Chapter 15

AIR POLLUTION STRESS

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1. INTRODUCTION

The atmosphere surrounding a plant is a complex mixture of gases and particles, some common and some present in only trace amounts. In addition to naturally-occurring gases such as N_2, O_2, CO_2, water vapor, and methane, the lower atmosphere also contains a wide array of natural and anthropogenic compounds whose presence in the air can strongly affect the growth of plants. Many of these potentially toxic compounds occur naturally in the lower troposphere, but when present at concentrations significantly in excess of background concentrations they are classified as air pollutants. Industrialization and urbanization over the past 200 years have greatly increased the concentrations of these toxic compounds in the atmosphere, increasing the potential for adverse effects of air pollution on the growth and productivity of crop plants and forests (Heck *et al.*, 1988; Smith, 1990).

Primary air pollutants are those that are emitted directly into the atmosphere, often from industrial processes or combustion, and are usually highly local in origin and effects. Examples of primary gaseous air pollutants are sulfur dioxide (SO_2), from combustion of sulfurous coal or oil, and smelting and refining of metal ores; hydrogen fluoride (HF), emitted from aluminum refineries; ethylene (CH_2CH_2) emissions from polyethylene plants; or carbon dioxide (CO_2) and oxides of nitrogen (NOx) from a variety of combustion sources. Such pollutants can also arise from small local sources, such as accidental spills of liquid ammonia (NH_3) or chlorine compounds (Cl_2, HCl). Primary particulate air pollutants include fugitive dust from exposed soil and roadways, abrasion products such as automobile tire and brake particles, construction and demolition dusts, and even salt from marine spray and industrial cooling towers.

Secondary air pollutants are those produced in the atmosphere by reactions among natural or anthropogenic precursor compounds. The most significant secondary air pollutant for vegetation is tropospheric ozone (O_3) produced by photochemical reactions among O_2 and NO_x from natural sources and from autos and other combustion sources, and organic compounds emitted from auto exhaust, industrial processes, solvents, and vegetation. Ozone is produced in great quantities in the upper atmosphere through the photolysis of molecular oxygen, and in small amounts in the lower troposphere, by lightning or similar high-energy events. However, O_3 is sufficiently chemically reactive that natural background concentrations in the lower troposphere are low, and without the presence of excess NO_x and hydrocarbons, O_3 concentrations would be self-limiting (Finlayson-Pitts and Pitts, 1986). Other secondary air pollutants include peroxyacetyl nitrate (PAN), H_2O_2, and a variety of other oxygenated compounds. These are less abundant than ozone, but mole for mole may be more phytotoxic.

Polluted atmospheres also contain aerosols, particles, heavy metal vapors, acid precipitation, pesticides, herbicides, and numerous other potentially toxic materials. With a few exceptions, the discussion of air pollution effects in this chapter will be confined to the major gaseous air pollutants, particularly O_3 and SO_2. Descriptions of injury, lists of susceptible plant species, and color photographs of injury symptoms produced by exposure to these and to the minor air pollutants can be found in *Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas* (Flagler, 1998).

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2. PLANT EXPOSURE TECHNIQUES

Our current state of knowledge of the effects of air pollutants on plants has been obtained from the integration of information derived across the spectrum of plant sciences applied to all levels of organization in the plant and its environment. The greatest progress in advancing the state of the science has come when techniques for the exposure of whole plants or plant parts have been applied at the appropriate level of organization within the plant. Information on the effects of acute (*i.e.,* high concentration, short-term) exposures on biochemical or physiological processes, such as photosynthesis, has primarily been obtained using leaf cuvettes. In cuvettes, whole leaves can be exposed to known concentrations of pollutants, and photosynthetic rates can be measured under defined environmental conditions (Legge *et al.*, 1979). Intermediate-term (days or weeks) exposures to study the effects of air pollutants on whole-plant processes, such as carbon partitioning or water relations can be carried out in growth chambers or in continuously-stirred tank reactor (CSTR) chambers, specifically designed for air pollution research (Rogers *et al.*, 1977; Heck *et al.*, 1978). Information on the effects of chronic exposures (several months or growing seasons) to low, medium, or high levels of pollutants to study effects on growth and yield of agronomic crops has been obtained primarily from open-top chamber studies, conducted in the field on plants growing directly in the ground or in large pots. One typical open-top chamber design (Heagle *et al.*, 1973), widely used in the National Crop Loss Assessment Network (Heck *et al.*, 1988), was 3 m in diameter, 3 m high, and was open at the top to allow natural precipitation and pollinators to enter (Fig. 15-1). Pollutant concentrations are controlled by filtering the air entering the chamber through activated charcoal to remove ambient pollutants, and then adding known amounts of pollutants such as O_3 or SO_2 to yield the desired concentrations and exposure regimes within the chambers.

All plant exposure systems come with a suite of advantages and disadvantages (Hogsett *et al.*, 1987; Manning and Krupa, 1992). For example, temperature and humidity in open-top chambers are often slightly higher than field conditions, and light intensity a shade lower. To date, the open-top chamber is the most widely used and accepted technique for the exposure of plants to gaseous air pollutants in the field. Most studies of the effects of air pollutants on the growth and yield of cotton have used open-top chambers, while physiological studies have often been conducted in CSTR and closed field exposure chambers (Musselman *et al.*, 1986).

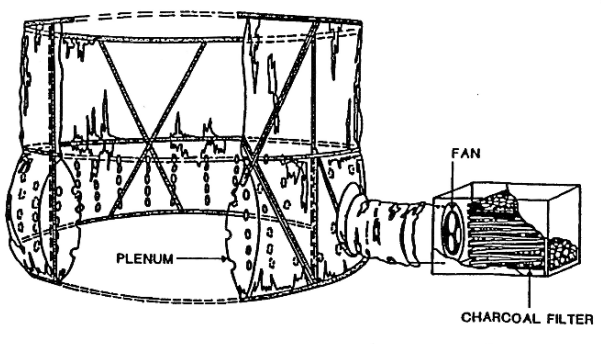

Figure 15-1. Diagram of an open-top chamber used to assess the effects of air pollutants on the growth and yield of field-grown crops. The open-top chambers used in the National Crop Loss Assessment Network were 3 m in diameter by 3 m high. (Diagram adapted from Heagle *et al.,* 1973)

3. OZONE

3.1 Origin and Distribution.

Ozone is a colorless, odorless gas that is only moderately soluble in water. As mentioned earlier, excess tropospheric O_3 is formed by photochemical reactions among O_2, NO_x, and reactive hydrocarbons. Both NO_x and hydrocarbons are produced by the combustion of gasoline, so the production of excess O_3 is often associated with dense automobile traffic. Other factors that contribute to the formation of high concentrations of O_3 include the presence of an inversion layer or stagnant air mass, which traps the precursor and reaction product pollutants, and high temperature and light intensity, which increase the rate of the photochemical reactions (Finlayson-Pitts and Pitts, 1986). Although these conditions are normally associated with Los Angeles-type smog, high O_3 pollution occurs in major parts of the cotton-growing regions of the U.S., including the southern San Joaquin Valley, south-central Arizona, southeastern and central Texas, and the Cotton Belt from Alabama to North Carolina (Lefohn, 1992).

No specific information is available on O_3 concentrations in other cotton-growing regions of the world. However, given the close association among O_3 formation, high temperatures and light, and stagnant air masses (Finlayson-Pitts and Pitts, 1986), the potential is present for production of phytotoxic concentrations of O_3 in cotton-growing areas downwind of major urban areas around the world (Schenone, 1993).

Other photochemical oxidant air pollutants, particularly peroxyacetyl nitrate (PAN) are produced in polluted urban atmospheres if sufficiently high concentrations of precursor molecules are available. Cotton is resistant to the effects of PAN (Taylor and MacLean, 1970), and no known instances of PAN injury to cotton have been recorded in the field.

3.2 Entry of Ozone into the Leaf and Initial Toxicity

3.2.1 Transport

Ozone moves from the bulk atmosphere to the sites of action inside the sub-stomatal cavity by eddy transport in turbulent air and by molecular diffusion near the leaves.

Dry deposited O_3 is removed from the atmosphere through decomposition on soil and external plant surfaces, and on plant interior surfaces through oxidation of metabolites and enzymes and by degradation in the extracellular fluid. Ozone enters the plant through the stomata, and the rate of entry can be predicted from models of stomatal conductance and ambient O_3 concentrations (Grantz *et al.*, 1994; Musselman and Massman, 1999).

Cotton in the San Joaquin Valley of California was found to remove 32.5 mg m^{-2} of O_3 per day (Grantz *et al.*, 1994), based on aircraft measurements using the eddy covariance technique. This is a relatively high flux, attributed to the large stomatal conductance of cotton relative to other vegetation. For example, in the same study, orchards and vineyards removed about 25.8 mg m^{-2} per day (Grantz *et al.*, 1994). This removal by dry deposition to vegetation and other surfaces was sufficient to alter regional O_3 concentrations in large-scale model simulations. In general the stomatal pathway dominates O_3 removal from the atmosphere, and thus stomatal regulation is key to determination of total O_3 dose to a plant (Baldocchi *et al.*, 1987). The aircraft-based measurements of O_3 deposition to an extensive cotton field were closely related to directly measured stomatal conductance (Fig. 15-2). In general a correlation exists between radiation, stomatal opening, and atmospheric transport efficiency (Grantz *et al.*, 1997), which strengthens the relationship between stomatal conductance and O_3 deposition velocity (ratio of O_3 flux to ambient concentration). This may facilitate prediction of O_3 uptake by the cotton canopy.

3.2.2 Initial Toxicity

The site of action and the mode of initial toxicity of O_3 have been investigated for over four decades, yet these issues remain unresolved. Studies with animal cells demonstrate that little, if any, O_3 reaches the cytoplasm (Pryor, 1992), due to the reactivity of O_3 and its interactions with extracellular fluids. In plants, O_3 reacts with the cell wall and antioxidants and enzymes present therein. However, reaction products of O_3 with plant metabolites may be less reactive and thus penetrate to the cell membrane and possibly into the cytoplasm. That the oxidizing potential of O_3 is somehow communicated to the cytoplasm is made clear by the rapid changes observed in the chloroplasts. Chlorophyll fluorescence kinetics, mRNA for the primary carboxylase, RUBISCO, and ultrastructure of the thylakoid membranes, all change rapidly in response to O_3 exposure (Heath, 1988).

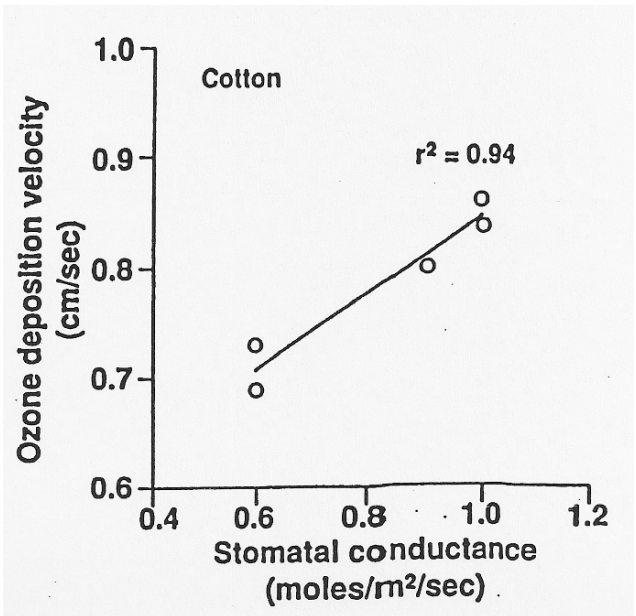

Figure 15-2. Relationship between ozone deposition velocity (total conductance for uptake) and stomatal conductance in upland cotton. (Data from Grantz *et al.,* 1994)

The plasma membrane, located just inside of the cell wall, is a logical site of initial phytotoxicity, either of the toxic reaction products or of O_3 itself (Heath, 1987). It is not clear how much O_3 actually survives the tortuous diffusion pathway through the wall space to the plasmalemma, given the reactivity of O_3 with aqueous solutions, and the presence of ascorbate, polypeptides, and other reactive compounds (Castillo and Greppin, 1988). Unsaturated lipids in the plasma membrane are highly reactive with O_3 in vitro, however, the significance of these reactions in vivo has been questioned. Similarly, hydrophilic domains of membrane-bound and surface proteins are reactive with O_3 (Heath, 1987) and could represent sites of primary toxicity.

Despite the uncertainties surrounding the mechanism of attack of O_3 on the plasma membrane, impacts on key membrane functions are observed very quickly following O_3 exposure. The increased permeability of the plasma membrane to monovalent cations was observed very early in studies of O_3 effects (Heath and Frederick, 1979; Evans and Ting, 1973). More recently (Castillo and Heath, 1990) the role of divalent cations, notably calcium, has been emphasized, as the importance of calcium as a messenger metabolite has become recognized in many plant processes (Leonard and Hepler, 1990). Exposure of isolated protoplasts to O_3 in solution (*e.g.,* guard cell protoplasts of *Vicia faba*; Torsethaugen *et al.*, 1999) significantly perturbed plasmalemma transport of monovalent cations. The inward rectifying K^+ channel that mediates guard cell swelling and stomatal opening was impaired, while the outward K^+ channel was unaffected. However, the divalent cation, Ca^{++}, may also be involved, if it serves as an intracellular second messenger of O_3 attack as it does for other abiotic stresses affecting stomatal conductance. Elevated cytosolic Ca^{++} concentration leads to an inhibition of the inward K^+ channel (*e.g.,* McAinsh *et al.*, 1996) similar to that observed in the O_3-treated guard cell protoplasts.

Indirect evidence for O3 interaction with the plasmalemma is the rapid impact in intact plants on phloem loading of recent photoassimilate in Pima cotton (Grantz and Farrar, 1999). This putative effect on sugar transport across the plasma membrane may be related to whole plant effects mediated by altered carbohydrate translocation (Cooley and Manning, 1987; Grantz and Yang, 1996). Oxidation

of some yet unidentified membrane component, following initial oxidation of some compound in the cell wall space, is the likely initial phytotoxic effect of O_3 (Heath, 1987). Identification of these initial targets of O_3 action remains a high research priority.

3.3 Physiological Effects of Ozone

3.3.1 Visible Injury

The appearance of visual O_3 damage is not a good indicator of physiological or agronomic damage. Yield and visible symptoms are not strongly correlated in general (Runeckles and Chevone, 1992).

The visible injury responses of cotton foliage to O_3 are typical of most broad-leaved dicots. Rapid absorption of high concentrations of O_3 by cotton leaves produces an acute injury response characterized by degradation of cellular membranes, loss of membrane integrity, loss of turgor pressure, and cell death, particularly of palisades cells. The visible manifestation of this cellular injury is the appearance of small, irregularly-shaped chlorotic or necrotic lesions on the upper leaf surface, a symptom usually called chlorotic stipple or chlorotic flecking. As these lesions age, their color changes from yellow to various shades of reddish-purple to brown, as anthocyanin pigments accumulate in and around the lesion. Continuous exposure of cotton leaves to lower, but still toxic concentrations of O_3 produces a chronic injury condition. Ozone injury is usually most apparent on older leaves, as injury accumulates in response to continual exposure to O_3 and loss of chlorophyll, accelerated senescence, and premature leaf abscission on the older leaves that have already progressed toward senescence. The very youngest leaves do not exhibit much O_3 sensitivity, often exhibiting enhanced, possibly compensatory, rates of photosynthetic gas exchange. However, leaves approaching full expansion are more susceptible to O_3 injury than older leaves. In cotton, specifically, the period of maximum leaf susceptibility to O_3 follows maximum leaf expansion rates and the period of maximum cellular surface-to-volume ratio, and precedes cell wall lignification and secondary cell wall synthesis (Heath, 1975). For cotton, this period of maximum foliar susceptibility corresponds to a leaf age of 2 to 3 weeks (Ting and Dugger, 1968).

Symptoms of acute foliar injury on cotton may appear within 5 to 7 days following O_3 exposure of 0.25 ppm or higher for several hours (Taylor and Mersereau, 1963). Chronic O_3 injury symptoms, including chlorosis and accelerated foliar senescence and abscission, have been observed during several weeks or months of exposure to a daylight average of < 0.07 ppm in CA (Temple *et al.*, 1985) and < 0.05 ppm in the more humid areas of the southeast (Heggestad and Christiansen, 1982; Heagle *et al.*, 1986). Foliar O_3 injury symptoms on field-grown cotton have been observed in the San Joaquin Valley and in experimental cotton plantings in Riverside and Indio, CA, Phoenix, AZ (Taylor and Mersereau, 1963; Brewer and Ferry, 1974; Temple *et al.*, 1988a), and Raleigh, NC (Heagle *et al.*, 1986).

3.3.2 Gas Exchange

Photosynthetic and respiratory gas exchanges are susceptible to O_3-inhibition. Plants susceptible to O_3, such as cotton, may experience a relatively rapid reduction in rates of net photosynthesis (Pn) when exposed to elevated concentrations of O_3 for short periods of time, even in the absence of visible foliar injury symptoms (Darrall, 1989; Runeckles and Chevone, 1992). Exposure to >0.20 ppm O_3 for >2 hr. induced a rapid, but reversible, reduction in Pn in susceptible plants (Hill and Littlefield, 1969; Dann and Pell, 1989). The direct effect of O_3 on Pn is presumed to occur through oxidative attack on the plasmalemma as discussed above, by O_3 or its cell wall space-derived free radical by-products, leading potentially to increased membrane permeability, loss of K^+ from cells and organelles, and loss of the pH gradient across the chloroplast membrane (Heath, 1987). As O_3 concentrations increase, or length of exposures increases, the ability of cells to repair this damage decreases, and the reduction in Pn could become irreversible. An additional, indirect, impact of chronic O_3 exposure on Pn has been suggested (Grantz and Farrar, 1999, 2000). End product inhibition and down regulation of photosynthetic enzymes may suppress Pn following O_3-inhibition of phloem loading and carbohydrate export from photosynthesizing leaves. In short term experiments (45 min exposure), carbohydrate export was inhibited considerably more than was Pn. In any case, the permanent reduction in Pn is associated with decreased RUBISCO concentrations, loss of leaf chlorophyll, increased chlorosis, accelerated leaf senescence, and foliar abscission (Pell *et al.*, 1992).

Reductions in Pn in response to increased exposure to O_3 have been documented for cotton and a number of other crop species in the field (Darrall, 1989; Runeckles and Chevone, 1992). Measurements of Pn on the youngest fully-expanded leaves of field-grown SJ-2 cotton were made on 2 September 1986, after three months of exposure to four levels of O_3 in open-top chambers in Riverside, CA (Temple *et al.*, 1988b). No visible O_3 injury was present on these leaves. Exposure to elevated O_3 significantly reduced Pn and the reduction in Pn was proportional to the O_3 dose (Fig. 15-3). However, while rates of carbon assimilation are clearly associated with plant growth and yield, direct correlation between leaf or whole canopy Pn and growth or yield is not straightforward (see Chapter 14). Measurements of whole-canopy photosynthesis of wheat exposed to a gradient of O_3 concentrations correlated well with grain yield reductions, but measurements of Pn on individual plant parts underestimated yield reductions (Amundson *et al.*, 1987). For field-grown soybeans (*Glycine max* Merr.), Pn of upper-canopy leaves was reduced proportionally more by O_3 in well-watered plants than was bean yield, but in drought-stressed plants yield was reduced more than Pn at low O_3 concentrations and slightly less at high O_3 concentrations

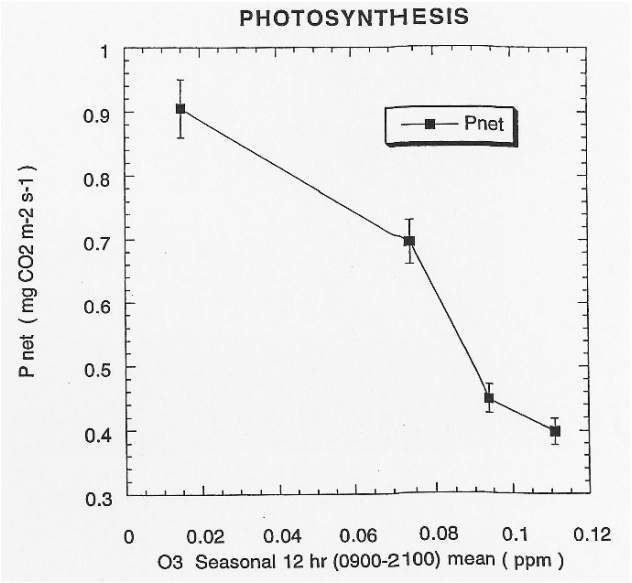

Figure 15-3. Reductions in net photosynthetic rate (Pn) of the youngest fully-expanded leaf on the main-stem of Acala (cv. 'SJ-2') cotton exposed to a range of ozone concentrations in field exposure chambers. Each data point is the mean of three plants from two replicate chambers at each level of ozone, +/- one standard error. Measurements were made in September, following three months (chronic) exposure to ozone. (Data from Temple *et al.,* 1988b)

(Miller, 1988). In the SJ-2 cotton study mentioned above, lint yield was highly correlated with reduced Pn as O_3 exposures increased in well-watered plants (R^2=0.94), but in severely drought-stressed plants the correlation between Pn and lint yield was not significant (Temple *et al.*, 1988b). This interaction reflects both the dominant effect of water deficit on yield, independent of O_3 exposure, and a reduced effective dose (uptake) of O_3 in drought stressed plants because of stomatal closure. As moisture deficit is variable across a field and unpredictable over time, these studies suggest that measurements of Pn on field-grown cotton may not be useful in predicting the effects of O_3 on yield.

Measurements of g_s in cotton leaves exposed to chronic doses of O_3 have shown significant reductions in g_s both on Upland cotton (SJ-2) growing in the field (Temple, 1986; Temple *et al.*, 1988a) and in closed field exposure chambers (Grantz and McCool, 1992) and in Pima cotton (*G. barbadense* L. cv. S-6) in CSTR experiments (Grantz and Yang, 1996). However these instantaneous measurements following long term exposure to O_3 do not distinguish direct effects of O_3 on stomatal guard cell function from indirect effects on g_s mediated by mesophyll metabolites or elevated intercellular CO_2 concentrations resulting from decreased Pn. A kinetics experiment, designed to determine the direct effects of O_3 on stomatal function in cotton found no effect of O_3 exposure on rates of stomatal opening or closing in response to step changes in photon flux density (Temple, 1986). Diurnal courses of g_s in O_3-exposed and control leaves revealed similar rates of opening and closing, and similar complete closure at night. The major difference was that daily maximum g_s was significantly reduced in the O_3-injured cotton plants. These results suggest that reduced g_s may be an indirect effect of O_3-induced inhibition of Pn.

The role of stomatal conductance (g_s) in O_3-induced reduction in Pn has not been resolved. Experiments in which g_s has been measured simultaneously with Pn during and following plant exposure to O_3 have found in some instances concurrent reductions in both parameters [*e.g.* sunflower (*Helianthus annuus* L.), Furukawa *et al.*, 1984], suggesting increases in both stomatal and non-stomatal limitations to Pn. Other studies have demonstrated a clear reduction in Pn with no concomitant reduction in g_s [*e.g.* wheat (*Triticum aestivum* L.), Lehnherr *et al.*, 1988]. This difference in O_3 response is not necessarily species-specific, as two out of three lines of hybrid poplar (*Populus* spp.) exposed to O_3, exhibited parallel reductions in g_s and Pn while in the third Pn declined with no immediate effect on g_s (Furukawa *et al.*, 1984). In *Vicia faba* (Torsethaugen *et al.*, 1999) exposure to 0.10 ppm O_3 for 4 hr reduced g_s and the rate of stomatal opening from darkness, without affecting Pn at saturating intracellular CO_2 concentration. However, increasing the O_3 concentration to 0.18 ppm inhibited both Pn and g_s.

Relatively few studies have been conducted on the effects of O_3 on plant respiration, and none on cotton. In general, respiration in leaves increases in response to acute or short-term (hours) O_3 exposures, particularly when leaf injury occurs (Amthor, 1988; Dugger and Ting, 1970; Pell and Brennan, 1973). This increase is not well understood but is attributed to increased metabolic repair processes, enzyme and antioxidant synthesis, and membrane reconstruction and energization (Chevrier *et al.*, 1990; Sutton and Ting, 1977). Long-term exposure to O_3 may increase or decrease (Miller, 1988; Runeckles and Chevone, 1992) respiratory rates, both in leaves (Lehnherr *et al.*, 1988) and roots (Hofstra *et al.*, 1981). Reductions may reflect decreased availability of carbohydrate substrate in leaves with impaired photosynthetic capacity and, in the case of roots, reductions in phloem transport of carbohydrates from leaves to roots (see below). Effects of O_3 on photorespiration (competitive fixation of O_2 rather than CO_2 by the photosynthetic carboxylase, RUBISCO) are largely unknown (Runeckles and Chevone, 1992).

3.3.3 Leaf Area Responses: Senescence and Abscission

Exposure of cotton to mean daylight O_3 concentrations of >0.05 ppm for several weeks can induce accelerated senescence and abscission of older leaves of well-watered plants (Miller *et al.*, 1988; Temple *et al.*, 1988b). Leaf area duration of 'McNair 235' grown in open-top chambers in Raleigh, NC was reduced by 13 % at a seasonal O_3 concentration of 0.051 ppm and 28.5% at 0.073 ppm, compared with charcoal-filtered air (CF) controls (Miller *et al.*, 1988). Rates of foliar abscission for four cultivars of cotton grown in Riverside, CA averaged 51% in CF open-top chambers,

60% in chambers receiving ambient O_3, and 75% in chambers receiving added O_3 (Temple, 1990b). Foliar abscission was significantly less in drought-stressed cotton exposed to O_3 in studies conducted in CA using cv. SJ-2 (Temple *et al.*, 1985; Temple *et al.*, 1988a). However, leaf abscission in response to O_3 in drought-stressed 'McNair 235' in Raleigh, NC did not differ from that of well-watered plants (Miller *et al.*, 1988). In the latter study, Miller *et al.* (1988) compared the relative loss of photosynthetic tissue due to foliar injury and leaf abscission with total lint and seed yield in response to O_3. They concluded that yield losses in cotton exposed to O_3 could not be explained solely on the basis of reductions in leaf area due to foliar abscission, but were also attributable to reductions in efficiency of net CO_2 assimilation in remaining leaves exposed to O_3.

Seedlings of Pima cotton (cv. S-6) exhibited a substantial decline in leaf area development and retention in response to O_3. Mechanical reduction of leaf area to simulate O_3-induced loss of photosynthetic tissues also showed that O_3 had other systemic effects, and plant responses were not mediated solely by loss of photosynthetic leaf area (Grantz and Yang, 1995). In these studies O_3 exposure and mechanical leaf area reduction produced similar reductions in whole plant biomass, but O_3 mediated an additional reduction in root system development on a leaf area basis that was not reproduced by an equivalent mechanical reduction in photosynthetic source tissue. The resulting O_3-specific effect on root hydraulic properties suggested a possible direct effect on carbohydrate translocation to the roots.

Plants can compensate for loss of photosynthetic tissues in a number of ways (Pell *et al.*, 1994). Increased g_s and Pn have been observed in the newly expanding leaves following O_3-induced foliar abscission (Beyers *et al.*, 1992; Greitner *et al.*, 1994). Plants exposed to O_3 also retain greater amounts of carbohydrates in leaves and stems, for use as substrate for repair and growth of new photosynthetic tissues (see below). For example, four cotton cultivars averaged 15.5 main-stem leaf nodes in open-top chambers equipped with charcoal filters (CF) to remove ambient O3, 16.0 in chambers supplied with ambient air, and 18.0 in O_3-added chambers (Temple, 1990b). The leaves produced on cotton plants exposed to O_3 are proportionally larger but thinner than those on control plants, as shown by the increase in specific leaf area on 'McNair 235' at elevated O_3 concentrations, relative to controls (Miller *et al.*, 1988). However, cotton cultivars can differ in their compensatory responses to O_3 injury. Increased branching in response to O_3 injury was observed in an indeterminate Acala cv. 'SJ-2' (Oshima *et al.*, 1979), but the determinate cvs. 'GC510' and 'SS2086' increased the number of main-stem leaves and showed no tendency to increase branching in response to O_3 (Temple, 1990b). Because boll yield in cotton is a function of number of sympodial branches (Oosterhuis and Urwiler, 1988), this difference in compensatory strategies among cotton cvs. may favor boll production in indeterminate cvs. growing in areas with high ambient O_3, though reduced carbohydrate availability may lead to abscission and incomplete development of these additional bolls.

3.3.4 Carbon Allocation and Biomass Partitioning

Plants can respond to environmental stressors that limit growth by altering patterns of carbon allocation and partitioning to favor maximum capture and utilization of resources (Chapin, 1991). In response to limited below-ground resources, such as water or nutrient deficiencies, plants alter shoot-root ratios (SRR) to favor the growth of roots. Plants growing under limiting light conditions, or those that have lost leaf tissue through herbivory, retain greater amounts of carbohydrates in shoots, to support the growth of new photosynthetic tissues. In this respect, O_3 is similar to other above-ground stressors in that O_3-induced reductions in growth are proportionally more severe on roots than on shoots, so that SRR of O_3-injured plants are often higher than those of controls (Cooley and Manning, 1987; Reiling and Davison, 1992). The mechanism of this effect of O_3, and of allocation in general, remains very poorly characterized. A variety of experimental manipulations with CSTR-grown Pima (Grantz and Yang, 2000) ruled out two simple hypotheses. Source limitation caused by O_3 damage to Pn was simulated by progressive leaf pruning, but reduced total productivity without reproducing the enhanced SRR observed following O_3 exposure. Similarly, SRR was determined in plants of different sizes (various ages) chosen to correspond to the different sizes of plants of uniform age exposed to different O_3 concentrations. These studies showed that O_3-induced changes in SRR were not due to changes in rate of plant development during which changes in SRR are expected to occur.

The retention of newly-fixed carbon in the stems of plants exposed to O_3 was demonstrated by carbon isotope studies, in which beans (*Phaseolus vulgaris* L.) exported up to 57% less carbohydrate from leaves exposed to O_3 compared with controls (McLaughlin and McConathy, 1983). Primary bean leaves retained greater amounts of carbohydrates, thereby reducing the amount exported to roots, but upper-stem trifoliate leaves exported greater amounts of carbohydrates towards the shoot apex, increasing the amount of substrate available for growth (Okano *et al.*, 1984). The kinetics of export of recent photoassimilate were inhibited in Pima cotton by 45 min exposure to a range of high concentrations of O_3 (0 to 0.8 ppm; Grantz and Farrar, 1999, 2000). Compartmental analysis indicated that vacuolar storage and tonoplast function were not affected by O_3. In contrast, the labile carbohydrate, presumably the cytoplasmic pool mediated by plasmalemma transport, was substantially impacted. At the highest O_3 concentration the carbohydrate available for export to the root system was inhibited by about 80%, of which 20% was due to reduced Pn and about 60% due to impaired phloem transport.

Reduced root growth and increased SRR in cotton exposed to chronic O_3 have been demonstrated in a number of pot studies, both on Acala (Oshima *et al.*, 1979) and on Pima (Grantz and Yang, 1996). In a field study using 'SJ-2' cotton, root biomass of well-watered (WW) plants was reduced 20% more than stem biomass in the high-O_3 treatment, relative to CF controls. The SRR increased from 7.5 in CF controls to 11.1 in plants exposed to a seasonal mean O_3 level of 0.111 ppm. In severely drought-stressed (DS) plants, SRR increased from 3.8 in CF to 5.4 in the high-O_3 treatment (Temple *et al.*, 1988b). However, not all studies have reported significant effects of O_3 on SRR. In a field study using 'McNair 235', Miller *et al.* (1988) reported reduced root growth of plants exposed to elevated O_3, but no significant effect of O_3 on SRR. They attributed this difference to accelerated leaf abscission, which balanced losses in stem biomass with reductions in root growth so that SRR did not change across O_3 treatments.

Allocation of carbon to carbohydrate pools in plants exposed to O_3 has been investigated in a number of studies, but results appear to depend upon species, experimental design, and sampling procedures (Miller, 1988). Miller *et al.* (1989) reported that high O_3 reduced concentrations of reducing sugars, sucrose, and starch particularly in stems and roots of field grown well-watered (WW) 'McNair 235'. However, the reduction was dependent upon stage of growth of the plants, and was statistically significant only at mid-growing season. Starch levels in stems and roots showed the most consistent reductions throughout the growing season. Drought-stressed cotton plants generally had higher carbohydrate concentrations than WW, and no consistent effects of O_3 were observed in DS plants or in the combination O_3 x drought treatment.

In young source leaves of Pima cotton exposed to O_3 in CSTRs (Grantz and Yang, 2000), starch declined with increasing chronic exposure to O3, while soluble sugars increased. Over a range of acute exposures to O_3 (Grantz and Farrar, 2000) soluble sugars increased proportionally to the O_3 concentration except at the extreme value of 0.8 ppm a smaller increase was observed. These results are generally consistent with the model that exposure to O_3 reduces total carbohydrate pools in source leaves of cotton, and the available carbohydrates are partitioned primarily to repair and growth of new photosynthetic tissues. Reduced export of carbohydrate to distant sinks, including roots, seems to reflect a type of source limitation associated with inhibited phloem export from O_3-impacted source leaves.

Reduction in carbohydrate partitioning to roots and reduced root growth in plants exposed to O_3 can have significant effects on root physiology. Reduced nodulation in *Rhizobium*-infected legumes exposed to O_3 (Blum *et al.*, 1983), and reduced rates of endomycorrhizal infection in O_3-injured plants (McCool *et al.*, 1982) have been reported. Reductions in rates of infection and growth of root symbionts may reflect lower pools of available carbohydrate in roots of these O_3-injured plants (McCool, 1988).

3.3.5 Water Relations: Drought Stress, Root Hydraulics

Root Hydraulic Conductance. The inhibition of carbohydrate partitioning to roots that is induced by O_3 exposure undoubtedly has functional effects on root system performance. As 85% of the 20 species surveyed exhibited such reduction in root/shoot biomass ratio (Cooley and Manning, 1987), this effect seems to be of general importance. Potential effects of reduced root biomass are related to concomitant reductions in root surface area for absorption of water and mineral nutrients, and reduced exploration of the soil volume for acquisition of these soil resources. Other possible consequences of impaired root growth or function in plants exposed to O_3, such as reduced production of hormonal transducers such as cytokinins and abscisic acid (ABA), have not been investigated.

Ozone exposure may lead to an increase in hydraulic conductance per unit root biomass, as storage tissue in primary roots is reduced more than surface area of fibrous roots and root hairs [*e.g.,* red spruce (*Picea rubens* Sarg.), Lee *et al.*, 1990; Pima cotton, Grantz and Yang, 1996). Fibrous roots and root hairs are also substantially reduced by O_3 (Ogata and Maas, 1973). On a whole plant basis, however, the smaller root system and reduced conductive tissue lead to reduced hydraulic conductance following O_3 exposure.

A functional measure of root system performance is hydraulic conductance per unit transpiring leaf area (Yang and Tyree, 1993). This reflects the root system capacity to supply water (and by inference mineral nutrients and phytohormones) to the remaining leaf area. This parameter is reduced substantially by O_3 exposure in Pima cotton (Fig. 15-4; circles), even though leaf area expansion and retention are themselves reduced by O_3. While reduced root hy-

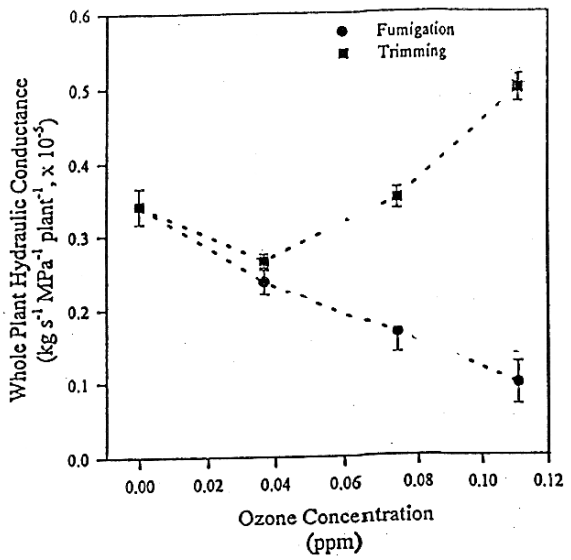

Figure 15-4. Effect of ozone on plant hydraulic conductance on a leaf area basis in Pima cotton grown in CSTRs. (Data from Grantz and Yang, 1995)

draulic conductance with reduced plant size are commonly observed on a per plant basis, the O_3 effect seems to be unique in inducing a similar relationship on a leaf area basis (Grantz and Yang, 1996). Following leaf pruning (Grantz and Yang, 1995, 2000) reduced photosynthetic capacity resulted in reduced whole plant biomass similar to that induced by a range of O_3 exposures. However, effects on root hydraulic conductance per leaf area were minimal (about 15%), and not similar to the large reductions (about 50%) induced by O_3 exposure.

Interactions of Ozone and Drought Stress. The common assumption that plants exposed to elevated O_3 may be more susceptible to drought because of reduced root growth has not been confirmed in the field. It is clear that drought stress (Temple *et al.*, 1985), like low nitrogen stress (Grantz and Yang, 1996), and elevated CO_2 (Heagle *et al.*, 1999), reduces the relative effect of O_3 exposure on yield and biomass production. As exposure to O_3 causes short-term stomatal closure in many cases, entry of O_3 is reduced and consequent phytotoxicity is minimized. However, the limitations posed by drought may sufficiently reduce plant performance to the extent that the relative benefit of increased resistance to O_3 is trivial in a practical sense.

Available evidence is conflicting regarding the effect of O_3 on transpiring leaf water potential, a potential marker for drought stress. Reduction in root hydraulic capacity might be expected to reduce water availability to the shoot, reducing midday water potential (increasing tissue water deficit and stress). On the other hand, many studies have shown that reduced root hydraulic conductance leads to nearly immediate and persistent reductions in stomatal conductance (*e.g.,* Fig. 15-5a), which reduces transpiration and maintains or improves shoot water status. In Pima cotton (Fig. 15-5b; Grantz and Yang, 1996), and Upland cotton (Temple, 1986, 1990a) leaf water potential increased or was stable with increasing exposure to O_3. Field-grown 'SJ-2' cotton exposed to a range of O_3 concentrations in open-top chambers maintained the same leaf water potentials in high-O_3 exposures as in control plants throughout the growing season (Temple, 1990a). It appeared that the lower canopy leaf area and lower rates of stomatal conductance and transpiration of the cotton plants in the high-O_3 treatment balanced the smaller root systems so that the plants were able to maintain adequate water supplies to the leaves (Temple *et al.*, 1988b; Temple, 1990a). This reflects integrated whole plant function. It is not clear whether this homeostasis of leaf water potential would be observed under non-experimental conditions of variable soil moisture supplies, particularly in environments subject to rapidly changing evaporative demand.

It is commonly considered that reductions of net carbon assimilation following exposure to O_3 lead directly to stomatal closure and to reduced carbon allocation to the roots. However, compensatory photosynthesis in newly emerging leaves may restore much of the whole plant carbon assimilatory capacity (Pell *et al.*, 1994). Long-term reduction of stomatal conductance may not be mediated by direct effects

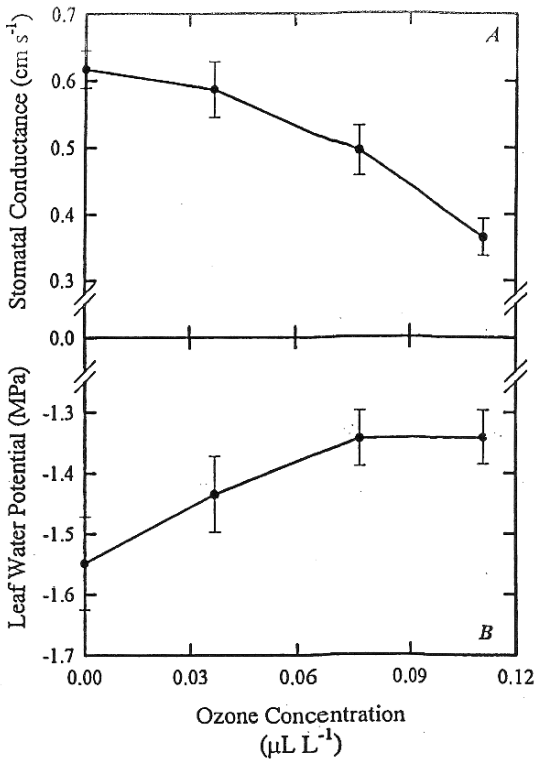

Figure 15-5. Effect of ozone on stomatal conductance (a) and transpiring leaf water potential (b) of Pima cotton grown in CSTRs. (Data from Grantz and Yang, 1996)

of O_3 on the components of leaf gas exchange. A recent modeling exercise has demonstrated that the O_3 effect on root hydraulic properties is sufficient to mediate observed reductions in stomatal conductance during chronic exposure to O_3 (Grantz *et al.*, 1997; Grantz and Yang, 2000). In this case, no direct effects on leaf gas exchange were incorporated into the model. Postulating a stomatal sensitivity to leaf epidermal water status and parameterizing this water potential in terms of leaf to air vapor pressure difference, soil water content, and root hydraulic conductance, resulted in model output that closely reflected observations of stomatal conductance. Scaling these observations to the canopy level, using a comprehensive soil-plant-atmosphere transport sub-model, reproduced O_3 fluxes observed in the field. While the mechanism of O_3 action on whole plants remains elusive, it is clear that a holistic, integrated view of the plant will be required.

3.4 Effects on Yield

The adverse effects of O_3 on yield of cotton have been under investigation for the past 30 years. Brewer and Ferry (1974) placed pairs of ventilated charcoal-filtered (CF) and non-filtered (NF) plastic-covered greenhouses over plots of field-grown 'SJ-1' at several locations in the southern San Joaquin Valley, CA in 1972 and 1973. Number of bolls and

weight of lint plus seed were reduced 20% in NF plants compared with CF in areas with the highest ambient O_3 concentrations, and proportionally less in areas with lower ambient O_3. No effects of O_3 on fiber quality were observed in this study. However, in these experiments growth and yield of cotton plants inside the greenhouses differed from that of plants growing outside, prompting concerns that the reductions in yield may have been an artifact of the greenhouse enclosure. A two year study of the effects of O_3 on cotton yield was conducted on 'SJ-2' in Shafter, CA in 1981 and 1982, using open-top chambers to minimize differences between open and chambered plots (Temple *et al.*, 1985). This study was conducted as part of the National Crop Loss Assessment Network (NCLAN) program, designed to assess the economic impacts of air pollution on the major agronomic crops in the U.S. (Heck *et al.*, 1988). In the NCLAN experimental protocol the crops were exposed to a range of O_3 concentrations, from sub-ambient to levels 1.5 or 2.0 times greater than ambient. Ozone exposure-yield response data were then analyzed by regression analysis to produce exposure-response regression equations. Results from this NCLAN study were similar to those reported earlier: yields of well-watered (WW) plants in NF chambers were reduced 15 to 20%, relative to CF controls. Drought-stressed (DS) cotton had relatively little yield losses, except at O_3 levels higher than expected in the San Joaquin Valley. Yield losses to O_3 varied from year to year, being higher in a cool, humid summer, when plants were more susceptible to O_3, than in a year with a hot, dry growing season. Yield losses in cotton exposed to O_3 were attributable primarily to fewer numbers of bolls rather than reduction in weight per boll.

Ozone had no effect on fiber quality or in ratio of lint to seed weight in either year of the experiment, similar to results reported for 'SJ-2' in a greenhouse study (Oshima *et al.*, 1979). In Pima S-6 (Grantz and McCool, 1992) increasing ozone exposure led to a substantial decrease in yield and in fiber quality. Micronaire, length, and length uniformity were particularly impacted. An NCLAN study conducted on 'SJ-2' grown under three different irrigation regimes in Riverside, CA showed that ambient O_3 levels reduced lint yield of WW cotton 26.2% relative to CF plants. Ozone concentrations were higher in Riverside than in Shafter during the earlier study. Cotton grown with one-third less irrigation showed a 19.8% reduction in lint yield, and severely drought-stressed cotton had a 4.7% yield reduction compared with CF plants (Temple *et al.*, 1988a). As with previous studies with 'SJ-2', yield losses were due to fewer numbers of bolls per plant, and not reduction in weight per boll. Yield losses at ambient O_3 levels were also reported for two cultivars of cotton in NCLAN studies conducted in Raleigh, NC. Yields of the cv. 'Stoneville 213' declined 11% in NF relative to CF chambers (Heagle *et al.*, 1986). The cv. 'McNair 235' had a 15% reduction in number of harvested bolls and a 20% reduction in seedcotton weight in ambient O_3 chambers compared with CF plants. Drought-stressed plants showed no reductions in yield at comparable O_3 exposures. Unlike results with 'SJ-2' in California, reductions in yield of both 'Stoneville 213' and 'McNair 235' were attributable both to fewer numbers of harvested bolls and to reductions in weight per boll. Minor changes in fiber quality were also observed at high O_3 concentrations in both cvs.

Relative seedcotton yield losses for the three cotton cultivars used in the five NCLAN studies are plotted in Fig. 15-6. Yield losses at a mean seasonal O_3 concentration of 0.06 ppm ranged from near zero to over 26%, relative to cotton grown at a background O_3 level of 0.025 ppm. The cv. 'SJ-2' had both the lowest loss, on DS plants in 1981, and the highest loss, on both WW and DS plants in 1982. This large difference in the yield response of a single cotton cultivar to similar concentrations of O_3 was attributable to weather conditions, which increased stomatal conductance and rendered the plants more susceptible to O_3 in 1982 than in 1981 (Temple *et al.*, 1985). The Deltapine cvs. 'Stoneville-213' and 'McNair-235' appeared to be intermediate in susceptibility, with somewhat greater losses in 'McNair'. Seasonal mean daylight O_3 concentrations between 0.05 and 0.06 ppm are common in many cotton-growing regions of the U.S. (Lefohn, 1992). This suggests that annual yield losses due to ambient O_3 for cotton cvs. similar in susceptibility to those used in the NCLAN studies may average between 10 to 15%. However, the variability in these data demonstrates the difficulty of providing accurate predictions of cotton yield losses, without reference to internal and external conditions that alter the physiological susceptibility of the plants to O_3 uptake and injury. Elevated concentrations

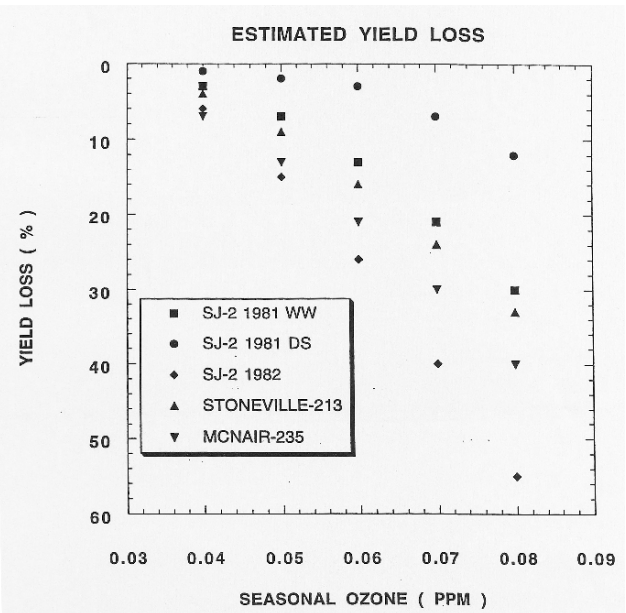

Figure 15-6. Estimated seedcotton yield losses of three cultivars of cotton from five NCLAN studies across a gradient of mean seasonal daylight ozone concentrations, relative to yields at a background ozone level of 0.025 ppm. WW = plants with optimum irrigation; DS = drought-stressed plants, with one-third less irrigation water. In 1982, WW and DS cotton did not differ in yields. (Data from Heck *et al.,* 1988)

of CO_2 in the atmosphere, for example, may reduce the potential impact of O_3 on growth and yield of cotton (Heagle *et al.*, 1999).

3.5 Relative Susceptibilities of Cotton Cultivars to Ozone

Variability in cotton cultivar responses to O_3 has been investigated in a small number of experiments. Growth and yield of eight cotton cultivars were compared in greenhouses supplied with ambient air or charcoal-filtered air in Beltsville, MD (Heggestad and Christiansen, 1982). The Acala type 'SJ-1' was the most resistant of the eight cultivars to O_3-induced yield losses, but the Delta type 'Stoneville-213' was almost as resistant as 'SJ-1'. The upland cultivar 'Paymaster-202' was the most susceptible, and had a 40% reduction in yield in the ambient-air greenhouse. Field trials conducted in the southern San Joaquin Valley have shown large differences in susceptibility to O_3 among CA cultivars (Brewer, 1979). The cv. 'SJ-2' had greater yield reductions in response to ambient O_3 than did the *Verticillium*-resistant 'SJ-5', and 'SJ-2' appeared to be slightly more resistant to O_3 than the earlier-released 'SJ-1'. Of the two Deltapine cvs. used in NCLAN experiments in NC, 'McNair-235' appeared to be more susceptible to O_3-induced yield losses than 'Stoneville-213', although environmental conditions during the two growing seasons may account for some of these differences (Heagle *et al.*, 1988). In a field study conducted in Riverside, CA, susceptibility to O_3-induced yield losses of four cotton cultivars was directly correlated with degree of determinance, so that the cvs. ranked in order of increased determinance and increased susceptibility to O_3: SJ-2 < C1 < GC510 < SS2086 (Temple, 1990b).

The physiological and biochemical mechanisms that determine cultivar susceptibility or resistance to O_3 are largely unknown. Because of the importance of stomata in regulating gas exchange it is reasonable to assume that differences in rates of stomatal conductance among cultivars could account for differences in relative susceptibility to O_3 or other air pollutants (Runeckles and Chevone, 1992). Indeed, greater susceptibility to O_3 among cultivars of common bean (*Phaseolus vulgaris* L.) (Knudson-Butler and Tibbets, 1979; Temple, 1991), tobacco (*Nicotiana* sp., Turner *et al.*, 1972), and petunia (*Petunia hybrida* Vilm., Thorne and Hanson, 1976) has been associated with higher rates of stomatal conductance in these cvs. However, no clear association between rates of stomatal conductance and susceptibility to O_3 injury or yield losses was observed in a field study of four cotton cvs. in Riverside, CA. Instead, differences in susceptibility to O_3 were attributed to differences in degree of earliness, because the more determinate cvs. flowered and set bolls earlier in the summer, when O_3 levels were highest and their short growing season did not allow recovery from O_3 injury, as did the indeterminate lines (Temple, 1990b). Pima cotton (*G. barbadense* L.) cv. S-6 appeared to be more susceptible to O_3 than the cv. SJ-1 (*G. hirsutum* L.), although the mechanisms for these differences have not yet been established (Grantz and McCool, 1992). *G. hirsutum* lines developed in the San Joaquin Valley of California were less susceptible than lines developed in the Mississippi Delta (Grantz, unpublished data). In general, lines developed in areas with low ambient O_3 concentrations, and introduced into production areas characterized by high ambient O_3 concentrations, such as the San Joaquin Valley may lack the constitutive O_3-resistance mechanisms present in cvs. selected for high yield potentials in areas with high ambient O_3 pollution.

Among the resistance predictors currently being studied intensively is ascorbic acid concentration in photosynthetically active leaves. In soybean lines exhibiting sensitivity (cv. Forrest) and resistance (cv. Essex) to O_3, the concentrations of total leaf ascorbate were greater in Essex. In addition the fraction of total ascorbate maintained in its reduced and thus protective form during exposure to O_3 was higher in Essex (Robinson and Britz, 2000). Recent simulations (Plochl *et al.*, 2000) suggest that interception and detoxification of O_3 in the cell wall apoplast by ascorbic acid, prior to its attack on the plasmalemma, could mediate plant resistance to O_3. In this case, a number of physiological or anatomical factors, including total leaf concentration of ascorbate, factors regulating diffusion, and capacity for cytosolic regeneration of ascorbic acid, might be related to the relative O_3 resistance of advanced cultivars of various species, including cotton.

3.6 Economic Losses

Translating crop yield reductions into economic losses is a complex problem in agricultural economics (Adams and Crocker, 1988). Reduction in total crop yield may increase crop unit value, so that the burden of economic losses due to O_3 air pollution falls disproportionally on consumers and not on producers of agricultural commodities. Indeed, models of economic losses from the impact of O_3 on major crops have estimated that consumer surplus losses comprise 50 to 100 % of total losses (Adams *et al.*, 1988). Regional differences in levels of O_3 pollution mean that the costs and benefits of improving air quality are not distributed evenly (Howitt and Goodman, 1988). Distortions in the free market economy introduced by government agricultural support programs also increase the difficulty of estimating the benefits to agriculture from cleaner air (McGartland, 1987). For cotton, benefits to the California economy from improvements in O_3 air quality have been estimated at a 12.4% increase in base benefits at a seasonal mean O_3 level of 0.06 ppm, to a 48% increase in benefits at a background O_3 level of 0.025 ppm (Howitt and Goodman, 1988). For the U.S. economy as a whole, the annual economic benefits accrued from a 25% reduction in O_3 concentrations in the major crop-growing regions of the country, including CA, the Mid-West, and the South-East were estimated at \$1.7 x 10^9 (Adams *et al.*, 1986).

3.7 Agricultural Practices

Relatively little can be done to protect cotton from the adverse effects of O_3. Because O_3 flux is dependent upon the rate of stomatal conductance, it is theoretically possible to withhold irrigation water prior to a predicted O_3 episode, to close stomates and exclude O_3. This tactic seems unrealistic in common agricultural practice, and would not be available for non-irrigated cotton. Several agricultural chemicals have been developed that provide some protection from O_3 injury, including fungicides such as benomyl that provide ancillary protection from O_3, and various anti-oxidants such as citrate and ascorbate, that act as free-radical scavengers (Pryor *et al.*, 1982). The most widely studied "antioxidant" chemical is ethylene diurea [N-2-(2-oxo-1-imidazolidinyl)ethyl]-N'-phenylurea] or EDU. This chemical, supplied either as a soil drench or foliar spray, can provide many plant species almost complete protection from foliar O_3 injury (Carnahan *et al.*, 1978; Manning and Krupa, 1992). Apparently, EDU increases cellular activity of superoxide dismutase and catalase, thereby protecting foliage from O_3-induced accelerated senescence (Bennett *et al.*, 1984). However, because of its expense and the difficulty in adjusting dose (EDU may be toxic at high concentrations (Kostka-Rick and Manning, 1993)), and because the foliar O_3 protection afforded by EDU did not translate into significant increases in yield in many field trials (Heagle, 1989), EDU has not proven useful as a standard agricultural chemical.

Leaf wetness from dewfall was found to reduce ozone deposition to cotton (Grantz *et al.*, 1997), apparently by occlusion of adaxial (upper) stomatal pores. This suggests a possible management strategy in fields in which overhead sprinklers are available, and reliable air quality forecasts can be obtained. The blockage of upper stomata is a conjecture based, in part, on the opposite effect observed following dewfall in hypostomatous grape (Grantz *et al.*, 1995), in which leaf wetness increased ozone deposition to the canopy, apparently by increasing the reactivity of the upper surface of the leaves.

Breeding cotton lines for increased resistance to O_3 may have already occurred, inadvertently. Cultivars selected for optimum growth and yield in areas of relatively high ambient O_3, such as the San Joaquin Valley, have been selected under the environmental conditions prevalent in the area, including O_3. Cultivars originally developed in areas of low ambient O_3, such as Pima cotton, may suffer significant yield losses when introduced into high O_3 regions (Grantz and McCool, 1992). The increased susceptibility of highly determinate cotton lines to O_3-induced yield losses has already been discussed. In addition, breeding for one specific desirable characteristic, such as resistance to O_3, may not translate into increases in yield if the mechanism of resistance incurs metabolic costs, such as increased allocation of carbon into defensive antioxidant compounds, or reductions in assimilated carbon because of reduced stomatal conductance. Molecular engineering, involving transfer of genes for O_3-resistance to new cotton lines, is theoretically possible, and may become more economically viable if O_3 pollution increases in cotton-rowing regions of the world. The first stages in such a program would be to identify the biochemical and physiological mechanisms of susceptibility and resistance to O_3 in cotton, and then to identify and isolate the genes regulating those mechanisms. Given the complexities of the effects of O_3 on the different levels of organization within the plant (Darrall, 1989; Heath, 1988), this would appear to be a daunting task.

4. SULFUR DIOXIDE

4.1 Origin and Distribution

Sulfur dioxide (SO_2) is an extremely acrid, irritating gas, emitted into the atmosphere from the combustion of sulfur-containing ores during roasting and smelting processes, and from the combustion of sulfur-contaminated fossil fuels, such as coal and natural gas. Natural emissions of SO_2 also occur during volcanic eruptions and from fumaroles and vents in tectonically-active regions around the world. With the exception of these natural emissions of SO_2, high concentrations of SO_2 are generally found around point sources, in the vicinity of ore-processing facilities or large coal-fired power plants. In the 19th and early 20th century unregulated emissions of SO_2 caused widespread damage to vegetation around these large industrial sources, most notably around Sudbury, Canada and Copper Basin, TN (Hursh, 1948).

The advent of environmental regulations and strict emission controls in North America and western Europe has substantially reduced the impact of SO_2 on vegetation, to the extent that SO_2 is no longer considered to be a significant air pollutant in most agricultural areas (Rosenbaum *et al.*, 1994). In some areas of the developed world sulfur deficiencies are again being observed in sensitive crops. In eastern Europe and in many developing countries, SO_2 continues to be a threat to agricultural crops and forest vegetation.

4.2 Mechanisms of Toxicity and Symptomatology

Sulfur dioxide enters plant leaves through stomata, and because of its high degree of solubility in water, it immediately dissolves on the thin aqueous film surrounding cells in the sub-stomatal cavity (Mudd, 1975). The sulfite ion is readily oxidized to sulfate, and if SO_2 is absorbed slowly, it can act as a source of S nutrition for the plant (Thomas *et al.*, 1943; Olsen, 1957). If high concentrations of SO_2 (*e.g.,* 0.10 to 0.50 ppm for 8 hours) are absorbed rapidly by the plant, sulfite accumulates, and because sulfite is 30 times

more toxic to plant cells than sulfate (Thomas *et al.*, 1943), it rapidly reaches toxic concentrations. Cotton leaves are highly susceptible to the effects of SO_2, and rapid absorption of the gas can produce plasmolysis of mesophyll cells directly underneath the lower epidermis, leading to a glazed or silvery appearance of the adaxial leaf surface (Barrett and Benedict, 1970). Continued exposure to toxic concentrations of SO_2 leads to interveinal tissue chlorosis and necrosis, producing bleached light tan to dark brown interveinal markings.

Foliar injury symptoms resembling SO_2 injury are occasionally observed on irrigated cotton. The symptoms usually appear shortly after irrigation, if the plants are subjected to periods of high temperatures and low humidity, producing rapid rates of transpiration. Results of chemical analyses of foliage show high concentrations of salts, particularly calcium sulfate (Barratt and Benedict, 1970). Because these SO_2-mimicking symptoms are often observed on plants growing on soils with high gypsum (hydrous calcium sulfate) content, rapid absorption of sulfate, translocation to leaves, and accumulation of sulfate salts at the termini of transpirational streams appears to be the mechanism of toxicity.

4.3 Effects on Yield

Brisley *et al.* (1959) conducted an extensive series of experiments on cotton (cv. 1517-C) determining the relationship between amount of leaf area injured by exposure to SO_2 and subsequent effects on yield. They concluded that exposure to concentrations of SO_2 that did not produce visible foliar injury had no effect on yield. Reductions in yield of cotton due to SO_2 injury resulted from fewer numbers of squares produced, because SO_2 injury had no effect on boll development or weight of seed cotton per boll. Exposures to SO_2 had no effect on incidence of plant diseases, particularly *Verticillium* wilt. The relationship between amount of leaf tissue injured by SO_2 (X) and percent of total seed cotton yield (Y) was: $Y = 99.26 - 0.68\ X$. Fiber quality was unaffected by SO_2 injury to foliage (Davis *et al.*, 1965). A later NCLAN study found no effect of SO_2 on yield of 'Stoneville-213' at concentrations up to 0.35 ppm, and no interaction between SO_2 and O_3 on growth or yield of cotton (Heagle *et al.*, 1986). These data indicate that ambient levels of SO_2 in agricultural regions of the U.S. should have no effect on growth or yield of cotton. However, effects of SO_2 on cotton in developing countries are largely unknown.

5. SUMMARY

A review of the effects of air pollution on cotton growth and yield has been presented. This included a general description of the sources of air pollutants, both primary air pollutants from industrial porcesses or combustion and secondary pollutants from natural or anthropogenic precursor compounds. Methods of exposing plants to air pollutants and for measurement of the effects on growth and yield were described. The bulk of the review focused on ozone: it's origin, entry into the plant, toxic effects, and a detailed account of the physiological effects of ozone including reductions in gas exchange, leaf area responses of senescence and abscission, altered carbon allocation and biomass partitioning, reduced hydraulic conductance, and the resulting detrimental effects on yield. A final mention was made of the origin, toxicity, and symptomology and effects on yield.