COMPARATIVE PHYSIOLOGY OF RADISH POPULATIONS WITH DIFFERENTIAL SENSITIVITY TO O_3 AND SO_2

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(ABSTRACT)

Radish plants (*Raphanus sativus* L. cv Cherry Belle) were exposed to 0.10 μl l^{-1} ozone (O_3) or 0.50 μl l^{-1} sulfur dioxide (SO_2) for 4 h d^{-1}, 3 d wk^{-1} for 3 weeks. From these fumigated plants, individuals were selected that were resistant or sensitive to these pollutants. The selected plants were used as parental material in a breeding program to produce lines differing in resistance to O_3 and SO_2. Non-selected (NS) plants from the original populations served as controls.

F_1 populations were raised and exposed to O_3 or SO_2 with the same fumigation regime used for the parents. The plants were harvested 30 days after emergence and dry weights were determined. Plants selected for O_3 resistance (O_3R) weighed significantly more than either plants selected for sensitivity to O_3 (O_3S) or NS plants when exposed to either O_3 or SO_2. The hypocotyl was most affected by pollutant exposure, leading to reduced root/shoot ratios. Plants selected for resistance or sensitivity to SO_2 generally had biomass production similar to that of NS plants. Growth analysis at early stages of growth indicated that both O_3R and O_3S plants had less growth under O_3 fumigated conditions; however, by maturity

O_3R plants had similar amounts of growth under fumigated or non-fumigated conditions. Ozone fumigations tended to decrease free sugar concentrations in leaves at early stages of growth in both O_3R and O_3S plants, and caused some accumulations of carbohydrates during late stages of growth in O_3S plants. Allocation of ^{14}C was significantly lower to hypocotyls and roots of O_3 fumigated O_3S plants. Allocation to hypocotyls of O_3R plants was not affected, although both O_3R and O_3S plant groups had lower photosynthetic rates due to O_3 fumigation. Ozone did not significantly affect chlorophyll concentrations in leaves of either sensitive or resistant plants, nor was the time of new leaf production affected by fumigation.

These experiments demonstrated the potential of O_3 to influence the composition of sensitive plant populations. However, SO_2 was a much less powerful influence on the composition of these populations.

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1. Literature Review

1.1 Ozone

1.1.1 Growth Effects

Nationally, ozone (O_3) is considered to be the most prevalent gaseous air pollutant. It is classified as a photochemical oxidant since it is formed in the atmosphere through a series of reactions involving nitrogen dioxide and hydrocarbons from fuel combustion, sunlight, and atmospheric oxygen. During the past few years it has been demonstrated that O_3 can reduce crop growth not only when it is present at high concentrations, but also at the ambient levels found over vast areas (Heck *et al.* 1983). Characteristics of plants exposed to O_3 are initially viewed as water soaking, followed by stippling and then necrosis of large leaf areas. Such visible injury is not always a reliable indicator of actual effects of O_3 on growth and yield of the plants since significant growth and yield reductions can occur in the complete absence of any visible injury (Heagle, Body, & Neely 1974).

Annual agricultural yield losses due to O_3 are estimated to amount to $3 billion. Reich and Amundson (1984) used a field fumigation system to measure the effect of low level O_3 exposure upon growth and yield of soybean (*Glycine max* cv Hark). At concentrations of 0.06 to 0.08 $\mu l\ l^{-1}$ for 5 h/day over a period of 16 days, total yield (seed dry mass) per plant was reduced from 10-25%. Seed size was significantly reduced as well. Studies using higher O_3 concentrations have also demonstrated similar findings (Howell, Koch, & Rose 1979, Kress & Miller 1983). Results from greenhouse investigations are in general agreement with those done in the field. Ozone levels of 0.07 or 0.097 $\mu l\ l^{-1}$ for a total of 341 h reduced the yield of

Conroy soybeans in terms of total seed weight per plant and in total plant biomass accumulation. However, exposure to 0.046 $\mu l\ l^{-1}$ slightly stimulated total seed weight per plant (Endress & Grunwald 1985).

Phaseolus vulgaris is another species which has been extensively fumigated with different concentrations of O_3. Even at ambient levels, growth of *Phaseolus* may be reduced. Amthor (1988) exposed *P. vulgaris* L. cv Pinto to ambient O_3 levels using an open-top chamber system. Exposures lasted for twelve hours, and the mean daily O_3 concentrations were 0.043 and 0.080 $\mu l\ l^{-1}$ for ambient and 2X ambient treatments. The leaf relative growth rate (RGR) was significantly reduced in both treatments as compared to control plants. This decrease was attributed to an increase in total respiration. Kidney bean plants (*P. vulgaris*) are sensitive to O_3 as well. Continuous exposure for 2, 4 or 7 days to moderate (0.02 or 0.04 $\mu l\ l^{-1}$) O_3 concentrations significantly reduced plant dry weight (Ito *et al.* 1985).

The timing of the pollutant exposures may be important in determining the plant response. Repeated 3 h exposures of kidney bean to 0.30 $\mu l\ l^{-1}$ O_3 resulted in growth reductions only if the second exposure was at least three to five days after the initial fumigation (McCool *et al.* 1988). This indicates that the plants may develop a temporary resistance to O_3 which appears to be lost in a short period of time. Ozone also has the ability to cause stomatal closure in many species, such as tobacco (*Nicotiana tabacum*) (Turner, Rich, & Tomlinson 1972) and tomato (*Lycopersicon esculentum*) (Leone 1976).

The radish (*Raphanus sativus*) cultivar Cherry Belle, developed in 1949 for its uniformity of size at a specific harvest date of 30 days from germination, is widely grown on a commercial basis. It has been demonstrated that this cultivar is quite

sensitive to air pollutants. Reinert *et. al.* (1972) exposed nine different radish cvs to a single, 1.5 hour treatment of ozone at 0.35 $\mu l\ l^{-1}$ and then evaluated the plants three days later for the amount of visible injury. The cv Cherry Belle had the highest amount of visible injury of any of the cvs tested, with 35.7% of the upper leaf surface showing injury as compared to only 18% for the most resistant cv, Icicle. Growth was also reduced. Long term fumigations (40 h/week for 5 weeks) at 0.05 $\mu l\ l^{-1}$ altered total plant fresh weight, although dry weight was not significantly reduced (Tingey, Heck, & Reinert 1971). Similar experiments, differing mainly in the concentration of O_3 used, have demonstrated a negative impact of the pollutant on total plant growth (Reinert & Sanders 1982, Reinert, Shriner, & Rawlings 1982). Environmental factors may influence radish response to O_3. Leaves of the Cherry Belle cv are more sensitive to O_3 if they are grown at relatively low (day/night 20/15 C) temperatures, although roots are more sensitive at either high or low temperatures (Adepipe & Ormrod 1974).

The most detailed growth study of the Cherry Belle cultivar in response to O_3 was conducted by Walmsley, Ashmore & Bell (1980). In this study, plants were subjected to continuous O_3 exposure at a mean concentration of 0.17 $\mu l\ l^{-1}$, beginning only five days after germination and continuing until harvest at thirty days. A variety of growth parameters was measured during the experiment, including dry weight of various plant parts, net assimilation rate (NAR) and RGR. The data showed that plant response changed dramatically over the course of the experiment. The first leaves that developed during the fumigation period were small and showed a great amount of visible injury. These leaves tended to senesce prematurely. Newer leaves, however, were much larger and generally devoid of injury symptoms. These leaves also emerged earlier than the same leaves on non-fumigated plants. The third

leaf pair, for example, emerged on day 22 as compared to day 29 for the non-fumigated plants. Leaf size was also increased. Hypocotyl growth did not exhibit this pattern; it was always less for the fumigated plants as compared to non-fumigated plants. The authors concluded that the radish plants, although sensitive to O_3 exposure, had the ability to adapt to continuous exposure by shifting allocation patterns to alleviate inhibition of shoot growth.

From the studies cited here, it is apparent that O_3 is a potent stress factor that has the ability to restrict growth. The increasing presence of O_3 over large portions of the U.S. may be significantly reducing crop and tree growth. Studies have shown a potential 10-25% reduction in crop yield at present O_3 levels (Adams, Hamilton, & McCarl 1984). Growth, however, can be viewed as merely the final product of a series of physiological perturbations, which taken as a whole, reduce growth. Some of these physiological processes will be discussed next.

1.1.2 Biomass Partitioning

Probably the most consistent effect seen in plants fumigated with O_3 is the alteration in biomass partitioning. There tends to be an increase in shoot growth at the expense of root growth. This alteration may be due to a reduction in the amount of available assimilate in the leaves; such a reduction has been implicated in causing changes in allocation patterns (Wardlaw 1968). Root growth is much more affected by O_3 stress than shoot growth, even though roots themselves are not directly affected by O_3, as it is unable to penetrate the soil (Blum & Tingey 1977). Experiments with Cherry Belle radish have clearly demonstrated the effects of O_3 on partitioning (Tingey *et al.* 1971, Walmsley *et al.* 1980). Ozone decreased the dry weight of both the shoot and the root system by 10 and 50%, respectively, in the

experiments done by Tingey. Bennett and Oshima (1976) reported a 46% decrease in the growth of carrot (*Daucus carota*) roots with O_3 exposures of 0.25 $\mu l\ l^{-1}$, while shoot growth actually showed a slight increase. Similar results were noted in parsley (*Petroselinum crispum*), fescue (*Festuca arundinacea*), and soybean (*G. max*) (Tingey *et al.* 1973, Oshima, Bennett, & Braegelmann 1978, Flagler & Younger 1982). Oshima *et al.* (1979) carried out a sequential harvest experiment with greenhouse grown cotton (*Gossypium hirsutum*) exposed to 0.25 $\mu l\ l^{-1}$ O_3 for six hours twice per week. As in the study done by Walmsley *et al.* (1980) with radishes, there were indications in this experiment that the plants were able to adapt to the treatment conditions. Initially, there was a reduction in the number of leaves and leaf area of the O_3 treated plants. However, this was followed by a period in which leaf production was stimulated beyond that of the control plants, compensating to some extent for the earlier loss. Although O_3 did decrease shoot dry weight throughout the experiment period, these reductions were proportionally less than those of other parts, particularly roots and bolls. Leaf area ratio, the proportion of leaf area to total plant dry weight, was increased in the fumigated plants, indicating that these plants had shifted their allocation patterns. This shift was accompanied by a decrease in the NAR. By increasing the leaf area ratio, the plants may have been able to offset the decrease in the NAR. It is of interest to note that boll mass was reduced approximately to the same degree as root dry weight. Thus, it appears that while the root/shoot ratio is decreased by pollutant treatments and top growth of the plant is generally favored, fruit production in this case is not. Reallocation, therefore, seems to be selective. Sources of carbon uptake benefit the most from these reallocation shifts.

Atkinson, Robe, & Winner (1988) used the Cherry Belle radish in a dose response study. Plants were exposed to 0, 0.06, 0.12, and 0.20 $\mu l\ l^{-1}$ O_3 on a regular basis, 20 h/week for 4 weeks. The lowest O_3 concentration did not affect root/shoot ratios at either of the two harvest dates (25 and 37 days after germination). The two higher concentrations, however, reduced this ratio by approximately 25 and 60%, respectively.

1.1.3 Stomatal Responses

The sensitivity of stomata to oxidative pollutants was first observed in the early 1950's in a study done by Koritz and Went (1952). They observed that transpiration decreased in plants grown in polluted air due to stomatal closure. Since that time, stomata have been the subject of a wide range of studies. Hill & Littlefield (1969) exposed a variety of crop species to acute doses of O_3 and measured stomatal closure by taking epidermal strips. Although this was a rather crude measure, they were able to consistently observe stomatal closure and apparent recovery once the pollutant stress was removed. Photosynthesis and transpiration decreased and recovered simultaneous with stomatal response. Grape (*Vitis sp.*) leaves exhibited a similar response, closing in response to O_3 fumigations (Rosen, Musselman, & Wender 1978). The benefit of stomatal closure to the plant is apparently a reduction in the amount of pollutant absorbed, although closure has other adverse affects, primarily the reduction in uptake of carbon dioxide (CO_2) for photosynthesis. Other environmental factors which can modify stomatal opening can also affect plant response to pollutants. Pea (*Pisum sativum*) plants grown under constant moisture stress exhibited greater stomatal closure in response to a 2 h exposure of 0.23 $\mu l\ l^{-1}$ O_3 than did plants grown at field capacity. Necrosis of leaves on the water stressed

plants was significantly lower than that on the well watered plants. Thus, moisture stress gave the plants a degree of resistance to O_3 (Olszyk, & Tibbitts 1981).

Similar observations were made in a field study of cotton (Temple 1986). Open-top chambers were employed to regulate pollutant conditions. Several pollutant treatments were used: charcoal filtered air, non-filtered air, non-filtered air X 2.0, and ambient air (no chamber). The mean (7 hour) ambient O_3 concentration was 0.077 $\mu l\ l^{-1}$. Plants were grown over an entire growing season under either normal irrigated conditions (watered at -1.8 MPa) or under water stressed conditions (watered at -2.0 MPa). Measurements of stomatal conductance and transpiration were taken at monthly intervals. Diurnal measurements were made during one of the selected days. Water stressed plants had stomatal conductance value approximately 30% lower than those of normal irrigated plants. Transpiration was reduced approximately 17%. Ozone caused some additional closure, particularly in the 2X non filtered treatment. Stomatal function was apparently not impaired, as stomata of severely O_3 stressed plants responded at the same rate to light as did the plants in the charcoal filtered chambers. Decreased photosynthesis, which would increase internal CO_2 concentrations, was suggested as the cause of stomatal closure in this experiment. Reduced photosynthesis leads to increases in the internal CO_2 concentration (C_i), which can in turn cause stomatal closure. There was little evidence to support the idea that stomata respond directly to O_3; stomatal response appeared to be a secondary response, dependent upon other O_3 induced changes in leaf physiology.

The experiment by Walmsley *et al.* (1980) demonstrated that stomatal closure may be of little importance in a plant's adaptive response to O_3. As previously discussed, radishes exposed to O_3 on a continuous basis showed an adaptation to the

exposure conditions, in which shoot growth of exposed plants was equal to that of non-fumigated plants by the end of the experiment. There were no differences in stomatal aperture between fumigated and non-fumigated plants at this time, indicating that avoidance of pollutant uptake was not an important feature of the adaptive response. However, non-fumigated plants subjected to O_3 only two days prior to conductance measurements had greatly reduced apertures, an indication that the level of O_3 used was sufficient to cause closure.

1.1.4 Photosynthesis

Carbon dioxide fixation is responsible for the majority of plant dry weight, and as such is an area of great interest in regard to the effects air pollutants have upon this process. From past studies it has been learned that photosynthesis is an extremely O_3 sensitive process. One of the earliest studies was made by Hill and Littlefield (1969). Using a growth chamber into which O_3 could be delivered, photosynthetic measurements were made by monitoring the amount of CO_2 required to maintain the CO_2 concentration within the chamber at 325 $\mu l\ l^{-1}$. Several crop species were used, and photosynthetic reductions varied from 20-80%, with O_3 concentrations ranging from 0.40 to 0.90 $\mu l\ l^{-1}$. These effects were generally reversed within a few hours of removing the stress. Although this can be considered a crude study, it did reveal a consistent effect of O_3 upon photosynthesis across a broad range of plant species.

More recent studies have also documented the adverse effects O_3 may have. There appears to be a point at which O_3 effects on photosynthesis become irreversible. Photosynthesis of faba beans (*Vicia faba*) decreased upon O_3 exposure of 0.151 $\mu l\ l^{-1}$ for four hours, but recovery occurred within one day. At higher

concentrations, > 0.217 $\mu l\ l^{-1}$, recovery did not occur, indicating permanent damage to the photosynthetic apparatus (Black, Ormrod, & Unsworth 1982). Reich (1983) made light response curves for various aged hybrid poplar (*Poplulus deltoides* x *trichocarpa*) leaves in response to low level O_3 exposures given for approximately 5.5 h each day. For six day old leaves, there was little difference between those exposed to 0.025 (control) or 0.125 $\mu l\ l^{-1}$ O_3. However, from 24 to 58 days, the maximum photosynthetic rate was decreased for leaves exposed to either 0.085 or 0.125 $\mu l\ l^{-1}$ O_3. Quantum yield in the 58 day old leaves was reduced as well. Measurement of leaf chlorophyll indicated leaf aging was accelerated due to O_3 exposure, and that the reduction in photosynthesis may be a result of the accelerated aging.

A similar study by Reich, Schoettle, & Amundson (1986) involved the effects of low concentrations of O_3 on photosynthesis of sugar maple (*Acer saccharum*) seedlings. A linear decline of photosynthesis with increasing O_3 concentrations ranging from 0.03 to 0.12 $\mu l\ l^{-1}$ was observed. However, in this experiment, chlorophyll concentrations in the leaves were increased by fumigation with the pollutant, suggesting that an acceleration of aging did not occur in the sugar maple leaves. Reich & Amundson (1985) summarized their work with a wide variety of both tree and crop species by reporting that any increase in O_3 concentration was observed to reduce net photosynthesis, and that the declines could ultimately be related to reductions in growth or yield. The relationship shown between photosynthesis and yield was based upon relatively little data, and does not take into account many factors, such as duration of exposure, or other environmental factors which may influence response. In fact, the experiment done by the authors with sugar maple did not show any decline in growth despite apparent reductions in net photosynthesis.

1.1.5 Translocation

The effects of O_3 on photosynthesis have been extensively studied. It is highly capable of restricting the rate of CO_2 assimilation, through effects on a variety of systems, ranging from stomatal closure to membrane damage and enzyme inhibition. When CO_2 assimilation is altered, translocation of assimilates may also be affected. This is true, regardless of how photosynthesis is altered; a reduction in light intensity will result in a change in the translocation rate as will exposure to a pollutant. Ozone seems to have a large influence on translocation rates. A number of studies have documented decreases in translocation from source leaves to non-photosynthetic parts of the plant, in particular roots and other below-ground parts (Tingey *et al.* 1971, Oshima *et al.* 1978, McCool & Menge, 1983, Okano *et al.* 1984). However, there is no direct effect on the below-ground parts, as ozone has been shown to be unable to penetrate soil (Blum & Tingey 1977). The fate of carbon taken in by the plant during photosynthesis is dependent upon several factors. Assimilated carbon in the leaves can go to one of two main carbohydrate pools, sucrose or starch. Sucrose synthesized during the day is generally transported quickly out of the source leaf to a sink and is the dominant form of carbon transported. Starch, on the other hand, is an insoluble storage carbohydrate that accumulates when sucrose demands are being met. Starch synthesized during peak photosynthetic periods serves as a carbon reserve that can be utilized during dark or low light periods. Regulation of sucrose/starch synthesis is of prime importance to the plant and is dependent upon plant morphology, age and environmental conditions.

Although inhibition of photosynthesis by O_3 has been shown in a wide variety of plant species, the effects on translocation have yet to be thoroughly investigated. Probably the biggest stumbling block has been the difficulty in eliminating the effects

that changes in photosynthesis can have on translocation. Okano *et al.* (1984) conducted experiments with kidney bean (*P. vulgaris*) plants at a stage in which the primary leaves had reached full maturity and the first trifoliate leaf was still expanding. Translocation studies with ^{13}C indicated that the primary leaves were a sink mainly for the roots, while the trifoliate leaf was feeding the upper parts of the plant. Besides providing carbon to different parts of the plant, the leaves also differed in their susceptibility to ozone. First, photosynthesis was measured for each leaf. O_3, given continuously for 4 days at a level of 0.20 $\mu l\ l^{-1}$, decreased photosynthesis 62% in the primary leaf, but only 24% in the trifoliate leaf. This would seem to be one indication of greater sensitivity in the primary leaf. Also, the rate of translocation was measured by following movement of the ^{13}C, using infrared spectrophotometry. In the primary leaf, the percent of labeled carbon exported from the leaf in relation to the total amount taken up was decreased, while in the first trifoliate leaf, a greater percent was exported as compared to non-fumigated plants. Comparisons of the levels of photosynthetic and translocation inhibition were made. Photosynthesis was inhibited slightly more than translocation in the primary leaf, but translocation in the trifoliate leaf was inhibited to a much greater degree than was photosynthesis. Besides changes in the amount of translocation, the patterns were affected as well. In both the primary and trifoliate leaves, the carbon that was translocated tended to go more towards the shoot than the root. Overall then, the effect of O_3 was to reduce growth more in root of the plant than in the shoot by inhibiting translocation of assimilated carbon to the root. The results of this experiment fit in well with other results seen in a number of experiments. The ability of air pollutants, in particular O_3, to reduce root growth even though it does not directly affect it, has been observed in a number of species.

McLaughlin & McConathy (1983) investigated the effect of O_3 on allocation in two varieties of *P. vulgaris* which differed in their sensitivity to O_3. A short-term (4 h) acute dose of O_3 (0.4 μl l^{-1}) reduced ^{14}C translocation to roots in the resistant variety, but did not affect yield. Studies with the sensitive variety revealed that long-term exposures at lower concentrations altered allocation patterns, particularly in primary leaves. Similar results were seen with tomato plants exposed to 0.35 μl l^{-1} O_3 once per week for 9 weeks. More ^{14}C was retained in the labeled leaves in the fumigated plants and less was allocated to the roots. There was no indication of whether other sinks received more ^{14}C as a result of the decrease to the roots (McCool & Menge 1983). Only a few studies have attempted to look at the changes in the plant which result in this, and they have usually focused on translocation of carbon. Reductions in translocation have been observed, but there has been no firm hypothesis to suggest why translocation to the root is reduced to a greater extent than the shoot. The work by Okano *et al.* (1984) gives credence to the idea that this phenomenon may be related to the allocation patterns of different leaves and differences in susceptibility between them. Radishes have a greatly different morphology than do kidney beans, but there are indications that their carbon allocation patterns are similar. Several species in which leaves are separated by very short internodes, such as grasses, displayed allocation patterns similar to other, more elongated plants in that the older leaves allocated primarily to lower portions of the plant while younger leaves serve the upper portions (Wardlaw 1968). A similar pattern has been observed for sympton expression. Rosette plants typically exhibited pollutant injury initially on the oldest, outer leaves with the injury progressing inward. Tingey *et al.* (1971) found chlorotic bleaching occurring primarily on older leaves of radish in response to O_3 or O_3 + sulfur dioxide (SO_2) fumigations.

1.1.6 Carbohydrate Level Studies

Transport of carbon from the leaves is mainly in the form of sucrose. Inhibited translocation could result from a decrease in the amount of available sucrose, resulting from reduced carbon fixation or a favoring of starch biosynthesis. Barnes (1971) exposed five pine (*Pinus spp.*) species to either 0.05 or 0.15 $\mu l\ l^{-1}$ O_3 continuously for 5-22 weeks. Analysis of primary needles indicated an increase in total soluble sugars as a result of the fumigations. Tingey, Wilhour, & Standley (1976) found much the same results in ponderosa pine (*P. ponderosa*) exposed to 0.10 $\mu l\ l^{-1}$ O_3 for variable periods up to twenty weeks. Not only did soluble sugar levels increase in the needles and stems, but starch levels also increased. Barnes (1971) had suggested that the increase in soluble sugars may be a result of increased starch hydrolysis, but the results from Tingey's study indicate that this does not occur. A more likely explanation is that translocation of the sugars from the needles is reduced. Measurements of root carbohydrate levels from the second study in fact showed a decrease in both soluble sugars and starch in the roots. There is a lack of information relating differences in plant susceptibility to air pollutants to the effects pollutants have on sugar/starch ratios. There is some evidence indicating that pollutant injury may occur preferentially when sugar levels are low (Koziol & Jordan 1978). However, a study by Dugger, Koukul, & Palmer (1966) found that the ozone sensitive tobacco variety Bel-W3 had higher levels of soluble sugars in older leaves than did a more resistant variety.

1.2 Sulfur Dioxide

1.2.1 Growth

Due to plant requirements for sulfur, the effects of SO_2 on plant growth are much more complex than those of O_3. Sulfur dioxide is a primary pollutant emitted directly from the combustion of fuels. Although levels of SO_2 are quite low in most areas, they can reach exceptionally high levels in areas surrounding point sources of SO_2, such as those areas around power plants or ore smelters. At the high levels found in these areas, SO_2 can have a impact on plant growth. Low levels of SO_2 are also able to reduce plant growth in many situations, but this is complicated by the nutritive capabilities of sulfur, particularly in soils where sulfur is limited in availability. Sulfur dioxide appears to have less of an effect on the Cherry Belle radish in terms of growth, but damage can still occur. Tingey *et al.* (1971) determined that there was no reduction in shoot dry weight after long-term exposure to SO_2, but there was a significant reduction in the dry weight of the fleshy hypocotyl (root). Reinert & Sanders (1982) observed decreases in both shoot and hypocotyl dry weight in response to 0.30 $\mu l\ l^{-1}$ SO_2 exposures. Continuous exposure to 0.20 $\mu l\ l^{-1}$ SO_2 for 20 days reduced hypocotyl dry weight and root/shoot ratio (Godzik, Ashmore, & Bell 1985).

Growth of many other species is sensitive to SO_2 as well. Generally, a higher dose of SO_2 is required than O_3 to cause a similar level of injury. Reich & Amundson (1984) found that exposure of soybeans to 0.06 or 0.11 $\mu l\ l^{-1}$ SO_2 resulted in a slight, but significant decrease in mass per seed. However, other measurements of growth and yield did not reveal any other significant reductions. Jack pine (*P. banksiana*) exposed to a mean SO_2 concentration of 0.30 $\mu l\ l^{-1}$ for 3 h/day on a

regular basis for up to 8 weeks showed decreases in both leaf and stem dry weight, although there was considerable variation in plant response (L'Hirondelle, Addison, & Huebert 1986). Olszyk *et al.* (1986) found a decrease in lettuce yield in response to continuous SO_2 exposures using an open-top chamber system. This occurred only if a high (0.15 $\mu l\ l^{-1}$) concentration was used; lower concentrations did not significantly affect yield.

Differences in species sensitivity to SO_2 was demonstrated by Maas *et al.* (1987). Clover (*Trifolium pratense*), soybean (*G. max*), and kidney beans (*P. vulgaris*) were all exposed to 0.25 $\mu l\ l^{-1}$ SO_2 for two weeks. Fresh weight was not affected in either clover or kidney bean, but fresh weight decreased in soybean.

1.2.2 Biomass Partitioning

While SO_2 does not seem to have as dramatic an effect on root/shoot ratios and biomass partitioning as O_3, it can have significant effects. Jones & Mansfield (1982) exposed timothy grass (*Phleum pratense*) to SO_2 using both long and short-term fumigations. Long-term studies were either 0.06 $\mu l\ l^{-1}$ for eleven weeks or 0.11 $\mu l\ l^{-1}$ for six weeks. Short-term studies were either 0.40 or 0.80 $\mu l\ l^{-1}$ given for 1.5-3.0 hours every day for one week; this was meant to simulate periodic SO_2 episodes which occur in the field. All the treatments significantly reduced root/shoot ratios, although changes in this ratio were not evident in the long-term studies for the first three weeks. Shoot growth was never affected by the SO_2 treatments nor was total plant dry weight.

Oats (*Avena sativa*) were found to be sensitive as well (Heck & Dunning 1978). Short-term exposures to a high level of SO_2 (0.40 $\mu l\ l^{-1}$) reduced root growth

by 12% while shoot growth was unaffected. Flagler and Younger (1982) conducted a much longer term experiment (twelve weeks at 0.10 μl l^{-1} SO_2) and obtained much the same result: shoot dry weight was the same as untreated plants but root dry weight decreased 11%.

Sulfur dioxide can also interact with other pollutants in disturbing root/shoot ratios. Gould and Mansfield (1988) exposed winter wheat (*Triticum aestivum*) to a combination of SO_2 and nitrogen dioxide (NO_2), both at 0.08-0.10 μl l^{-1} for four weeks. After three weeks, root dry weight was decreased, although neither shoot dry weight nor yield was affected. After four weeks, pollutant treatment had decreased all these parameters. The same experiment, except for conditions of lower light in the growth chamber in which the plants were raised, magnified these effects. Root growth was affected after only two and yield after three weeks . Shoot growth remained unaffected throughout the experimental period. Low light may intensify the shifts in allocation seen with air pollutants in order to counteract reduced assimilation of carbon.

1.2.3 Stomatal effects

Sulfur dioxide effects on stomata are dependent on the dose of SO_2, the plant species involved, and the environment. Increased stomatal opening caused by SO_2 has been reported for corn (*Zea mays*) (Unsworth, Biscoe & Pinckney 1972), radish and tobacco (Black & Unsworth 1980), *P. vulgaris* (Ashenden 1979), and *Atriplex* spp. (Winner & Mooney 1980b). In most cases this response occurs at low doses of SO_2 and usually leads to a reduction in plant growth greater than that of plants which do not respond in this manner to increased SO_2 uptake. For example, in the study by Winner & Mooney, SO_2 concentrations of 0.2, 0.5, and 0.9 μl l^{-1} for 8 h increased

stomatal conductance of two different *Atriplex* species, one a C_3 and the other a C_4 plant. Exposure of the C_4 species resulted in stomatal closure. Stomatal closure has been reported in a number of other species as well. These include jack pine (L'Hirondelle & Addison 1985), birch (*Betula papyrifera*) (Norby & Kozlowski 1982), radish (Black & Unsworth 1980), *Heteromeles* and *Diplacus* (Winner & Mooney 1980a), and sunflower (*Helianthus annus*) (Omasa *et al.* 1985). In general, this response to SO_2 is seen at higher doses, but can occur when SO_2 is present at low concentrations as well. A 30 h 0.2 or 0.8 $\mu l\ l^{-1}$ SO_2 exposure caused stomatal closure in *Betula*, particularly when grown under a high humidity regime. Both *Heteromeles* and *Diplacus* exhibited an almost linear decrease in stomatal conductance in relation to the level of SO_2 employed. Leaf conductance of two pea cultivars differing in sensitivity to SO_2 decreased within 75 minutes of the initiation of a 0.80 $\mu l\ l^{-1}$ fumigation (Alscher, Bower, & Zipfel 1987). Kropff (1987) observed a decrease in conductance of faba bean exposed to SO_2. This decrease occurred only after a decrease in photosynthesis was observed, leading to the conclusion that the decrease in conductance was a result of photosynthetic depression.

Although stomatal responses are often postulated as a means of resistance to pollutants, little evidence has been given for such a mechanism in radish. Sulfur dioxide, in fact, has been shown to stimulate stomatal opening in the cv Champion, while O_3 causes stomatal closure in this cv, and a mixture of the two pollutants creates a stomatal resistance greater than that of O_3 alone (Beckerson & Hofstra 1979).

1.2.4 Photosynthesis

Sulfur dioxide also has effects on biochemical processes within the plant, although the exact nature of these effects is still being investigated. Net photosynthetic rates of many plants have been shown to decrease during SO_2 fumigations. Species include *Picea sativus* (Bull & Mansfield 1974), and *V. faba* (Black & Unsworth 1979). As with O_3, even when stomatal factors affecting photosynthesis have been eliminated, photosynthesis declines are still seen. This was shown for *Heteromeles arbutifola* and *Diplacus auranticus* through the use of CO_2 curves; even at high CO_2 levels at which stomatal effects on internal CO_2 concentrations were reduced, photosynthetic declines were still observed for both of these species while under SO_2 stress (Winner & Mooney 1980a). Atkinson, Winner, & Mooney (1986) reported similar reductions in photosynthesis of *Heteromeles* using a different gas exchange system under a similar exposure regime. A similar situation was seen in faba bean, in which photosynthetic declines were linearly related to the rate of SO_2 uptake. Again, CO_2 curves were employed to segregate stomatal and non-stomatal factors. At a given C_i value, photosynthetic rates for non-fumigated plants were higher than for fumigated plants, indicating stomatal closure alone could not account for the observed decreases in photosynthesis (Kropff 1987). Muller, Miller, & Sprugel (1979) observed reduction in soybean photosynthesis exposed to high concentrations of SO_2 while lower levels either had no effect or slightly increased photosynthetic rates.

Carlson (1983) measured soybean photosynthetic response to SO_2 fumigations at different concentrations. Concentrations as low as 0.2 $\mu l\ l^{-1}$ for 2 h caused inhibition of photosynthesis, and inhibition quickly increased as SO_2 levels increased. Reduction in photosynthesis was found to be highly correlated with the

total amount of SO_2 absorbed. Radish leaves are also sensitive to SO_2. Light and CO_2 curves of radish leaves exposed to filtered air or 0.40 $\mu l\ l^{-1}$ SO_2 for 10 h/day every day revealed that SO_2 reduced both light and CO_2 saturated photosynthetic rates, and increased the light compensation point (Mooney *et al.* 1988).

1.2.5 Translocation and Carbohydrate Studies

Koziol & Cowling (1980) exposed ryegrass, (*Lolium perenne*) to 0.15 $\mu l\ l^{-1}$ SO_2 for a period of approximately two months. The amount of carbon, in the form of free carbohydrate, increased in the leaves, while storage carbohydrate levels decreased. Bitter lemon (*Citrus limon*) leaves exposed to either O_3 or SO_2 had increased sugar and decreased starch levels (Dugger & Palmer 1969). This increase in sugar levels may be a response to the decreased levels of photoassimilate available due to pollutant effects on photosynthesis. Leaf demand for sucrose would be such that a greater percentage of carbohydrate is required for immediate use, making less available for storage. This could also account for the decreased amount of carbon translocated.

Noyes (1980) attempted to circumvent this problem in a study involving *P. vulgaris* and the effects of SO_2 on translocation. The methodology employed in this experiment was to first find a pollutant level at which there was no photosynthetic depression, and then determine if translocation was affected at this level. Sulfur dioxide levels of 3.0 and 1.0 $\mu l\ l^{-1}$ for short periods (< 2 h) were found to greatly inhibit photosynthesis, but 0.10 $\mu l\ l^{-1}$ SO_2 had no apparent effect during a two hour exposure. Estimates of translocation indicated a significant decrease in carbon export from the fumigated leaf. Autoradiographs made of the leaf showed that much of the label remained in the minor veins of the leaf. Noyes concluded that phloem

loading was the limiting step in this process and may be where SO_2 was affecting the translocation process rather than decreasing the amount of translocatable material. Unfortunately, this experiment did not determine where labeled carbon was going, only the rate at which the amount of activity in the labeled leaf decreased. As a result, it was not determined if translocation to various sinks was affected equally.

A similar study involving *P. vulgaris* also found that translocation may be sensitive to SO_2 beyond what would be predicted from merely measuring photosynthetic rates (Teh & Swanson 1982). In this experiment, an exceedingly high level of SO_2 was used (2.9 μl l^{-1}). Photosynthesis of the primary leaf decreased 75% and translocation rate decreased by 45%. Using a regression equation for translocation rate on net photosynthetic rate, it was determined that the observed inhibition of translocation was about 25% greater than the predicted inhibition. It was again suggested that phloem loading may be the site of action for SO_2 effects on translocation. However, this level of SO_2 would seemingly also have a great potential to severely damage or kill the leaf completely. It is not clear whether similar effects would be seen at more realistic SO_2 levels.

One hypothesis concerning the reduction in translocation due to air pollutant exposure concerns the differences in susceptibility of young and old leaves of plants. Various studies have generally shown that leaves at or near full maturity are more sensitive to O_3 or SO_2. Craker and Starbuck (1973) found that intermediate age tobacco leaves had higher rates of uptake of either O_3 or SO_2 than young or old leaves. The intermediate age leaves also had the greatest amount of visible injury. In *P. vulgaris*, translocation of ^{14}C was reduced more in the older primary leaves than in the trifoliate leaves (McLaughlin & McConathy 1983).

1.3 Genetics

Intraspecific variation to air pollutants is well known for many species including radish and lettuce (Reinert *et. al.* 1972), *Pinus strobus* (Houston & Stairs 1973), *Geranium carolinianum* (Taylor 1978), cucumber (*Cucumis sativus*) (Bressan *et al.* 1981), and *P. vulgaris* (Butler & Tibbits 1979a). Genetic studies of these plants have shown that control of resistance appears to be multigenic due to the quantitative rather than qualitative distribution of resistance characters in a population. Plants tend to have a degree of resistance rather than either absolute resistance or sensitivity, as there is usually a complete range of plant responses seen, ranging from near absence of injury to plants which have severe signs of injury. Mechanisms of resistance remain unclear; differences in the ability to avoid pollutant uptake have been implicated in resistance mechanisms for some species (Butler & Tibbits 1979b), but they do not appear to be important in others (Taylor & Tingey 1981).

Naturally occurring resistance to pollutants, particularly SO_2, has been observed in many species. Five species of pasture grass growing in a highly SO_2 polluted area of Britain were compared with populations of the same species growing in non-polluted areas. Prolonged exposure with SO_2 in a controlled fumigation system indicated that on a dry weight basis, the grass populations from the polluted site were more resistant to SO_2 than the populations from the clean areas (Ayazloo & Bell 1981).

It was determined that selection for SO_2 resistance in *P. pratense* and *Lolium perene* could occur within four to five years for these annuals (Bell, Ayazloo, & Wilson 1982, Wilson & Bell 1985). Several physiological and morphological surveys

were made on these populations in order to determine possible tolerance mechanisms. Neither stomatal size nor stomatal frequency could be useful in distinguishing the relative tolerance of the plants. However, in clean air, tolerant plants of each species had lower stomatal conductance values than did the sensitive plants. Under SO_2 exposure conditions, there was no difference in conductance between tolerant and sensitive plants. Other measurements, including membrane permeability and uptake of both $^{14}CO_2$ and $^{35}SO_2$ revealed little insight into the differences which may account for the differential response observed in these grasses (Ayazloo, Garsed, & Bell 1982).

The most intensive breeding study of air pollution resistance involved the annual weed species *G. carolinianum*, collected from sites near a Georgia power plant which was a source of high levels of SO_2. Populations of this species located varying distances from the point source were collected and then inbred for five generations until their response to SO_2 fumigations were predictable based on visible injury. Crosses were then made between parents of varying resistance or sensitivity to SO_2, and the resulting F_1's were then tested for their response to the same pollutant. Crosses of two highly resistant parents resulted in a distribution of progeny exhibiting a positive skewness toward resistance. When two sensitive parents were crossed, the offspring showed a complete range of SO_2 responses from highly resistant to highly sensitive. Regression analysis indicated that fifty percent of observed variation in these plants was due to genetic effects. It was also concluded that SO_2 resistance was probably a multigenic factor due to the continuous distribution of responses exhibited in these populations (Taylor & Murdy 1975, Taylor 1978).

Gas exchange studies of resistant and sensitive plants of this species were carried out in order to evaluate pollutant flux into the leaves. There was little difference in SO_2 flux between resistant and sensitive plants, and resistant plants had to absorb 30% more SO_2 before showing an injury response similar to the sensitive plants. This implies that the genetically differential response in *G. carolinianum* is due not to stomatal factors which could limit pollutant uptake, but is a result of a physiological or biochemical process in the plant interior (Taylor & Tingey 1981). Later work with these same populations further elucidated potenially important differences. Photosynthesis of the sensitive ecotype was sensitive to lower doses of SO_2 than was the resistant ecotype. The degree of inhibition was also less in the resistant ecotype for SO_2 exposures of 5 h at concentrations below 0.60 $\mu l\ l^{-1}$. These alterations in photosynthesis were apparent in the changes seen in biomass. Resistant plants exposed to 0.45 $\mu l\ l^{-1}$ SO_2 had 22% greater biomass than control plants, whereas biomass of the sensitive plants decreased 10% under fumigated conditions (Taylor, Tingey, & Gunderson 1986).

1.4 Objectives

The specific objectives of these studies were:

1. To determine whether resistance and sensitivity of radish plants to O_3 and SO_2 was heritable from generation to generation.

2. To determine the effect of O_3 and SO_2 on biomass allocation in radish populations with differential sensitivity to these pollutants.

3. To determine the effect of O_3 on the concentrations of leaf carbohydrates in resistant and sensitive populations.

4. To determine how transport and allocation of ^{14}C is affected in the two radish populations when exposed to O_3.

5. To determine the gas exchange responses of differentially sensitive radish populations exposed to O_3.

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2. Breeding Program for Development of Differentially Sensitive Radish Populations

2.1 Introduction

Cultivated radish (*Raphanus sativus* L.) is a member of the Cruciferae family, which also includes *Brassica spp.* Several members of this family, including *Raphanus*, have a strong sporophytic self-incompatibility system, making the production of inbred lines difficult. Although techniques for production of inbreds exist, they tend to be labor intensive and seed production per plant is greatly reduced. The cv Cherry Belle is typical of most radish cultivars in that it is produced through cross-pollination. This cv is produced through open pollination, in which individual plants are selected for the desired characters and these plants are then allowed to interbreed at random with all other selected plants. This process is repeated through as many generations as needed until stable lines develop.

Dating back to the early 1970's, this particular cultivar has been the subject of several experiments involving plant-air pollutant interactions. Reinert, Tingey, & Carter (1972) compared the response of several commercial radish cultivars to single 1.5 h ozone (O_3) exposures of 0.35 $\mu l\ l^{-1}$. The Cherry Belle cultivar was among the most sensitive of the cultivars examined in terms of visible leaf injury. Experiments from the early 1980's found a great degree of variability of response within this cultivar when exposed to either O_3 or sulfur dioxide (SO_2). This made for difficulty in determining plant response to these pollutants. It was decided to attempt to exploit this variability in an attempt to develop populations of radish from the cv Cherry Belle for their relative sensitivity to these two pollutants.

2.2 Materials and Methods

2.2.1 Parent Propagation and Selection

Seeds of radish cv Cherry Belle were used in this study because this plant has been previously shown to be sensitive to air pollutants, and it can be rapidly brought to flower and seed. Seeds were obtained from Park Seed Co. (Greenwood, SC).

In order to reduce possible sources of variation in growth of the plants only seeds between 2.00 and 2.36 mm diameter were used. Previous work with wild radish (*R. raphanistrum*) has demonstrated a correlation between seed size and eventual plant size (Stanton 1984). Several seeds were sown in one liter pots containing a 3:1 (v:v) mixture of vermiculite and clay with 6 g of Osmocote (14:14:14) fertilizer (Sierra Chemical Co., Milpitas, CA) added to each pot. Seedlings were thinned to one per pot five days after emergence. Plants were maintained in a greenhouse equipped with high pressure sodium 1000 W lamps set to provide a 16 h daylength. These lamps maintained a minimum intensity of 250 μmol m^{-2} sec^{-1} PAR. Radishes are long day plants. Flowering was initiated by increasing the daylength to 24 h beginning the first day after air pollution exposures were completed. Bolting began to occur approximately 40-45 days after emergence, the first flowers appeared within 10-14 days following bolting.

2.2.2 Air Pollutant Exposures

Ozone and SO_2 exposures were carried out in continuous stirred tank reactors (CSTR). Fumigations were conducted three days each week for four hours

per day beginning ten days after emergence and continuing until the plants were fully grown, thirty days from emergence. Chamber lighting, provided by 1000 W metal halide lamps, was approximately 600 μmol m^{-2} sec^{-1} PAR. Compressed SO_2 (1.7% in N_2) was supplied from a cylinder and diluted to a level of 0.50 μl l^{-1}, while O_3 was generated from compressed O_2 with a Welsbach Model T-408 ozone generator (Welsbach Ozone Systems Corp., Philadelphia, PA) at a level of 0.10 μl l^{-1}. These exposures were not intended to simulate ambient conditions but rather to characterize the response of plants to air pollutant concentrations that produce obvious adverse growth effects. Sulfur dioxide was measured by a Series 43 pulsed fluorescent analyzer (Thermo-Electron Corp. Hopkinton, MA), O_3 by a Model 8002 O_3 analyzer (Bendix Corp. Lewisburg, WV). Calibrations of the instruments were made at intervals of two weeks along with single span checks every few days. For the SO_2 monitor, calibrations were made with an SO_2 calibrator (model CS10-2, Meloy Laboratories Inc., Springfield, VA) with a certified permeation device (Dynacal, Vici Metronics, Santa Clara, CA). The O_3 monitor was calibrated with a CSI Model 3000 O_3 Calibrator (Columbia Scientific Instruments Corp., Austin, TX)

2.2.3 Parental Screening and Selection

One hundred fifty plants were exposed to either O_3 or SO_2 and were moved daily from the greenhouse to the fumigation chambers. Three chambers were used for each pollutant. Sixty plants, divided between two chambers, were exposed to filtered air and used as control plants. Plants were rotated daily between chambers to reduce chamber effects. Two days after the final exposure, all plants of the parent populations were visually rated on the basis of hypocotyl size and visible leaf injury

as a means of selecting resistant and sensitive individuals to each pollutant. Visible injury was estimated as the percent of total leaf area showing symptoms typical of the particular pollutant. Hypocotyl size was visually estimated and ranked on a scale of 1 (smallest) to 4 (largest). For each of the two treatments (O_3 and SO_2), the fifteen most resistant, those with large hypocotyls and fifteen most sensitive, those with small hypocotyls, individuals were selected. These four groups were then physically isolated from one another in order to avoid unwanted cross pollinations and allowed to flower. Unless insects, the main vector for transmission of crucifer pollen, are present, such cross pollinations are unlikely to occur as pollen from radish is heavy and not wind borne.

2.3.3 Cross Pollinations

Cross pollinations were made by hand between individuals within each of the four parent groups on a daily basis to promote a large seed set. Pollinations were generally made in the morning. Individual stamens were removed from newly opened flowers using forceps. Prior to removing the stamens from the flowers, the forceps were dipped in alcohol to kill any stray pollen on them and allowed to thoroughly dry. Pollen from this stamen was then transferred to stigmas on other plants within the same group by rubbing the anther directly on the stigma. Approximately thirty days after pollination the siliques, the fruiting bodies, matured and were harvested. They were dried, and seeds were collected from all parents within a group.

2.3 Results

The degree of variation in hypocotyl size among all the plants exposed to O_3 or SO_2 was greater than that among non-fumigated plants. Visible leaf injury, particularly in the form of necrosis, was evident on the majority of exposed plants. Average percent leaf injury of all plants treated with O_3 was 50%, while plants treated with SO_2 averaged 20%. Plants receiving filtered air lacked any visible injury symptoms commonly associated with either of these pollutants (Table 1).

Within the O_3 and SO_2 treatment groups, plants were selected for their resistance or sensitivity to the pollutants. Those selected for resistance to SO_2 had very little visible injury (10%) and hypocotyls which were ranked in the largest size class (Table 1). Several other plants also exposed to SO_2 were not selected as resistant even though they had similarly large hypocotyls, because they had slightly more leaf injury than those selected. Even plants selected for sensitivity to SO_2 had less visible injury than the mean for all plants treated with O_3. There were few plants treated with O_3 which had hypocotyls in the largest size class (size = 4), even among those selected as resistant. It was thus somewhat easier to rely on hypocotyl size for selection of resistant plants in the O_3 treatment group. Comparison of the mean hypocotyl size in the O_3, SO_2, and FA treatments shows that O_3 had a much more dramatic effect on hypocotyl size than did SO_2. The mean hypocotyl size for all SO_2 treated plants was only slightly less than that for all FA treated plants.

The total number of siliques and seeds collected from each of the four selected groups were counted (Table 2). Fewer siliques were collected from the

Table 1. Visible leaf injury and hypocotyl size of *Raphanus sativus* L. cv Cherry Belle plants exposed to 0.10 $\mu l\ l^{-1}$ O_3, 0.50 $\mu l\ l^{-1}$ SO_2, or filtered air (FA). Values represent the mean ± SD.

Selected Group	*n*	Visible Leaf Injury (%)	Hypocotyl Size
O_3R	15	30 ± 13	3.7 ± 0.46
O_3S	15	75 ± 12	1.5 ± 0.50
SO_2R	15	10 ± 3	4.0 ± 0.00
SO_2S	15	40 ± 15	2.6 ± 0.49
All O_3 treated	150	50 ± 17	2.5 ± 0.76
All SO_2 treated	150	20 ± 13	3.5 ± 0.61
All FA treated	60	0 ± 0	3.7 ± 0.54

Table 2. Number of siliques, seeds, and seeds/siliques collected from the selected plant groups following pollination.

Group	# of Siliques	# of Seeds	Seeds/Silique
O3R	643	1627	2.53
O_3S	947	1829	1.93
SO_2R	931	1728	1.86
SO_2S	937	1645	1.76

O_3R group than the others. Some plants from each of the groups died or were injured before producing seed. The cause of death appeared to be by either a fungus or bacteria entering the plant through the hypocotyl, which tended to split open as plant size increased. As a result, the number of seed producing plants from each group was not equal, making comparisons of actual seed production difficult.

Seed numbers were approximately equivalent for all groups, although the sensitive groups were originally comprised of smaller plants. However, after the pollutant treatments stopped, the sensitive plants appeared to catch up in growth to the resistant plants even before flowering occurred. Thus, there was generally little apparent difference in size of the plants by the time pollinations began.

2.4 Discussion

In the past, there have been some attempts to breed plants resistant to air pollutants. However, these tended to be strictly for commercial purposes and few if any physiological measurements were made on the resulting plants (Menser & Hodges 1972, Butler, Tibbitts, & Bliss 1979, Guri 1983). Many of these experiments were actually screening studies in which a number of cultivars were exposed to a particular pollutant, ranked for sensitivity based on visible leaf injury and then bred with other cultivars. The resulting offspring were then screened in order to see how they compared with the two parents.

Another group of studies, primarily in Great Britain, involved the development of tolerance to SO_2 in a number of pasture grass plants. The initial studies were not breeding studies, but were ecological studies concerned with the

development of resistant populations in areas affected by SO_2 (Bell & Clough 1973), similar to the development of heavy metal tolerant populations found near mine trailings (Antonovics, Bradshaw, & Turner 1971). Later work with some of these grass species did involve breeding for resistance and some physiological investigations into the differences among various differentially sensitive populations (Ayazloo & Bell 1981, 1982).

The only data from this experiment are observational, being in the form of visible leaf injury and hypocotyl size, neither of which was quantitatively measured. There was some relation between the two data sets however. The selected sensitive plants had on average more visible leaf injury than the other plants, while the resistant ones had less. This is expected since leaf injury was one of the selection factors. The work with *Geranium carolinianum* has shown a relationship between visible injury by SO_2 and plant growth. Originally, the differentially sensitive ecotypes were selected based on visible injury (Taylor & Murdy 1975). Later work revealed that there were significant differences based on growth as well (Taylor, Tingey, & Gunderson 1986). The emphasis for selection, though, was hypocotyl size. The reductions in hypocotyl size were much more evident in the O_3 treated than in the SO_2 treated plants (Table 1). Also, the range of hypocotyl sizes was greater in the O_3 treatment. the difference between the mean for resistant and sensitive plants was 2.22 in the O_3 treatment, but only 1.43 in the SO_2 treatment. Selection of the most resistant or sensitive plants is to some extent dependent upon the degree of stress present. If there is little stress, then the effect of the stress on the plant may not be sufficient enough to differentiate between resistant and sensitive plants. If

there is too much stress, then resistance in all plants may be broken, again making it difficult to distinguish among differing levels of resistance.

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3. Development of Populations of Radish Differing in Resistance to O_3 and SO_2

3.1 Introduction

Plant species have long been known to differ in physiological, growth, and foliar injury responses to air pollutants. Several studies have shown that individual plants within a species may also differ in these responses to toxic chemicals, including air pollutants (Taylor & Murdy 1975, Karnosky 1977, Alscher, Bower & Zipfel 1987). Some intraspecific differences in air pollution responses are thought to be genetically determined and therefore heritable traits. Such differential responses are of interest because they can ultimately affect fitness of individuals and exert selective pressure, leading to development of resistant populations.

The capacity for plant populations to evolve resistance to toxic substances has been best demonstrated with studies of weeds growing on mine waste, rich in heavy metals, (Antonovics, Bradshaw & Turner 1971). These metals act as a strong selective force, leading to development of resistant populations within a few generations. In a non-stressed environment, resistant individuals comprise only a small fraction of the total population.

Plant populations within species have also been found to differ in air pollution resistance. For example, ryegrass (*Lolium perenne*) growing in regions of Great Britain with high SO_2 concentrations was found to be more productive in SO_2 exposure experiments than populations taken from less polluted environments (Bell & Clough 1973). Ayazloo, Garsed & Bell (1982) collected this species as well as several other grasses from both polluted and non-polluted sites in northern England

and again found greater SO_2 resistance in the populations collected from the polluted areas. In another example, selection pressure from SO_2 seemed to account for differences in SO_2 responses observed for *Geranium carolinianum* populations found growing along a SO_2 gradient away from a power plant in Georgia (Taylor & Murdy 1975). The SO_2 resistant population produced SO_2 resistant offspring, indicating heritability of the resistance trait. Uptake of SO_2 by resistant and sensitive plants was similar, suggesting that either a detoxification or repair mechanism accounted for resistance (Taylor & Tingey 1983).

The possibility that plants can evolve resistance to gaseous air pollutants is important because selection processes of plant populations over wide areas may be altered. For example, although SO_2 is known to act as a selective agent, little is known about the capacity for O_3 to act as a selective pressure. Ozone is of particular importance because it is known to reduce growth of crops and trees, it is common over vast regions of North America and other industrialized areas, it is present in higher concentrations than SO_2, and it is more phytotoxic than SO_2.

In this study, an approach was developed for exploiting intraspecific differences in response to SO_2 and O_3 found in radish plants. First identified were individual plants that were either resistant or sensitive to SO_2 or O_3. These plants were then raised to flowering, cross pollinated within selected groups, and the seed collected. The seeds were then germinated, the seedlings fumigated, and plant growth analyzed, in order to determine whether resistance to SO_2 or O_3 was heritable after one generation. More specifically, since root growth is thought to be more sensitive to air pollution stress than shoot growth (Oshima *et al.* 1979), I wanted to determine whether selection for air pollution resistance would result in reduced

pollutant effects on root growth or whether resistance would be observed in shoot growth alone. These studies have been designed so as to indicate the potential for SO_2 and O_3 to act as selective agents, to identify the nature of inherited resistance, and the ability of these pollutants to alter the genetic structure of vegetation on a regional scale.

3.2 Materials and Methods

3.2.1 Parent Propagation and Selection

Radish cv "Cherry Belle" was used in this study because this plant has been previously shown to be sensitive to air pollutants, and it can be rapidly brought to flower and seed. Seeds were obtained from Park Seed Co. (Greenwood, SC).

Previous work with wild radish (*Raphanus raphanistrum*) has demonstrated a correlation between seed size and eventual plant size (Stanton 1984), so only seeds between 2.00 and 2.36 mm diameter were used. Seeds were sown in one liter pots containing a 3:1 (v:v) mixture of vermiculite and clay with 6 g of Osmocote (14:14:14) fertilizer (Sierra Chemical Co., Milpitas, CA) added to each pot. Seedlings were thinned to one per pot five days after emergence. Plants were maintained in a charcoal filtered greenhouse equipped with supplemental lighting (1000 W high pressure sodium lamps) set to provide a 16 h daylength with a minimum intensity of 250 μmol m^{-2} sec^{-1}. Plants were induced to flower by increasing daylength to 24 h one day after air pollution exposures were completed.

3.2.2 Air Pollution Exposures

Ozone and SO_2 exposures were carried out in continuous stirred tank reactors (CSTR). Exposures were given three days each week for four hours per day beginning ten days after emergence and continuing until the plants were fully grown (thirty days from germination in the parent population, thirty-five days in the F_1 population). Chamber lighting, provided by 1000 W metal halide lamps, was approximately 600 μmol m^{-2} sec^{-1}. Compressed SO_2 (1.7% in N_2) was supplied from a cylinder and diluted to a level of 0.50 μl l^{-1}, while O_3 was generated from compressed O_2 with a Welsbach Model T-408 ozone generator (Welsbach Ozone Systems Corp., Philadelphia, PA) at a level of 0.10 μl l^{-1}. These exposures were not intended to simulate ambient conditions but rather to characterize the response of plants to air pollutant concentrations that produce obvious adverse growth effects. Sulfur dioxide was measured by a Series 43 pulsed fluorescent analyzer (Thermo-Electron Corp. Hopkinton, MA), O_3 by a Model 8002 O_3 analyzer (Bendix Corp. Lewisburg, WV). Calibrations of both instruments were made at intervals of two weeks along with single span checks every few days.

3.3 Experimental Approach

3.3.1 Parental Screening and Selection

A protocol was devised for examining the capacity of O_3 and SO_2 to act as selective agents (Figure 1). One hundred fifty plants were exposed to either O_3 or SO_2 and were moved daily from the greenhouse to the fumigation chambers. Three chambers were used for each pollutant. Plants were rotated daily between chambers to reduce chamber effects. Two days after the final exposure, all plants of the parent

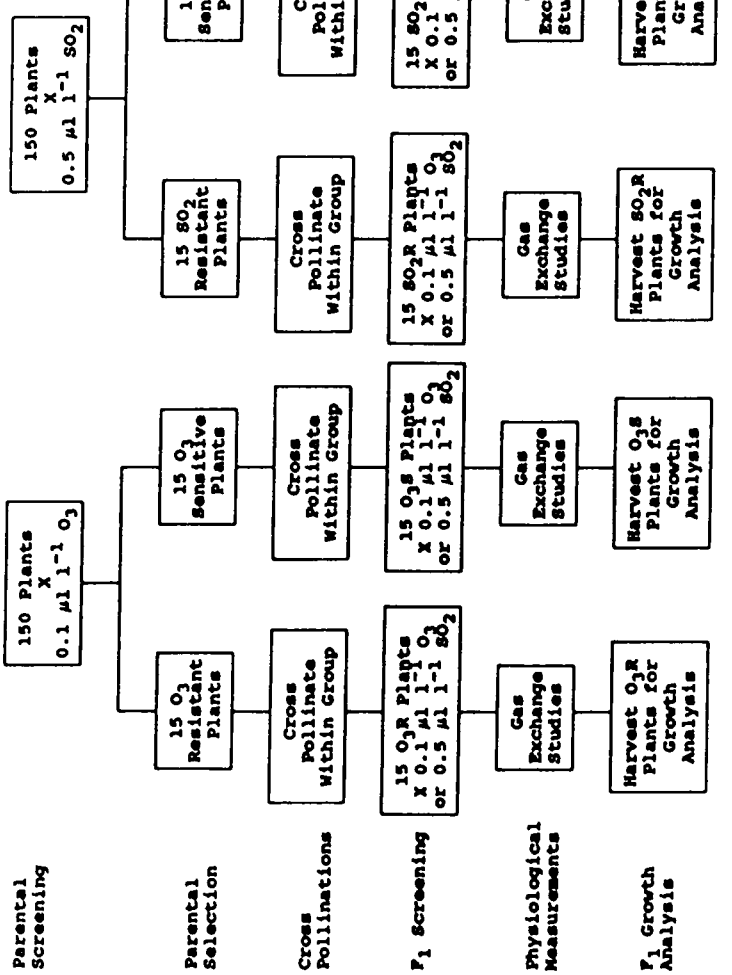

Figure 1. Experimental approach for determining the heritability of resistance and sensitivity of *Raphanus sativus* to O_3 and SO_2.

populations were visually rated on the basis of hypocotyl size and visible leaf injury as a means of selecting resistant and sensitive individuals to each pollutant. For each of the two treatments (O_3 and SO_2), the fifteen most resistant and fifteen most sensitive individuals were selected. These four groups were then isolated from one another and allowed to flower.

3.3.2 Cross Pollinations

Cross pollinations (Figure 1) were made by hand between individuals within each of the four parent groups on a daily basis to promote a large seed set. Thirty days after pollination the siliques matured and were harvested. They were dried, and seeds were collected from all parents within a group.

3.3.3 Screening of F_1 Plants

Four different populations were raised from the collected seed: O_3 resistant (O_3R), SO_2 resistant (SO_2R), O_3 sensitive (O_3S), and SO_2 sensitive (SO_2S), along with a non-selected 'Cherry Belle' population (NS) from the same seed source as the parents (Figure 1). Plants were grown and fumigated under the same conditions as the parent population. A total of twenty-five plants, five from each population, were placed in a chamber and exposed to either 0.10 $\mu l\ l^{-1}$ O_3, 0.50 $\mu l\ l^{-1}$ SO_2, or charcoal filtered air. Three chambers per treatment were used. Due to the occurrence of lower greenhouse temperatures than when the parent population was grown, plant growth rates were slower so plants were allowed to grow for thirty-five days before harvesting in order for them to reach a developmental stage similar to the previous generation.

3.3.4 Gas Exchange

Net photosynthesis and stomatal conductance were measured during the final two fumigations of the F_1 plants (Figure 1). Measurements were taken during the final hour of the fumigations, using a LI-COR 6000 gas exchange system (Lambda Instruments Inc., Lincoln NE) with a one liter cuvette. The mean photon flux during measurements was approx. 400 μmol m^{-2} s^{-1} and relative humidity was approx. 30%. Fully expanded leaves from the second leaf pair of five plants on both days from each group within each treatment were used, for a total of ten measurements per treatment per group.

3.3.5 Harvest

Two days after the final exposure in the F_1 population, all plants were harvested (Figure 1). Broken roots were collected by sieving the soil and were washed to remove any attached soil particles. Leaf area was measured with a LI-COR 3050 leaf area meter (Lambda Instruments Inc., Lincoln, NE), and plants were then divided into shoots (leaves), hypocotyl, and roots. All parts were then oven-dried at 60 C for one week before dry weights were measured. Analysis for statistical significance was performed with the Statistical Analysis System (SAS Institute, Raleigh, NC) using contrasts between means within treatments for comparisons.

3.4 Results

3.4.1 Whole Plant Responses

Differences in growth responses among the F_1 groups were apparent from the fumigations with either O_3 or SO_2 (Figure 2). In general, there was little or no

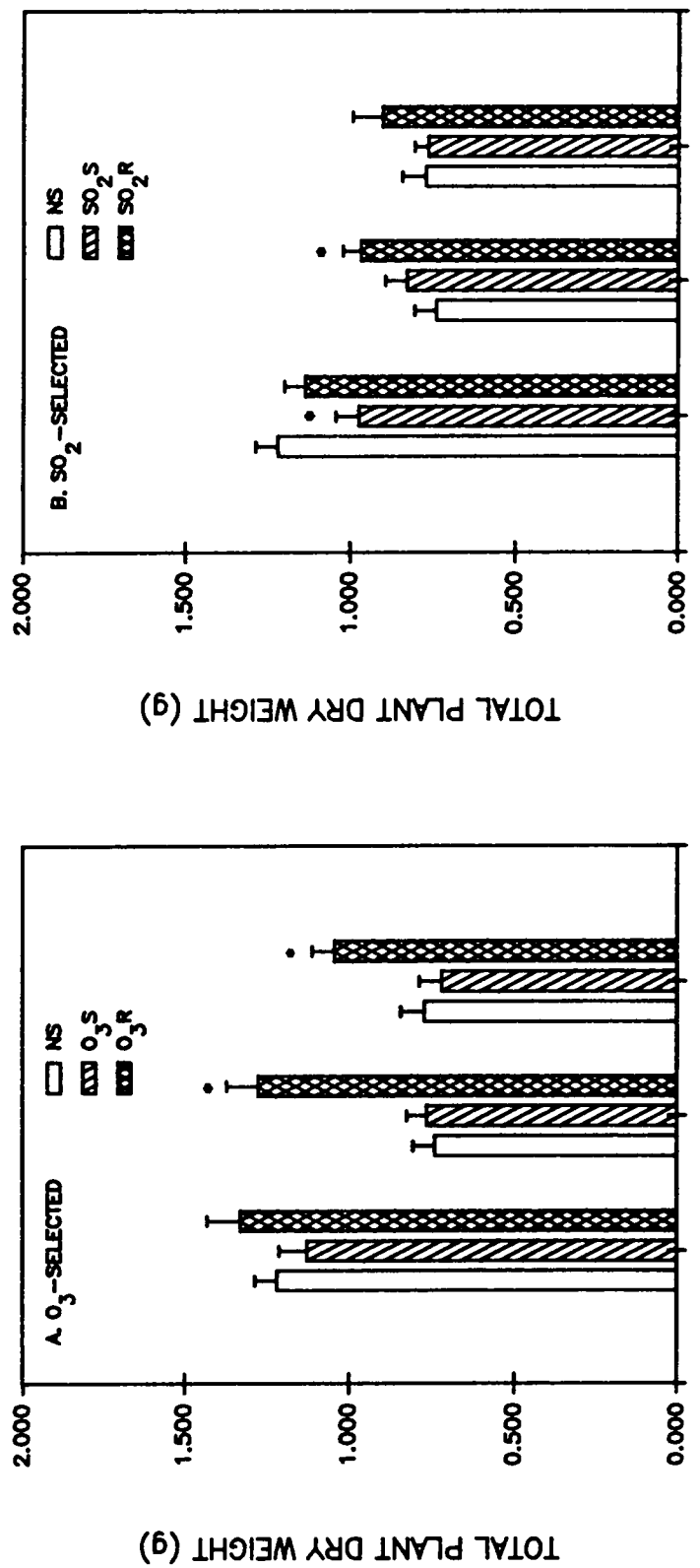

Figure 2. Total plant dry weight of A) O_3 selected and B) SO_2 selected plants to 0.1 $\mu l\, l^{-1}$ O_3, 0.5 $\mu l\, l^{-1}$ SO_2, or filtered air (FA). (Values are mean ± SE, n = 15, * = means significantly different than non-selected (NS) at the $P < 0.05$ level).

difference among the plant groups in the filtered air treatments. Total plant dry weight was reduced by both pollutants, but O_3R plants were apparently more resistant to both O_3 and SO_2 than either the O_3S or NS plants (Figure 2A). The SO_2-selected groups responded similarly except that the SO_2R plants did not have greater resistance than the NS plants to SO_2, only to O_3 (Figure 2B).

3.4.2 Shoot Responses

The air pollution-caused reductions in plant dry weights were in part related to reductions in shoot growth (Figures 3A and 3B). Even though shoot growth reductions did not account for all of the air pollution effects on plant biomass, distinctions could still be made among plants selected for air pollution resistance and either NS plants or plants selected for air pollution sensitivity. O_3R plants had greater shoot dry weights (Figure 3A) than the O_3S or NS plants following either O_3 or SO_2 fumigations. These differences were particularly striking in the O_3 treatment. SO_2R plants tended to be resistant to both pollutants, but shoots of the SO_2S plants also appeared fairly resistant to O_3, as both shoot dry weight and leaf area were similar in FA or O_3 treatments (Figures 3B and 4B). Although the NS population showed effects of fumigations, the shoot dry weight of neither the SO_2R nor the SO_2S groups appeared to be greatly affected by fumigations with either O_3 or SO_2.

Reduction in shoot dry weight could be due to either reduced leaf area or reduced leaf thickness. Measurements showed that air pollution-caused changes in plant leaf area were much the same as those of shoot dry weight. There was little, if any reduction of leaf area for the O_3R plants exposed to the pollutants, in contrast to the reductions observed in either the O_3S or NS plants (Figure 4A). Such

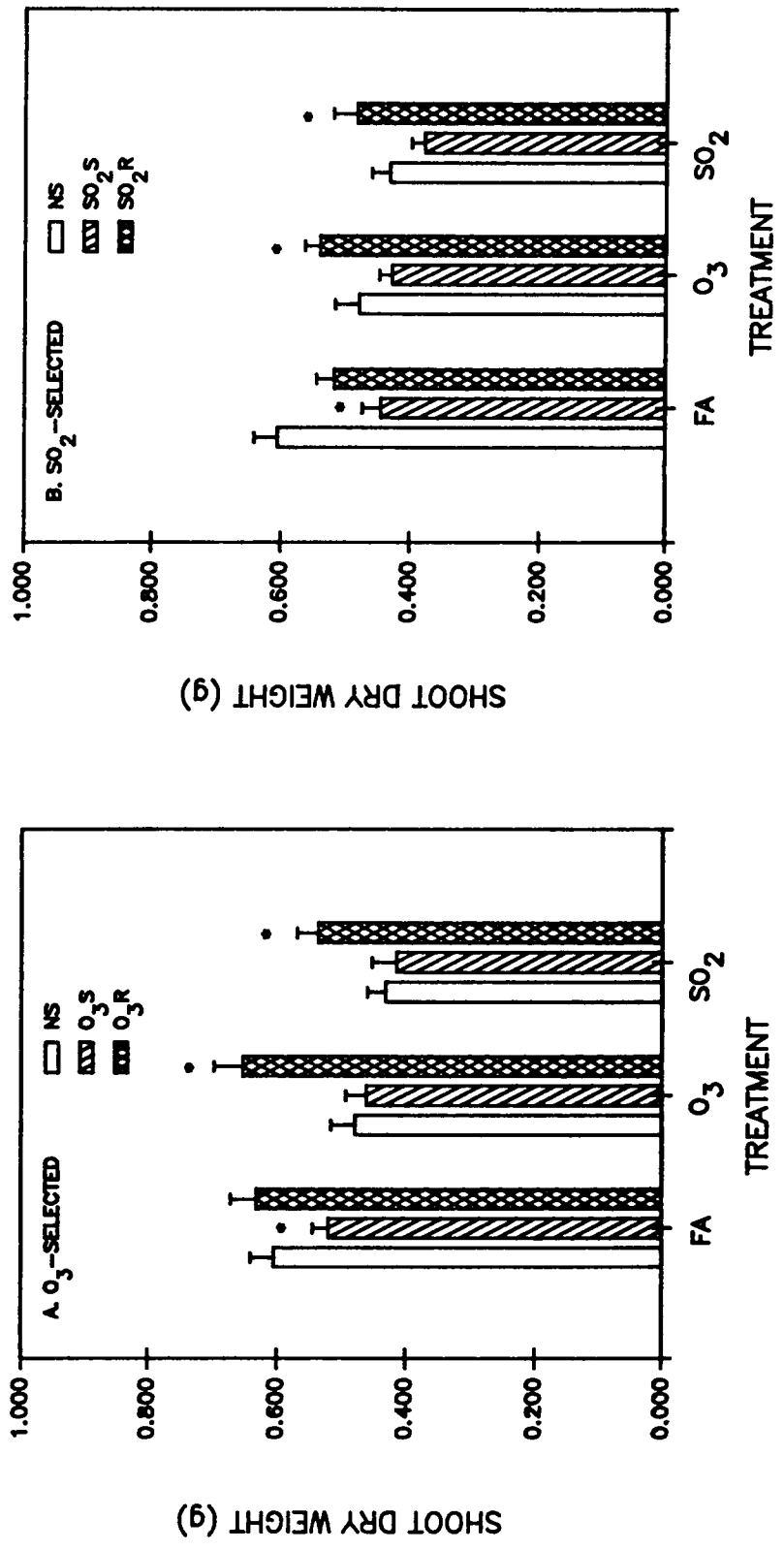

Figure 3. Shoot dry weight of A) O_3 selected and B) SO_2 selected plants to 0.1 μl l^{-1} O_3, 0.5 μl l^{-1} SO_2, or filtered air (FA). (Values are mean ± SE, n = 15, * = means significantly different than non-selected (NS) at the $P < 0.05$ level).

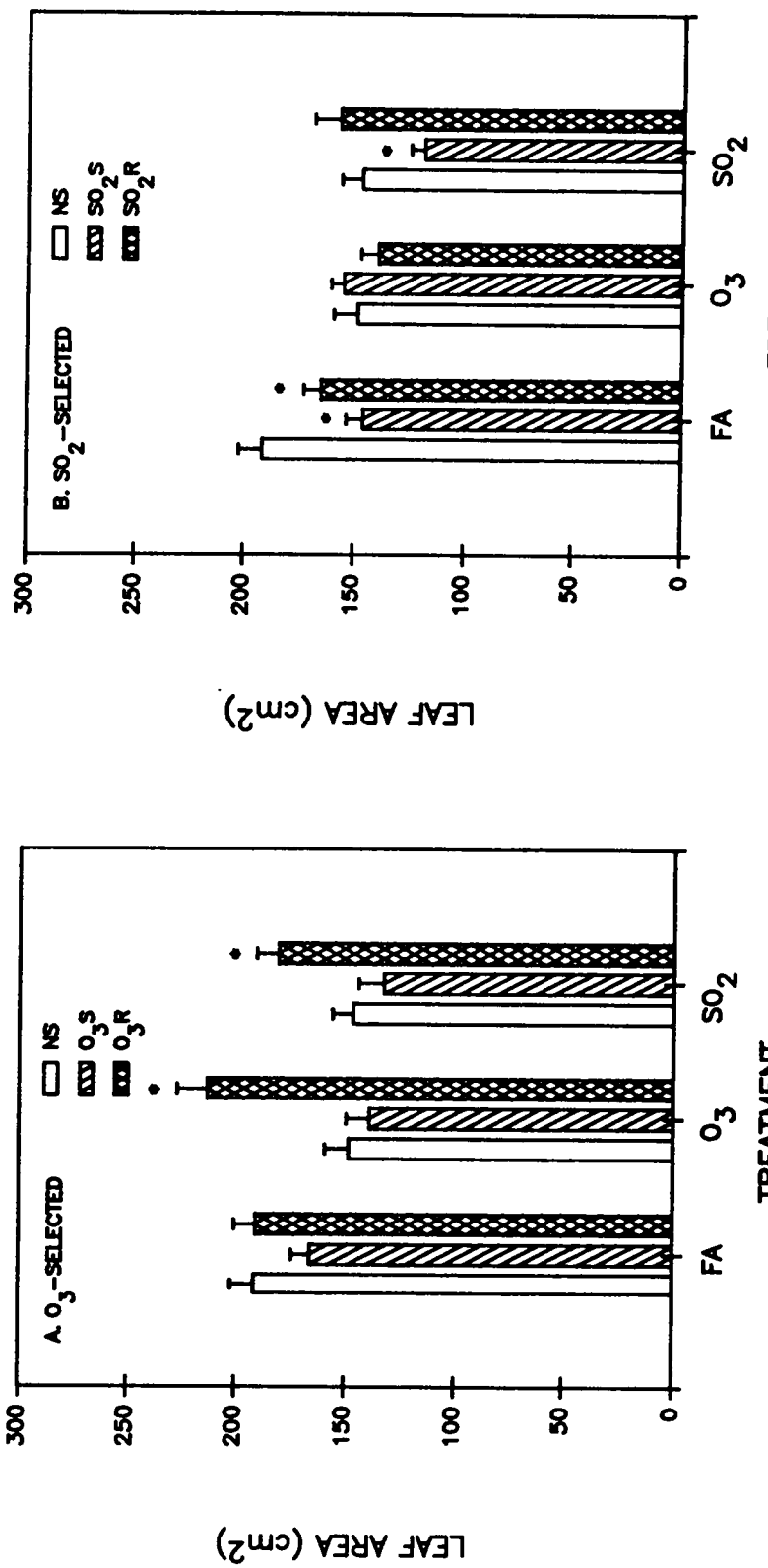

Figure 4. Leaf Area of A) O_3 selected and B) SO_2 selected plants to 0.1 $\mu l\, l^{-1}$ O_3, 0.5 $\mu l\, l^{-1}$ SO_2, or filtered air (FA). (Values are mean ± SE, n = 15, * = means significantly different than non-selected (NS) at the $P < 0.05$ level).

differences were not seen in the SO_2 selected plants, as leaf area in either the SO_2R or SO_2S plants was apparently little affected by either O_3 or SO_2 (Figure 4B).

3.4.3 Hypocotyl and Root Responses

The hypocotyl was generally very sensitive to pollutant exposure, with a few important exceptions (Figures 5A and 5B). Hypocotyl dry weight was more than 50% greater in the O_3R plants exposed to O_3 than either the NS or O_3S plants (Figure 5A). The O_3R hypocotyls exhibited little if any growth effects from O_3 fumigation. A similar situation was apparent in SO_2 fumigations. O_3R hypocotyl dry weight was reduced more by SO_2 than O_3, but the reduction was still far less than that of the other two groups. There was no difference between NS and the O_3S plants.

For the SO_2 selected plants, the general sensitivity of hypocotyl growth was apparent, but there was no difference between the two selected groups in either the O_3 or SO_2 treatments (Figure 5B). These data are complicated by the greater hypocotyl growth in O_3 of both of these groups than the NS plants. Both the SO_2R and SO_2S groups show resistance to O_3 in terms of hypocotyl growth as compared to the NS group.

True roots comprised less than 5% of the total plant dry weight, but were analyzed separately from the hypocotyl because of their biological importance. Root dry weight was affected similarly to hypocotyls in the O_3 exposures (Figure 6A). Growth of O_3R roots was slightly greater in the O_3 treatment whereas the roots of the other two groups was reduced slightly. No differences in root growth among the three groups was evident from SO_2 exposure (Figure 6A). Thus, selection for O_3

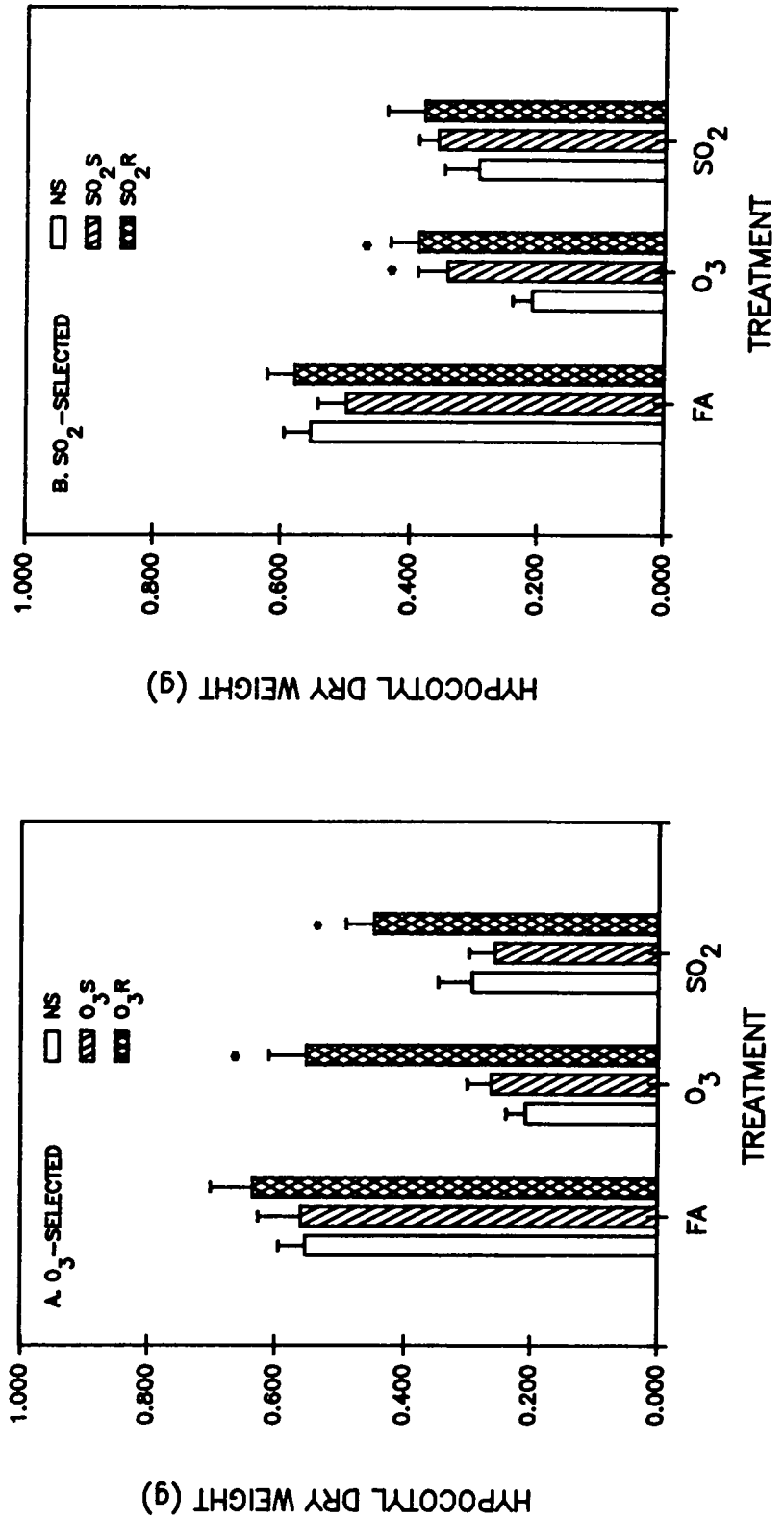

Figure 5. Hypocotyl dry weight of A) O_3 selected and B) SO_2 selected plants to 0.1 μl l^{-1} O_3, 0.5 μl l^{-1} SO_2, or filtered air (FA). (Values are mean ± SE, n = 15, * = means significantly different than non-selected (NS) at the $P < 0.05$ level).

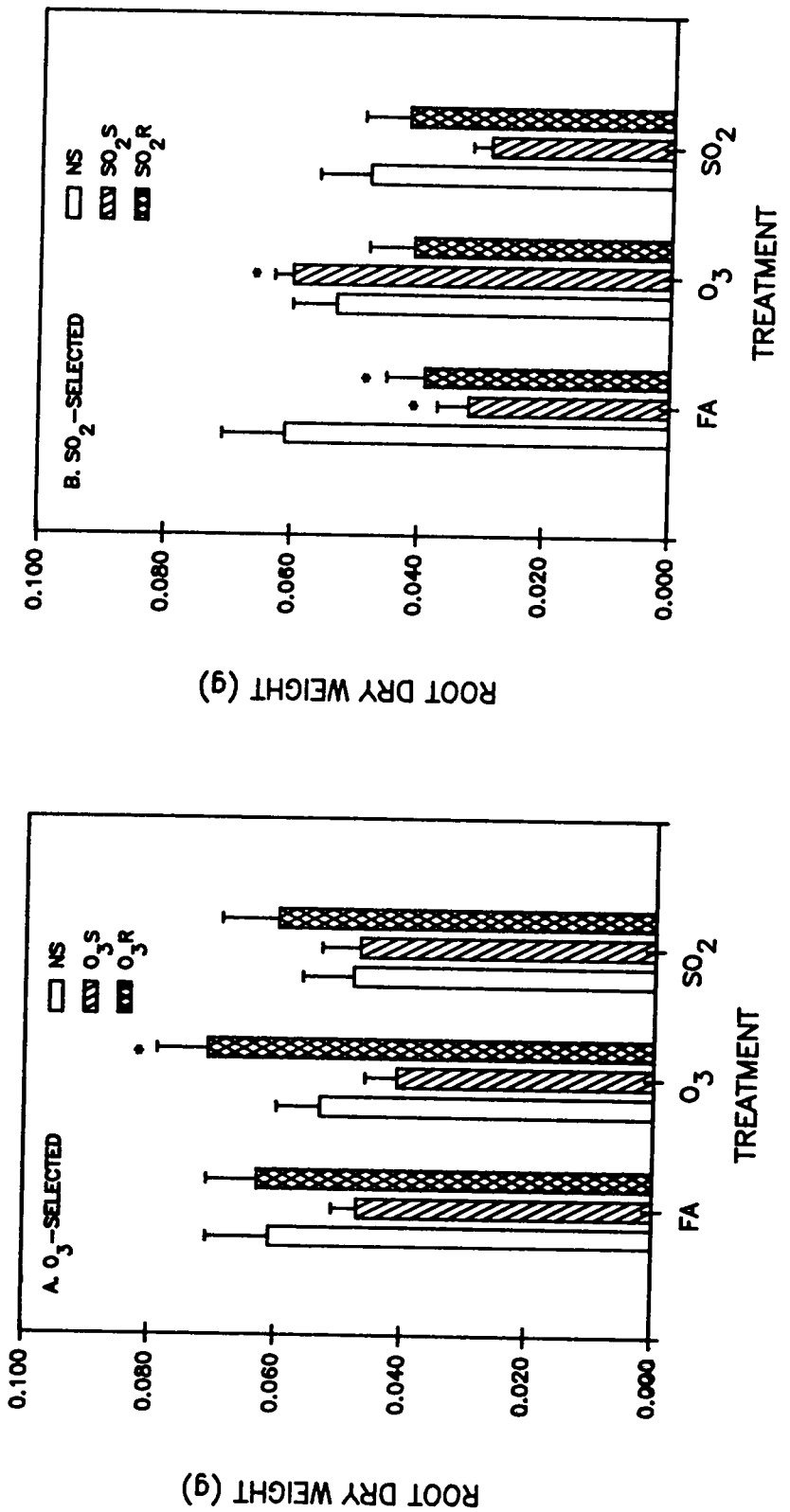

Figure 6. Root dry weight of A) O_3 selected and B) SO_2 selected plants to 0.1 μl l^{-1} O_3, 0.5 μl l^{-1} SO_2, or filtered air (FA). (Values are mean ± SE, n = 15, * = means significantly different than non-selected (NS) at the $P < 0.05$ level).

resistance yielded plants that produced roots resistant to O_3 exposures. Pollutant effects on root growth of the SO_2 selected groups varies markedly from group to group with no consistent pattern (Figure 6B). There is no apparent indication of resistance or sensitivity in any of these groups.

Because of the greater effect of air pollutants on below ground growth, root/shoot ratios tended to decrease as a result of pollutant exposure. As this measure incorporates all components of plant growth, it may be a particularly good indicator of plant resistance to stress. Steep declines in this ratio were seen in the O_3S and NS groups treated with either O_3 or SO_2, indicating sensitivity to the treatments (Figure 7). However, the O_3R plants had relatively unchanged root/shoot ratios, significantly greater in the O_3 treatment than the other two groups. This is in marked contrast to the SO_2 selected groups (Figure 7B), where all groups showed decreased root/shoot ratios from exposure to either O_3 or SO_2.

3.4.4 Gas Exchange Parameters

Net photosynthesis and stomatal conductance were measured on both fumigated and non-fumigated plants in the CSTR chambers in order to determine the effects of genetic selection and air pollution treatments on leaf gas exchange parameters. Ozone reduced stomatal conductance of both O_3R and O_3S plants (Table 1). One important effect is that O_3 reduced conductance of the O_3R plants by more than 50%. Thus, O_3 resistance may be due in part to O_3 exclusion during fumigation. Sulfur dioxide had no apparent effects on conductance although mean values for NS and SO_2S plants were higher in SO_2.

Photosynthesis values did not differ with either genetic selection or with treatment (Table 2). Apparently the stomatal response observed here neither had

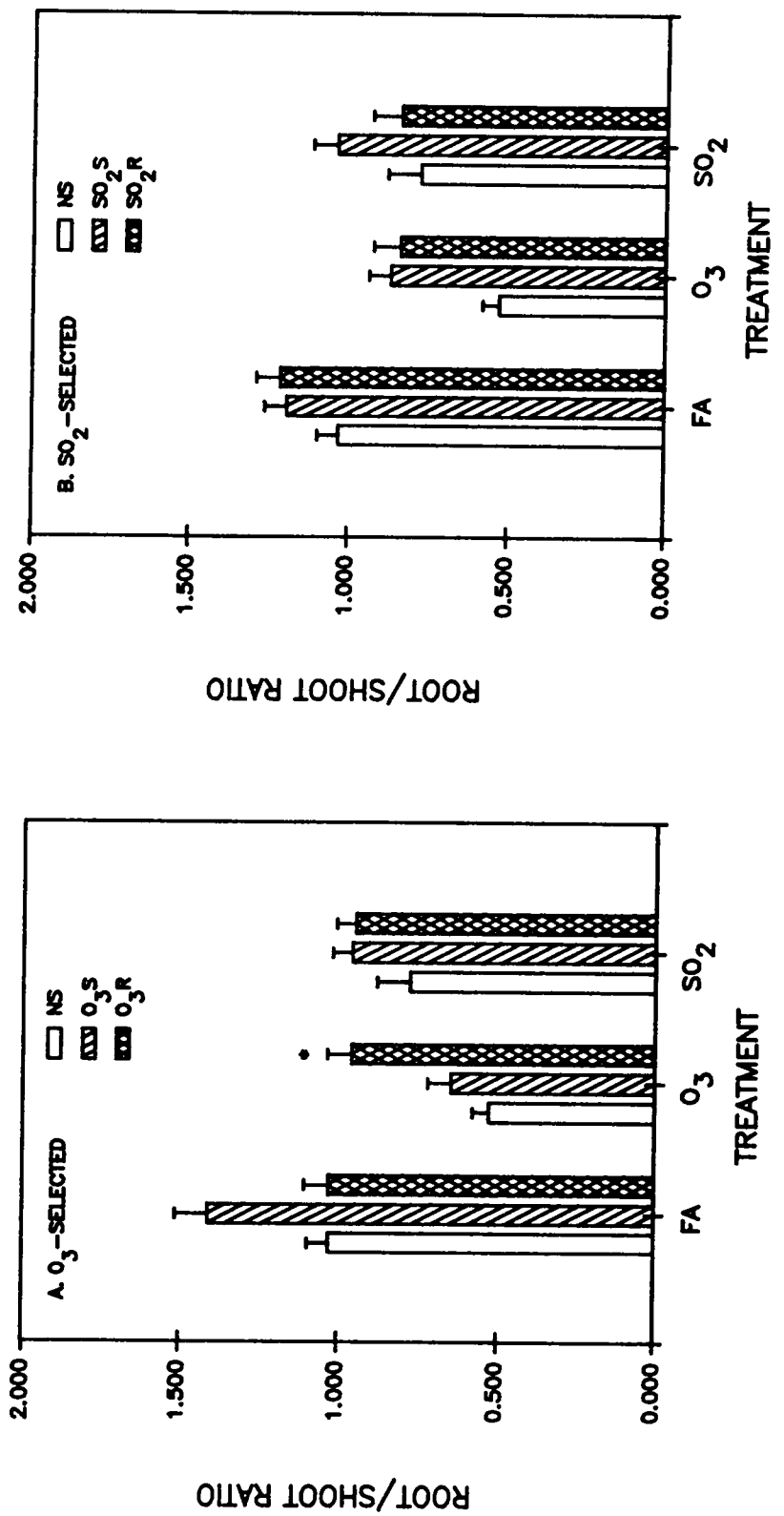

Figure 7. Root/Shoot Ratio of A) O_3 selected and B) SO_2 selected plants to 0.1 μl l^{-1} O_3, 0.5 μl l^{-1} SO_2, or filtered air (FA). (Values are mean ± SE, n = 15, * = means significantly different than non-selected (NS) at the $P < 0.05$ level).

Table 1. Stomatal conductance (mol H_2O m^{-2} s^{-1}) of leaves from 2nd leaf pair of plants at end of a four hour fumigation. Values are means ± SE, n = 10.

Plant Type	Treatment		
	O_3	FA	SO_2
O_3R	0.195 ± 0.026	0.467 ± 0.081	-
O_3S	0.446 ± 0.046	0.670 ± 0.078	-
NS	0.404 ± 0.087	0.334 ± 0.052	0.382 ± 0.052
SO_2R	-	0.420 ± 0.046	0.366 ± 0.063
SO_2S	-	0.347 ± 0.044	0.403 ± 0.048

Table 2. Net photosynthesis (μmol CO_2 m^{-2} s^{-1}) of leaves from 2nd leaf pair of plants at end of a four hour fumigation. Values are means ± SE, n = 10.

Plant Type	Treatment		
	O_3	FA	SO_2
O_3R	7.02 ± 0.66	6.81 ± 0.53	-
O_3S	6.76 ± 0.56	6.36 ± 0.79	-
NS	7.52 ± 0.41	5.91 ± 0.74	7.54 ± 0.84
SO_2R	-	6.90 ± 0.67	8.10 ± 0.92
SO_2S	-	6.95 ± 0.39	8.05 ± 0.62

an effect on photosynthesis nor was it photosynthetically mediated. There appeared to be increases in net photosynthesis from SO_2 exposure, but these were not significant.

3.5 Discussion

3.5.1 O_3 and SO_2 as Selection Agents

Ozone acted as a much stronger selection agent for resistance than did SO_2 in these experiments. This may be due to differences in the degree of stress from the two pollutants. Previous studies with radish have shown that at equal concentrations ($0.12\ \mu l\ l^{-1}$), O_3 is much more phytotoxic than SO_2 (Atkinson, Robe & Winner 1988). Thus, the $0.1\ \mu l\ l^{-1}$ O_3 treatment may have been severe enough for selection to occur and be seen through growth response analysis. Although the SO_2 treatment was five fold higher in concentration, it may not have been high enough to strongly force selection and suppress growth. Alternatively, there may be some genetic basis that accounts for the fact that O_3 resistance is more readily heritable than SO_2 resistance. Differences in heritability may be due to the number of loci involved in resistance, or the amount of genetic control for a specific mechanism for resistance.

Past work has demonstrated that SO_2 can exert a selective pressure in plant populations (Taylor & Murdy 1975). Dueck, Dil & Pasman (1987), in a study involving development of tolerance in grasses, considered O_3 and SO_2 to have important influences on selection. However, development of stable populations has generally required several generations. Wilson & Bell (1985) estimated that at least three to four generations were needed before populations of SO_2 resistant grasses

were seen. In comparison, we were able to observe O_3 selected resistance in a single generation.

Selection for sensitive lines was not as successful as selection of resistant lines for either pollutant. This may be the result of other factors such as mechanical injury limiting the growth of certain plants which may have been erroneously selected as sensitive to the pollutant, or there may not be a strong correlation between large amounts of visible injury and large growth reductions. The population selected for O_3 resistance also exhibited resistance to SO_2. Exposure of this population to either pollutant gave similar results. Other studies have shown cross-resistance of certain species to more than one pollutant. Trembling aspen clones selected for sensitivity to SO_2 were found to be sensitive to O_3 as well, in terms of visible leaf injury (Karnosky 1977). The "Cherry Belle" cultivar of radish used in this study has been shown to be sensitive to both O_3 and to SO_2 (Reinert, Tingey & Carter 1972, Tingey *et al.* 1971).

3.5.2 Nature Of Resistance

Much effort has been made to determine the factors important for resistance to pollutants. Some mechanisms may be found as a physiological property of foliage, while others may be more related to whole plant processes.

A first line of defense against gaseous air pollutants is the response of the stomata; some species exhibiting resistance to pollutants appear to have low conductance values, either in response to a pollutant or as a regular feature of their physiology. This may give them protection against foliar absorption of toxic pollutant levels. *Heteromeles arbutifolia*, an evergreen shrub with a low conductance rate, is more resistant to SO_2 exposure than is *Diplacus aurantiacus*, a deciduous shrub with

a higher conductance rate (Winner & Mooney 1980). Differences in SO_2 sensitivity between different genotypes of several grass species appear to be partially due to a greater ability to close stomata during SO_2 exposure and reduce uptake of the pollutant (Ayazloo *et al.* 1982). A similar difference in conductance values was observed between O_3R and O_3S plants in this study. Conductance of O_3S plants decreased significantly upon exposure to O_3, but only to a value similar to that of non-fumigated O_3R plants, while conductance of O_3R plants decreased to an even lower value upon exposure to the pollutant. Not only is the physiology of the leaf response important in determining resistance, but also the physiology of that leaf in normal conditions.

Other mechanisms recognized as possibly being important in differential sensitivity include 1) pollutant detoxification 2) pollutant threshold levels and 3) repair or compensation processes (Taylor & Tingey 1983). The differences in sensitivity of two pea cultivars to SO_2 may be a result of the first mechanism, through differences in glutathione levels (Alscher *et al.* 1987), whereas variation between ecotype response in *Geranium carolinianum* to SO_2 was attributed to the latter two mechanisms (Taylor & Tingey 1983). At this point, it is difficult to positively assign one or more of these mechanisms to the plants in our study. Moreover, because the O_3R plants exhibited resistance to SO_2 as well as O_3 but not to the same degree more than one mechanism may be involved.

In addition to foliar processes, whole plant carbon allocation patterns may reflect air pollution resistance. Ozone and SO_2 are both known to reduce root/shoot ratios (Tingey, Heck & Reinert 1971, Okano *et al.* 1984, Godzik, Ashmore & Bell 1985), with O_3 having larger effects on this parameter (McLaughlin & McConathy

1983). This is mediated through the leaf, as O_3 does not directly affect below ground parts of the plant (Blum & Tingey 1977). Air pollution resistant plants would not be expected to have changed root/shoot ratios in response to air pollutants; this was the case for plants in this study selected for O_3 resistance. Since air pollution-caused reductions in root/shoot ratios are likely caused by inhibition of carbohydrate translocation processes, the maintenance of root/shoot ratios following fumigations could be attained several ways. For example, the processes involved in carbohydrate translocation may become resistant to air pollutants, providing the plant with air pollution tolerance. On the other hand, plants which excluded absorption of air pollutants by closing stomata during fumigations could also maintain unchanged root/shoot ratios through avoidance. This could only be the case if the periods of stomatal closure were brief, if the stomata recovered quickly after the fumigation period, and if the degree of closure was sufficient to reduce air pollution absorption to rates below critical thresholds. If air pollutants caused long term reductions in conductance, whole plant changes in growth would result.

3.5.3 The Significance of Selection

The fact that O_3 resistance was expressed within one generation has meaning for both crop and native plants. Crops that have been genetically manipulated for increased productivity are possibly indirectly increasing in O_3 resistance as well. This is known to occur inadvertently in pasture grasses growing in areas of high SO_2 concentrations (Bell & Clough 1973, Horsman, Roberts & Bradshaw 1978). It should be noted that resistance to SO_2 develops more quickly in response to acute rather than chronic exposures (Wilson & Bell 1986). While it is not known whether or not this holds true for O_3 as well, it appears from this study that O_3 resistance

develops more rapidly than does SO_2 resistance. Ambient O_3 may also act as a selective pressure and contribute to the evolution of O_3 resistance in native plant populations. Although this has been known to occur for SO_2 in small areas near SO_2 sources, this study suggests that O_3, which is found at phytotoxic concentrations over much vaster areas than SO_2, has the potential to influence plant genetics on a wide regional scale. One possible implication is a change in plant community structure, as sensitive individuals are put at a competitive disadvantage. Although effects of gaseous pollutants are normally considered for crop plants on the basis of growth or yield, a principal resource of native populations is their diversity. Westman (1979) implicated airborne oxidants as the most likely cause of a reduction in species diversity in California coastal sage scrub communities. Results from the experiments presented here indicate that gene pool structure within a species may also be influenced by air pollutants.

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4. Effect of O_3 on Leaf Carbohydrate Concentration in Two Different Population of Radish

4.1 Introduction

Ozone (O_3) affects a variety a plant processes, especially those involved in the carbon balance of the plant. These processes include photosynthesis, leaf carbohydrate metabolism, and carbon allocation. The carbohydrate content of a leaf is an important link between photosynthesis and allocation. Numerous experiments have detailed the response of leaf carbohydrate content to air pollutant stress. From these, it is apparent that plant response is quite varied. There are reports of increased leaf soluble carbohydrate content (Adepipe & Ormrod 1974), decreased total nonstructural carbohydrate content (Blum, Smith, & Fites 1982), and of plants in which the effect of sulfur dioxide (SO_2) depended on the age of the plants (Koziol & Jordan 1980). Other factors, such as leaf age, may have a role in the great variety of results seen.

The effects of O_3 on carbon allocation are also well researched. In contrast to studies of carbohydrate content, the data on carbohydrate allocation are consistent. Ozone, as well as SO_2, has been found to inhibit carbon allocation to lower parts of the plant such as roots. The means by which O_3 actually inhibits translocation is not known. Noyes (1980) found that phloem-loading was inhibited in response to high-level SO_2 exposures. However, it has been suggested that shifts in carbon allocation may be more of a plant response to pollutant exposure than actually caused by the presence of the individual pollutant. The plant may merely be compensating for a reduced carbon supply by allocating a greater percentage of available carbohydrate into photosynthetic tissue (Mooney *et al.* 1988).

In this study, the link between alterations in leaf carbohydrate content and changes in plant carbon allocation was studied. Also, I examined the leaf free sugar concentrations in two plant populations that differed in pollutant sensitivity. Sequential harvests of the plants were made in order to document the change in carbon allocation and leaf carbohydrate concentrations over the life cycle of the plant.

4.2 Materials and Methods

From previous studies, seeds of populations of radish (*Raphanus sativus* L.), originally developed from the cv Cherry Belle, with differential sensitivity to O_3 were available. The differences between these populations were based on final plant dry weight. Plants from both an O_3 resistant (O_3R) and an O_3 sensitive (O_3S) population were used as well as non-selected (NS) Cherry Belle radishes.

Seeds were sown in one liter pots in a 3:1:1 mixture of peat moss, vermiculite, and Weblite^R (v/v/v) with 6 g of a 14:14:14 controlled release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA) added to each pot. Prior to seeding, the soil mix was watered with a micronutrient solution. Seedlings were thinned to one per pot 3-5 days after emergence. Plants were grown in a charcoal filtered greenhouse with daylength maintained at 16 h using 1000 W high pressure sodium lamps, which provided a minimum of 250 μE m^{-2} s^{-1} PAR. Average day/night temperatures in the green house over the entire growing period were 29/23 C.

Fumigations were made in a Continuous Stirred Tank Reactor System (CSTR), beginning ten days after emergence. A fumigation regime of 3 days per week, 4 hours per day for 3 weeks was maintained. Plants were moved from the greenhouse to the CSTR immediately prior to the fumigation and returned

immediately after. Ozone, generated from O_2 passed through a UV light source (Welsbach Model T-408, Welsbach Ozone Systems Corp., Philadelphia, PA), was supplied at 0.1 μl l^{-1}. Pollutant concentration was regulated by mass flow controllers connected to a data acquisition system. Ozone concentrations in the chambers were monitored on a time-share basis with a TECO Model 49 photometric O_3 analyzer (Thermo-Electron Corp., Hopkinton, MA). The monitor was calibrated on a regular basis with a CSI Model 3000 O_3 calibrator (Columbia Scientific Instruments, Austin TX). Chamber lighting, provided by 1000 W metal halide lamps, ranged from 450-600 μE m^2 s^{-1} PAR. Initially, 45 plants per chamber were used with three replications. Fifteen plants from each chamber group were sacrificed at each harvest date. Harvests were made at 20, 25, and 30 days after germination. On these days, the plants were fumigated first and harvested immediately after the fumigation was completed. One leaf from each of the first two leaf pairs was removed from the plant. Leaf area was measured with a LI-COR 3050 meter (Lambda Instruments Corp, Lincoln, NE), fresh weight was recorded, and the leaf was stored in liquid N_2 prior to oven drying. The remainder of the plant was divided into shoot, hypocotyl and root and dried in a forced air oven at 60 C.

The carbohydrates of the sampled leaves were assayed using a modification of the method of Wolf and Ellmore (1975). For tissue preparation the dried samples were ground and weighed. Due to the small amount of plant material available, particularly from the first harvest, the entire leaf sample was used for analysis. The tissue was then extracted in 25 ml H_20 at 100 C for 1 h. The samples were allowed to cool before a 3 ml aliquot was transferred to the sample vials. Starch concentration was determined by adding dilute HCl having a concentration sufficient to give 0.4 N after mixing. This sample was then boiled at 100 C for 1 h in order to solubilize the

starch, and another 3 ml aliquot was taken for measurement. The concentration of starch was determined from the difference of the two measurements made on the sample. An Autoanalyzer system (Technician Instrument Corp., Tarrytown, NY) was used for actual sugar measurements. A colorimetric assay was used with potassium ferricyanide as the reagent to determine sugar levels in the samples through comparison with a series of glucose standards run through the system prior to the actual samples.

The remaining plant material was weighed after complete oven drying and used for growth analysis. Tests for significant differences were made using a 1-way analysis of variance with the ANOVA procedure on SAS (Statistical Analysis System, Raleigh, NC). LSD tests were used to distinguish differences between treatment means.

4.3 Results

Suppression of growth of fumigated plants was evident at the first harvest (Figure 1). Overall, the greatest number of significant differences was seen on the first harvest. This occurred within all the groups, regardless of the level of resistance to O_3. By day 20, shoot growth accounted for approximately 80% of total plant dry weight, and the majority of O_3-induced growth reduction was seen here (Figure 2). Although hypocotyl growth reductions were small on an absolute basis as compared to the shoot, the percent reduction was much greater (Figure 3). In particular, the O_3S plants, which were larger overall than the other plants, and had higher root/shoot ratios (Figure 4), showed this effect. This indicates that even at this point in the experiment, when carbon allocation to the below ground parts was relatively small, O_3 was causing the plant to reallocate the carbon supply.

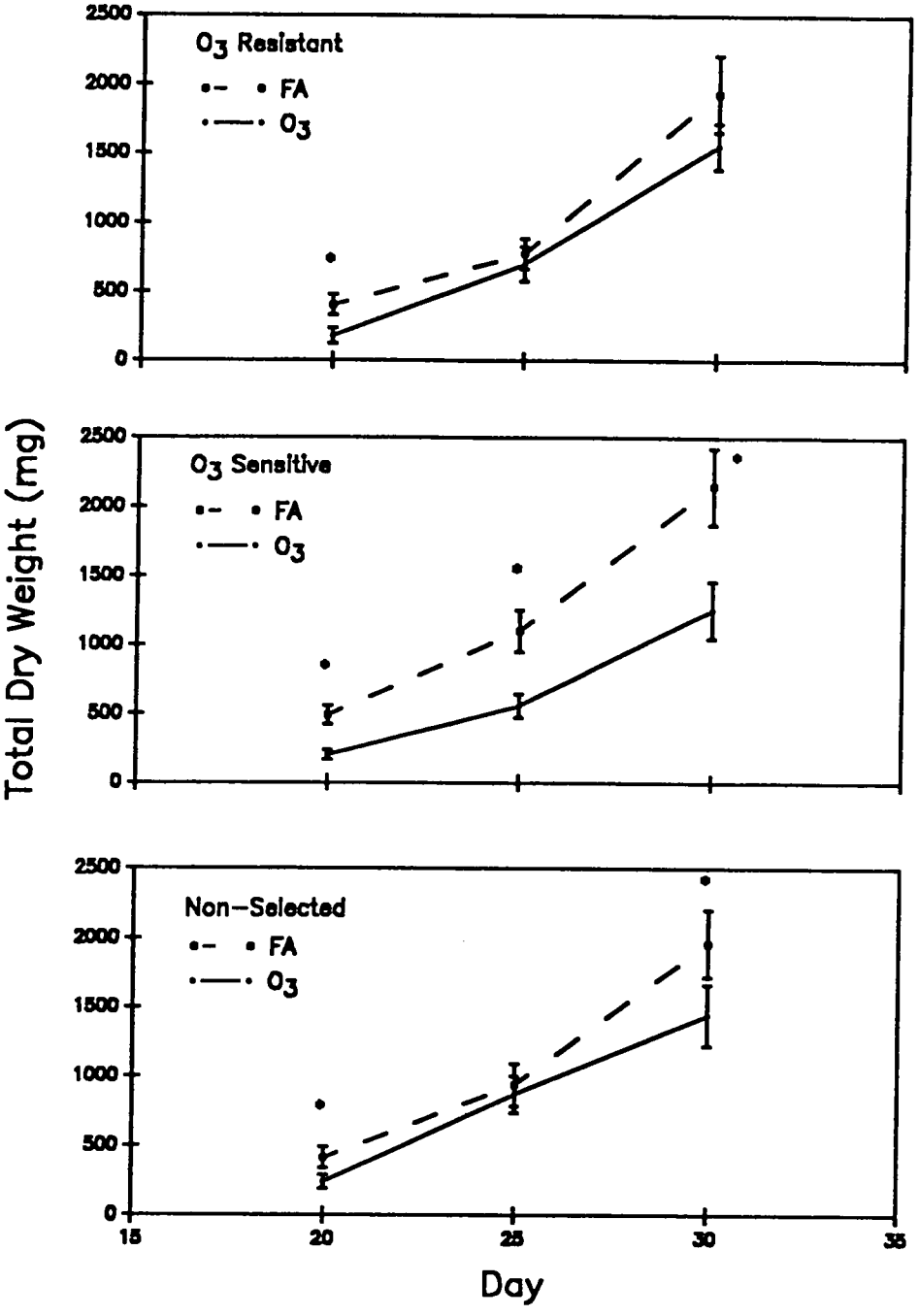

Figure 1. Total plant dry weight at three different harvest dates of A) O_3 resistant (O_3R), B) O_3 sensitive (O_3S) and C) non-selected (NS) plants exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are means of 15 samples ± SD, an * indicates means significantly different at the $P < 0.05$ level.

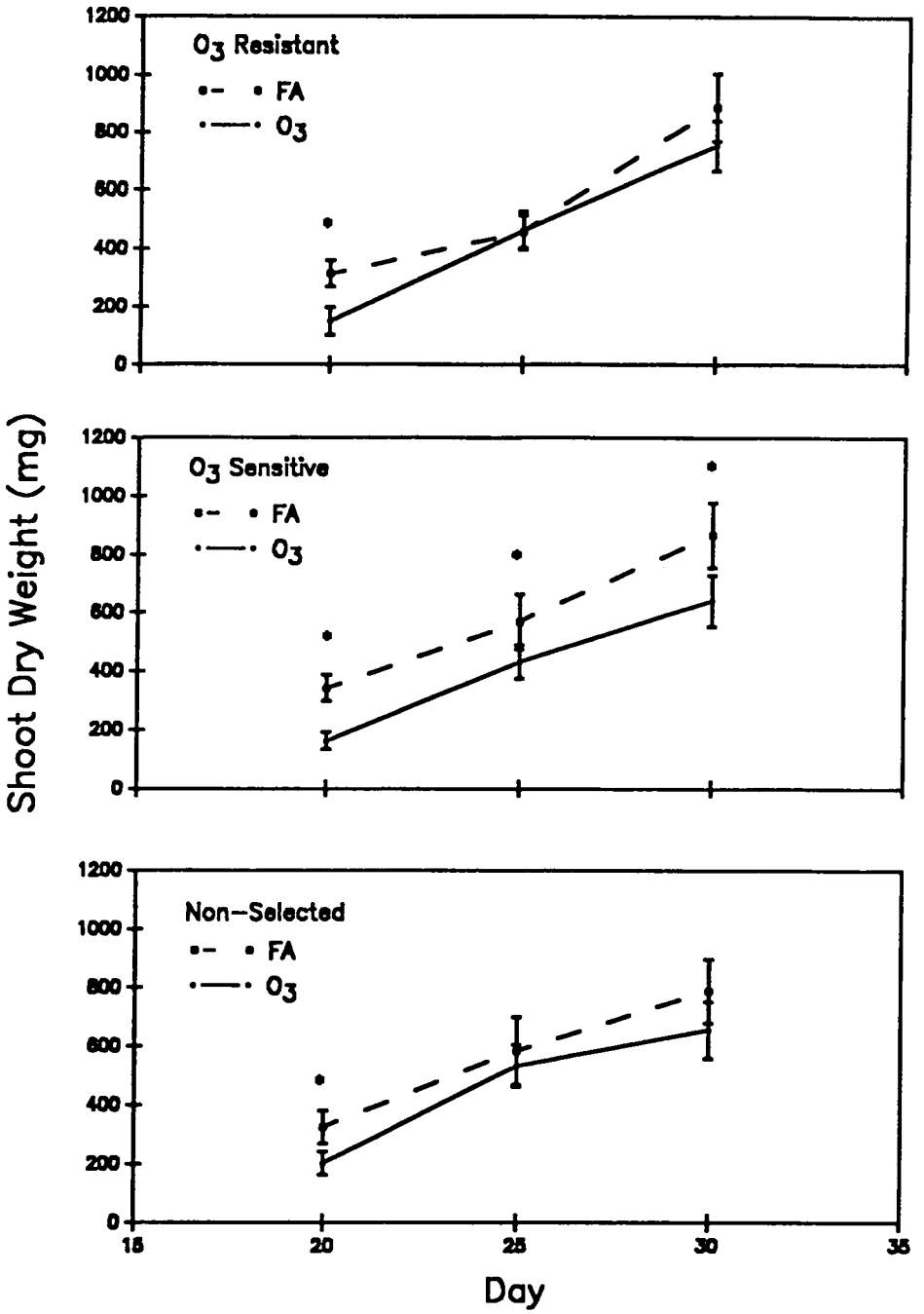

Figure 2. Shoot dry weight at three different harvest dates of A) O_3 resistant (O_3R), B) O_3 sensitive (O_3S) and C) non-selected (NS) plants exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are means of 15 samples ± SD, an * indicates means significantly different at the $P < 0.05$ level.

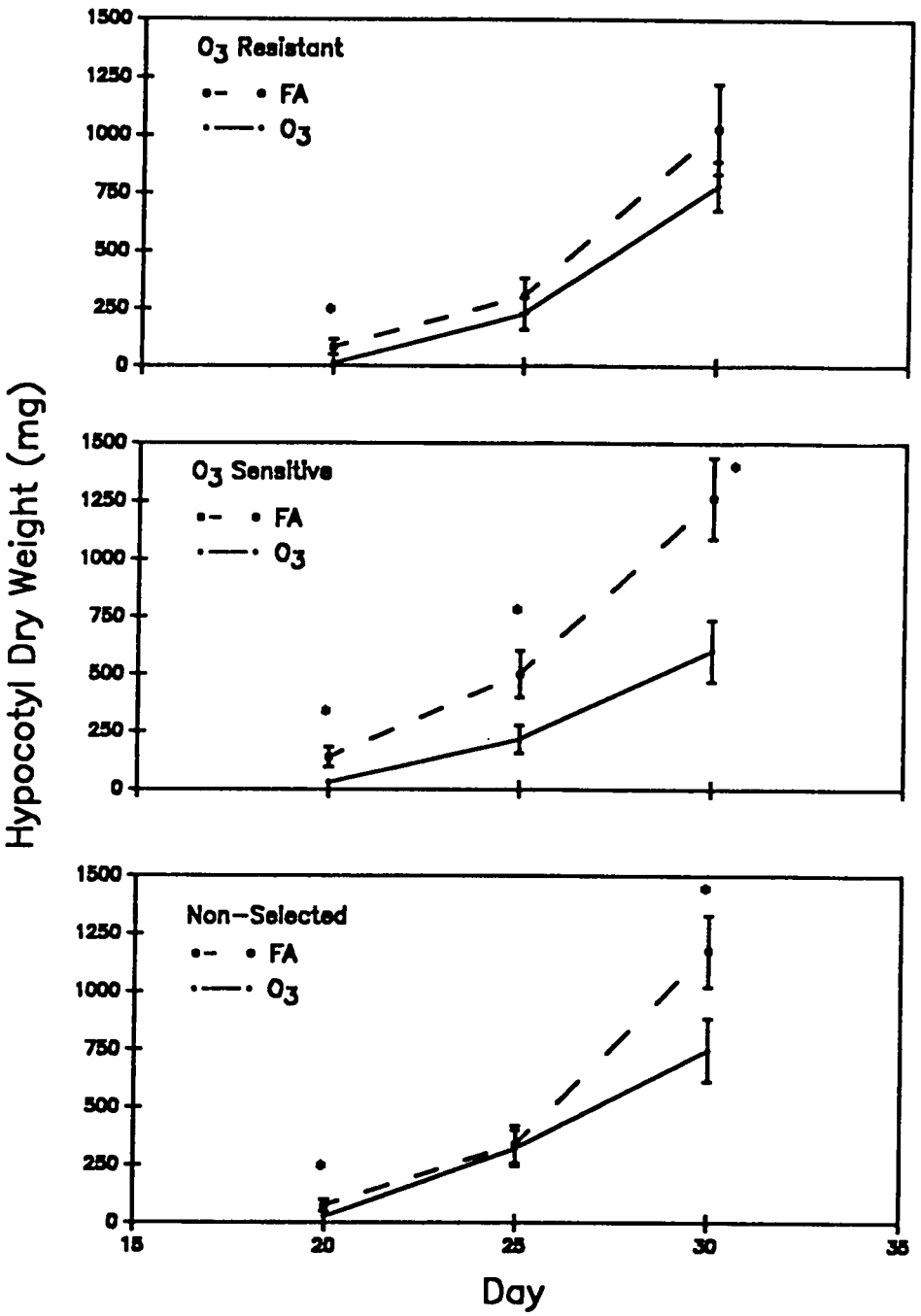

Figure 3. Hypocotyl dry weight at three different harvest dates of A) O_3 resistant (O_3R), B) O_3 sensitive (O_3S) and C) non-selected (NS) plants exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are means of 15 samples ± SD, an * indicates means significantly different at the $P < 0.05$ level.

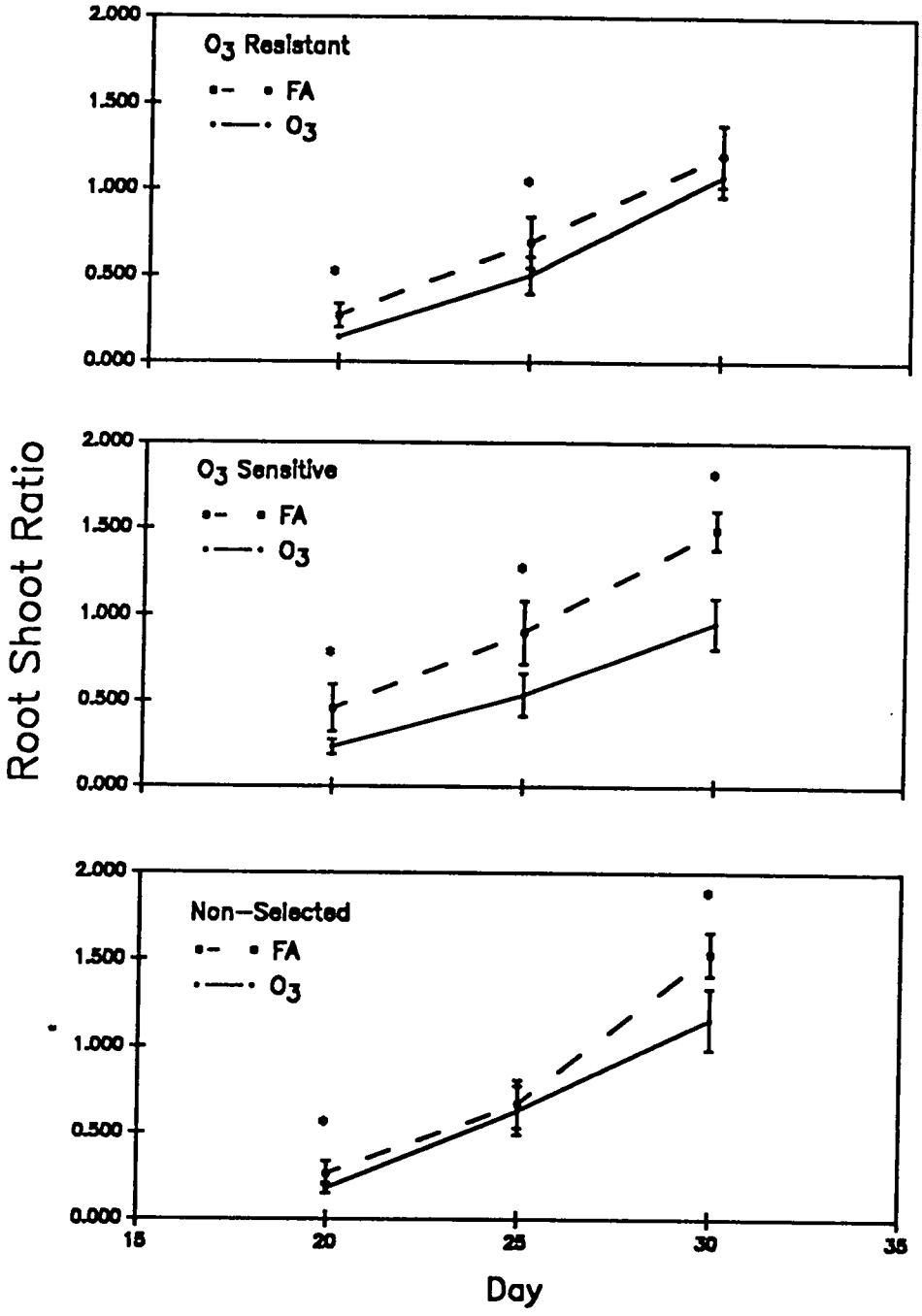

Figure 4. Root/Shoot ratios at three different harvest dates of A) O_3 resistant (O_3R), B) O_3 sensitive (O_3S) and C) non-selected (NS) plants exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are means of 15 samples ± SD, an * indicates means significantly different at the $P < 0.05$ level.

Ozone did not significantly affect carbohydrate content of the first leaf pair (Figure 5). Free sugar levels were approximately the same in all plant types regardless of treatment. Samples of the second leaf pair, taken from fumigated plants, did have slightly reduced carbohydrate levels (Figure 6). This was apparent from both the O_3R and O_3S plant analyses. These data at Day 20 do not give indications of greater resistance of the younger second leaf pair.

By Day 25, visual signs of growth depression of the plants were apparent, particularly in hypocotyl dry weight (Figure 3). Clear distinctions among the fumigated plant groups could be made in regard to both visible injury and growth. While neither O_3R nor NS plants showed significant decreases in total plant growth due to O_3 fumigation, O_3S plants did (Figure 1). Fumigated O_3S plants were noticeably smaller, having only about 50% of the total plant dry weight of the non-fumigated O_3S plants. The non-fumigated O_3S plants were still larger than those of the other groups.

Ozone effects on shoot growth at Day 25 were less pronounced (Figure 2). Only O_3S plants had significantly reduced shoot growth. Even this reduction was smaller than it had been at the initial harvest. Apparently, a shift in allocation had occurred between the first and second harvests, allowing shoot growth to be maintained at near its normal level. The effects of this shift were particularly obvious in the O_3S plants; hypocotyl growth was decreased greatly in the treated O_3S plants (Figure 3). Neither O_3R nor NS had significantly reduced hypocotyl growth.

Root/shoot ratios followed the same pattern as hypocotyl growth (Figure 4). Ratios were lower in the fumigated O_3S plants, again indicating a shift in allocation patterns away from the root to the shoot. This change was not seen in the other plant groups.

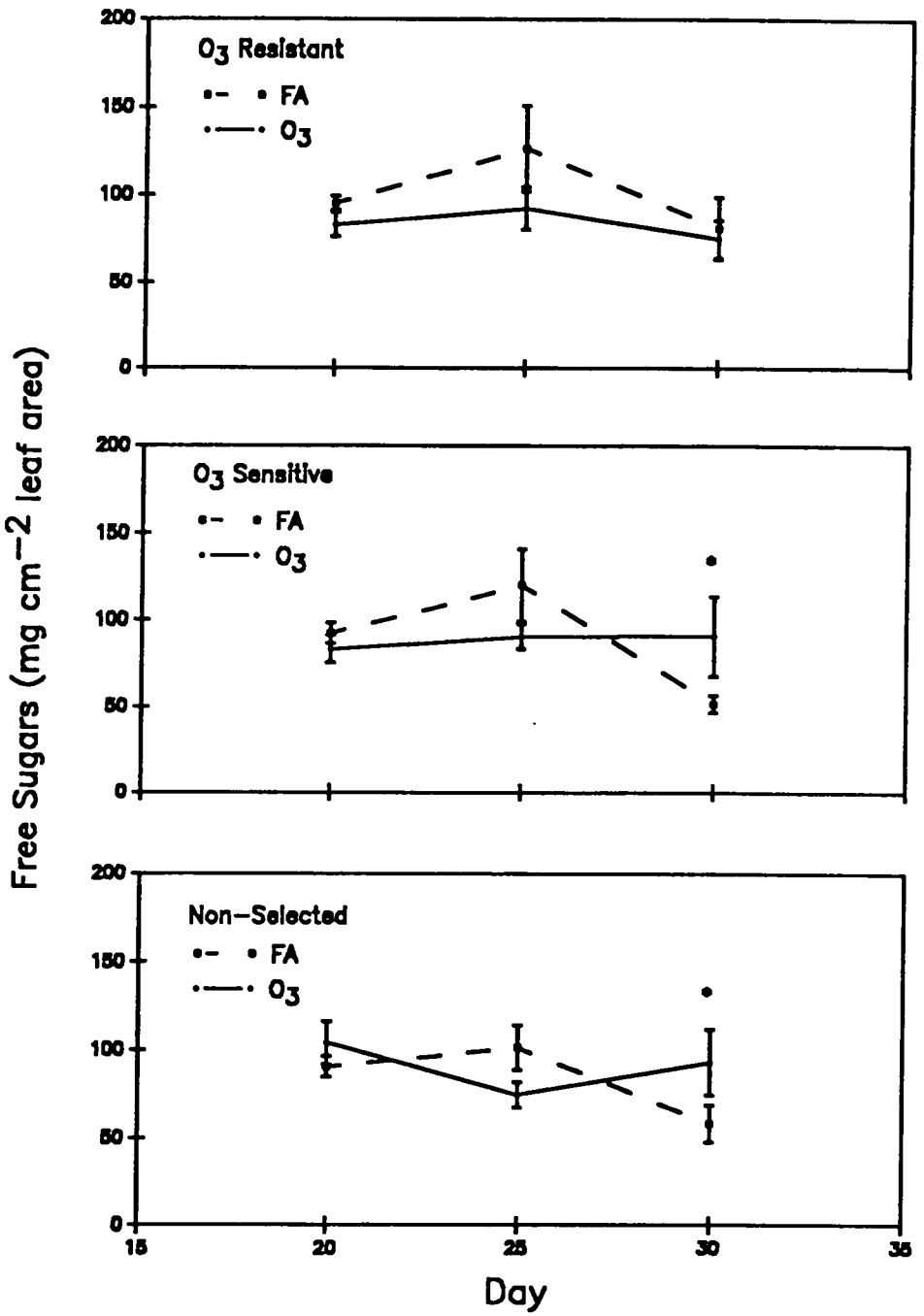

Figure 5. Total free sugar concentration of one leaf from the first leaf pair at three different harvest dates of A) O_3 resistant (O_3R), B) O_3 sensitive (O_3S) and C) non-selected (NS) plants exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are means of 8 samples ± SD, an * indicates means significantly different at the $P < 0.05$ level.

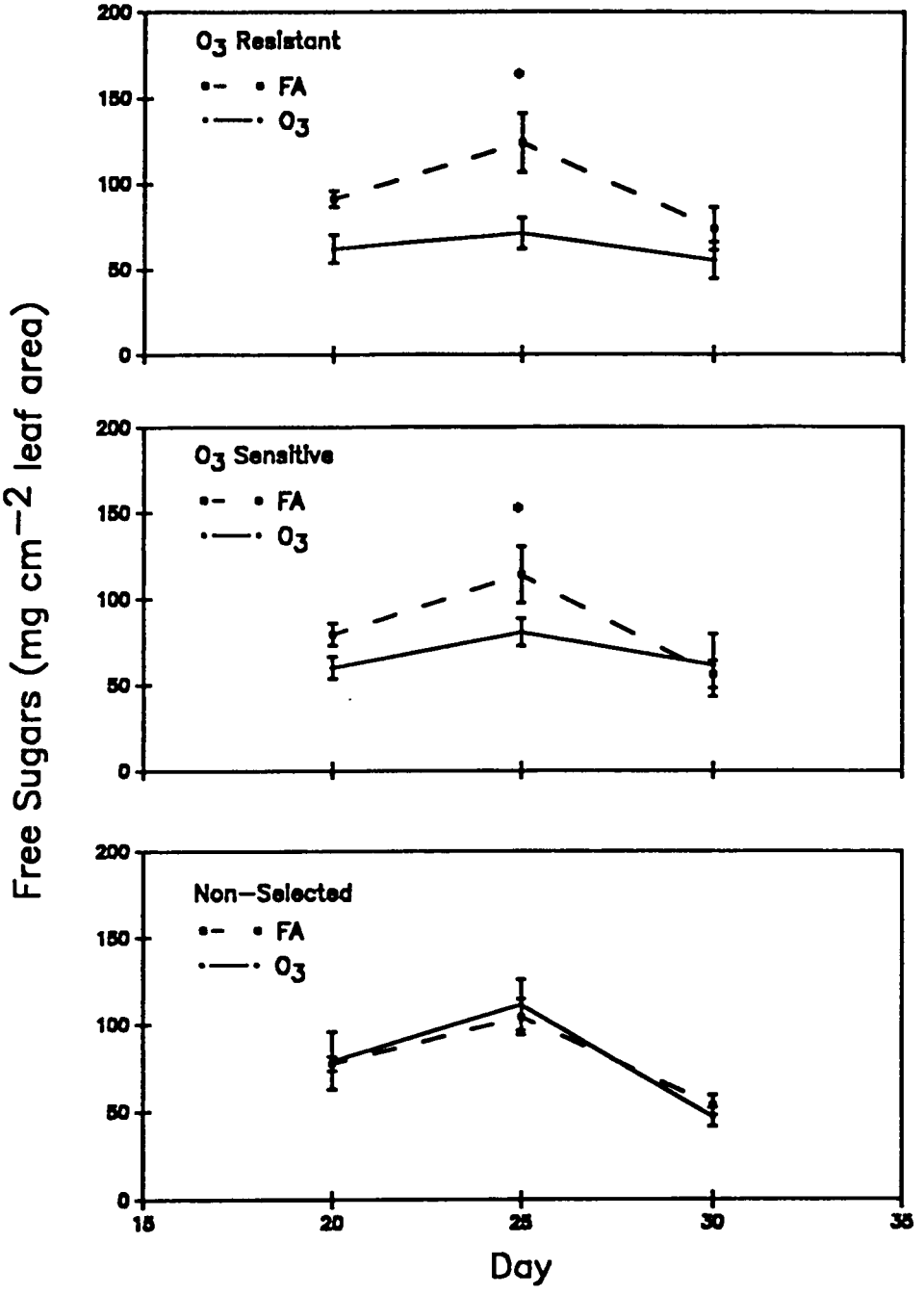

Figure 6. Total free sugar concentration of one leaf from the second leaf pair at three different harvest dates of A) O_3 resistant (O_3R), B) O_3 sensitive (O_3S) and C) non-selected (NS) plants exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are means of 8 samples ± SD, an * indicates means significantly different at the $P < 0.05$ level.

Leaf carbohydrate content was altered in all the groups (Figures 5 & 6). In the first leaf pair, which had been unaffected at the first harvest, O_3 affected these leaves by the second harvest. Whereas the free sugar pool increased in the non-fumigated leaves, in fumigated leaves it remained at or near the level of the first harvest. This was also true of the O_3R and O_3S leaves from the second leaf pair. There was apparently little difference in response between either the different age class leaves or the different resistance types based on free sugar levels. Levels of starch in these levels was difficult to measure accurately due to the low levels found and the degree of error in the measurements. Mean values for starch averaged only between 2-5 mg cm^{-2} leaf area, as compared to levels of 50-100 for sugars. Using this method of extraction, no significant differences between treatments was found for leaf starch concentrations.

On the final harvest day (Day 30), the greater resistance to O_3 of the O_3R plants was apparent. At this point, both the O_3S and the NS plants had growth reductions caused by O_3. Total plant dry weights were reduced by 41 and 28% respectively (Figure 1).

Shoot growth was still the least sensitive to fumigation at day 30 (Figure 2). Only O_3S plants had significantly reduced shoot weights, and the percent reduction was less than that of the total plant dry weight. The other groups did not experience significant reductions in shoot dry weight. Hypocotyl dry weight was much more sensitive to O_3 fumigations (Figure 3). Both O_3S and NS plants showed significant hypocotyl growth reductions resulting from O_3 stress. The O_3S were in particular sensitive, with a 52% reduction in hypocotyl dry weight as opposed to non-fumigated conditions.

Root/shoot ratios continued to follow a pattern similar to that of the hypocotyl (Figure 4). While O_3R plants were relatively unaffected, the other two groups showed indications of a shift in biomass allocation. This was a continuation of the trend for O_3S plants, as it appeared that there was an increasing shift from the hypocotyl to the shoot over all three harvests. NS plants, which did not appear affected at day 25, now had significantly reduced root shoot ratios.

By the final harvest, the levels of free sugars even in the non-fumigated plants had begun to decline, possibly an indication of approaching senescence (Figures 5 & 6). This was the only harvest at which there was any indication of accumulation of sugars, seen in the first leaf pairs of both the O_3S and NS plants. The younger second leaf pairs did not show the same trend, as there was no difference between fumigated or non-fumigated leaves. This could indicate an effect of leaf age, as older leaves are often considered to be more sensitive to O_3, particularly when leaf sugar levels are low (Ting & Mukerji 1971).

4.4 Discussion

4.4.1 O_3 Effects on Growth

The growth analysis data from this experiment demonstrate the differential sensitivity of the selected radish populations. Growth differences were apparent by the first harvest date of the experiment. In the previous experiment, growth differences were observed, but only a final harvest was made. Another difference from the previous growth experiment is the sensitivity of the O_3S plants to O_3 in this experiment. Presumably this is a result of the variability present in these populations, particularly in the NS plants. In the first experiment, there were obvious

differences between the O_3R plants and either the O_3S or NS plants. However, NS and O_3S plants had similar growth. There were differences in the growth environment between this experiment and the first, particularly in greenhouse temperatures, which would affect the growth of the populations. Possibly the NS plants responded more favorably to the warmer temperatures of this experiment than did the other populations. Also, just the variability of response of the NS population may lead to growth differences from experiment to experiment. This would not be surprising, as one of the main purposes of breeding differentially sensitive lines was to eliminate the extreme variability found in the Cherry Belle radish in response to air pollution treatments.

The difference in growth among the three populations was not visibly apparent by the first harvest (day 20). All the populations had reduced growth, in terms of total growth. One of the reasons for this is the proportion of total growth at this point allocated to the shoot. As shown previously (Atkinson, Robe & Winner 1988), shoot growth is the least sensitive to air pollutant exposure, as well as many other environmental stresses. Translocation has been shown to be altered to favor shoot growth in times of stress, such as SO_2 (Mooney *et al.* 1988). However, at day 20, hypocotyl and root growth were minor compared to the shoot, indicating there was little allocation of photosynthate to these parts. Thus, event though an allocation occurred which favored shoot growth, the amount of carbohydrate available to be reallocated from the hypocotyl and root was insufficient to completely offset O_3 induced growth reduction. This may be why shoot growth was affected in all populations at this point, but was not affected in any of them by the second harvest, when hypocotyls were much larger.

The development of the individual population during this experiment revealed how resistance or sensitivity can develop. At the first harvest, differentiating between resistant and sensitive populations was difficult to identify because all plants were exhibiting signs of pollutant stress. By the second harvest, O_3S plants were distinguishable from the others due to the greater degree of growth suppression of these plants. This trend continued through the next experiment and became magnified, particularly with hypocotyl growth. Walmsley, Ashmore & Bell (1980) followed growth of radish plants under continuous O_3 exposure. These plants showed initial signs of growth depression and injury, but recovered so that there were little differences in terms of growth by the end of the experiment. The O_3R plants and to a lesser extent, the NS plants behaved similarly. Despite early effects on growth, these plant seemingly recovered by the final harvest so that growth reductions were eliminated or minimized compared to the first harvest. NS plants were much less affected at the second harvest; in fact, their growth was very similar to the O_3R plants at this stage. By the last harvest, however, they showed obvious growth reductions in all parts except the shoot. The development at this point was very similar to that of the O_3S plants at day 25. O_3R plants were not negatively affected by O_3 even at the final harvest. These results would seem to point to a threshold of pollutant uptake, which if exceeded, leads to growth reductions. O_3S plants would have the lowest threshold, followed by the NS plants.

4.4.2 O_3 Effects on Leaf Carbohydrates

The data presented in this experiment show either no change or a decrease in the amount of free sugars present in the source leaves. The wide range of response is not uncommon; Ito *et al.* (1985) found that concentrations of soluble sugars varied

without a consistent trend in kidney bean leaves exposed to either O_3 or NO_2. Koziol and Jordan (1978) found a similar trend in *Phaseolus vulgaris* plants exposed to high concentrations of SO_2. Whereas low SO_2 exposures seemed to stimulate sugar concentrations in plant tissue, high doses caused a decrease, which was attributed to decreased photosynthesis and increased respiration. The SO_2 concentration at which sugar levels began to decrease rather than increase was also the concentration at which visible injury began to occur on the leaves. This connection between decreases in sugar levels and leaf injury holds up in this experiment as well, as visible leaf injury was apparent on the radish leaves, principally on the O_3S plants.

As there was no increase in the growth of any sinks (hypocotyls, roots, new leaves) in the radish plants exposed to O_3, the decrease in leaf sugar levels is apparently not due to acceleration of translocation, as seen in the primary leaves of *Phaseolus vulgaris* (Okano *et al.* 1984). Several studies have shown that translocation is inhibited with O_3 fumigation (McLaughlin & McConathy 1983, Okano *et al.* 1984). Generally, this is regarded as a consequence of reduced photosynthetic rates. Even at relatively low concentrations, O_3 can have adverse effects on photosynthesis. This has a direct impact on the amount of assimilate available to the plant and can reduce the free sugar supply. However, it appears that many plants have an ability to compensate for a reduced photosynthetic ability by reallocating the carbohydrate supply in favor of free sugars rather than starch. Koziol and Jordan (1980) examined the carbohydrate content of ryegrass exposed to either high (0.15 $\mu l\ l^{-1}$) or low (0.02 $\mu l\ l^{-1}$) SO_2 concentrations. At the initial harvest, more carbohydrate was found in the free sugar pool at the expense of storage carbohydrates. Faroq *et al.* (1985) documented an increase in both free and total sugar supply in response to an

extreme SO_2 exposure. Starch, however, was found to decrease. In the present experiment, there was a general trend towards a decrease in free sugars in the radish leaves. The proportion of sugar to starch was not measured, as the starch levels in the leaf were extremely low and difficult to measure with precision. It does not seem likely, given the small amount of starch measured, that there would have been a major impact on free sugar levels if indeed there was some alteration made in the relative sizes of the available carbohydrate pools.

Other possibilities for the observation of reduced free sugar levels could be a shift in partitioning to storage carbohydrates such as starch, although as mentioned previously the reverse process has been observed more often. Also, the use of available carbohydrate for synthesis of cell walls or used in repair processes may act to reduce free sugar concentrations found in the leaf although this has yet to be demonstrated conclusively (Alscher & Amthor 1988).

4.4.3 The Potential Role of Leaf Age

Although relationships between leaf age and pollutant sensitivity have been made (Ting & Mukerji 1971, Tingey *et al.* 1973), attempts at linking this relationship to growth effects are less common. Okano *et al.* (1984) demonstrated that older leaves are more sensitive to O_3 exposure and that the subsequent alterations in translocation have more impact on below ground parts. While I did not find a similar response in radish, perhaps due to architectural differences between bean and radish plants, this experiment demonstrated the differential sensitivity of the pollutant selected radish population. It did not, however, provide an explanation for the differences in growth observed among the populations.

A factor that may be important is the early growth rates of the different plant populations. O_3S plants grew much faster early in the experiment under non-fumigated conditions than did either of the other two groups. They also had much higher root/shoot ratios at the first harvest. Thus, the O_3S plants were apparently more stressed by the first harvest than the other plant groups; this may be a factor in determining overall sensitivity.

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5. Effect of O_3 on ^{14}C Allocation and Gas Exchange in Two Populations of Radish

5.1 Introduction

Ozone (O_3) can significantly impact plant productivity by both reducing carbon gain and by altering the partitioning of assimilated carbon within the plant. Because of changes in allocation, O_3 affects various plant parts differently. Non-photosynthetic sinks, such as roots, tend to experience greater growth reductions than do leaves or shoots (Tingey, Heck, & Reinert 1971, Walmsley, Ashmore, & Bell 1980). Experiments with both sulfur dioxide (SO_2) and O_3 have shown that translocation from source leaves is reduced by pollutant exposure (Noyes 1980, McLaughlin & McConathy 1983, Okano *et al.* 1984). The means by which these pollutants actually inhibit translocation are not known. Noyes (1980), used autoradiographs to determine that in bean plants phloem-loading was inhibited in response to high-level SO_2 exposures. However, ryegrass has been shown to have increased levels of free carbohydrates in leaves after being exposed to SO_2 (Koziol & Cowling 1980). This suggests that inhibition of translocation by pollutants is not absolutely tied to reductions in photosynthesis. Teh & Swanson (1982) observed inhibition of translocation in bean plants exposed to high levels of SO_2 beyond that which would be expected from the decreases in photosynthesis. However, it has been suggested that shifts in carbon allocation may be more of an adaptive plant response to pollutant exposure than a direct effect of the pollutant. The plant may merely be compensating for a reduced carbon supply by allocating a greater percentage of available carbohydrate to produce more photosynthetic tissue (Mooney *et al.* 1988).

In this investigation, the effects of fumigations with O_3 on both gas exchange and carbon allocation have been measured in two lines of radish, one resistant to O_3 (O_3R) and one sensitive (O_3S). These two populations differ in their overall growth response to O_3, with the resistant plants having greater biomass, particularly in the lower parts of the plants such as the hypocotyls and roots. Because differences in either carbon gain, through photosynthesis, or carbon allocation, or both, may account for the observed differences in growth of these two populations, the possible relationship between resistance to air pollutants, carbon gain and allocation has been examined in these two lines of radish.

5.2 Materials and Methods

From previous studies, populations of radish (*Raphanus sativus* L. cv Cherry Belle) with differential sensitivity to O_3 were available. Differences between these populations were based on final plant dry weight following fumigation with O_3 had been demonstrated. Plants from both an O_3 resistant (O_3R) and an O_3 sensitive (O_3S) population were used.

Seeds were sown in fifteen cm pots in a 3:1:1 mixture of peat moss, vermiculite, and Weblite^R (v/v/v) with 6 g of a 14:14:14 controlled release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA) added per pot. Prior to seeding, the soil mix in the pots was watered with a micronutrient solution. Seedlings were thinned to one per pot 3-5 days after emergence. Plants were maintained in a charcoal filtered greenhouse with daylength maintained at 16 h using high pressure sodium lamps approximately one meter above the benches.

Fumigations were made in a Continuous Stirred Tank Reactor System (CSTR), beginning ten days after emergence, at which point the first leaf pair on the

plants was just beginning to develop. A fumigation regime of 3 days per week, 4 hours per day for 3 weeks was maintained. Plants were moved from the greenhouse to the CSTR immediately prior to the fumigation and returned immediately afterwards, except for gas exchange studies, in which the plants to be measured were moved to the fumigation chambers the night before measurements were made in order to allow for acclimation to occur. Ozone was generated by a Welsbach Model T-408 Ozone Generator (Welsbach Ozone Systems Corp., Philadelphia, PA) from O_2 passed through a UV light source, and was supplied to each chamber at a concentration of 0.1 μl l^{-1}, which was maintained by mass flow controllers connected to a data acquisition system. Ozone concentrations in the chambers was monitored on a time-share basis with a TECO Model 49 photometric O_3 analyzer (Thermo-Electron Corp., Hopkinton, MA). The monitor was calibrated on a regular basis with a CSI 3000 O_3 calibrator (Columbia Scientific Industries Corp., Austin TX). Chamber lighting ranged from 450-600 μE m^2 sec^{-1} PAR through the use of 1000 W metal halide lamps above each chamber.

Gas exchange of fumigated and non-fumigated plants was measured twenty-seven days after emergence. Three plants from both plant groups from both treatments were measured immediately prior to fumigation, at one hour intervals throughout the fumigation process, and one hour after completion of the fumigation. Measurements were made inside the CSTR chambers with a LI-COR 6200 Photosynthesis System with an one liter cuvette (Lambda Instruments Corp., Lincoln, NE). The relative humidity ranged from 62-72%, air temperature from 21-26 C, and lighting from 430-500 μE m^{-2} sec^{-1} PAR. A leaf from the third leaf pair of each plant was selected for measurement. These leaves were reaching full expansion when the measurements were made.

Immediately following fumigation on day 29, six plants from both groups per treatment were removed from the CSTRs, taken to the greenhouse and labeled with $^{14}CO_2$, using a modification of the method of McCool & Menge (1983). A test tube containing 10 μCi of [^{14}C]sodium bicarbonate (specific activity = 55 mCi mmol^{-1}) was placed inside a polyethylene bag and sealed with a screw top lid on the outside of the bag. A small hole in the side of the tube allowed for escape of the generated gas. The bag was then placed over an individual leaf and sealed around the petiole. The labeled CO_2 was generated by injecting 10% sulfuric acid into the test tube through a hole in the lid and the hole sealed with tape. Leaves from the second leaf pair (Leaf 2) or from the third leaf pair (Leaf 3) were labeled. The labelling period was for one hour, after which an excess of 1 N NaOH was added to the tubes to capture excess $^{14}CO_2$. The bags were removed within 15 minutes. A chase period of 21 hours then followed before plants were harvested and divided into four parts: labeled leaf, hypocotyl and root, leaves less than 4 cm in length (Shoot A), and leaves greater than 4 cm in length (Shoot B). Two groups of leaves were included so as to determine the partitioning between leaves of different ages. Four cm was chosen as the cut-off point between the 2 groups as radish leaves have generally completely unfolded by this length. These parts were then freeze dried and dry weights were recorded. Each sample was then ground to pass through a 10 mesh screen.

The ground tissue (up to 400 mg per sample) was then combusted for thirty seconds in a Packard Model 306, Tri-Carb sample oxidizer (Packard Instrument Company, Downers Grove, IL). The $^{14}CO_2$ was absorbed into Carbosorb^R and placed into scintillation vials with Permafluor V scintillation cocktail. Activities of the samples were then assayed using an LS 5000TA Scintillation counter (Beckman Instruments, Fullerton, CA). Counting efficiency ranged from 71-80%. CPMs were

converted into DPM, background radiation was subtracted from these values, and DPM mg^{-1} dry weight calculated.

5.3 Results

Net photosynthesis (Pn) was lower in both O_3R and O_3S plants fumigated with O_3 than in non-fumigated plants (Figure 1). This was evident in the initial measurements (hour 0) made prior to the fumigation. This initial depression is likely due to the previous exposures to O_3 of these plants. At the time the measurements were made, the plants had been given eight 4 h fumigations. The difference in Pn between fumigated and non-fumigated O_3R plants increased during the fumigation period, going from an initial difference of about 7 to 15% at the end of the fumigation period (Figure 1A). There was no indication of recovery of Pn between hour 4 and 5, the first hour after the fumigation had ended.

Although Pn was lower prior to the fumigation in treated O_3S plants (Figure 1B), little further inhibition was seen during the fumigation period. Initially, Pn was 11% higher in the control plants; after four hours of exposure to O_3 the difference was 7%. Thus, while O_3 had an apparent long-term effect, little response could be seen during the actual fumigation period.

Stomatal conductance was initially higher in both O_3R and O_3S non-fumigated plants compared to the fumigated plants (Figure 2). Conductance of the non-fumigated O_3R plants increased during the fumigation period, while that of the fumigated O_3R plants remained stable. The increase seen in the control plants appears to be part of the normal diurnal pattern as maximum conductance was reached at midday (hours 3 to 4). For O_3S plants, there was little difference in stomatal response seen between fumigated and non-fumigated plants. Conductance

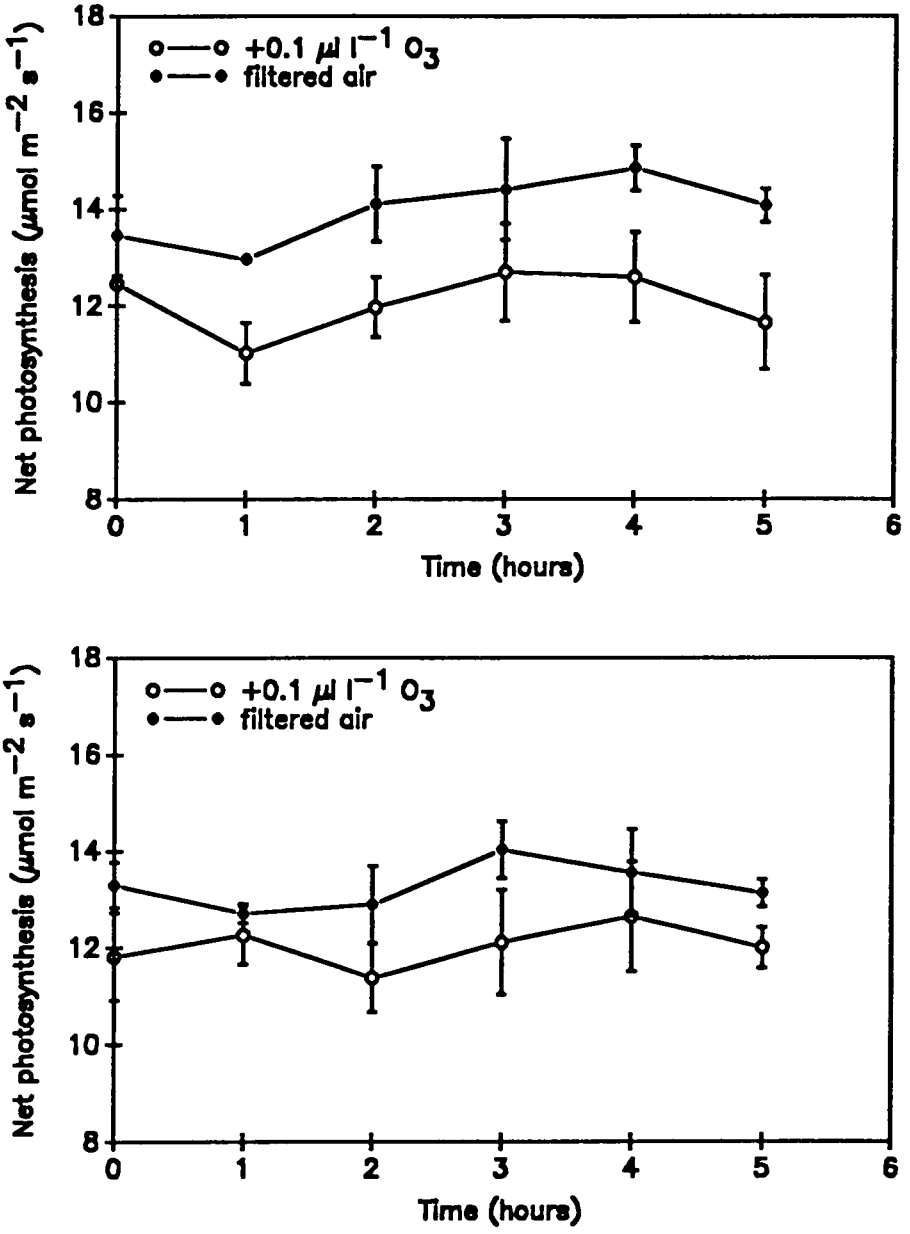

Figure 1. Net photosynthesis (μmol CO_2 m^{-2} s^{-1}) of A) O_3R and B) O_3S *Raphanus sativus* leaves exposed to 0.10 μl l^{-1} O_3 or filtered air. Fumigation was initiated immediately following the measurement at hour 0 and were completed at hour 4. Each point represents the mean of three measurements ± 1 sd.

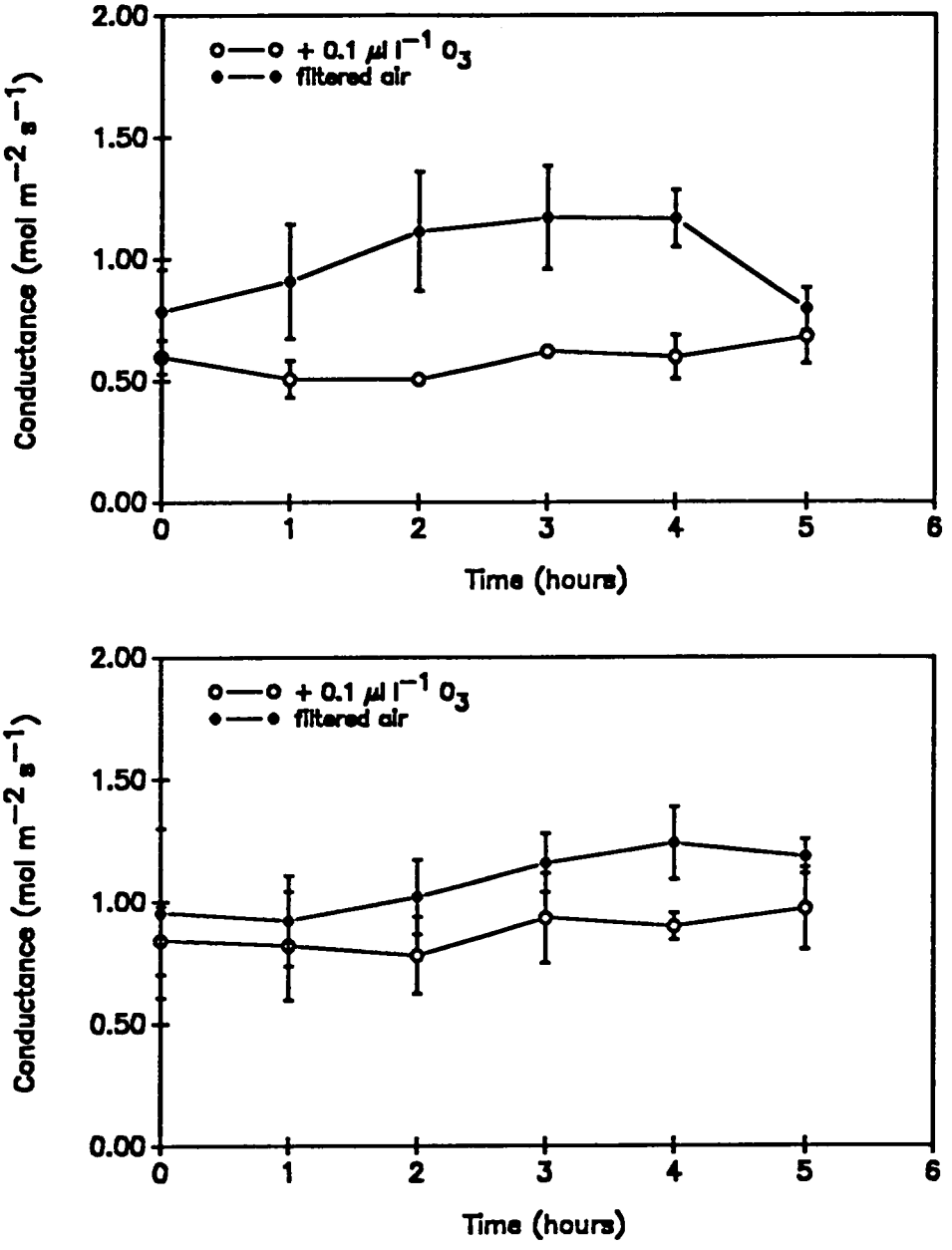

Figure 2. Stomatal conductance (mol H_2O m^{-2} s^{-1}) of A) O_3R and B) O_3S *Raphanus sativus* leaves exposed to 0.10 μl l^{-1} O_3 or filtered air. Fumigation was initiated immediately following the measurement at hour 0 and were completed at hour 4. Each point represents the mean of three measurements ± 1 sd.

in both cases increased slightly during the fumigation period. Stomata of O_3R plants may be more responsive to fumigations on a short-term basis, as they responded differently than non-fumigated plants during the fumigation. Both O_3R and O_3S plants may have long-term responses as seen by the differences seen in the initial measurements.

Similar to conductance, transpiration (Figure 3) was initially higher in the fumigated plants. However, during the fumigation period these differences narrowed, particularly in the resistant plants apparently because of the changes that were observed in the conductance of fumigated and non-fumigated plants. Fumigated sensitive plants always had higher transpiration rates than did the control plants, even though conductance values were very similar. As a result, water use efficiency (WUE) of the fumigated O_3S plants was lower than that of the non-fumigated plants (Figure 4B). WUE was also slightly lower in the O_3R plants fumigated with O_3 (Figure 4A). In this case, WUE was affected by the lower photosynthetic rates of the fumigated plants, not higher transpiration values.

The majority of ^{14}C found in the plants was in the hypocotyls, due to the plants being in a period of rapid hypocotyl growth when the leaves were labeled (Table 1). No significant differences in total uptake of labeled CO_2 were observed based on total DPMs recovered from the different groups (Table 2). Sink strength was strong in both groups of leaves sampled. Overall, carbon allocation was relatively unaffected by O_3 exposure in O_3R plants. The only significant effect seen was an reduction in ^{14}C transported out of the labeled leaf 3. This was the only indication of O_3 induced retention of assimilate observed. However, the reduction in transport appeared to affect all sinks equally, as no other differences were noted between fumigated and non-fumigated plants.

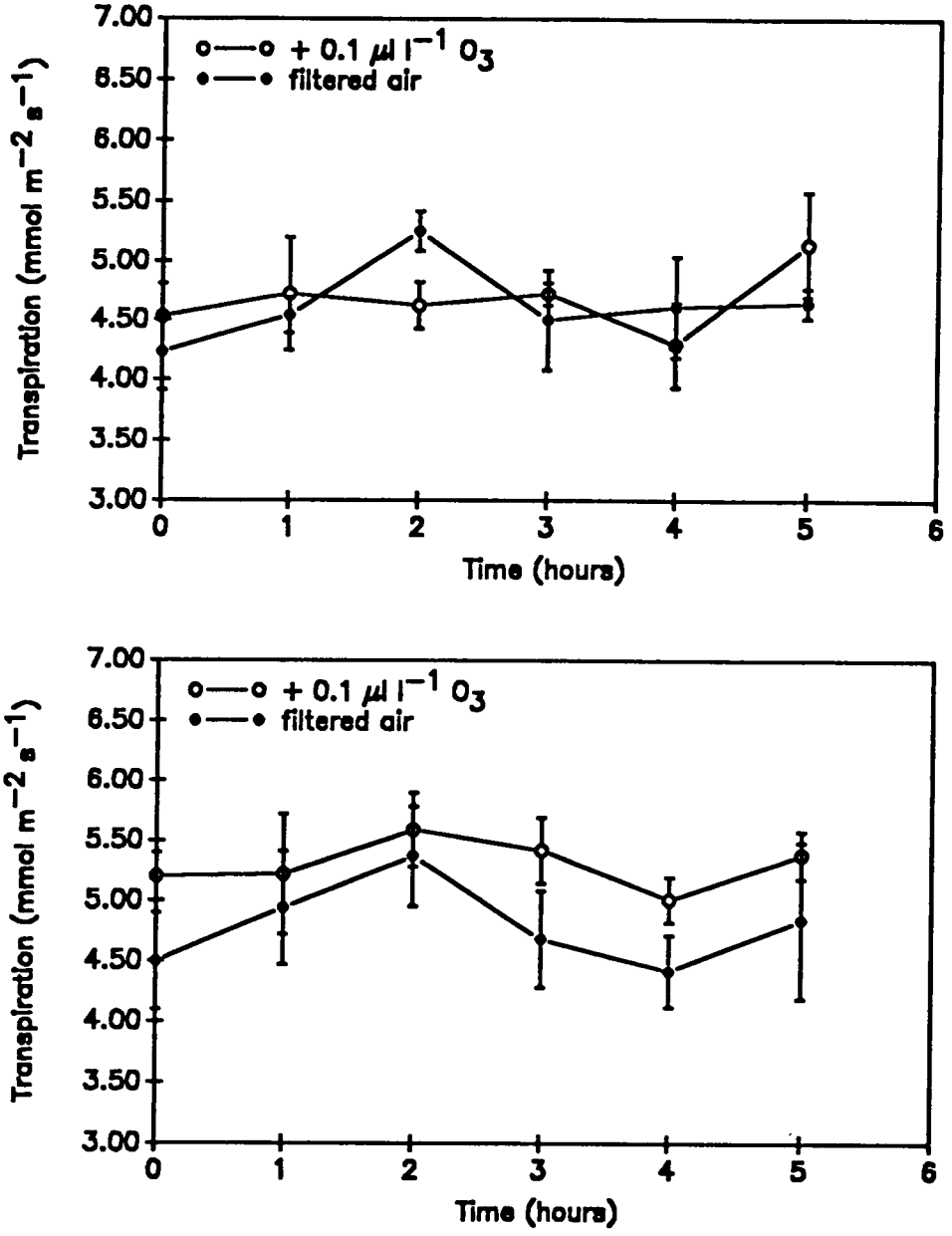

Figure 3. Transpiration (mmol H_2O $m^{-2}s^{-1}$) of A) O_3R and B) O_3S *Raphanus sativus* leaves exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air. Fumigation was initiated immediately following the measurement at hour 0 and were completed at hour 4. Each point represents the mean of three measurements ± 1 sd.

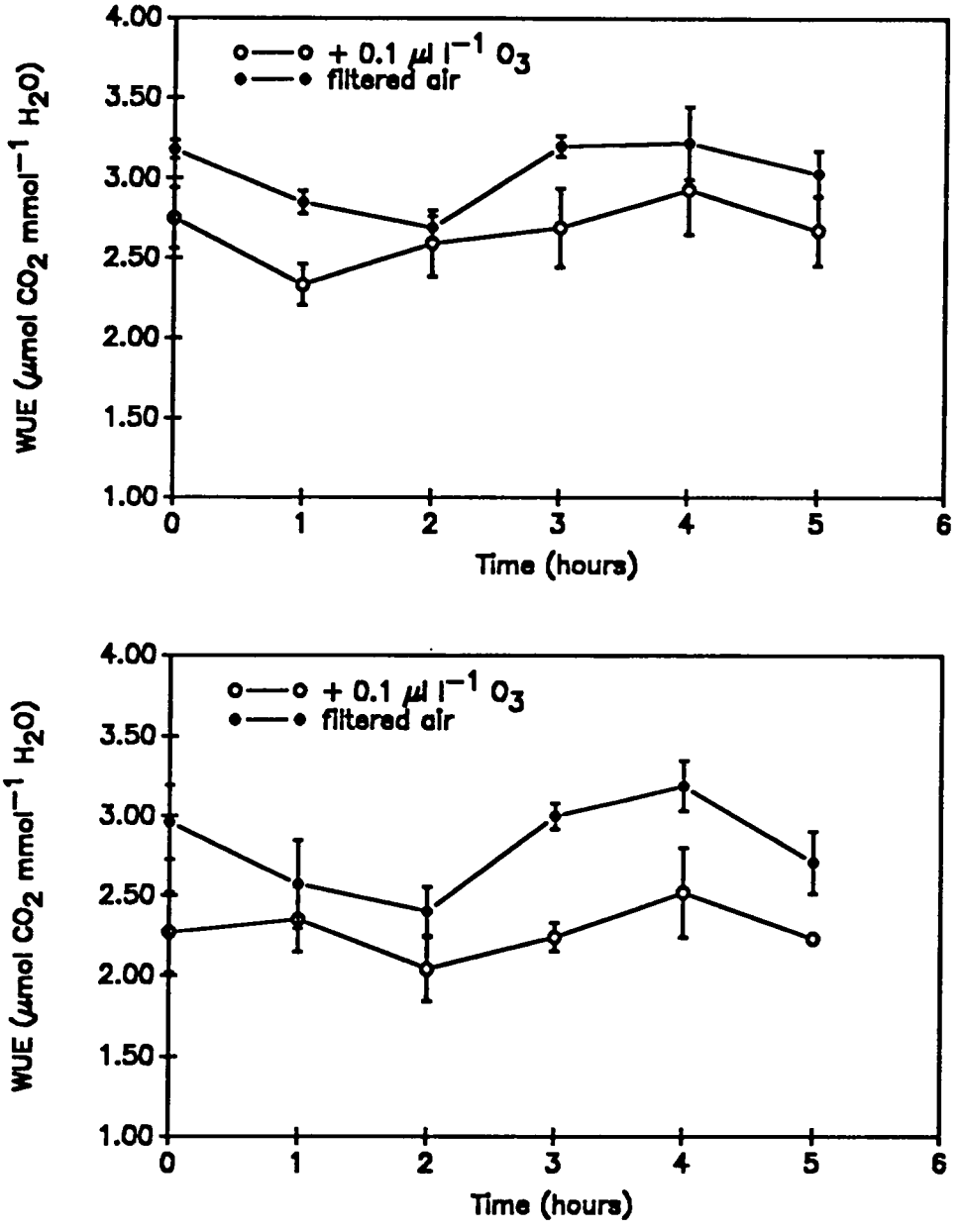

Figure 4. Water Use Efficiency (μmol CO_2 $mmol^{-1}$ H_2O) of A) O_3R and B) O_3S *Raphanus sativus* leaves exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air. Fumigation was initiated immediately following the measurement at hour 0 and were completed at hour 4. Each point represents the mean of three measurements ± 1 sd.

Table 1. Effect of 0.10 $\mu l\ l^{-1}$ O_3 on distribution of ^{14}C (% of total label found in the plant) in the various parts of 25 day old radish plants. Values are means ± SD of 3 samples, an * indicates a mean significantly different from the control value at the $P < 0.05$ level, ** at the $P < 0.01$ level.

		Leaf 1		Leaf 2	
Group	Plant Part	Control	O_3	Control	O_3
O_3R	Labeled Leaf	9.5±6.87	10.9±5.49	5.9±2.78	24.6±5.78*
	Shoot B	8.1±4.69	13.4±6.12	26.1±7.63	15.0±6.28
	Shoot A	13.2±6.79	6.4±3.17	10.8±5.56	10.4±2.85
	Hypocotyl/Root	69.3±6.58	69.4±2.54	57.2±5.56	50.0±7.35
O_3S	Labeled Leaf	6.4±1.52	9.7±5.45	8.3±6.21	8.3±3.79
	Shoot B	7.4±2.59	27.2±3.58**	10.7±3.77	13.5±0.68
	Shoot A	9.0±4.07	3.9±1.91	6.2±2.71	17.6±3.74*
	Hypocotyl/Root	77.2±5.34	59.2±6.45*	74.9±12.5	60.6±6.34*

Table 2. Total DPMs recovered from radish plants exposed to $^{14}CO_2$ following exposure to 0.1 μl l^{-1} O_3 or filtered air (FA). Single leaves of each plant were exposed for 15 minutes to the label. A 21 h chase period followed. Values are means ± SD.

Group	Labeled Leaf	Treatment	
		FA	O_3
O_3R			
	Leaf 3	1186410±206819	711630±285420
	Leaf 2	1309670±320050	1199540±291648
O_3S			
	Leaf 3	1237380±413814	1213170±161932
	Leaf 2	1025660±158548	1112650± 66463

O_3S plants were much more sensitive to O_3 fumigation than the O_3R plants. In particular, carbon transport to the hypocotyl was reduced by fumigation with O_3. This was not due to inhibition of transport from the labeled leaf, as seen in other studies with both O_3 and SO_2 (Jones & Mansfield 1982, Okano *et al.* 1984), but rather due to redistribution of assimilate. Instead of a greater proportion of label remaining in the labeled leaf, there was redistribution of label to the other leaves. The redistribution was dependent upon the age of the labeled leaf. When Leaf 3 was labeled, more label was found in the Shoot A sample, and when Leaf 2 was labeled, more was found in the Shoot B sample (Table 1).

This study indicates that both gas exchange and carbon allocation can play important roles in determining resistance to gaseous air pollutants. Gas exchange parameters of resistant plants responded more to O_3 exposures than did those of O_3S plants. This may lead to less pollutant damage within these plants. Based on the amount of DPMs recovered from the plants, uptake of CO_2 was not different between fumigated and non-fumigated plants of either O_3R or O_3S populations. However, the plants were moved from the chambers to the greenhouse immediately prior to labeling, and gas exchange of the leaves was likely affected by this movement. Examination of allocation patterns revealed differences. Hypocotyl sink strength in sensitive plants was markedly reduced by O_3 exposure, whereas allocation patterns in resistant plants were relatively unchanged.

5.4 Discussion

In this experiment, gas exchange of both O_3R and O_3S plants was affected by exposure to O_3. Both long-term and short-term effects were observed. By the time the actual gas exchange measurements were made, the fumigated plants had been

repeatedly exposed to O_3. Thus, the pre-fumigation measurements reflect the effects of these previous fumigations. The measurements made during the fumigation represent an immediate plant response, although the previous exposures can influence this immediate response as well. McCool *et al.* (1988) found that a second exposure of kidney bean plants to O_3 was less likely to cause detrimental growth effects if it came within 3-5 days of the first exposure. The interval between fumigations in this investigation was only one or two days, so there may be a strong influence of previous fumigations on succeeding ones. There is even evidence suggesting that adaptation of stomatal responses may occur under continuous fumigation. Radishes exposed to a mean 0.17 $\mu l\ l^{-1}$ O_3 exposure for thirty days had conductance values equivalent to plants grown in filtered air, similar to the stomatal response of sensitive plants in this experiment. Plants exposed for only two days had significantly lower conductance values (Walmsley, Ashmore, & Bell 1980).

Pre-fumigation measurements revealed that net photosynthesis of treated O_3R plants was less than that of the plants exposed to filtered air (Table 1). Although Pn was less in O_3S plants treated with O_3, this was not a significant difference. Other studies have documented the detrimental effects of long-term O_3 exposure on photosynthesis. Reich and Amundson (1985) found decreased Pn in a number of tree and crop species exposed to levels of O_3 below that used in this experiment, although the fumigation periods were longer. However, the work of Barnes (1972) indicated that 0.150 $\mu l\ l^{-1}$ O_3 affects Pn in some species of *Pinus*, but not in others. The level of inhibition seen in this study in the O_3R plants ranged from about 7 to 15% as compared to control values, so the effect of O_3 was not dramatic, but consistent.

During the actual fumigation, Pn of the O_3 exposed O_3R plants decreased more relative to the initial rate more than that of the non-treated plants, while that of the O_3S plants was similar to that of the controls. Thus, it seems that Pn of O_3R plants is more sensitive to O_3 than is that of the O_3S plants. But previous growth studies have demonstrated that in fact plant biomass is greater in the resistant plants. Utilization of photosynthate presumably is different between resistant and sensitive plants under O_3 stress. Taylor and Tingey (1983) have outlined possible responses to pollutant stress and injury. These include avoidance, compensation, or repair. Both avoidance and repair reduce carbon allocated for growth. Avoidance may be a factor in the greater growth of the O_3R plants, as they had lower conductance under O_3 stress, while the O_3S plants had similar conductance in either fumigated or non-fumigated conditions. Repair processes may well be increased more in the sensitive plants, although there is no direct evidence for this. Other processes related to carbon use in the plant were altered, even in the O_3S plants. Labeling of the plants was conducted at a time during plant development in which the hypocotyls were rapidly increasing in size. The subsequent finding of a large part of the label in the hypocotyl and root fraction is not surprising. The only change found in the O_3R plants was increased retention of label in the younger labeled leaves. This is in contrast to some other studies. McLaughlin & McConathy (1983) reported that primary leaves of *Phaseolus vulgaris* had a greater tendency to retain carbon upon exposure to O_3 than did first trifoliate leaves. This led to greater relative reductions in root growth, as the primary leaves were the primary sources of carbon for the root. Older leaves generally are more important sources for lower plant parts, but this may be, in part, due to their position along the stem. In this experiment, there was less indication of older leaves being so tightly linked as carbohydrate sources for the

hypocotyl and root, possibly due to the rosette form of the radish plant, in which all leaves are relatively the same distance from various sinks. While the older leaves in the O_3R apparently translocated more carbon to the hypocotyl and roots than did the younger leaves, in the O_3S plants, the percent of ^{14}C reaching the lower portions of the plant were similar for both old and young leaves.

Overall, though, translocation was affected more by O_3 in O_3S plants than in O_3R plants. Hypocotyl and roots had reduced label when either first or second leaves were labeled, and there were subsequent increases in the amount of label in the leaves. In comparison to O_3R plants, this would not appear to be an adaptive response, as the O_3S plants have been shown to have reduced growth, including leaves. O_3R plants are able to grow larger (more biomass) under O_3 stress without such large shifts in carbon allocation patterns.

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6. Effects of O_3 on Leaf Development and Chlorophyll Concentrations in Two Populations of Radish.

6.1 Introduction

In many areas of the U.S., the ambient levels of ozone (O_3) inhibit growth of several plant species. While leaves may not be the most sensitive part of the plant, alterations in leaf development, physiology, and biochemistry often result from exposure to O_3. Mooi (1980) reported an acceleration of leaf senescence in hybrid poplars fumigated with 0.4 μl l^{-1} O_3 over several months, while Noble & Jensen (1980) observed similar results with hybrid poplar treated with high concentrations of O_3. Physiological responses associated with accelerated senescence include decreases in net photosynthesis (Reich 1983) and reduced amounts of chlorophyll (Reich *et al.* 1986).

In this study, development of individual leaf pairs was monitored in two lines of radish (*Raphanus sativus* L.) differing in their sensitivity to O_3. Previous studies had indicated that O_3 reduced leaf growth in the sensitive line. Leaf growth of the resistant line was apparently unaffected based on measurements taken at maturity. Growth analysis measurements indicated that even in resistant plants in the early stages of growth, leaf growth was reduced after fumigation compared to control plants. This suggests that the resistant plants are in some way able to accommodate O_3 stress and minimize its effects on leaf growth. Two possible mechanisms were considered in this study: the increased production of new leaves and the production of larger leaves. The ability of plants to adapt to stresses by producing more leaf

biomass has been shown in both low light and pollutant stress studies (Oshima, Bennett, & Braegelmann 1978, Mooney *et al.* 1988).

6.2 Materials and Methods

From previous studies, populations of radish (*Raphanus sativus* L. cv Cherry Belle) with differential sensitivity to O_3 were available. Differences between these populations based on final plant dry weight had been demonstrated. Plants from both an O_3 resistant (O_3R) and an O_3 sensitive (O_3S) population were used.

Seeds were sown in fifteen cm pots in a 3:1:1 mixture of peat moss, vermiculite, and Weblite^R (v/v/v) with 6 g of a 14:14:14 controlled release fertilizer (Osmocote, Sierra Chemical Co., Milpitas, CA) added per pot. Prior to seeding, all pots were watered with a micronutrient solution. Seedlings were thinned to 1 per pot 3-5 days after emergence. Plants were maintained in a charcoal filtered greenhouse with daylength maintained at 16 h using high pressure sodium lamps approximately 1 meter above the benches. Average day/night greenhouse temperatures during this study was 27/22 C.

Fumigations were made in a Continuous Stirred Tank Reactor System (CSTR), beginning 10 days after emergence. A fumigation regime of 3 days per week, 4 hours per day for 3 weeks was maintained. Plants were moved from the greenhouse to the CSTR prior to the fumigation and returned immediately afterwards.. Ozone was generated by a Welsbach Model T-408 Ozone Generator (Welsbach Ozone Systems Corp., Philadelphia, PA) from O_2 passed through a UV light source, and was supplied to each chamber to maintain a concentration at 0.1 μl l^{-1}. Pollutant concentration was maintained by mass flow controllers connected to a data acquisition system. Ozone concentrations in the chambers were monitored on a

time-share basis with a TECO Model 49 photometric O_3 analyzer (Thermo-Electron Corp., Hopkinton, MA). The monitor was calibrated on a regular basis with a CSI 3000 O_3 calibrator (Columbia Scientific Industries Corp., Austin TX). Chamber lighting ranged from 450-600 $\mu E\ m^2\ sec^{-1}$ through the use of 1000 W metal halide lamps above each chamber.

Determinations of leaf emergence and length were made every other day beginning 6 days after emergence. Length was measured from the base of the petiole to the tip of the largest leaf from each leaf pair. Leaf area was also measured 30 days from emergence on the same plants used for leaf length measurements. These leaves were removed with a razor blade and area was determined for each leaf pair using a LI-COR 3050 Leaf Area Meter (Lambda Instruments Corp., Lincoln, NE). Using the leaf measurements and leaf areas measured on Day 30, a first order regression was made in order to be able to estimate leaf area from the previous measurements of leaf length. The regression had an r^2 value of 0.943. The estimated leaf areas were then used to develop curves of leaf area over time. Differences in final leaf area between the two plant lines in either O_3 or filtered air were tested using t-tests. Two replicates, each consisting of four samples, were run.

Leaf chlorophyll concentrations were determined using the methodology of Moran & Porath (1980). One leaf from each of the second and third leaf pairs was removed immediately following fumigation and weighed. The whole leaves were then placed in 10 ml of *N,N*-dimethylformamide. The samples were covered and placed in a refrigerator for 48 h. A ml sample was then taken for analysis with a Beckman UV-6 Scanning Spectrophotometer (Beckman Instruments, Fullerton, CA). Absorbance readings were taken at 647 and 664 nm. concentrations ($\mu g\ ml^{-1}$)

of chlorophyll a (chl*a*) and chlorophyll b (chl*b*) were calculated according to the equations of Moran (1981):

$$Chla = 12.64(A_{664}) - 2.99(A_{647})$$

$$Chlb = -5.6(A_{664}) + 23.26(A_{647})$$

6.3 Results

While air pollutants directly affect aerial parts of plants such as leaves, indirect effects of pollutants may also influence these plants parts. In this experiment, changes in leaf area could be the result of either direct or indirect effects of O_3. For O_3R plants, there was no difference in final leaf area between the two treatments, either on a single leaf pair or total leaf area basis (Table 1). There were, however, differences in leaf area of O_3S plants between treatments. Leaf area of the third leaf pair and total leaf area was less in the O_3 treatment than in filtered air.

Patterns of leaf development also indicate some differences, particularly between the two plant lines. The first leaves (Figure 1) developed similarly for all treatment groups; these leaves began to develop prior to or just as the fumigations began. Thus, they may have been able to develop fully without any reduction in leaf size because the dose of O_3 was insufficient during their development to cause such an effect.

The next leaf pair, though, did not begin development until after the fumigations were started (Figure 2). Leaves of both control and fumigated O_3R plants appeared to develop ahead of those of the O_3S plants, although the time of new leaf initiation was similar. Differences between treatments were not observed for either O_3R or O_3S plants.

Table 1. Leaf area (cm^2) of *Raphanus sativus* L. plants 30 days after emergence exposed to 0.10 $\mu l\ l^{-1}$ O_3 or filtered air (FA). Values are the mean ± sd of 8 plants. Numbers followed by an * are significantly different at the $P < 0.05$ level.

Group	Treatment	Leaf Pair				
		1	2	3	4	Total
O_3R	FA	45.51±5.34	103.44±11.96	82.70±10.38	14.44±11.63	246.31±30.33
	O_3	46.68±6.74	101.02±14.27	78.35± 8.43	19.20± 2.34	246.58±22.12
O_3S	FA	44.33±7.47	91.64±8.98	79.26±11.02	11.48± 8.67	226.7±15.38
	O_3	44.40±4.97	84.36±9.35	65.23±9.65*	20.88± 9.12	201.6±11.12*

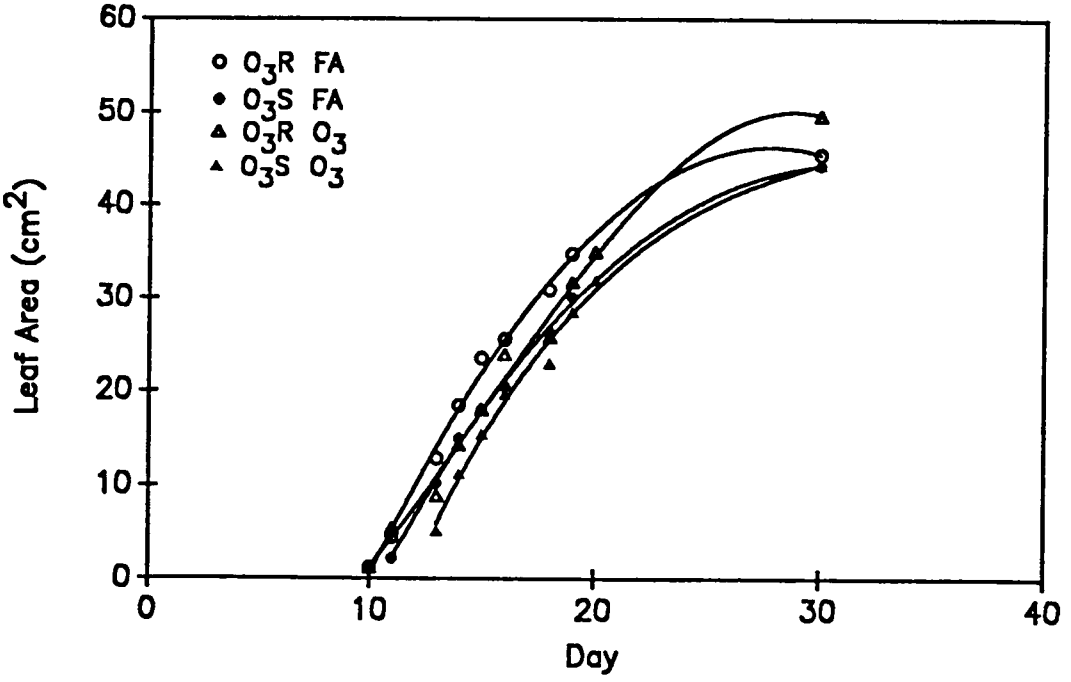

Figure 1. The change in leaf Area (cm^2) of one leaf from the first leaf pair of radish plants through time. Each point represents the mean of eight plants.

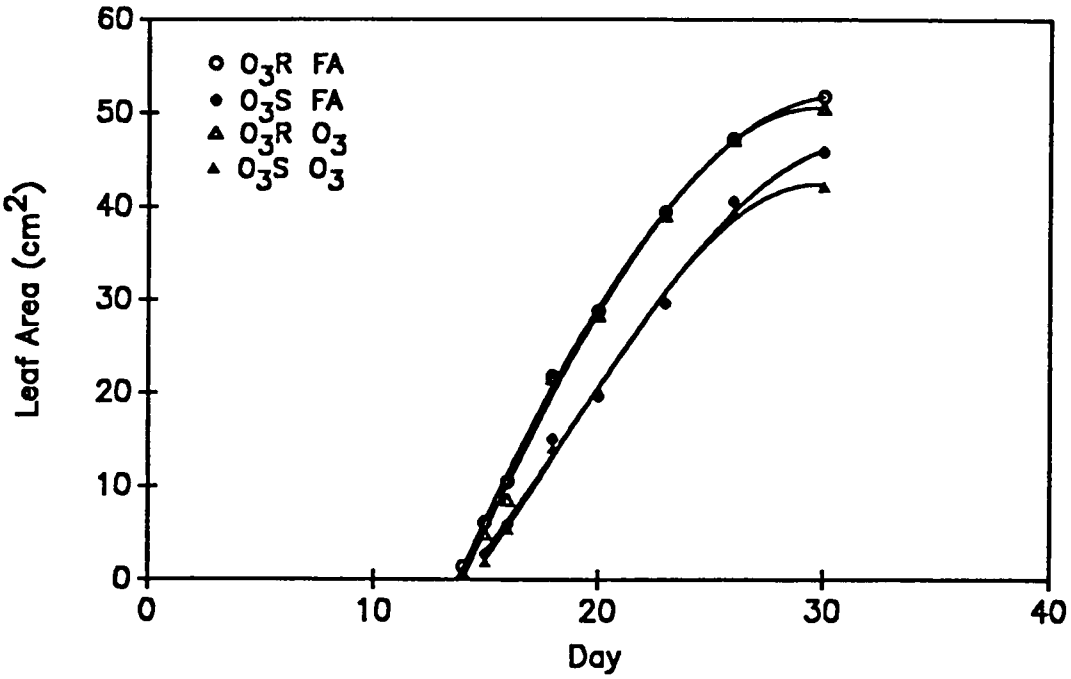

Figure 2. The change in leaf Area (cm^2) of one leaf from the second leaf pair of radish plants through time. Each point represents the mean of eight plants.

The last leaf pair to develop to any great extent during the life of these plants was the third leaf pair (Figure 3). Like the previous two pairs, the O_3R plants were slightly ahead, developmentally, of the O_3S plants. This occurred even though the leaves would emerge on roughly the same day in both O_3R and O_3S plants. Thus, O_3 did not seem to affect the time of leaf emergence. For this leaf pair, O_3 affected only the leaf area in O_3S plants. Although leaf area of the fourth leaf pair was slightly higher in treated plants, the leaves were still small by day 30 and the differences were not significant. The number of leaf pairs present at the end of the experiment was equivalent between treatments for both O_3R and O_3S plants (Figure 4). While some plants had produced a fifth leaf pair, the majority of plants had produced only four pairs by the final fumigation date.

Chlorophyll concentrations were related to leaf age. Older leaves had higher a/b ratios than did young leaves (Table 2), as a result of reduced levels of chl *b* in the older leaves. Ozone did not appear to have any obvious effect upon leaf chlorophyll concentrations in either group. No significant differences were found between O_3R and O_3S plants in either young or old leaves.

6.4 Discussion

Leaves are the almost exclusive entry point for gaseous pollutants, and the fate of pollutants is generally determined within the leaves. Thus, the way in which leaves respond to pollutants has great bearing on the whole plant response. In these experiments, O_3 had a negative impact upon the area of leaves of O_3S plants, but only the leaves of the second leaf pair. Uptake of gaseous pollutants tended to be greatest in intermediate age class leaves (Craker & Starbuck 1973), which leads to those leaves being the most sensitive. Three leaf pairs in this experiment approached

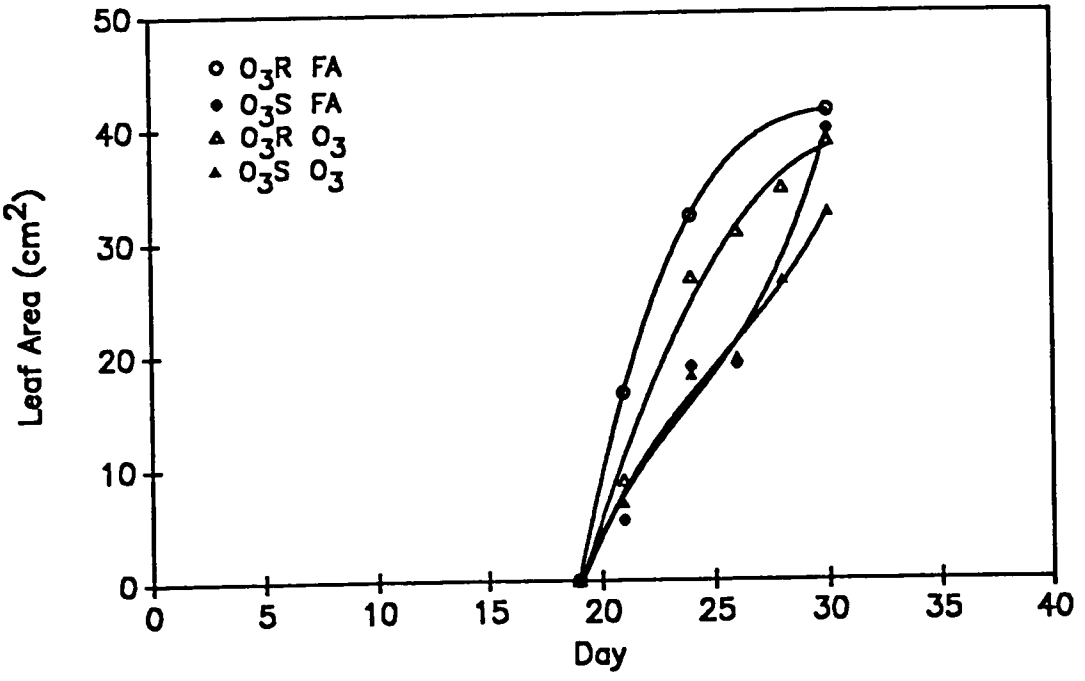

Figure 3. The change in leaf Area (cm^2) of one leaf from the third leaf pair of radish plants through time. Each point represents the mean of eight plants.

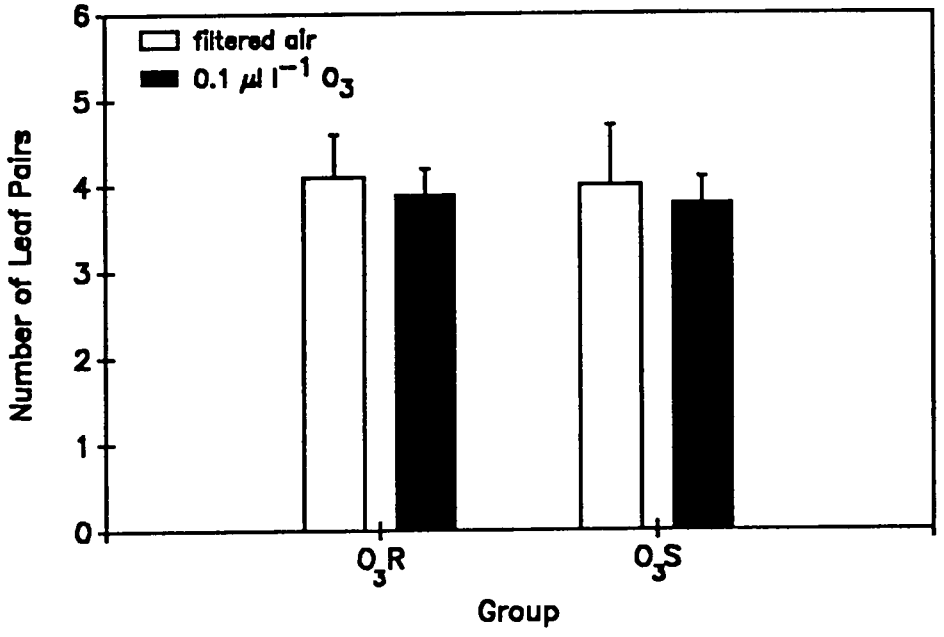

Figure 4. Number of leaf pairs 30 days after emergence on radish plants exposed to 0.10 μl l^{-1} O_3 or filtered air (FA). Values are the mean ± sd of 8 plants.

Table 2. Leaf chlorophyll concentrations ($\mu g\ mg^{-1}$ fresh weight) and chlorophyll a/b ratios measured on Day 30 of young (3rd leaf pair) and old (2nd leaf pair) leaves. Values are the mean of 4 measurements ± sd.

Leaf Pair	Group	Treatment	chl *a*	chl *b*	a/b
Young	O_3R	FA	0.602±0.084	0.902±0.191	0.667
		O_3	0.418±0.113	0.760±0.180	0.550
	O_3S	FA	0.590±0.212	0.914±0.133	0.646
		O_3	0.603±0.219	0.801±0.074	0.753
Old	O_3R	FA	0.538±0.050	0.567±0.189	0.949
		O_3	0.664±0.140	0.732±0.464	0.907
	O_3S	FA	0.662±0.132	0.468±0.124	1.415
		O_3	0.488±0.135	0.485±0.199	1.006

or reached near full expansion, but only the third pair in the sensitive plants appeared to be affected. The effects of O_3 on this leaf pair were not obvious until the leaves were reaching full expansion (Figure 2), when they may be most sensitive. Leaf area of leaves on the O_3R plants did not appear to be affected. The reason why only one pair of leaves was sensitive may be related to an adaptive response to O_3. Walmsley, Ashmore, & Bell (1980) found that under continuous exposure conditions, succeeding pairs of radish leaves tended to be more resistant to O_3 than previous ones. The first two leaf pairs in both lines were apparently unaffected by fumigation with O_3 These leaves may have been able to develop without any loss of growth before the O_3 dose was sufficient to cause damage. The fourth leaf pair was far from reaching full expansion, making an assessment of the effect of O_3 on this leaf pair difficult. It would be interesting to determine whether this leaf pair was affected by O_3 or whether an adaptive response might be seen.

A characteristic of plants given long-term O_3 fumigations is the ability to accelerate the production of new leaves. Walmsley *et al.* (1980) observed this in radishes and hybrid poplars also do this (Reich & Lassoie 1985). In both cases the authors attribute the onset of new leaves to the increase in leaf senescence, particularly in the poplars which are known to initiate new leaves as old ones drop. From the data here, neither leaf senescence nor new leaf initiation was affected by O_3. New leaves began to develop at approximately the same time whether or not they were exposed to the pollutant and all plants had 4-5 leaf pairs by the end of the experiment. The plants still retained all their leaves at the end of the experimental period. The dose of O_3 used in this experiment was possibly insufficient to cause the onset of leaf senescence, which may be required for increased leaf production to occur.

Chlorophyll concentrations were measured in order to determine whether it may be a good measure of pollutant sensitivity. Reich (1983) found significant reductions in both chl*a* and chl*b* in leaves of hybrid poplar (*Populus deltoides* x *trichocarpa*) given a chronic low dose of O_3, as did Adepipe, Fletcher, & Ormrod (1973) in O_3 exposed tobacco leaves. Lenherr *et al.* (1988) also detected decreases in total chlorophyll of wheat leaves, but only if the dose of O_3 was above a certain level. Ozone did not alter chlorophyll concentrations in either of the leaf pairs measured in this experiment. The lack of effect may be due to the level of O_3 used. Low doses of O_3 in the experiment by Lenherr did not affect leaf chlorophyll concentrations and fumigations in that experiment lasted for more than one month. Although there were signs of visible injury on some of the radish leaves, in general the plants appeared healthy, even in the O_3 treatment.

Ozone will accelerate leaf aging and senescence (Jensen 1973) and chlorophyll concentrations are dependent upon leaf age. Chlorophyll a is generally more sensitive to pollutant exposure than is chlorophyll b, creating a decrease in the a/b ratio (Fangmeier 1989). There was a marked difference in the a/b ratio between young and old leaves; however, O_3 did not cause a similar effect in either resistant or sensitive plants.

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7. Summary and Conclusions

These experiments demonstrate the potential of ozone (O_3) to influence the composition of O_3 sensitive plant populations. Using a concentration of O_3 (0.1 μl l^{-1}) commonly reached in many parts of the U.S., development of a resistant population was achieved through selection of resistant individuals and subsequent breeding among the resistant individuals. On the other hand, sulfur dioxide (SO_2) proved to be a much less potent selective force. A very high concentration (0.5 μl l^{-1}), well above commonly found ambient levels, was required to cause a degree of growth inhibition equivalent to that caused by O_3. Even at this level, growth analysis of the resulting F_1 plants indicated that a stable SO_2 resistant population did not result from the selection and breeding process.

Some possible physiological mechanisms related to the differences in growth of the O_3-selected populations were examined. From the initial growth studies, it was apparent that the root/shoot ratios of O_3 resistant (O_3R) plants was much less affected by O_3 than those of either the non-selected (NS) or O_3 sensitive (O_3S) plants. Thus, the focus of the following experiments was on the mechanisms involved in biomass partitioning. A more detailed growth analysis study, involving sequential harvests, was made. At early growth stages, all three populations exhibited reduced growth in response to O_3 fumigation; however, the O_3R plants were able to overcome the early growth reductions so that by maturity, the plants were similar in size to non-fumigated plants. Fumigated O_3S plants did not recover and differences in hypocotyl growth between fumigated and non-fumigated plants increased as plants reached maturity.

Analysis of leaf carbohydrate levels revealed some changes caused by O_3 fumigation. During the earlier stages of growth, there were lower concentrations of free sugars in the fumigated leaves in both resistant and sensitive plants. These lower concentrations may be due to greater partitioning of assimilated carbon into starch, but could also be due merely to reduced amounts of carbon available, through a decreased photosynthetic rate or greater use of available carbohydrates for repair processes. Measurements made as the plants reached maturity indicate that there were higher levels of sugars in the older leaves of sensitive plants. Because these plants also had reduced hypocotyl biomass, the increase of sugars in the older leaves could be a product of reduced translocation.

In the next series of experiments, ^{14}C was used as a tracer to follow the movement of carbon assimilated into the plant through photosynthesis. This study indicated that there was, in fact, reduced carbon flow to the hypocotyl of fumigated O_3S plants when older leaves were labeled with ^{14}C. When younger leaves were labeled, the amounts of label found in the hypocotyl were similar in both fumigated or non-fumigated plants. Gas exchange measurements made during this study indicate that photosynthesis was reduced, particularly in O_3R plants. These differences in photosynthesis were increased during actual fumigations. Photosynthetic inhibition generally results from one of two causes: closure of stomata or an increase in the mesophyll resistance. Stomatal closure was apparently not a factor in the initial low photosynthetic values, as conductance was higher in the fumigated plants. However, during the actual fumigation period, the increase in differences in photosynthetic rates between fumigated and non-fumigated plants is more likely due to stomatal responses. Conductance of the control plants increased in a typical diurnal response, while those of the fumigated plants remained fairly

stable. Due to generally lower photosynthetic rates and higher rates of transpiration, water use efficiency was reduced in the fumigated plants.

The final experiments attempted to elucidate the effects of O_3 on leaf development and aging. While leaf area was reduced in fumigated O_3S plants, there was no indication of an acceleration of leaf aging as determined by measurements of leaf chlorophyll, or the subsequent increase in the production of new leaves as detailed in other studies. The same experiment done on a larger scale, perhaps with sequential harvests to better measure leaf area and leaf development, may provide a more clear indication of the influence of O_3 on leaf development and aging.

Overall, these experiments provide an initial step into understanding the potential not only for gaseous pollutants to influence plant populations, but also the ways in which plants adapt to the presence of the pollutants. The O_3R radish population was able to better maintain normal patterns of carbon partitioning when put under O_3 stress. It has been suggested that an adaptive response may occur which results in an increase in partitioning to shoots at the expense of roots. Sensitive plants did this to a greater extent than did resistant ones, so this would not appear to be a particularly successful response, as total biomass was reduced. Interestingly, fumigated resistant plants had greater leaf area and biomass than did fumigated sensitive plants, despite less severe decreases in assimilate partitioning to roots. This would seem to indicate that there may be additional demands for carbon in the shoots and leaves of sensitive plants, possibly for repair mechanisms.

The ability of radish plants to dynamically respond to pollutant stress has been demonstrated in these experiments. With O_3 becoming an increasingly widespread problem, plant resistance to O_3, whether produced by human design or unintentionally by selection, may become more common, as have SO_2 resistant grass

populations in Britain. These studies indicate that O_3 resistant populations may develop very quickly and that the resistant individuals are able to accommodate ambient pollutant stress without dramatic changes in growth.

Appendix. List of Abbreviations

C_i	Internal CO_2 concentration
FA	Filtered air
NAR	Net assimilation rate
NO_2	Nitrogen dioxide
NS	Non-selected population
O_3	Ozone
O_3R	Ozone resistant population
O_3S	Ozone sensitive population
PAR	Photosynthetically active radiation
Pn	Net photosynthesis
RGR	Relative growth rate
SO_2	Sulfur dioxide
SO_2R	Sulfur dioxide resistant population
SO_2S	Sulfur dioxide sensitive population
Ts	Transpiration
WUE	Water use efficiency

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