, are significant because the slope of the line differs from zero. Significant reductions in RGR were observed in sunflower (cv. Russian Mammoth) exposed to 0.1 (15%) and 0.2 ppm (19%) ozone (12 h/d, 6 d) (Shimizu et al, 1984a).

The reduction in RGR at 0.2 ppm was accompanied by a reduction in the net assimilation rate, and attributed to a reduction in photosynthesis.

In the present study, greater injury was observed at high, than at low relative humidity (significant reductions in RGR, leaf dry weight, and LWR were induced at 90%, but not at 30% RH), a result of higher ozone flux permitted by lower leaf resistance. It has previously been shown that stomatal resistance in bean plants is increased by ozone at low, but not high relative humidity (Rich and Turner, 1972). As ozone concentration increased, leaf resistance in kidney bean plants increased more at low, than at high relative humidity in (McLaughlin and Taylor, 1980). In agreement with these results, total leaf resistance (calculated as the change in water vapor gradient /transpiration) in the present study was increased more by ozone at low, than at high relative humidity (these values were derived, not measured, and therefore used for comparison only).

Similarity in the slopes of growth rate and dry weight reductions vs. ozone flux at 30% and 90% RH (Fig. 9), suggests that the amount of reduction per unit ozone flux is the same regardless of relative humidity. This would indicate that relative humidity does not affect ozone flux through altered internal leaf resistance, but through altered stomatal resistance.

Recovery from ozone-induced injury is suggested by

the increases in RGR, compared to controls, during the 3-day post-exposure period in all ozone treatments. At 0.1 and 0.2 ppm (60% RH), growth rates over the 7-day period exceeded that of the control. A similar increase observed in the RGR of sunflower seedlings (cv. Russian Mammoth) exposed to 0.1 ppm ozone (14 h/d, 12 d) was attributed to an increase in net assimilation rate during the exposure period, and it was suggested that low concentrations of ozone may accelerate photosynthetic rates (Shimizu et al, 1984b). Bennett et al (1974) suggested that plants may be adapted to low levels of naturally existing ozone, or that some crops may have been bred in areas where low pollutant levels of ozone exist.

II. Validity of the model.

Models for predicting pollutant flux based on Fick's law of diffusion have previously been described (e.g., Bennett, Hill and Gates, 1973; Unsworth, Biscoe and Black, 1976; O'Dell, Taheri and Kabel, 1977; Black and Unsworth, 1979). These models calculate ozone flux from leaf conductance or resistance to water vapor, and the concentration gradient of ozone.

The present model calculates ozone flux from transpiration rate rather than resistance, with an adjustment for the concentration gradient of water vapor. Consequently, predicted ozone flux varies linearly with transpiration at constant relative humidity, and with the leaf-air water

vapor concentration gradient. Predicted ozone flux also varies proportionately with ozone concentration, but deviations from linearity may occur as a result of changes in transpiration.

The validity of the predicted linear relation between transpiration and ozone flux is supported by the appearance of this relationship in studies in which ozone flux was measured (Thorne and Hanson, 1972; Omasa et al, 1980). Some studies report a deviation from linearity at high ozone concentrations, due to ozone-induced stomatal closure (Townsend, 1972; McLaughlin and Taylor, 1980). The significant reduction in transpiration rate at 0.2 ppm, resulting in the slightly lower than proportional increase in ozone flux between 0.1 and 0.2 ppm observed in the present study, were probably due to partial stomatal closure, which may be induced by ozone (Hill and Littlefield, 1969; Omasa et al, 1980; Olszyk and Tibbits, 1981; Amiro and Gillespie, 1985).

Ozone fluxes predicted by the present model are similar to those measured and predicted in previous studies (Table 4).

III. Model assumptions.

The model assumes that the diffusion pathways for water vapor and ozone are the same, that transpiration and ozone flux are regulated by the same leaf resistances, that leaf resistance varies linearly with the vapor pressure deficit, and that the concentration of ozone at

Table 4. Comparison of ozone fluxes estimated in the present study with those predicted or measured in other studies.

O3 Flux ($umol/m^2/h$)	Transp. (mol/h)	O3 (ppm)	RH (%)	Plant	Reference
33-98	7.0-8.1	0.1-0.3	60	sunflower	present study
140	15.4	0.3	30	"	"
409	5.8	"	90	"	"
100	4.5	0.2	70	sunflower	Omasa et al (1980)
110-210	3.0-4.0	0.4	70	"	"
100	12.3	0.3	66	soybean	Yingjajaval (1976)
0-10		0.052-0.145	35	bush bean	McLaughlin & Taylor (1980)
31-58		"	75	"	"
18-3		0.035-0.113	70	bean	Amiro et al (1984)
123		0.05	50	alfalfa	Hill (1971)
22-60		0.04-0.10	-	corn	Leuning et al (1979)
16-45		0.25	65	petunia	Elkiey & Ormrod (1981)

the surface of the substomatal cells is zero.

The assumption that ozone and water vapor follow the same diffusion pathways may result in a slight overestimation of ozone flux by the model, because ozone flux does not occur through the cuticle (Leuning et al, 1979), although low rates of transpiration may (Slatyer, 1967).

The assumption that ozone and water vapor fluxes are regulated by the same leaf resistances is supported by several studies. Rich et al (1970) measured leaf resistance to water vapor and found that it was nearly equal to leaf resistance to ozone, calculated from ozone flux. They concluded from this equality that the concentration of ozone at the surfaces of the substomatal cells is very low (since the water vapor concentration is nearly 100%), and that the diffusion pathway is essentially the same for ozone and water vapor. These results also indicate that stomates are the predominant factor in controlling ozone flux, because transpiration is affected by stomatal, intercellular air space, and boundary layer resistances, but intercellular air space resistance is constant (Nobel, 1983), and boundary layer resistance is proportional for all gases.

Water stress, induced by osmotically lowered soil-water potential, reduces plant sensitivity to ozone by decreasing leaf conductance (Tingey et al, 1982). Reductions in leaf turgidity induced by dry soil conditions result in reduced stomatal aperture, which reduces plant sensitivity to ozone (Adedipe et al, 1973). In plants

whose stomates have been chemically closed, water-stressed and non-water-stressed plants are equally tolerant to ozone, suggesting that stomatal aperture, rather than physiological changes induced by water stress, is more important in controlling plant ozone flux (Tingey and Hogsett, 1985).

However, some studies suggest that an internal, or residual resistance to ozone exists in addition to the leaf resistances that affect water vapor (Yingjajaval, 1976; McLaughlin and Taylor, 1980; Taylor et al, 1982).

The assumption that leaf resistance varies linearly with vapor pressure deficit is supported by several studies. Leaf resistance has been shown to decrease as relative humidity increases in bean (Wilson, 1948), and the decrease is linear as the leaf-air vapor pressure deficit decreases, without inducing a reduction in leaf water potential, in sunflower (cv. Single Tall, Black, 1979; Aston, 1976).

However, in contrast to these studies, stomatal resistance in sunflower (cv. Hysun 30) remained constant asthe vapor pressure deficit decreased (Rawson & Begg, 1977). Constant stomatal resistance over a range of water vapor deficits would result in constant predicted ozone flux over a range of relative humidities (at constant ozone concentration). Observations of a positive correlation between relative humidity and injury do not support the

possibility of constant ozone flux at varying relative humidity.

Although some evidence is controversial, it appears that the assumptions made by the model are valid. The ozone and water vapor diffusion pathways may not be exactly identical, and possible differences should be taken into account when using the model to predict ozone flux. Many studies support the concept that the diffusion resistances regulating ozone and water vapor are the same. If a resistance to ozone exists in addition to resistances to water vapor, the model will overestimate ozone flux by an amount proportional to the percentage of the total leaf resistance comprised by the residual resistance. Most studies support the assumption that stomatal resistance changes as the leaf-air vapor pressure deficit changes, whether induced by water stress, or relative humidity. Ozone flux will not be accurately predicted in plants, or under conditions, where stomatal resistance does not change as relative humidity changes.

IV. Conclusions.

1. Visible foliar injury, reductions in growth rate, and reductions in biomass accumulation were strongly correlated with predicted ozone flux.

2. Plant response to low ozone flux included biomass repartitioning -- an increase in leaf biomass at the expense of the roots -- accompanied by reduced root, but

not total plant dry weight. Visible foliar injury and reduced total plant dry weight were induced by higher ozone flux. Only at the highest ozone fluxes were leaf growth rate (except at 30% RH), and stem and leaf (except at 30% RH) dry weight reduced, although reduction in leaf growth rate was strongly correlated with low, as well as high ozone flux.

3. High relative humidity induced greater plant response than low relative humidity, but results suggest that the rate of reduction (growth and biomass) per unit ozone flux was the same at low and high relative humidity.

4. Recovery occurred following all ozone treatments, and growth rate stimulation may have occurred at 0.1 and 0.2 ppm (60% RH).

5. This study shows that a simple model based on gas diffusion laws, such as the one developed in this study, can be used to estimate ozone fluxintoplants. Despite possible limitations imposed by model assumptions, the validity of the model is indicated by the high correlation between predicted ozone flux and injury.

6. This model may be useful in predicting injury to crops in the field, once the crop's injury-response is experimentally correlated with predicted ozone flux. Measurement of only three environmental variables, ozone concentration, air temperature, and relative humidity, and two plant parameters, leaf temperature and transpiration rate, are necessary.

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APPENDICES

Appendix A

CERL EPA Greenhouse Nutrient Solution*

	Stock Solution (g/l)	Working Solution (ppm)
Solution "A":		
Magnesium Nitrate	13.0	Mg 12.32
		N 14.20
Calcium Nitrate	32.0	Ca 54.31
		N 37.96
Sequesterone 330 Fe	5.0	Fe 5.00
Solution "B":		
Potassium Nitrate	20.25	N 28.00
Ammonium Nitrate	8.0	N 28.00
Potassium Phosphate:		
monobasic	2.4	K 6.89
		P 5.46
dibasic	2.8	K 6.28
		P 4.98
Potassium Sulfate	3.0	K 13.46
		S 5.52
Sodium Sulfate	3.4	Na 11.01
		S 7.67
Methylene Blue	0.0125	
Minor Elements:		
Boric Acid	0.140	B 0.24
Molybdic Acid	0.001	Mo 0.005
Hampene Zinc	0.009	--
Hampol Manganese	0.0945	--
Hampol Copper	0.006	--

* Stock solutions are proportioned at the rate of 1 ml of A and 1 ml of B to 1 l of water. Nutrient solution content obtained from R. Field.

Appendix B

Calibration of Ozone Analyzers*

The Monitor Labs Ozone Analyzer (Model 8410) is an ozone measuring device which is calibrated against a known standard (McElroy, 1979), to relate its output to independently measured ozone concentrations. There are two basic methods of relating the ozone analyzer response to a controlled source of ozone: (a) dynamic calibration, and (b) zero/span checks. Dynamic calibration refers to a complete multipoint definition of the Model 8410 with an ozone standard. More reliable operation of the Model 8410 is further ensured by performing weekly zero/span checks, which consist of a zero baseline check with zero air and a one-point span check. Ozone readings within 15% of the concentration generated by the calibrator are accepted.

To generate accurate calibration levels from an ozone calibrator, it is necessary to know the exact flowrate of the sample. The flowrate of the calibrator was measured using a bubble flowmeter and stopwatch. The ozone concentration generated was measured by the standard ozone analyzer, and the three analyzers used in the study, as follows:

CALIBRATOR (Model 8500) Flowrate (ml/min)	STANDARD ANALYZER (Model 1008PC) Reading (ppm)	THREE ANALYZERS USED IN STUDY (Model 8410) Reading (ppm)		
3333	0.638	0.640	0.620	0.640

The Monitor Labs Calibrator (Model 8500) employs UV lamps, the most reliable ozone source, to generate ozone. The calibrator directly provides a .05 -1.0 ppm ozone supply. During dynamic calibration, the following ozone standard concentrations were generated with the calibrator: 100%, 50%, 10%, and 5%, $\pm$ 5% of full scale of the Model 8410 0.5 ppm range, and zero air.

The Dasibi Ozone Analyzer (Model 1008PC), the ozone standard, was calibrated annually to EPA standards (Paur and McElroy, 1979) by an independent auditor. During calibration of the three Monitor Labs Ozone Analyzers (Model 8410) used throughout the study, concentrations generated by the ozone source, measured by the three Model 8410 analyzers, were compared to measurements made by the Model 1008PC. The Model 8410 analyzers measure ozone concentrations of up to 5 ppm, however, only concentrations of 0.10 - 0.40 ppm were used in the study. The following measurements were made during dynamic calibration in the 0.5 ppm range:

STANDARD ANALYZER (Model 1008PC) (ppm)	THREE ANALYZERS USED IN STUDY (Model 8410) (ppm)		
0.512	0.512	0.513	0.512
0.259	0.260	0.263	0.263
0.088	0.089	0.095	0.093
0.035	0.033	0.043	0.041
-0.002	-0.004	0.006	0.002

The error percentages were < or = 0.20% at 0.5 ppm, 1.54% at 0.25 ppm, 7.95% at 0.1 ppm, and 22.86% at 0.035 ppm. Error percentages at the ozone concentrations used in the study were less than 15%, and considered acceptable by EPA standards.

Operational Tests

The Monitor Labs Ozone Analyzer Model 8410 is a gas phase chemiluminescent detection device which performs a continuous dry analysis of ozone. Its operation is based on the chemiluminescence of an activated aldehyde molecular species produced by the chemical reaction between ozone and ethylene. A photomultiplier tube measures the chemiluminescent emission intensity.

Front panel and remote controlled test functions allow quick checks on electrical and optical continuity. The electrical test function imposes an electrical signal at the pre-amp input of the photomultiplier tube/pre-amp assembly, and the optical test imposes a light signal at the burner window. The signal continuity from the burner to the recorder is checked to determine faulty operation of the photomultiplier tube.

The optical and electrical tests were made in range 5 (0-5 ppm). The front panel optical and electrical test buttons were depressed and the readings recorded for subsequent comparison. Front panel meter readings during subsequent tests throughout the use of the analyzer were approximately the same as during the initial test, indicating correct functioning of the photomultiplier tube. The ethylene flowrate was measured by a bubble flowmeter, and also recorded for subsequent comparison. The flowrate was measured prior to, and throughout, the experiments, and remained at the recommended rate of 30 ml/min.

* Assistance in calibration procedures provided by J. Miller.