In order for ozone to cause damage to a leaf, it must diffuse through the stomates. It has been suggested that closure of stomates in the presence of ozone can protect plants from ozone damage. It has further been suggested that differences in stomatal response may be in part responsible for varietal differences in susceptibility to ozone damage. The objective of the present study was to determine stomatal control of the entry and exit of $H_2O$, $CO_2$, $O_3$ into and out of leaf structures. These measurements were made at different leaf ages for two soybean varieties.

Transpiration rate, net photosynthesis rate, and rate of ozone uptake were measured at five ozone concentrations, ranging from 25 to 58 pphm during a 4-hour exposure period. These measurements were made on the first trifoliate leaves of two soybean cultivars, namely Dare, a sensitive variety and Hood, a resistant variety at the
leaf ages of 14 days representing the expanding phase of growth and 20 days representing the mature growth phase.

Ozone reduced both Tr and Pn rates during the period of exposure. At ozone concentration lower than 30 pphm, the percentage decrease of Tr was greater than that of Pn. When ozone concentrations exceeded 40 pphm, the percentage decrease in Pn was as great as that of Tr. The decreases in Tr and Pn exceeded 50 percent at the end of the four hour exposure period at the highest O₃ concentration. The 14-day old leaves recovered completely within 24 hours following termination of fumigation at all concentrations used. No damage was visible in these leaves. Older leaves were more permanently damaged as indicated by necrotic spots. These leaves failed to regain photosynthetic capacity fully.

The first effect of ozone was on stomatal resistance. The closure of stomates, however, was small and did not effectively eliminate ozone uptake by the leaves. When the ozone concentration was raised to 40 pphm, stomatal resistance increased to about 3 times its original level with concomitant increases in both mesophyll resistance and CO₂ compensation point Γ. The CO₂ accumulation in the substomal cavity led to the conclusion that control of stomatal opening might be twofold, namely by an effect on the guard cells and indirectly by increased CO₂ concentration inside the leaf. Increase in Γ was correlated with the cell damage and occurrence of necrosis.
The substantial increase in $r_s$ when exposed to high ozone concentration restricted subsequent ozone absorption by the leaf. But the increase in $r_s$ occurred as a result of increases in $r_m$ and $\Gamma$ after the cells were already damaged. Consequently, if the closure of stomates was to be useful in excluding the pollutant and thus serving as a protective mechanism, it has to take place before the exposure to ozone.

By applying the resistance model of CO$_2$ diffusion to the diffusion of ozone into the leaf, the concentration of ozone inside the leaf at the mesophyll-intercellular space interface was computed. The magnitude of stomatal resistance was about the same for both leaf ages and for the two cultivars at each ozone concentration used.

The accumulation of ozone inside the 14-day old leaves indicated that the decomposition of ozone inside these leaves proceeds at a slower rate than inside the 20-day old leaves. The difference in leaf sink strength for ozone rather than the difference in stomatal resistance appears to account for the differential ozone absorption by the leaves of the two varieties. The 20-day old leaves of the tolerant (Hood) cultivar absorbed more ozone than the corresponding leaves of the sensitive (Dare) cultivar. Yet the tolerant leaves showed less damages to the rates of $T_r$ and $P_n$ with better recovery capability. They also were less sensitive to changes in $r_s$, $r_m$ and CO$_2$ compensation point.
Ozone Inhibition of Transpiration and Photosynthesis of Two Soybean (Glycine max. [L.] Merr.) Cultivars

by

Suntaree Yingjajaval

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Master of Science

June 1976
APPROVED:

Redacted for Privacy

Professor of Soil Science

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Redacted for Privacy

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Redacted for Privacy

Dean of Graduate School

Date thesis is presented ______________ October 6, 1975 ______________

Typed by Clover Redfern for ______________ Suntaree Yingjajaval ______________
ACKNOWLEDGMENTS

In this vast field of scientific research, one must remain humble in the face of what one does not know, and be grateful for the wisdom of those who do know. I should especially like to thank Dr. Larry Boersma for his interest, guidance, and inspiration throughout my graduate studies. His invaluable effort in acquainting me with scientific methodology of attacking problems and rational deductions towards the solutions is deeply appreciated.

The assistance and counsel of staff and fellow graduate students at Oregon State University is greatly acknowledged.

I should also like to thank Dr. D. T. Tingey for his contributions to the development of the experimental scheme and his generous provisions for plant materials and ozone analyzer.

In addition, I sincerely wish to thank

Dr. C. T. Youngberg, and Dr. H. H. Wickman for their willingness to serve on my graduate committee,

Dr. E. W. R. Barlow and Mr. Gary Jarman for their patience in helping me solve the technical problems which occurred with the equipment used in this study.

Financial support from the Environmental Protection Agency is greatly appreciated.

I also owe a debt of gratitude to my aunt, Veena, for all her kindly concerns in my welfare and all the helps to the unexpected problems arising during my stay away from home.
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25. Cumulative ozone uptake by the leaves of the Hood cultivar during four hour exposures to several ozone concentrations.
INTRODUCTION

Plant injury resulting from oxidants in photochemical smog resulting from sunlight induced chemical reactions of components of automobile exhaust, has been known to occur for at least 25 years. Ozone, a major component of photochemical smog, is an important phototoxicant responsible for damage to field crops representing losses valued at millions of dollars each year. It is, therefore, desirable that the effect of ozone on the life processes of plants be investigated.

Many experiments lead to the general conclusion that ozone enters leaves through the stomates. This gave rise to the suggestion that closure of stomates in the presence of the oxidant should exclude subsequent uptake into the leaf, thereby serving as a protective mechanism for the plant. Other researchers concluded however, that the closure of stomates by itself cannot account for observed differences in damage upon ozone exposure between cultivars of the same species.

Diffusion processes are well described and resistance models for water vapor and carbon dioxide into and out of leaf structures have been well established. It was hypothesized that the resistance models might be used to describe ozone diffusion into the leaf. It was
proposed to measure simultaneously the transpiration rate, the photosynthesis rate, and the ozone uptake rates of leaves exposed to a range of concentrations of ozone in the ambient air. This information may offer a better understanding of the control of stomates on ozone entry. By using several plant varieties and plant ages it should be possible to demonstrate differential sensitivity to ozone damage and to verify whether the stomatal control on ozone uptake could result in differences in degree of leaf injury due to differences in tissue susceptibility. The objectives of the experiments reported here were to study (i) the changes in transpiration and net photosynthesis rates during ozone exposure, (ii) the differences in such changes due to leaf ages and leaf sensitivity at several ozone concentrations, and (iii) to relate these changes to the amount of ozone absorbed into the leaves.
Approximately 125 million tons of gases and particles of air pollutants are emitted into the air in the United States every year from five major sources (Table 1). These pollutants have been divided into two categories, namely, general air pollutants and those which are phytotoxic (USDA Forest Service, 1973).

<table>
<thead>
<tr>
<th>Source</th>
<th>All major pollutants</th>
<th>Phytotoxic pollutants</th>
</tr>
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<tbody>
<tr>
<td>Transportation</td>
<td>60%</td>
<td>28%</td>
</tr>
<tr>
<td>Industry</td>
<td>18%</td>
<td>30%</td>
</tr>
<tr>
<td>Generation of electricity</td>
<td>14%</td>
<td>26%</td>
</tr>
<tr>
<td>Space heating</td>
<td>5%</td>
<td>9%</td>
</tr>
<tr>
<td>Refuse disposal</td>
<td>3%</td>
<td>7%</td>
</tr>
</tbody>
</table>

The two important photochemical phytotoxicants that cause the greatest damage to plants are ozone ($O_3$) and peroxyacetyl nitrate (PAN).

Ozone is a natural component of the air, existing in the outer layers of the stratosphere, about 15-30 miles above the earth surface where it absorbs and breaks down ultraviolet radiation resulting in
formation of heat. Ozone absorbs the high energy of short-wave radiation. The energy causes excitation of ozone molecules and upon returning to ground state, the radiation energy is transformed into heat. Short-wave, high energy, radiation such as ultraviolet light is absorbed by the ozone layer and only longwave, low energy, radiation is transmitted to the earth's surface. Turbulence and vertical down-drafts occasionally bring down quantities of O$_3$ to ground level where it is usually diluted in the ambient air and ozone concentration greater than 0.01 ppm does generally not result. It is not known whether ozone originating in the stratosphere is ever sufficient by itself to damage crops (Wanta, Moreland and Heggestad, 1961). Ozone results also from electrical discharges such as lightning flashes. It is possible that violent thunderstorms may produce enough ozone to cause injury in plants located far from population centers. Another possible source of ozone is the action of ultraviolet light on the volatile hydrocarbons produced by large masses of vegetation such as forests. Went (1955) suggested that ozonides and peroxides may arise in this way.

These sources are of minor importance, however, when compared to the amounts of ozone formed in urban environments. The atmospheric chemistry of nitrogen dioxide, nitric oxide, and ozone is usually discussed within the context of the photochemical smog common in those urban areas that have (i) high automobile traffic densities, (ii) frequent periods of atmospheric stability or topographic
traps, and (iii) abundant sunlight (Butcher and Charlson, 1972). But elevated ozone concentrations are not restricted to cities. Ozone concentrations exceeding EPA standard of 0.08 ppm are not uncommon in non-urban areas of several states.

Photochemical smog is produced from auto emissions while the air from the cities moves into the non-urban areas. Oxidants may actually reach higher concentrations many miles downwind from cities than in the cities themselves. This is so because the rates of the reactions leading to ozone are slow, therefore the concentrations gradually increase while the air masses move away from the city. Also, city air contains materials that react with ozone and continuously break it down into molecular oxygen. Other possible sources are the stratospheric layer and electrical discharges (Marx, 1975).

Man-produced air pollution is usually not a problem when the pollutants rise and disappear into the thin envelope of air surrounding the earth. However, the air is only a temporary transport medium. The ultimate sinks are the oceans, the soil, and the vegetation. Air serves to dilute and disperse pollutants before they come back to these sinks.

The temperature of the air near the ground normally decreases with altitude. Inversions occur when a layer of warm air occurs in the normally cool upper levels of air. Warm air rising from ground level is blocked from further upward movement when it meets this
layer of warm air. Ground level inversions occur when the air immediately above the ground becomes warmer with an increase in altitude. When such inversions occur in a basin, or narrow valley where horizontal dispersion is limited, severe air pollution episodes may occur (Battan, 1966).

The concentration of ozone in nature generally does not exceed 1 pphm. The American Industrial Hygiene Association has set the upper limit for an exposure period of 40 hrs per week--8 hrs/day for 5 days per week at 0.2 mg O₃/m³ of air (0.1 pphm), beyond which ozone will cause eyes and skin irritations and be hazardous to the respiratory system. The concentration of ozone produced by photochemical smog may exceed 50 pphm and levels exceeding 20 to 30 pphm occur frequently for a few days or sometimes for weeks during the four to five months of the summer season in some cities. Table 2 shows a survey of ozone concentrations in selected cities (Bond and Straub, 1972).

The concentration of ozone in photochemical smog is determined by a sequence of reactions involving interactions between ozone, nitrogen dioxide, and nitric oxide which are compounds produced in the smog. Three of the more important reactions linking NO₂, NO and O₃ at concentrations generally observed in the atmosphere (Leighton, 1961; Butcher and Charlson, 1972) are:
Table 2. Oxidant concentrations in selected cities, 1964-67.

<table>
<thead>
<tr>
<th>Station</th>
<th>Days of observation</th>
<th>Percentage of total days with maximum hourly average equal to or greater than</th>
<th>Maximum hourly average</th>
<th>Peak concentration</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>0.15 ppm</td>
<td>0.10 ppm</td>
<td>0.05 ppm</td>
</tr>
<tr>
<td>Pasadena</td>
<td>728</td>
<td>41.1</td>
<td>55.1</td>
<td>75.0</td>
</tr>
<tr>
<td>Los Angeles</td>
<td>730</td>
<td>30.1</td>
<td>48.5</td>
<td>74.0</td>
</tr>
<tr>
<td>San Diego</td>
<td>623</td>
<td>5.6</td>
<td>20.9</td>
<td>70.6</td>
</tr>
<tr>
<td>Denver</td>
<td>285</td>
<td>4.9</td>
<td>17.9</td>
<td>79.3</td>
</tr>
<tr>
<td>St Louis</td>
<td>582</td>
<td>2.4</td>
<td>10.1</td>
<td>62.2</td>
</tr>
<tr>
<td>Philadelphia</td>
<td>556</td>
<td>2.3</td>
<td>10.9</td>
<td>41.9</td>
</tr>
<tr>
<td>Sacramento</td>
<td>711</td>
<td>2.3</td>
<td>14.6</td>
<td>62.3</td>
</tr>
<tr>
<td>Cincinnati</td>
<td>613</td>
<td>1.6</td>
<td>9.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Santa Barbara</td>
<td>723</td>
<td>1.5</td>
<td>10.5</td>
<td>70.5</td>
</tr>
<tr>
<td>Washington, D. C.</td>
<td>577</td>
<td>1.2</td>
<td>11.3</td>
<td>54.2</td>
</tr>
<tr>
<td>San Francisco</td>
<td>647</td>
<td>0.9</td>
<td>4.5</td>
<td>28.6</td>
</tr>
<tr>
<td>Chicago</td>
<td>530</td>
<td>0</td>
<td>4.5</td>
<td>50.8</td>
</tr>
</tbody>
</table>
\[ \text{NO}_2 + h\nu(300-400 \text{ nm}) \xrightarrow{\phi k_a} \text{NO} + \text{O} \quad (1) \]

\[ \text{O} + \text{O}_2 + \text{M} \xrightarrow{k_3} \text{O}_3 + \text{M} \quad (2) \]

\[ \text{O}_3 + \text{NO} \xrightarrow{k_1} \text{NO}_2 + \text{O}_2 \quad (3) \]

\[ \phi k_a = 0.25 \text{ hr}^{-1}, \quad k_3 = 8.9 \times 10^{-4} \text{ ppm}^{-1} \text{ hr}^{-1}, \quad k_1 = 1320 \text{ ppm}^{-1} \text{ hr}^{-1} \]

These reactions require sunlight. There is no net chemical change

sunlight \xrightarrow{\text{heat}}

although a small steady-state concentration of ozone occurs:

\[ \text{hv} \quad \text{NO}_2 + \text{O}_2 \xrightarrow{h\nu} \text{NO} + \text{O}_3 \quad (4) \]

The factors \( k_a, k_3 \) and \( k_1 \) are rate constants which determine how fast the reactions occur. The factor \( \phi \) is a function of sunlight intensity. The \( \text{M} \) stands for some unknown compound which serves as a combination site for \( \text{O} \) and \( \text{O}_2 \). At the same time, other reactions involving these three constituents also take place but at a much slower rate. The above reactions are dominant in the chemistry of photochemical smog. According to Equations (1) and (2) the rate of production of oxygen atoms from the formation and decomposition reactions is:
\[ \frac{d[O]}{dt} = \phi k_a [NO_2] - k_3 [O][O_2][M] \]  \hspace{1cm} (5)

and the rate of ozone production according to Equations (2) and (3) is given by:

\[ \frac{d[O_3]}{dt} = k_3 [O][O_2][M] - k_1 [O_3][NO] \]  \hspace{1cm} (6)

If it is assumed that a dynamic equilibrium or steady state is reached, then the decomposition rate equals formation rate and \( \frac{d[O]}{dt} = 0 \). Equation (5) then becomes:

\[ \phi k_a [NO_2] = k_3 [O][O_2][M] \]  \hspace{1cm} (7)

Substituting the left hand side of Equation (7) into Equation (6) gives the following result:

\[ \frac{d[O_3]}{dt} = \phi k_a [NO_2] - k_1 [O_3][NO] \]  \hspace{1cm} (8)

Since the change of ozone concentration with time \( \frac{d[O_3]}{dt} \) is very small as it is in the atmosphere, \( \frac{d[O_3]}{dt} \) may be set equal to zero as a first approximation. Equation (8) then becomes:

\[ \frac{[NO][O_3]}{[NO_2]} = \frac{\phi k_a}{k_1} \]  \hspace{1cm} (9)
The equilibrium constant $\phi k_a /k_1$ depends on the radiation intensity. Equation (9) describes a photochemical equilibrium between these three reactants. It also indicates the concentrations at which nitric oxide and ozone can coexist in air. If the NO concentration is high, then less ozone is formed. On the other hand, if gaseous hydrocarbons are also produced in the smog from the automobile exhaust gases, they will be first oxidized by active O and O$_3$, and in turn oxidize NO to form O$_2$ as shown in Equations (10) and (11).

\[
\begin{align*}
\text{RC}(:\text{O})\text{OO} + \text{NO} & \rightarrow \text{RC}(:\text{O})\text{O} + \text{NO}_2 \\
\text{RC}(:\text{O})\text{O} + \text{NO} & \rightarrow \text{RO} + \text{NO}_2
\end{align*}
\]

According to Equation (9), the low concentration of NO and high concentration of NO$_2$ favor the accumulation of ozone. High concentration of ozone can therefore occur in the atmosphere that contains gaseous hydrocarbons contained in automobile exhaust gases.

Ozone does not only react with the oxides of nitrogen. Because of its strong oxidative property, ozone also reacts with olefin hydrocarbons forming aldehydes, ketones, acids, alcohols, peroxides, ozonides and hydrocarbons. At atmospheric temperatures, ozone undergoes no reactions of importance with either carbon monoxide or sulfur dioxide. Reactions with such substances as nitrous acid, organic nitrites, nitroso compounds, other hydrocarbon organic species, and with the particulate matter in polluted air occur but at an
unknown rate (Leighton, 1961). Only the reactions of ozone with nitric oxide, nitrogen dioxide, and the olefins, are definitely known to be fast enough to be of importance in air pollution.

Effects of Ozone on Plants

Foliar damage resulting from exposure of plants to smog has been known to occur for some time. But studies to quantify ozone damage were not initiated until 1954 when ozone was first identified as another major component of oxidants apart from peroxyacetylnitrate (PAN). The first report that ozone was the cause of grape stipple was published in 1958 (Richards, Middleton and Hewitt, 1958). It has since been shown that ozone is responsible for other plant diseases such as tobacco fleck (Heggestad and Middleton, 1959), white pine emergence tipburn (Berry and Ripperton, 1963), California conifer X-disease (Miller et al., 1963) and onion tipburn (Engle, Gabelman and Romanowski, 1965). Symptoms of the X-disease are a development of chlorotic mottles on the needles, terminal die-back, and abscission.

Environmental Effects

The extent of the damage caused by ozone depends on a number of environmental conditions during the growth of the plant. The effects have been primarily studied under laboratory conditions which emphasized only one or two factors without controlling the others.
This has produced fragmentary reports and contradictory results without giving a clear understanding of the possible interactions of factors such as temperature, light, humidity, and nutrition. In general, environmental conditions which tend to produce optimum growth and vigor of a plant are those which maintain full turgidity with wide open stomates and active gaseous exchange. These conditions make a plant most susceptible to ozone damage (Darley and Middleton, 1966).

Light Intensity. Pinto beans and tobacco grown under a low light intensity were severely damaged by ozone (Dugger et al., 1963; Heck and Dunning, 1967). This would be expected as it is known that irradiance partially controls physiological age by its influence on cell division and plant maturity via a phytochrome mediated reaction. Low light intensity retarded the maturation of these two plants. The young leaves were most susceptible to ozone. Physiological age rather than chronological age is the important factor determining differential biochemical responses to air oxidants and these two must be distinguished in order to predict plant sensitivity (Heck, 1964; Heck and Dunning, 1967). If a plant is put in the dark for a period of time before ozone exposure so that the stomates are partially closed, then its sensitivity decreases (Hull and Went, 1952; Koritz and Went, 1953). Juhren, Noble and Went (1957) found that sensitivity of annual bluegrass did not occur until the light intensity was raised above the threshold light intensity of 400 ft-c. which caused stomatal opening. Heck, Dunning
and Hindawi (1965) also reported a positive correlation between degree of injury and light intensity at the time of exposure over a range of light intensities up to the maximum intensity available. In addition, Heck and Dunning (1967) found that tobacco plants were less sensitive to ozone than were control plants when exposed to the high carbon dioxide concentration of 500 ppm which induced stomatal closure, immediately before and during exposure to ozone.

**Temperature.** The degree of ozone damage to plants is also influenced by temperature. Juhren, Noble and Went (1957) conducted extensive research on the relationship between temperature and the sensitivity of plants to oxidants. They found that plants were less sensitive when grown under hot conditions. The plants would gain sensitivity when transferred to cool conditions. The opposite was true for plants grown under cool conditions. These were initially sensitive but lost sensitivity when changed to hot conditions. They concluded that the smog sensitivity was not correlated with the size of plants, nor with the growth rate but with the degree to which the stomates were wholly or partly open. In their experiments stomates opened wider with increasing temperature up to 26 C (warm) but then started to close in the hot temperature region. They related the resistance of plants grown under hot conditions (30 C) to the closure of stomates. Generally, extreme low and high temperatures which induce stomatal closure tend to reduce plant sensitivity. In the

**Humidity.** There are reports stating that ozone-induced injury increases with the humidity of the ambient air (Hull and Went, 1952; Menser, 1962; Otto and Daines, 1969). Otto and Daines (1969) showed that tobacco plants fumigated at a relative humidity of 95 percent with 0.10 ppm ozone developed flecking equivalent to those fumigated at 0.30 ppm in air with a relative humidity of 26 percent. They measured the leaf permeability index by porometers and found it to increase with increased relative humidity. Stomates are known to close when exposed to relative humidities less than 50 percent. Therefore, very low relative humidity results in decreased gaseous transfer through the leaf.

**Water Stress.** The resistance to oxidant injury increases with increasing osmotic stress of the solution when plants are grown in saline nutrient solutions or in plants grown under water stress conditions (Middleton, 1956; Macdowell, 1965; Maas et al., 1973). Soil conditions that reduce water uptake and produce drought conditions within the plant may cause stomatal closure. These conditions would then favor resistance to oxidant pollutants (Heck, 1968).
Ozone-Induced Injury

Symptoms of Leaf Injury. Ledbetter, Zimmerman and Hitchcock (1959) described the characteristics of leaf injury caused by ozone. The damage varies from dark stippling or light flecks on the upper leaf surface to necrotic patches on both surfaces of the leaf and general chlorosis. They found that, anatomically, the dark stippling is due to a thickening of the palisade cell walls and pigmentation. The light flecking is due to collapse of the palisade cells without the formation of pigments. The necrotic patches occur when there is a general injury to mesophyll cells as well as to epidermal cells. In very severely damaged areas the guard cells degenerate and the stomata are pulled open by the drying epidermis. This greatly increases the drying of the injured cells within the leaf (Rich, 1964). Bystrom et al. (1968) found that the expanding leaves of sugar beets which were injured during exposure to auto exhaust pollutants showed definite changes in surface texture 24 hours to one week after exposure. They observed excessive extrusion of waxy material in irregular spots. The older leaf surfaces which were naturally coated with wax showed no such damage. They proposed that the incompletely waxed surfaces might be responsible for the susceptibility found in the young expanding leaves.
Effect of Tissue Maturity. One of the most important factors in determining the extent to which a plant can be damaged by exposure to ozone is the age of the plant tissue. On a given tobacco plant, leaves of different ages show varying degrees of damage. Very young leaves at the apex of the plant and old leaves at the base of the plant are not sensitive. Sensitivity of the expanding leaves varies between the young and old parts. Glater, Solberg and Scott (1962) noted that damage to young leaves appeared only at the tips, damage to somewhat older leaves appeared in the middle of the leaf, and damage to fully mature leaves appeared at the leaf base. Such localization of damage shows that ozone damage is a function of tissue maturity. In addition, the pattern of symptom development can be ascribed to the development of functional stomata (Glater et al., 1962; Menser et al., 1963).

The localization of damage is correlated with the gradient of cellular differentiation from tip toward base as the leaf matures. In the young region, stomatal initials are formed in the epidermis. As cells mature, the differentiation goes on until the guard cells are distinct and raised above the surface of the epidermal cells. The stomates are considered functioning when the guard cells reach their maximum size and the walls immediately surrounding the pore are heavily thickened. The damage occurs in the region of the leaf where stomata have just become functional and ambient polluted air can make
direct contact with interior leaf tissues by virtue of large substomatal chambers and intercellular air spaces.

Effects on Stomates. There is a general agreement that in order to cause damage, ozone must first enter through the stomates (Bobrov, 1952; Heggestad and Middleton, 1959). Therefore, any environmental factor that favors stomatal opening increases susceptibility to ozone. Ozone damage is most frequently observed in regions of the leaf where the stomates are most numerous (Homan, 1937) or more open (Juhren et al., 1957).

Ozone causes rapid and prolonged stomatal closure in many plant species. Koritz and Went (1953) fumigated tomato plants with a mixture of 0.1 ppm ozone and 1-n-hexene vapor for one hour and found the stomates to close as soon as fumigation was initiated and to remain closed for at least one hour after fumigation was terminated. If the treatment was repeated with the same plant on successive days, it was found that on the third day, most of the stomates were closed prior to the fumigation and were unable to reopen following the treatment, indicating permanent injury. Hill and Littlefield (1969) obtained similar results when fumigating oats with 0.65 ppm of ozone for an hour. Over half of the stomates were closed completely by the end of the ozone treatment. About 15 minutes after the exposure was discontinued the stomata began to reopen, but recovery was not complete. On the next day, visible leaf injury was evident. The stomata were only
partially open at the beginning of the subsequent ozone treatment. Apparently, the visible leaf injury indicates permanent damage where cells can no longer maintain the turgor pressure. The drying epidermis could cause the stomata to pull open and increase the drying of the injured cells within the leaf. However, Dugger et al. (1962) found no correlation between stomatal opening and leaf damage from ozone. Dugger and Ting (1970) then suggested that a major obstacle in understanding the relationship between stomatal opening and damage by oxidants seems to be the lack of appreciation of the functional relationship between stomatal opening *per se* and gas transfer. This is because gas transfer is a hyperbolic function of stomatal opening in most agronomic plants so that the stomates become limiting to the diffusion of gases into and out of the leaf only when the aperture is very small and the stomates do not have to be fully open in order to yield a maximum rate of gaseous diffusion.

Ozone-induced stomatal closure has been proposed as a possible explanation for the different ozone susceptibilities of various plant species. Ozone induced closure would exclude further entry of toxicant and thus decrease damage. On the other hand Todd and Propst (1963) suggested that such a protective mechanism could not explain the differences in ozone sensitivity between tomato, coleus and bean plants, when beans showed the greatest reduction in transpiration which inferred the highest degree of stomatal closure and also the
most damage when exposed to ozone. Other (Dugger et al., 1963; Heck, et al., 1965) suggested that stomates are not per se the primary mechanism controlling susceptibility to ozone, but serve only as a pathway for entrance of the toxicant. This does not mean that stomates are of no importance in the response of a plant to ozone. One of the major resistances to the entry of ozone into the interior spaces of the leaf is controlled by stomatal opening.

The mechanism by which ozone causes stomates to close remains unresolved. Lee (1965) suggested that, in tobacco leaves, the concentration of soluble sugars plays a dual role with respect to ozone susceptibility. At the very low and very high sugar concentrations of the leaves, the plants were damaged least. He suggested that high sucrose or reducing sugar content closed stomates as a result of turgor changes in the guard and adjacent epidermal cells caused by differential distribution of the soluble sugars. Lee did not have an explanation for the role of sugar at low concentrations.

Ozone-induced closure of stomates is generally accompanied by reduced transpiration and apparent photosynthesis rates (Hill and Littlefield, 1969). This raises a question as to which is the cause and which is the effect. Did stomatal closure reduce these rates or was there a direct effect of ozone on these rates. Using plants with non-functional stomata such as Lemna minor, Erickson and Wedding (1956) demonstrated that ozone did affect photosynthesis directly
rather than indirectly by stomatal movement. They used lesser
duckweed (*Lemna minor*) which has only nonfunctional stomates.
Therefore the stomatal control factor was not present. Todd (1958b)
showed that the respiration rate of pinto beans increased four times
when exposed to ozone, which could be interpreted to mean that
stomatal closure is caused by increased concentrations of carbon
dioxide in the interior spaces of the leaf. The decrease in photosyn-
thesis rate has also been related to chlorophyll destruction which is
a linear function of duration of exposure. The loss of photosynthetic
capacity has also been related to the increased permeability and
ultimate death of the cells (Erickson and Wedding, 1956).

**Effects on Physiology and Metabolism.** Once inside the leaf,
ozone attacks the palisade parenchyma first (Ledbetter *et al.*, 1959;
parenchyma cells are two-fold (Thomson, Dugger and Palmer, 1966).
The first phase involves changes in the chloroplast stroma consisting
of either a granulation and an increase in electron density or the
formation of ordered arrays of granules and fibrils. In the second
phase, a general disruption of the cellular membranes occurs when
organelles and cellular contents aggregate in the center of the cells.
Recently, the effect of ozone on the cell membrane permeability has
been studied. There is ample evidence showing that ozone increases
cell membrane permeability (Nobel and Wang, 1973; Evans and Ting,
Perchorowicz and Ting (1974). Coulson and Heath (1974) further investigated this using isolated spinach chloroplasts and concluded that ozone inhibits the electron transport by alteration of the transfer of photon energy from the collecting chlorophyll to the photosynthetic trap, resulting in photosynthesis inhibition.

One of the most probable explanations for the effect of ozone on membrane permeability is that ozone reacts with the double bonds of unsaturated lipids (Scott and Lesher, 1963). Rich (1964) proposed that ozone might not only react with the lipids but could also prevent the production of new lipids for membrane repair by destroying sulfhydryl groups of certain enzymes. Enzyme inactivation is due to oxidation of the sulfhydryl group of the enzyme (Todd, 1958a; Mudd, 1965; Tomlinson and Rich, 1970). Other enzymes are affected by ozone as well. Ordin and Altman (1965) showed that phosphoglucomutase, an enzyme system responsible for converting glucose 1-phosphate to glucose 6-phosphate was inhibited by ozone. This can cause an observable change in the pathways of carbohydrate metabolism studied by Tingey, Fites and Wickliff (1975). Ordin, Hall and Kindinger (1969) also observed an inactivation of cellulose synthetase by ozone.

Because of its high oxidation potential, +2.07 V, ozone can irreversibly oxidize the reduced forms of nicotinamide adenine dinucleotides (NADH and NADPH) (Siegel, 1962; Mudd, 1965). The
ozone oxidation products of nucleotides inhibit enzymatic activity in reactions in which these cofactors are required.

Mitochondria are also affected by ozone. Lee (1967) found that the oxidative phosphorylation system was quite sensitive to ozone and later (1968) observed that ozone induced rapid swelling of isolated mitochondria from tobacco tissue with an increase in membrane permeability. The rate of energy (ATP) production by mitochondria can be lowered by such damage (Tomlinson and Rich, 1968).

Mudd et al. (1970) reported on the susceptibility of amino acids and proteins to ozone damage. Cysteine is the most susceptible amino acid. King (1961) reported an increase in α-keto acid after solutions of pepsin and serum albumin were treated with ozone. He presented data to indicate that ozone reacted with the aromatic amino acids of proteins.

Many investigators correlated ozone sensitivity to the size of the leaf metabolite pools. The stage of maximum sensitivity to ozone in pinto beans corresponded to high levels of amino acids (Bennett, 1969). Ting and Mukerji (1971) on the other hand found maximum sensitivity to correspond to minimal levels in cotton. The period of maximum sensitivity was characterized by declining, but not minimal, levels of free amino acids in soybeans. Total soluble sugars and sucrose concentrations decreased but reducing sugar concentrations increased during the stage of high ozone sensitivity (Tingey, Fites and Wickliff,
This suggests that sensitivity to ozone is probably the result of interaction among several factors, including metabolite pools, enzyme activities, and morphological features such as the formation of cutin on the mesophyll cells. The lack of consistent trends in the correlation between size of metabolite pools and sensitivity among species lead Tingey et al. (1973) to conclude that metabolite pools do not play the primary role in governing ozone sensitivity.

Many environmental conditions cause symptoms on plants which mimic those caused by specific air pollutants. In response to physiological and environmental stress, as well as ozone, plants accumulate peroxidase isoenzymes (Curtis and Howell, 1971). Tingey et al. (1975) found an increase in phenol biosynthesis activities in leaves exposed to ozone. The oxidation of phenol to quinones is responsible for the brown pigment developed when plant cells are disrupted and exposed to the air.

**Growth and Yield Suppressions.** Most of the studies on ozone effects on plants based the injury assessment on the visible damage of ozone where it was apparent that leaf cells had been killed. However, the hidden damage not accompanied by visible injury should be of equal importance and must not be overlooked. High concentrations of ozone cause a temporary reduction in rate of apparent photosynthesis and rate of transpiration often with little or no visible injury (Hill and Littlefield, 1969; Middleton, 1961). Growth suppression of plants
exposed to natural photochemical smog has been reported for alfalfa, endive, sugar beet and tomato (Hull and Went, 1952), tobacco (Menser et al., 1964), grapefruit (Taylor, 1958) and lemon (Thompson et al., 1967). Hoffman, Maas and Rawlins (1973) showed that the cumulative effects of daily ozone exposures for 2 hour periods at ozone concentration as low as 0.15 ppm for about 60 days from the seedling stage to harvest, resulted in pinto bean yield reductions considerably greater than suggested by visual injury indices. Maas et al. (1973) reported that pinto bean plant injury and reduction in growth were sigmoidal functions of ozone dose. Daily exposures above threshold levels produced cumulative injury that progressed from the primary leaves to subsequent trifoliate leaves. Furthermore, they indicated that although the direct effect of ozone was on the leaves, growth reductions were greater for the roots. This was confirmed by Tingey, Heck and Reinert (1971) who showed that ozone reduced yields of rootcrops such as raddish by as much as 50 percent while the reductions in leaf weights were much smaller. They hypothesized that the roots, being dependent upon photosynthate, are affected by the impaired metabolism of the leaves. The rate of N₂-fixation by root nodules may also be restricted (Tingey and Blum, 1973). Ordin and Propst (1962) found that ozone could irreversibly inactivate the biological activity of indoleacetic acid (IAA) in vitro by breaking the indole ring of the compound. The inactivation has not been confirmed
in vivo. If such reactions occur, then the lack of this growth
promoting hormone would result in growth suppression. Growth
suppression might also result from a reduction in photosynthesis along
with an increase in respiration (Todd, 1958b; Todd and Propst, 1963).

Research on Protective Agents

Attempts have been made to develop anti-oxidants and
anti-ozonants to protect plants from ozone. Freebairn and Taylor
(1960) found that some reduction of the damage was achieved by spray-
ing with vitamin C or ascorbic acid. Jones (1963) tried the use of
particulate materials which react with or destroy ozone, but found no
practical means of application to leaf surfaces. Seidman, Hindawi
and Heck (1965) reported some protection from several stomate-
closing chemicals. Heck (1968) pointed out three general weaknesses
in any suggested spray program: (i) the frequency of spraying needed
for continued resistance, (ii) the cost, and (iii) the inability to pre-
dict the occurrence of high oxidant days accurately. Engle et al.
(1965) reported that ozone resistance of an onion variety based on the
stomatal closure mechanism was controlled by a single dominant gene.
Brewer and Ferry (1974) suggested that in order to grow plants in an
area covered by oxidants for prolonged periods of time, breeding for
oxidant resistant varieties seems to be the most practical means of
dealing with this problem.
Conclusion

Studies on plant damage induced by ozone have emphasized either the influence of environmental conditions or the metabolic activities and size of metabolic pools on the susceptibility of plant tissues. Even though the complex interactions of environmental factors such as temperature, light, humidity, and nutrition are still not clearly understood, there is general agreement that these factors exert their influence through their effect on the functioning of stomates and indirectly through their influence on predisposing plant tissues to be susceptible to the oxidant damage. Studies on the metabolic pools of plants gave inconsistent results among plant species making it impossible to specify any specific substrate to be responsible for plant sensitivity. Whether the sensitivity is due to the interactions between many metabolites in a series of complex processes or simply due to control by a single gene remains to be investigated. Many studies on metabolic changes are conducted. These render many pieces of information which lead to a better understanding of the mechanism of ozone damage. So far, it appears most likely that the primary effect of ozone is on the membrane by oxidizing the unsaturated bonds of lipids and the -SH groups of enzymes involved in production of new membrane components. The oxidation impairs the semipermeable nature of membranes and leads
to 'leaky membranes'. Ozone induced changes observed with other metabolites, substrates, or enzymes might only be secondary effects due to ionic and water imbalances of the cells resulting from the leaky membranes. Specific membranes such as those of chloroplasts and mitochondria not only function to retain ions inside the cells but also as the sites of energy production. It is conceivable that disturbance of these membranes would cause damage to many metabolic pathways, such as the Kreb cycle, CO$_2$ fixation, and lipid and protein synthesis. That is to say that the entire cell integrity is destroyed.

The more recent studies show that plants have differential responses to other oxidants, e.g., oxides of nitrogen and sulfur dioxide, present as components of smog. The effects of ozone and SO$_2$ turn out to be synergistic. Investigations will not be complete until the complete understanding of the actual mode of action of each individual oxidant is attained. Understanding of the interactions among the oxidants must be determined as well.
MATERIALS AND METHODS

Experimental Arrangements

Completion of the experimental objectives required the simultaneous measurement of rates of net carbon assimilation and transpiration of leaves exposed to air with specified ozone concentrations. Such measurements are normally obtained by measuring the inflow and outflow concentrations of CO₂ and H₂O of air flowing through an environmentally controlled chamber containing the foliage to be exposed. The experimental arrangement originally described by Bierhuizen and Slatyer (1964) was used for these experiments.

The experimental arrangement is shown schematically in Figure 1. It consists of a leaf chamber, pumps for maintaining a constant rate of air flow through the chamber, an infrared gas analyzer used in the differential mode for the measurement of changes in CO₂ concentration, a differential psychrometer for the measurement of changes in water vapor concentration, an ozone generator, and an ozone analyzer for the measurement of ozone concentrations.

Control of Air Flow

Air to be pumped through the cuvette was drawn from the roof by air pump P₁. The flow rate was regulated by pressure regulating valves V₁ and V₂. The use of two valves made it possible to keep the
Figure 1. Schematic diagram of the equipment used for the experiments.
flow rate constant. The flow rate should vary as little as possible to avoid errors in the calculation of transpiration and photosynthesis rates and for the control of the ozone concentrations. Precise control of the flow rate was particularly important for the determination of boundary layer resistance which was calculated using the difference between the transpiration rates at two leaf temperatures. The first pressure regulator \( V_1 \) was placed immediately following the air pump \( P_1 \). The second regulator \( V_2 \) was placed after the first drying column \( D_1 \) and column of active carbon \( C \) which served to trap oxidants in the air.

The temperature and humidity of the air were adjusted by successive passage through coils in two temperature controlled water baths, \( B_1 \) and \( B_2 \). The air was saturated with water vapor by bubbling it through distilled water contained in a diffuser submerged in water bath \( B_1 \). The air bubbling through the distilled water was saturated with water vapor at the temperature \( B_1 \). The amount of water vapor contained by the air could be adjusted by changing the temperature of bath \( B_1 \). Upon saturation the air passed through a copper coil in bath \( B_2 \) set at the temperature desired in the leaf chamber. The relative humidity of the air entering the leaf chamber thus equalled the ratio of the saturation vapor pressures at the temperatures of baths \( B_1 \) and \( B_2 \). The air stream was divided following water bath \( B_2 \). A reference air stream went directly toward one side
of a differential psychrometer via regulating valve $V_4$ through a copper coil in bath $B_2$. The second part was routed to the leaf chamber through regulating valve $V_5$ and flow meter $F_1$. The flow meter had a capacity ranging from 1 to 9 liters per minute. It was used to regulate and measure the flow rate to the chamber. The air leaving the leaf chamber was brought to the same temperature as the reference air by passing it through a parallel copper coil in bath $B_2$. The sample and reference air streams then followed parallel, but separate paths through the differential psychrometer to the drying columns $D_2$ and $D_3$. The drying columns were lucite containers filled with indicating drierite followed by anhydrous magnesium perchlorate.

After passing the drying columns the air streams were directed to an infrared gas analyzer using four-way switching valves $V_{10}$ and $V_{11}$. The flow rates were regulated with regulating valves $V_{12}$ and $V_{13}$ and measured with flow meters $F_2$ and $F_3$. Capacities of the two flow meters ranged from 0.1 to 1.5 l/min. They were needed to regulate the flow rate to the infrared gas analyzer. The measurements of these two meters were not used in subsequent calculations.

The infrared gas analyzer was modified for use in the differential mode. The reference air stream and leaf chamber air stream passed through the instrument with the output indicating the difference in the CO$_2$ concentration of the two samples.
Throughout the gas system, 0.635 mm O.D. (1/4 inch) copper tubing was used wherever possible. Where flexible connections were required, 0.635 mm O.D. (1/4 inch) high density nylon tubing was used. All connections were made with Swagelock gas-tight fittings.

Leaf Chamber

The leaf chamber, made from lucite, has inside dimensions of 20.5 x 16.5 x 2.5 cm (Figure 2). Water jackets, 1 cm thick, covered the chamber on the upper and lower surfaces. Water from bath B2 was circulated through these jackets by pump P2 to remove energy from the light source trapped in the chamber. Air entered and left the chamber through holes, 0.4 mm in diameter, spaced 1 cm apart along 16 cm long lucite distribution tubes placed on opposite sides of the chamber. Half the holes directed the air flow upwards and the other half downwards under 45 degree angles to achieve an equal flow rate across both sides of the leaf. Uniform air mixing within the chamber was further aided by an electrically driven fan installed at the center of the lower half of the chamber. The fan speed was controlled by a variable DC power source outside the chamber. The leaf was kept in the central plane position by nylon threads stretched across the chamber. A pass-through slot for the petiole was provided in the chamber wall. It was sealed by plasticine when a leaf was in position. An air tight seal between the upper and lower chamber
Figure 2. The leaf chamber, with the top removed, showing the central fan and the thermocouple for measuring leaf temperature.
halves was obtained with a 4 mm thick closed-cell neoprene rubber gasket. The chamber halves were held together with eight lightly tightened wing nuts.

**Ozone Generator**

Ozone was generated by passing air through a quartz tube, running parallel to an ultraviolet lamp about 24 cm long. The device used was a Model STC-4 ozone generator manufactured by Ultra-Violet Products. The concentration of ozone in the air stream can be varied by covering the UV lamp with a sliding metal tube. The low output of this generator makes it appropriate for experiments involving small leaf chambers. The generator was put in the system immediately following the flow meter $F_1$, so that the flow rate through the generator was kept constant and the ozone concentration produced was calibrated against the length of the exposed UV lamp. The possible change in relative humidity of the air stream during its pass through the generator was minimized by controlling the room temperature at the same level as the water bath $B_1$, which was 28 C.

**Ozone Analyzer**

Arrangements were made for the measurement of ozone concentrations in the air stream just prior to entering the leaf chamber and immediately upon leaving the leaf chamber (Figure 1). Glass
T's were installed at both points to allow small samples to be withdrawn from the air stream passing the chamber. Each sampling line connected to a common line to the ozone analyzer through glass stopcocks ($V_{14}, V_{15}$). Use of these stopcocks allowed either the influx or efflux air to be admitted to the ozone analyzer. Only teflon or glass tubes were used for connections following the ozone generator to avoid ozone destruction on exposed surfaces. When a sample was taken from the air flowing into the chamber, flow meter $F_1$ did not indicate the correct rate of air flow to the chamber since part of it was diverted. Therefore, only the ozone concentration of the chamber outflow was measured during the ozone exposure periods. This procedure was adopted after it was established by measurement that the ozone concentration of the inflow air remained constant during this time period.

The ozone analyzer used was a Mast model meter which measures the oxidation rate of a KI solution by ozone. The meter output depends on the rate of air flow through the meter. To assure a constant air flow rate the sampling air was routed to a reservoir consisting of a 250 ml Pyrex flask (Figure 1A). Sampling air filled this reservoir at a rate slightly exceeding the rate through the meter. Excess air was vented outside the room. A second outlet allowed the meter to draw sampling air at a constant rate. The flask was sealed by glass.
The concentration output of the ozone generator was calibrated periodically by spectrophotometric procedures using EPA standards. The analyzer was in turn calibrated against this ozone generator using dry compressed air from a reservoir tank before the start of each experiment. The reading was recorded on a Leeds Northrup recorder.

**Light Source**

The 2500 watt long-arc Xenon lamp described by Jarman, Barlow and Boersma (1974) was used as the light source. The light intensity used in the experiments was 308.26 W/m$^2$ measured inside the leaf chamber with the chamber cover in place. The quantum flux at the leaf surface equalled 680 μE/m$^2$/sec.

**Measurements**

**Growth Characteristics**

The objective of these measurements was to characterize development of Hood and Dare soybean plants by measuring leaf length and determining the plant plastochron index as a function of time.

The "plastochron index" was proposed by Erickson and Michelini (1957) as an index to define a developmental stage of plants.
As they pointed out that

... a plastochron is conventionally defined as the time interval between initiation of two successive leaves. It might be more broadly defined as the interval between corresponding stages of development of successive leaves, and one might choose initiation, maturity, or any intermediate stage of development as the stage of reference.

The plastochron index for leaf length is calculated from the equation:

\[ P.I. = n + \frac{\log L_n - \log a}{\log L_n - \log L_{n+1}} \]  

where "a" is a reference length, "n" is the serial number of the leaf which just exceeds the reference length "a," and \( L_n \) is the length of leaf "n" in mm. In this experiment 20 millimeter (mm) was used as the reference length. This is a useful value for soybeans since leaves of this length are growing exponentially while being large enough to be measured accurately and without injury to the shoots.

Seeds of soybean plants were planted in a mixture of perlite and jiffy (2:1 v/v) in 500 cm\(^3\) PVC pots, with a 10.3 cm diameter. Four pots were planted every day and put in a growth chamber, with a daytime temperature of 30°C, a nighttime temperature of 26.5°C, and relative humidities of 65 percent and 75 percent respectively. Fluorescent and incandescent lamps provided a light intensity of about 54 W/m\(^2\) (1860 ft.-c) in the plane 30 cm above the containers which
was about the middle of the height of the fully grown plants. The lamps were adjusted to come on at 6 a.m. and turn off at 6 p.m. The incandescent lamps were on for three hours during the middle of the night to keep the plants in a strictly vegetative growth phase.

Initially four seeds were planted in each pot. After eight days only the most uniform plant was retained. Plants were watered every day with NCSU phytotron nutrient solution (Table 3) and with distilled water once each week.

The total length, including the central leaflet and petiole of each leaf on each of four plants was measured daily at about 4 p.m. These measurements of length and age of plants were used to calculate a plastochron index for each day. Only the vegetative growth phase of plants ranging from 8 to 25 days after planting was studied.

Transpiration Rates

The rate of transpiration from the leaf in the leaf chamber was determined by measuring the difference between the water vapor density of the air passing over the leaf and the reference air stream using the differential psychrometer positioned in bath B₂. The basic design of the differential psychrometer was similar to that described by Bierhuizen and Slatyer (1964). The thermocouple output was measured with a Keithley 150 B microvoltmeter and recorded on a potentiometric recorder.
Table 3. Composition of NCSU phytotron nutrient solution.

<table>
<thead>
<tr>
<th>Stock Solution</th>
<th>Grams per liter stock solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stock Solution A</strong></td>
<td></td>
</tr>
<tr>
<td>Magnesium nitrate, Mg(NO$_3$)$_2$·6H$_2$O</td>
<td>65.0</td>
</tr>
<tr>
<td>Calcium nitrate, Ca(NO$_3$)$_2$·4H$_2$O</td>
<td>160.0</td>
</tr>
<tr>
<td><strong>Stock Solution B</strong></td>
<td></td>
</tr>
<tr>
<td>Ammonium nitrate, NH$_4$NO$_3$</td>
<td>80.0</td>
</tr>
<tr>
<td>Sequestrene 330 Fe</td>
<td>25.0</td>
</tr>
<tr>
<td>Potassium phosphate, KH$_2$PO$_4$ (monobasic)</td>
<td>12.0</td>
</tr>
<tr>
<td>Potassium phosphate, K$_2$HPO$_4$·3H$_2$O (dibasic)</td>
<td>14.0</td>
</tr>
<tr>
<td>Potassium sulfate, K$_2$SO$_4$</td>
<td>15.0</td>
</tr>
<tr>
<td>Sodium sulfate Na$_2$SO$_4$</td>
<td>17.0</td>
</tr>
<tr>
<td>Boric acid, H$_3$BO$_3$</td>
<td>0.70</td>
</tr>
<tr>
<td>Molybdic acid, MoO$_3$·2H$_2$O</td>
<td>0.005</td>
</tr>
<tr>
<td>Hampene Zn, 14.5%</td>
<td>0.045</td>
</tr>
<tr>
<td>Hampol Mn, 9.0%</td>
<td>0.63</td>
</tr>
<tr>
<td>Hampol Cu, 9.0%</td>
<td>0.03</td>
</tr>
<tr>
<td>Sequestrene Co, 14.0%</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**Directions:**

1. Use care not to overstir as excessive agitation may cause precipitation.

2. The minor elements are dissolved before adding to the "B" stock solution, the final pH should be 7.0.

3. Both stock solutions "A" and "B" are to be used at 1 to 500 or 200 ml stock per 100 liter water.
For this differential psychrometer arrangement the psychrometric equations for the air streams may be written (Bierhuizen and Slatyer, 1964) as:

\[
(e_{\text{wr}} - e_r) = A(t - t_{\text{wr}}) \quad (13)
\]

and

\[
(e_{\text{ws}} - e_s) = A(t - t_{\text{ws}}) \quad (14)
\]

where \( t \) is the temperature of the air stream which equals the temperature of bath \( B_2 \), \( t_{\text{wr}} \) and \( t_{\text{ws}} \) are the dew point temperatures of the reference and sample air streams respectively, measured by the differential psychrometer, and \( e_{\text{wr}} \) and \( e_{\text{ws}} \) are the saturated water vapor pressures corresponding to \( t_{\text{wr}} \) and \( t_{\text{ws}} \) as recorded in the Smithsonian Meteorological Tables (List, 1966), \( e_r \) and \( e_s \) are the actual vapor pressures to be calculated, and \( A \) is the psychrometric constant equal to 0.667 mb vapor pressure per degree C. Subtracting the two above equations yields:

\[
\Delta e = e_r - e_s = A(t_{\text{wr}} - t_{\text{ws}}) + (e_{\text{wr}} - e_{\text{ws}}) \quad [\text{mb}], \quad (15)
\]

with \( t_{\text{wr}} - t_{\text{ws}} \) derived direction from the output of the differential psychrometer and \( e_{\text{wr}} - e_{\text{ws}} \) obtained from tables. The vapor pressure difference \( \Delta e \) can be converted to water vapor concentration difference using the equation
\[ c = \rho \left( \frac{\Delta e}{e_s} \right) \, \text{mg/liter}, \quad (16) \]

where \( \rho \) (mg/l) and \( e_s \) (mb) are the saturation vapor density and water vapor pressure at the temperature of bath B.

The transpiration rate per unit leaf surface \( E \) is then calculated from

\[ E = \frac{c \times F}{S} \, \text{mg H}_2\text{O/cm}^2/\text{min}, \quad (17) \]

or

\[ E = \frac{c \times F}{S} \left( \frac{1}{0.06} \right) \, \mu\text{g H}_2\text{O/cm}^2/\text{s}, \quad (18) \]

or

\[ E = \frac{c \times F}{S} (6) \, \text{g H}_2\text{O/dm}^2/\text{hr}, \quad (19) \]

where \( S \) (cm\(^2\)) is the area of the leaf and \( F \) (liters/min) is the flow rate across the leaf chamber as measured with flow meter \( F_1 \).

Both thermocouples in the psychrometer and the one used for measuring the leaf temperature were calibrated with a quartz thermometer.

The conversion factor for the psychrometer was 39.94 \( \mu \)v per C.

Photosynthesis Rates

The rate of net photosynthesis was obtained by determining the difference in CO\(_2\) concentration \((\Delta \text{CO}_2)\) between the leaf chamber and reference air streams with a Beckman 215 infrared gas analyzer (IRGA).
The air streams to be analyzed were first dried because water molecules absorb infrared light and thus interfere with the measurement. They were then passed through two flow meters to maintain the same flow rate to each detecting cell. Following the IRGA the air was exhausted outside the room.

The photosynthesis rate was calculated using the equation

\[
P_n = \Delta CO_2 \times F \times 10.69/S \text{ mg CO}_2/\text{dm}^2/\text{hr},
\]  \hspace{1cm} (20)

or

\[
P_n = \Delta CO_2 \times F \times 10.69/360 \text{ S} \text{ \mu g CO}_2/\text{cm}^2/\text{s},
\]  \hspace{1cm} (21)

where \( \Delta CO_2 \) is the concentration difference (ppm), \( F \) is the flow rate measured with flow meter \( F_1 \) (l/min) and \( S \) is the leaf area (cm\(^2\)). The factor 10.69 accounted for the conversion from \( \mu l CO_2/cm^2/min \) at 28 C to \( \text{mg CO}_2/\text{dm}^2/\text{hr} \) at standard temperature and pressure (S.T.P.).

The IRGA was used in the differential mode. Originally, the IRGA was calibrated with a standard \( CO_2 \) mixture containing 290 ppm as the baseline and 370 ppm as the upper level. Then the \( CO_2 \) concentration of the sample air was measured using the reference ambient air stream as the baseline. This procedure was found to underestimate the actual values because most of the time the \( CO_2 \) concentration in the reference ambient air was higher than 290 ppm. The reference and sample air \( CO_2 \) concentrations were therefore measured.
separately by manipulating 4-way switching valves V₁₀, V₁₁ and exhaust valves V₈, and V₉. This procedure had the advantage of also producing a measurement of the CO₂ concentration of the reference air which was needed to calculate leaf diffusion resistances. Both measurements were made using the standard 290 ppm CO₂ mixture as a baseline. Each measurement consisted of obtaining the average of a continuously recorded concentration for 5 to 10 minutes. The differences between reference and sample air streams were used to calculate net photosynthesis. The usual range of CO₂ concentration encountered in the measurement was from 300 to 360 ppm. Over the narrow concentration range of 290 to 370 ppm the analyzer output was linear and no further calibration gas was required.

**Boundary Layer Resistances**

Determination of the boundary layer resistance \( r_a \) is required for the calculation of leaf resistances. The energy balance method was chosen for the measurement of boundary layer resistances.

The boundary layer resistance \( r_a \) of leaves can be calculated from a change in the transpiration rate resulting from a change in relative humidity of the ambient air (Jarvis, 1971). Under conditions of constant irradiance and ventilation, the transpiration rate of plants can be changed by varying the ambient water vapor
pressure or carbon dioxide concentration. Since the leaf chamber was supplied with air drawn from the outside which had a relatively constant CO₂ concentration, it was more appropriate to change the water vapor pressure by increasing or decreasing the temperature of the air. This could be accomplished by changing the temperature of bath B₁.

The energy balance of a leaf in an assimilation chamber with constant irradiance and ventilation at two rates of transpiration $E_1$ and $E_2$ can be written as follows:

$$Q_s + Q + 2\varepsilon\sigma T_1^4 = LE_1 + (T_1 - T_a)c_p \rho_a / r_a \quad (W/m^2) \quad (22)$$

and

$$Q_s + Q + 2\varepsilon\sigma T_2^4 = LE_2 + (T_2 - T_a)c_p \rho_a / r_a \quad (W/m^2), \quad (23)$$

where

- $Q_s =$ net short wave irradiance ($W/m^2$),
- $Q =$ thermal radiation influx from the environment ($W/m^2$),
- $E =$ transpiration rate ($kg/m^2/s$),
- $r_a =$ leaf boundary layer resistance ($s/m$),
- $T = \frac{1}{2}(T_1 + T_2) \quad (K)$,
- $T_1, 2 =$ leaf temperature ($K$),
- $T_a =$ air temperature ($K$),
- $\varepsilon =$ thermal radiation emittance of the leaf (about 1),
\[ \sigma = \text{Stefan-Boltzmann constant} \left( 5.67 \times 10^{-7} \text{ J/m}^2/\text{K}^4/\text{s} \right), \]

\[ L = \text{latent heat of vaporization} \left( 2.438 \times 10^6 \text{ J/kg (28 C)} \right), \]

\[ c_p = \text{specific heat of air} \left( 1013 \text{ J/kg/K} \right), \]

\[ \rho_a = \text{density of moist air} \left( 1.20 \text{ kg/m}^3 \right). \]

Subtraction of the two equations yields:

\[ 2\sigma (T_1^4 - T_2^4) + L(E_1 - E_2) + (T_1 - T_2)c_p \rho_a / \rho_a = 0. \quad (24) \]

Since,

\[ 2\sigma (T_1^4 - T_2^4) \approx \epsilon \sigma 8 T^3 (T_1 - T_2) \quad (25) \]

where \( T \) is the mean of \( T_1 \) and \( T_2 \). Equation (24) reduces to

\[ \rho_a = -\left[ \frac{c_p \rho_a (T_1 - T_2)}{\epsilon \sigma 8 T^3 (T_1 - T_2) + L(E_1 - E_2)} \right]. \quad (26) \]

The leaf resistance \( \rho_a \) can then be calculated by measuring \( E_1 \) and \( T_1 \) followed by the measurement of \( E_2 \) and \( T_2 \) at a new relative humidity in the chamber.

One day prior to the measurement, soybean plants were placed under the xenon lamp to allow acclimatization to the leaf chamber conditions. The temperature of the room was about 28 C and the humidity was about 65 percent. The temperatures of the water baths \( B_1 \) and \( B_2 \) were adjusted the following morning to attain the required air
temperature and water vapor concentration. The air flow system and measuring devices were initially calibrated without a leaf in the chamber. For these conditions the differential psychrometer could be considered as two separate thermocouple-psychrometers in the same air stream and they should give identical results. Any difference between the two psychrometers was applied as a correction factor. The experiment was initiated only when steady state conditions for the air flow rate, air temperature, and water vapor concentration were achieved. In addition the criterion was used that the output of the differential psychrometer should be constant and the difference should be less than 10 microvolt. Usually this condition was achieved in about one hour. When these conditions were established the first trifoliate leaf was inserted into the chamber. A T-type thermocouple was pressed against the lower surface of this leaflet to measure its temperature. The light intensity on the leaf surface was about 310 W/m$^2$ (2960 ft-c), the air flow rate through the chamber was maintained at 2.75 liters/min and the relative humidity of the air stream was 66%. A steady transpiration rate was usually attained in about one hour. The time when the leaf first reached a steady transpiration rate did not usually coincide with the time of attaining a constant leaf temperature. Transpiration rate measurements were started after the leaf temperature became constant. After these measurements were completed the relative humidity was lowered
to 46 percent by lowering the temperature of water bath $B_1$ and measurements were repeated. Afterwards, the leaf lengths and leaf surface areas were measured. The chamber was once again closed to obtain a measurement of the correction factor for the differential psychrometer at the new humidity of the air stream.

Leaf temperature enters the calculation of the boundary layer resistances (Equation 26). The leaf-temperature measurement requires a precision of $\pm 0.1 \, ^\circ C$.

**Compensation Points**

The compensation point was determined using the closed system as a modification of the normally used open system. The humidifier which normally received the outside air was switched by a three-way valve to connect to a small pump $P_3$. The air stream was still maintained saturated by passage through the water in the humidifier. The humidity was adjusted by passage of the air through the copper coil in water bath $B_2$. The air stream, which in the open mode would be split into a reference stream and a leaf chamber air stream, was now passed entirely to the leaf chamber by completely closing valve $V_4$. The air coming through the chamber was dried before passing to the IRGA. After the concentration of $CO_2$ was obtained by the IRGA, the air stream was passed through a connecting tube between pump $P_3$ and the IRGA exhaust tube to be recirculated.
The air was recirculated in the system until the CO$_2$ concentration reached a constant level.

The IRGA was calibrated to read in the range of 0-300 ppm. In this range the analyzer output is not linear. The reading was taken from a calibration curve, with the full span corresponding to a CO$_2$ concentration range of 0 to 600 ppm.

The flow rate through the chamber was 0.6 liters/min. When the leaves of the plant were slightly bigger than normal this flow rate was not sufficient. Water vapor from the leaves accumulated inside the chamber on the walls of the water-jacket. To avoid such difficulty, the flow rate was increased.

**Ozone Losses in the Chamber**

Ozone breaks down when it comes in contact with rough, dirty surfaces. Passage through the assimilation chamber which was made of plexiglass lowered the concentration of ozone in the ambient air before it reacted with plants. The rate of loss was obtained by measuring ozone concentrations just before entering and after leaving the empty chamber with environmental conditions identical to those of the experiments to be conducted. The losses were measured for several initial ozone concentrations.
Ozone Losses at the Leaf Surfaces

Ozone loss on a leaf surface occurs as a result of the dissociation of ozone on impact with the leaf surface and as a result of the absorption of ozone in the leaf structure. If the stomates were manipulated to close tightly then the total loss of ozone at the leaf surface would be strictly due to impact with the leaf surface. Stomatal closure was obtained by containing the leaf in complete darkness. Measurements of ozone loss were made with the outside of the leaf chamber wrapped in black paper held in place with black polyethylene tape to prevent intruding light. Experimental procedures were the same as for other experiments. The leaf was put in its place in the chamber with the lid open and kept in darkness for one day and one night. The following day, the chamber was closed. Since the plant did not receive any light, only the transpiration rate was measured to ensure that the stomates were closed. The ozone was not administered until one hour was allowed for the leaf to equilibrate. Then the ozone concentration of the efflux air was measured during a four hour period.

Experimental Procedure

The soybean plant was placed under the xenon light one day before initiating the treatment. On the morning of the experiment,
the plant was illuminated for about one hour. During this period the air flow and measurement system were calibrated. The temperatures of water baths $B_1$ and $B_2$ were adjusted so that the air entered the chamber at a temperature of $28 \, ^\circ C$ with a relative humidity of 65 percent.

The light intensity at the surface of the leaf in the chamber was kept constant during the experimental period at $310 \, W/m^2$.

The IRGA was calibrated in the range of 0 to 290 ppm. After the system was calibrated, the first trifoliate leaf was placed in the chamber. The petiole of the first trifoliate leaf of 11 day old seedlings was too short for passing the chamber wall. This difficulty was overcome by excision of other younger leaves. The cut surfaces were sealed with a 90 percent lanolin-10 percent bees-wax mixture to prevent water loss. This material is not toxic to the plants.

First the $CO_2$ compensation point of the leaf was determined using the system in a closed mode. The carbon-dioxide concentration usually decreased to a constant value within about one to two hours. Then the leaf was taken out and the system was changed to the open-mode of operation. The IRGA was recalibrated for the carbon-dioxide concentration range encountered in the determination of photosynthesis rates, namely 290 to 370 ppm. The differential psychrometer was calibrated and its correction factor determined. The ozone analyzer was calibrated for the measurement of ozone concentrations of the air
entering and leaving the chamber. After these calibrations were completed, the leaf was returned to the chamber and allowed to equilibrate for one hour. When equilibrium conditions were reached, measurements of initial rates of transpiration and photosynthesis were obtained. Then the ozone generator was turned on and the ozone concentration of the outflow air from the chamber was recorded. Starting from this time measurements of transpiration and photosynthesis rates and ozone concentration were obtained at half hour intervals over a four hour period.

After four hours, the ozone generator was shut off and ozone free air was allowed to pass through the chamber until ozone could no longer be detected. The system was then changed again to a closed mode to determine the CO$_2$-compensation point. Finally, the leaf length and leaf area were measured.

Calculations for Diffusion Resistances

Diffusion resistances are calculated according to methods described by Gaastra (1959). For the calculation of the mesophyll resistance the CO$_2$ concentration at the site of fixation, $[\text{CO}_2]_{\text{chl}}$, is assumed equal to the CO$_2$ compensation point $\Gamma$. In some leaves, the value of $\Gamma$ increased after exposure to ozone. Therefore, $\Gamma$ was measured at the beginning and at the end of each experiment. Intermediate values were obtained by linear interpolation between
these two points. Since the compensation point reflects a change in rate of photosynthesis, the interpolation should be in proportion to changes in \( P_n \). For example, \( \Gamma \) at the beginning of an experiment might be 40 ppm and at the end 60 ppm with corresponding photosynthetic rates of 100 percent and 50 percent respectively. Thus there was a 50 percent change in \( P_n \) with respect to a change of 20 ppm in \( \Gamma \). If after one hour, the net photosynthesis rate was 90 percent, the corresponding \( \Gamma \) change should be \((100-90)\left(\frac{20}{50}\right) = 4\) ppm so that \( \Gamma = 44 \) ppm.

Total resistance to diffusion of \( CO_2 \) in s/cm is calculated using the equation

\[
\Sigma r_{CO_2} = \frac{([CO_2]_a-[CO_2]_{chl})(0.001782)}{P_n},
\]

(27)

where \([CO_2]_{chl}\) is the \( CO_2 \) concentration at the compensation point and \([CO_2]_a\) is the average concentration of the influx and efflux air (ppm), \( P_n \) is the net photosynthesis rate of the leaf (\( \mu g/cm^2/s \)), and 0.001782 is the conversion constant from ppm to \( \mu g/cm^3 \).

The total resistance to water vapor transfer by the leaf is calculated using the transpiration rate as follows:

\[
\Sigma r_{H_2O} = \frac{[H_2O]_t-[H_2O]_a}{Tr},
\]

(28)
where $Tr$ is the transpiration rate ($\mu g/cm^2/sec$), $[H_2O]_t$ is the water vapor density at the temperature of the leaf assuming the air within the leaf to be saturated, and $[H_2O]_a$ is the concentration of water vapor in the ambient air taking as an average between those of influx and efflux air. The value of $[H_2O]_a$ is obtained from the equation:

$$[H_2O]_a = \frac{1}{2} \rho \left( \frac{e_{wr} + e_{ws}}{e_s} \right)$$

(29)

where $\rho$ and $e_s$ are as defined for Equation (16) and $e_{wr}, e_{ws}$ are as defined for Equation (15).

The boundary layer resistance $r_a$ was measured separately using the energy balance method. Subtracting $r_a$ from the total resistance $\Sigma r_{H_2O}$ yields stomatal resistance $r_s$ to water vapor transfer according to the equation

$$\Sigma r_{H_2O} = (r_s + r_a)_{H_2O}$$

(30)

Diffusion resistances to CO$_2$ transfer are calculated from the values obtained for water vapor as

$$r_{CO_2} = r_{H_2O} \left( \frac{D_{H_2O}}{D_{CO_2}} \right)$$

(31)
A value of 1.60 was used for the ratio $D_{H_2O}/D_{CO_2}$ (Fuller, Schettler and Giddings, 1966). The mesophyll resistances $r_m$ are obtained as the difference

$$r_m = \Sigma r_{CO_2} - \Sigma r_{H_2O} \left( \frac{D_{H_2O}}{D_{CO_2}} \right).$$

(32)
RESULTS AND DISCUSSION

Growth Characteristics

Measured leaf lengths and calculated plastochron indices of the plants are summarized in Tables 4 and 5. Each data point is the mean of 40 measurements, each on a different plant. The relationships between the length of the first trifoliate leaf and plant age are shown in Figure 3. Figure 4 shows the plastochron index as a function of plant age. Based on these results, it was decided to use the 14 day old plants as representative of the expanding phase. The size of the leaf at this age is big enough and the petiole is sufficiently long to put the entire trifoliate leaf inside the leaf chamber. The 20 day old leaf was chosen to represent the condition where the rate of expansion was greatly decreased or stopped. The leaf areas were not measured.

The leaves of the Dare cultivars are longer, but thinner and have bigger leaf surface areas at the same chronological age. The Hood cultivar has slightly higher plastochron index values at the same chronological age, indicating that this cultivar matures more rapidly than the Dare cultivar. However, at both ages of 14 and 20 days, the corresponding plastochron indices are not significantly different between the two cultivars. It was assumed that they were at the same developmental stage. Because the leaf plastochron index is a linear function of leaf age and has about the same value for both cultivars,
Table 4. Leaf lengths in millimeters of the Dare cultivar ranging in age from 8 to 25 days since planting.

<table>
<thead>
<tr>
<th>Plant age</th>
<th>Leaf serial number</th>
<th>Plastochron index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>23.8 ± 4.4</td>
<td>6.7 ± 1.6</td>
</tr>
<tr>
<td>10</td>
<td>45.0 ± 7.6</td>
<td>11.5 ± 3.0</td>
</tr>
<tr>
<td>11</td>
<td>74.7 ± 9.6</td>
<td>20.7 ± 5.3</td>
</tr>
<tr>
<td>12</td>
<td>103.5 ± 8.5</td>
<td>36.8 ± 9.4</td>
</tr>
<tr>
<td>13</td>
<td>127.6 ± 10.4</td>
<td>60.8 ± 15.7</td>
</tr>
<tr>
<td>14</td>
<td>147.3 ± 10.8</td>
<td>92.3 ± 19.8</td>
</tr>
<tr>
<td>15</td>
<td>161.8 ± 10.7</td>
<td>129.2 ± 20.9</td>
</tr>
<tr>
<td>16</td>
<td>173.3 ± 11.1</td>
<td>166.8 ± 18.4</td>
</tr>
<tr>
<td>17</td>
<td>180.9 ± 11.2</td>
<td>188.3 ± 15.5</td>
</tr>
<tr>
<td>18</td>
<td>185.5 ± 10.6</td>
<td>200.6 ± 13.0</td>
</tr>
<tr>
<td>19</td>
<td>187.4 ± 10.9</td>
<td>207.9 ± 11.5</td>
</tr>
<tr>
<td>20</td>
<td>190.5 ± 11.9</td>
<td>217.3 ± 12.4</td>
</tr>
<tr>
<td>21</td>
<td>191.4 ± 10.6</td>
<td>220.8 ± 13.1</td>
</tr>
<tr>
<td>22</td>
<td>190.7 ± 11.9</td>
<td>222.1 ± 14.4</td>
</tr>
<tr>
<td>23</td>
<td>191.7 ± 11.3</td>
<td>223.7 ± 14.4</td>
</tr>
<tr>
<td>24</td>
<td>191.6 ± 11.5</td>
<td>224.8 ± 14.5</td>
</tr>
<tr>
<td>25</td>
<td>191.6 ± 11.5</td>
<td>224.7 ± 14.8</td>
</tr>
<tr>
<td></td>
<td>12.2</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Leaf lengths in millimeters of the Hood cultivar ranging in age from 8 to 25 days since planting.

<table>
<thead>
<tr>
<th>Plant age (days)</th>
<th>Leaf serial number</th>
<th>Plastochron index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>12.5 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>22.1 ± 4.1</td>
<td>9.7 ± 1.6</td>
</tr>
<tr>
<td>10</td>
<td>37.4 ± 6.6</td>
<td>14.7 ± 4.0</td>
</tr>
<tr>
<td>11</td>
<td>59.3 ± 8.9</td>
<td>25.8 ± 7.2</td>
</tr>
<tr>
<td>12</td>
<td>81.7 ± 10.5</td>
<td>42.5 ± 11.6</td>
</tr>
<tr>
<td>13</td>
<td>102.8 ± 12.5</td>
<td>65.3 ± 15.6</td>
</tr>
<tr>
<td>14</td>
<td>119.8 ± 13.5</td>
<td>90.7 ± 18.4</td>
</tr>
<tr>
<td>15</td>
<td>130.0 ± 14.4</td>
<td>119.3 ± 20.1</td>
</tr>
<tr>
<td>16</td>
<td>134.7 ± 14.2</td>
<td>138.0 ± 18.4</td>
</tr>
<tr>
<td>17</td>
<td>137.7 ± 14.3</td>
<td>150.1 ± 16.6</td>
</tr>
<tr>
<td>18</td>
<td>138.6 ± 14.6</td>
<td>156.8 ± 16.1</td>
</tr>
<tr>
<td>19</td>
<td>137.6 ± 13.7</td>
<td>159.5 ± 14.9</td>
</tr>
<tr>
<td>20</td>
<td>136.6 ± 12.9</td>
<td>159.8 ± 14.9</td>
</tr>
<tr>
<td>21</td>
<td>137.0 ± 12.7</td>
<td>160.9 ± 14.7</td>
</tr>
<tr>
<td>22</td>
<td>137.1 ± 12.7</td>
<td>161.7 ± 14.6</td>
</tr>
<tr>
<td>23</td>
<td>137.1 ± 12.7</td>
<td>162.5 ± 14.3</td>
</tr>
<tr>
<td>24</td>
<td>137.1 ± 12.7</td>
<td>162.7 ± 14.2</td>
</tr>
<tr>
<td>25</td>
<td>137.1 ± 12.7</td>
<td>162.9 ± 14.1</td>
</tr>
</tbody>
</table>
Figure 3. Length of first trifoliate leaf as a function of days since planting for Dare and Hood soybean cultivars. (Each data point is the average of 40 measurements.)
Figure 4. Plastochron index as a function of days since planting for Dare and Hood soybean cultivars.
the leaf chronological age is then used to represent the developmental stage of leaf.

**Boundary Layer Resistances**

The leaf boundary layer is a thin layer of still air adjacent to the leaf surface, including a transition region to the fully turbulent conditions of the ambient air. Gaseous transfer across this layer is by diffusion. The thickness of such a layer is a function of windspeed and the length of the leaf in the direction parallel to the bulk air flow. The boundary layer diffusion resistance is in series with stomatal resistance and other resistances which are under physiological control. In order to observe physiological responses of leaves to imposed stress conditions with more sensitivity, it is advantageous to maintain the boundary layer resistance small in relation to total leaf resistance. This can be achieved in a leaf chamber by using an internal fan to assure that the air is well stirred.

The magnitude of the boundary layer resistance $r_a$ can be measured in several ways (Jarvis, 1971). The most widely used method is by determining the rate of water loss from leaf replicas made of blotting paper. This method has been shown to overestimate the boundary layer resistance, however. Difficulties often encountered are that either the leaf replica is too wet or that the water is lost before the measurement of the evaporation rate and
temperature can be completed. The energy balance method (Jarvis, 1971) was considered to be the most appropriate for the conditions employed in this investigation. A desirable aspect of this method is that the actual leaf itself is used. As mentioned earlier, the diffusion resistance is a function of leaf dimension and leaf surface characteristics. The real leaf should give a more accurate estimation than a replica.

Results of the boundary layer resistance measurements are summarized in Tables 6 and 7. Each data point represents the measurement of one sample. Since the resistances showed a correlation with leaf areas rather than leaf ages or plastochron indices, the replication of measurements was based on leaves of about the same size. The linear correlations between the $r_a$ values and leaf surface areas of both soybean cultivars are shown in Figure 5. The correlation coefficients are highly significant. It should be noted that although the Hood cultivar has smaller leaves than the Dare cultivar, it has a greater boundary layer resistance. This is probably due to the texture of the leaf surface.

General observation on leaf formation shows that the leaves of the Hood cultivar are thicker and greener and that the surface is rougher than the Dare cultivar. Undoubtedly, the occurrence of trichomes (hairs) on the leaf surface increase the thickness of the boundary layer. Apart from the trichomes, electron micrographs of
Table 6. Boundary layer resistances ($r_a$) of the first trifoliate leaf of the Dare cultivar as a function of leaf area.

<table>
<thead>
<tr>
<th>Age days</th>
<th>P.I.</th>
<th>Leaf area $^2$ cm</th>
<th>$r_a$ sec/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.75</td>
<td>14.39</td>
<td>0.02</td>
</tr>
<tr>
<td>11</td>
<td>1.76</td>
<td>21.31</td>
<td>0.07</td>
</tr>
<tr>
<td>12</td>
<td>2.26</td>
<td>32.29</td>
<td>0.13</td>
</tr>
<tr>
<td>16</td>
<td>3.38</td>
<td>33.43</td>
<td>0.22</td>
</tr>
<tr>
<td>14</td>
<td>2.97</td>
<td>35.89</td>
<td>0.15</td>
</tr>
<tr>
<td>12</td>
<td>2.14</td>
<td>39.59</td>
<td>0.24</td>
</tr>
<tr>
<td>14</td>
<td>2.79</td>
<td>43.09</td>
<td>0.24</td>
</tr>
<tr>
<td>19</td>
<td>4.15</td>
<td>47.07</td>
<td>0.27</td>
</tr>
<tr>
<td>16</td>
<td>3.32</td>
<td>54.64</td>
<td>0.28</td>
</tr>
</tbody>
</table>

$r_a = -0.0656 + 0.00686 \text{ (leaf area)} \ (r = 0.942)^{**}$

Table 7. Boundary layer resistances ($r_a$) of the first trifoliate leaf of the Hood cultivar as a function of leaf area.

<table>
<thead>
<tr>
<th>Age days</th>
<th>P.I.</th>
<th>Leaf area $^2$ cm</th>
<th>$r_a$ sec/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>2.09</td>
<td>9.47</td>
<td>0.03</td>
</tr>
<tr>
<td>12</td>
<td>1.88</td>
<td>23.49</td>
<td>0.15</td>
</tr>
<tr>
<td>12</td>
<td>2.09</td>
<td>23.77</td>
<td>0.16</td>
</tr>
<tr>
<td>19</td>
<td>4.08</td>
<td>33.33</td>
<td>0.31</td>
</tr>
<tr>
<td>14</td>
<td>2.65</td>
<td>33.90</td>
<td>0.29</td>
</tr>
<tr>
<td>16</td>
<td>3.91</td>
<td>54.26</td>
<td>0.33</td>
</tr>
<tr>
<td>17</td>
<td>3.96</td>
<td>59.00</td>
<td>0.36</td>
</tr>
</tbody>
</table>

$r_a = 0.0182 + 0.00634 \text{ (leaf area)} \ (r = 0.916)^{**}$
Figure 5. Boundary layer resistances of the Dare and Hood soybean cultivars as a function of leaf areas.
the leaf surfaces revealed conspicuous deposits of wax on the surfaces of the cuticle. These wax bodies may also determine in part boundary layer thickness. Magnified pictures of stomates and vicinity as shown in Figures 6 and 7. The most remarkable distinction between leaf age and plant cultivar is the difference in distribution of crystalline wax structures. The density is always higher on the youngest leaves. The wax bodies become sparse as the leaves mature and expand. The Hood cultivar had more wax deposits than the Dare cultivar. Figures 8 and 9 show a lower magnification indicating the differences in degree of wax covering. The 14 day-old leaf of the Hood cultivar had particularly dense deposits of surface wax.

The difference in wax density between cultivars is pronounced in the young leaves. For a leaf of small area, the covering with wax bodies on the epidermal cells which lie close to each other could be, in addition to the trichomes, obstructive to the flow of air over the leaf surface. As the leaf expands, however, size of the leaf and trichome density play a greater part in the determination of $r_a$. The importance of wax density becomes less prominent. The roles played by leaf size, density of trichomes, and wax deposits might explain the observed features of the two lines in Figure 5 where the values of $r_a$ show a greater difference between cultivars for small leaves. This difference decreases as the leaf size increases.
Figure 6. Electron micrographs of leaf surfaces (Dare cultivar) showing stomates and depositions of wax materials (X5000).
Figure 7. Electron micrographs of leaf surfaces (Hood cultivar) showing stomates and depositions of wax materials (X5000).
Figure 8. Electron micrographs of leaf surfaces (Dare cultivar) showing trichomes and epidermal cells with wax bodies (X400).
Figure 9. Electron micrographs of leaf surfaces (Hood cultivar) showing trichomes and epidermal cells with wax bodies (X400).
The carbon dioxide compensation points ($\Gamma$) of leaves vary with species, physiological conditions, environmental history, and current environment. The magnitude of $\Gamma$ has sometimes been used as an indication of the rate of photorespiration (Meidner, 1967) and it is often explicitly stated that a close relationship between the two exists (Heath and Orchard, 1968). Gabrielsen (1948) regarded the CO$_2$ compensation point as a threshold below which no assimilation occurs or the CO$_2$ concentration at which there is a balance between rates of photosynthesis and respiration. Plants which fix CO$_2$ via the C$_4$-dicarboxylic acid pathway have low CO$_2$ compensation points, while plants which use the C$_3$ pathway have high compensation points.

The compensation points of the cultivars used in these experiments were quite high confirming that soybean plants fix CO$_2$ via the Calvin cycle. The values were very high and inconsistent for the young plants as shown in Table 8 and Figure 10. The CO$_2$ compensation points rapidly decrease with plant age and reached a constant value after about 13 days. The compensation points of fully expanded leaves were found to be about 45 ppm. This is in good agreement with Curtis, Ogren and Hageman (1969) who reported an average value of 40 ppm for 36 varieties of soybeans. Dornhoff and
Shibles (1970) reported the same value for 20 varieties of soybeans studied by them. The compensation points of the Hood and Dare cultivars are about the same.

Table 8. Compensation points of Dare and Hood soybean cultivars as a function of days since planting.

<table>
<thead>
<tr>
<th>Age</th>
<th>CO₂ compensation points (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hood</td>
</tr>
<tr>
<td>days</td>
<td>- - - - - - - - - ppm - - - - - - - - - - -</td>
</tr>
<tr>
<td>10</td>
<td>114 ± 13.5*</td>
</tr>
<tr>
<td>11</td>
<td>56 ± 5.0</td>
</tr>
<tr>
<td>12</td>
<td>59 ± 13.4</td>
</tr>
<tr>
<td>14</td>
<td>47 ± 7.5</td>
</tr>
<tr>
<td>15</td>
<td>48 ± 10.0</td>
</tr>
<tr>
<td>20</td>
<td>44 ± 3.8</td>
</tr>
</tbody>
</table>

* X ± σ.

The CO₂ compensation points were also measured before and after ozone exposure. Table 9 summarizes the results. The compensation points did not change until the ozone concentrations reached about 40 pphm. Figure 11 shows that the change in CO₂ compensation point progressed with increasing ozone concentrations. The compensation points of young leaves of both cultivars were less effected by ozone than those of the old leaves. The greatest increase occurred in the mature leaves of the sensitive cultivar, i.e., Dare. The increases in CO₂ compensation point indicate that ozone damage changed the balance between rates of photosynthesis and respiration.
Figure 10. Compensation points of Dare and Hood soybean cultivars as a function of days since planting.
The initiation of an increase in compensation points corresponded with the subsequent appearance of necrotic patches on the leaf, inferring that cells had been damaged.

Table 9. CO₂ compensation points (Γ) before and after exposure to ozone for Dare and Hood cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant age</th>
<th>Ozone concentration</th>
<th>CO₂ concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Dare</td>
<td>14</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25</td>
<td>48</td>
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<td></td>
<td></td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>45</td>
</tr>
<tr>
<td>Hood</td>
<td>14</td>
<td>25</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>48</td>
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<td></td>
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<td>34</td>
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<td>50</td>
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<td>58</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>50</td>
</tr>
</tbody>
</table>
Figure 11. CO$_2$ compensation points after four hours of exposure to several ozone concentrations.
Transpiration and Photosynthesis Rates

Control Plants

Measurements of the transpiration and photosynthesis rates of control plants were initiated following an equilibration period of 1 hour in the leaf chamber. The data from differential psychrometer and IRGA are continuous. Subsequent calculations of $T_r$ and $P_n$ rates were made at one hour intervals or at times 1, 2, 3, and 4 hrs after initial measurement (Table 10). The rates of $T_r$ and $P_n$ were then calculated as percentages of the rate measured at time $t = 0$. It was decided that a comparison of relative rates would provide the best evaluation of treatment effects.

Both the transpiration rate and the rate of photosynthesis of the control plants increased during the four hour experimental period (Table 10). The increases were attributed to response of the leaf to the new environmental conditions when put in the closed cuvette. The leaves received more radiant energy in the position directly under the lamp and the fan caused air turbulence near the leaf surface. Both conditions favor a decrease in total resistances and induce opening of stomates. The younger leaves of both cultivars showed greater increases in $T_r$ and $P_n$ than the older ones. The increases in transpiration rates were more than 30 percent for the 14 day old plants but only about 10 percent for the 20 day old plants. The increase in
Table 10. Measured rates of transpiration and photosynthesis of control plants and the rates as percentages of the initial rates at $t = 0$.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant age</th>
<th>Elapsed time</th>
<th>Measured rates</th>
<th>Relative rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>hr</td>
<td>$\text{Tr} / \text{g/dm}^2 \text{/hr}$</td>
<td>$\text{Pn} \text{mg/dm}^2 \text{/hr}$</td>
</tr>
<tr>
<td>Dare</td>
<td>14</td>
<td>0</td>
<td>2.030</td>
<td>20.310</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>2.233</td>
<td>20.310</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>2.472</td>
<td>20.859</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>2.610</td>
<td>20.859</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>2.679</td>
<td>21.133</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0</td>
<td>1.998</td>
<td>17.737</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>2.121</td>
<td>18.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>2.203</td>
<td>18.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>2.265</td>
<td>18.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>2.254</td>
<td>18.048</td>
</tr>
<tr>
<td>Hood</td>
<td>14</td>
<td>0</td>
<td>1.848</td>
<td>17.201</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>2.106</td>
<td>18.243</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>2.368</td>
<td>19.286</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>2.533</td>
<td>19.286</td>
</tr>
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<td>2.566</td>
<td>19.286</td>
</tr>
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<td></td>
<td>20</td>
<td>0</td>
<td>2.576</td>
<td>23.683</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>2.677</td>
<td>23.683</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td>2.727</td>
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<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>2.777</td>
<td>23.683</td>
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<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>2.777</td>
<td>23.683</td>
</tr>
</tbody>
</table>
rate of photosynthesis was small for the 14 day old plants and zero for the 20 day old plants. The differences resulted from the greater decrease in leaf resistance of the younger leaf. The rate of transpiration can be enhanced considerably by the opening of stomates. The net photosynthesis also increases when the stomatal resistance decreases, but to a lesser degree because the mesophyll resistance remains constant.

Exposed Plants

Transpiration and photosynthesis rates of the exposed plants are shown in Table 11. Also shown are the ratios \( \frac{Tr}{Pn} \) calculated by using the measured rates, and the rates of \( Tr \) and \( Pn \) as a percentage of the initial rate at time \( t = 0 \).

To express treatment effects clearly the data needed further manipulation. It was necessary to compare transpiration and photosynthesis rates of treated and control plants. The transpiration rate of the control plants increased during the 4-hour period of measurement. The transpiration rate of the ozone exposed plants decreased during the measurement period. The treatment effect was quantified by calculating relative rates with respect to the control rates. For example, assume that during the first hour the transpiration rates of the control plant and exposed plant changed from 100 to 110 percent and from 100 to 90 percent respectively. Then the relative rate of
transpiration of the exposed plant with respect to the control plant was

\[
(\frac{90}{110} \times 100) = 81.8
\]  

and the treatment decreased the transpiration rate 18.2 percent. These calculations were made for all transpiration and photosynthesis measurements and for all calculated values of diffusion resistances.

Rates of \(T_r\) and \(P_n\) of ozone exposed plants as percentages of control plant rates are shown in Figures 12, 13, 14, and 15. The results show a definite trend of the ozone exposure and exposure duration effects. This trend was consistent for both ages and both cultivars used in the experiments. The experiments were not replicated due to time limitations. The experiments are very time consuming. A degree of confidence in the data is provided by the consistent trend of the data.

At low ozone concentrations both transpiration rate and rate of photosynthesis continued to increase during the experimental period, although at a slower rate than the control plants (Table 11). The younger plants were more severely affected than the older plants as indicated by the relative rates. Low concentrations of ozone caused small decreases in rates of transpiration and photosynthesis. The rate of decrease remained constant during the four hour exposure period. Concentration of ozone above 30 ppm caused an initial rapid
Table 11. Measured rates of transpiration (Tr) and net photosynthesis (Pn), ratio Tr/Pn. Tr and Pn as a percentage of the initial rates at t = 0 and Tr and Pn as a percentage of corresponding rates of control plants.

<table>
<thead>
<tr>
<th>Plant age</th>
<th>Ozone conc.</th>
<th>Elapsed time</th>
<th>Measured rates</th>
<th>Rates as percentage of initial rate at t = 0</th>
<th>Rates as percentage of corresponding control rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>pphm (v/v)</td>
<td>Tr/hr gH₂O/dm²</td>
<td>Tr/Pn %</td>
<td>Pn/hr mg CO₂/dm²</td>
</tr>
<tr>
<td>Dare cultivar</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>2.665</td>
<td>22.330</td>
<td>119</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.718</td>
<td>22.653</td>
<td>120</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2.927</td>
<td>23.301</td>
<td>126</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.035</td>
<td>24.272</td>
<td>125</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>2.982</td>
<td>24.272</td>
<td>123</td>
<td>112</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>2.111</td>
<td>22.293</td>
<td>95</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.210</td>
<td>22.880</td>
<td>97</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>2.350</td>
<td>23.760</td>
<td>99</td>
<td>111</td>
</tr>
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<td>3.0</td>
<td>2.391</td>
<td>23.466</td>
<td>102</td>
<td>113</td>
</tr>
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<td>4.0</td>
<td>2.033</td>
<td>20.826</td>
<td>98</td>
<td>96</td>
</tr>
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<td>34</td>
<td>0</td>
<td>3.044</td>
<td>22.432</td>
<td>136</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>3.044</td>
<td>22.432</td>
<td>136</td>
<td>100</td>
</tr>
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<td></td>
<td>2.0</td>
<td>2.820</td>
<td>21.413</td>
<td>132</td>
<td>93</td>
</tr>
<tr>
<td></td>
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Figure 12. Relative transpiration rates of the Dare cultivar as a function of time during four hours of ozone exposure at several ozone concentrations.
Figure 13. Relative transpiration rates of the Hood cultivar as a function of time during four hours of ozone exposure at several ozone concentrations.
Figure 14. Relative net photosynthesis rates of the Dare cultivar as a function of time during four hours of ozone exposure at several ozone concentrations.
Figure 15. Relative net photosynthesis rates of the Hood cultivar as a function of time during four hours of ozone exposure at several ozone concentrations.
decrease followed by a period of more gradual decline. Changes in the relative rates of $P_n$ were smaller than changes in relative transpiration rates (Table 11). But at high concentration, the photosynthesis rates decreased as much as transpiration rates. Carbon dioxide diffusion must overcome the mesophyll resistance in addition to the boundary layer resistance and the stomatal resistance. Decreasing stomatal width (increasing $r_s$) should have a greater relative effect on transpiration rate than on net photosynthesis rate. Consequently, a decrease in photosynthesis equivalent to or greater than transpiration indicates that the photosynthetic capacity must have been impaired apart from decreases due to the closure of stomates alone.

**Recovery from Ozone Damage**

The recovery of transpiration and photosynthesis was evaluated by measuring rates 24 hours after removal of the ozone treatment following a 4-hour exposure. These measurements were conducted only for plants exposed to ozone at concentrations of 40 and 58 ppmm. At lower concentrations, leaves showed no visible damage and were assumed to recover completely. The results (Table 11) clearly show that the 14-day old leaves recovered almost completely. The injury of the 20-day old leaves was more permanent. Yet, during the period of exposure, the rates of both $T_R$ and $P_n$ were reduced more in
the younger leaves than in the older ones. The data show that care must be exercised when judgments are made of pollutant induced damages at times after the termination of gaseous fumigation. Measurement at those times exclude the information on the immediate damage which occurred during the fumigation. The decrease in Tr and Pn during exposure, even though revealing no subsequent visible leaf injury, can lead to suppression of growth and reduced yields. Such damage has been recognized as "hidden" or "hold-back" damage.

The permanent damages in Tr and Pn are related to the ozone induced increase in CO₂ compensation point (Γ) of the leaves. The 20-day old leaf of the Dare cultivar which had the greatest increase in Γ (Figure 11) also had the largest decreases in both Tr and Pn. Conceivably, any damage which occurred to the photosynthetic apparatus could deprive the cell of energy required for synthesis of new compounds used for recovery. The 14-day old leaves, where Γ was not greatly affected, were able to recover almost completely despite a great decrease (~50%) in Tr and Pn during the ozone fumigating period.
Diffusion Resistances

Control Plants

The boundary layer resistance to water vapor transfer was calculated for each experiment using the relationship between $r_a$ and leaf area, $A$, established in earlier experiments (Tables 6 and 7). Using these data, the correlation equations

Hood: \[ r_a = 0.0182 + 0.00634A \text{ sec/cm} \] \hspace{1cm} (34)

and

Dare: \[ r_a = -0.0656 + 0.00686A \text{ sec/cm} \] \hspace{1cm} (35)

were obtained. It was assumed that these resistances remained constant during the period of measurement (Table 12).

The stomatal resistances to water vapor transfer for each measurement of transpiration rate were calculated using Equations (28) and (30). Actual values and values as a percentage of the initial rate at time $t = 0$ are shown in Table 12.

Mesophyll resistances were calculated using Equations (27) and (32). Results are shown in Table 12. The $r_m$ were also calculated as a percentage of initial values.

The stomatal resistance, $r_s$, of the control plants decreased during the experimental period. The decrease of stomatal resistance probably resulted from opening of stomates after the leaf was
Table 12. Calculated values of diffusion resistances of control plants and values of resistances as a percentage of values at time $t = 0$.

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subjected to the new environmental conditions of a higher light intensity and greater air flow rate inside the cuvette. Stomates are known to open in response to high light intensity. The young leaves had the greatest decrease in stomatal resistances. This could be due to the higher density of functioning stomates. Microscopic study showed that 14-day old leaves of Dare and Hood cultivars had about 50 to 60 percent more stomates per unit area than the 20-day old leaves. The younger leaves more actively absorb ions into the leaves to maintain high turgidity of cells and guard cells and are therefore able to respond more by changing the aperture of stomates. The high light intensity had more effect on stomatal resistance than on mesophyll resistance which remained constant throughout the experiment. This would be expected because the mesophyll resistance is made up of several components including carboxylation and photochemical reactions involved in the biochemical process of photosynthesis which require a longer period of time to respond to changes in environmental conditions.

Exposed Plants

Resistances of the exposed plants were calculated using the same methods as used for the control plants. In addition, the treatment effects were quantified by calculating relative rates with respect to the control rates. The procedure was the same as used for data
reduction of transpiration and photosynthesis measurements.

Absolute and relative values of the resistances of the exposed plants are shown in Table 13 and Figures 16 and 17. Exposure to ozone increased leaf resistances relative to the control plants. The relative change was greater for stomatal resistance than for mesophyll resistance. Clearly, stomatal response is more rapid than changes in enzymatic activities inside the mesophyll cells.

The effect on $r_s$ was about the same for both leaf ages of the Dare cultivar. This suggests that in this sensitive cultivar there is no difference in response of stomatal closure as a function of leaf maturity at high ozone concentrations. On the other hand the 20-day old leaves of the resistant cultivar, Hood, always had a smaller increase in $r_s$ than the 14-day old leaves.

At ozone concentrations lower than 30 pphm, the stomatal resistance increased while the mesophyll resistance remained unaffected. The effect on $r_m$ began to appear at exposures above 34 pphm and became significant at ozone concentrations greater than 40 pphm. It was also found that the increase in $r_m$ was greater in the 20-day old leaves than in the 14-day old ones. Specifically, the increase was greater in the sensitive cultivar (Dare), than in the resistant cultivar (Hood).
Table 13. Boundary layer resistances \((r_a)\), stomatal resistances \((r_s)\), and mesophyll resistances \((r_m)\). The resistances are also shown as percentages of the initial values and as percentages of the corresponding control plant values.

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Hood cultivar

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| 3.0 | 0.84 | 1.34 | 4.20 | 80 | 93 | 124 | 94 |
| 4.0 | 0.81 | 1.30 | 3.86 | 78 | 86 | 125 | 85 |

| 30 | 0 | 0.24 | 1.09 | 0.38 | 1.74 | 4.66 | 100 | 100 | 100 | 100 |
| 1.0 | 1.10 | 1.76 | 4.56 | 100 | 98 | 121 | 99 |
| 2.0 | 1.04 | 1.66 | 4.15 | 95 | 89 | 137 | 92 |
| 3.0 | 0.95 | 1.52 | 4.54 | 87 | 97 | 135 | 98 |
| 4.0 | 1.39 | 2.22 | 4.37 | 128 | 94 | 205 | 93 |

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| 2.0 | 1.30 | 2.08 | 3.14 | 110 | 80 | 158 | 82 |
| 3.0 | 2.00 | 3.20 | 2.80 | 171 | 72 | 265 | 73 |
| 4.0 | 2.14 | 3.42 | 2.78 | 182 | 71 | 292 | 70 |</p>
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Figure 16. Stomatal resistances (solid lines) and mesophyll resistances (broken lines) to CO₂ diffusion as a function of time during four hours of exposure to several ozone concentrations. Data shown are for the Dare cultivar at 14 and 20 days.
Figure 17. Stomatal resistances (solid lines) and mesophyll resistances (broken lines) to CO₂ diffusion as a function of time during four hours of exposure to several ozone concentrations. Data shown are for the Hood cultivar at 14 and 20 days.
The smaller increase in $r_m$ of the 14-day old leaves of both cultivars corresponded to a lesser change in CO$_2$ compensation point ($\Gamma$) which, on the other hand, indicated a better recovery capability of the plants. The sequence of change in $r_s$, $r_m$ and $\Gamma$ can be stated as follows: at low ozone concentrations ($\leq$ 34 pphm), the increase in $r_s$ occurred first and accounted for the decrease in Tr and Pn with the relative change in Pn smaller than the relative change in Tr. During this time, $r_m$ and $\Gamma$ did not change much. Ozone first induced closure of stomates without causing much damage of the internal leaf structures. As the concentration of ozone was increased, both $r_m$ and $\Gamma$ began to increase notably. Apparently, at the high ozone concentrations, CO$_2$ utilization was restricted and Pn started to decrease as much as Tr. The resulting CO$_2$ accumulation in the substomal cavity could have caused guard cells to close further. Such a sequence of events suggests that at advanced stages of ozone induced damage, the closure of stomates could be caused by both ozone and CO$_2$ accumulation in the intercellular spaces.

The Fate of Ozone

The dissipation of ozone in the chamber is the result of three processes, namely, the decomposition on impact with the chamber surfaces, the decomposition on impact with the leaf surfaces, and the
diffusion into the leaf structures.

Bennett, Hill and Gates (1973) described a model simulating pollutant exchange with isolated leaves. The model was based on an electrical analogue. Resistances of upper and lower surfaces were considered separately in their model. In the calculation here, a composite resistance for both surfaces was obtained. Tingey, Fites and Wickliff (1973) used a ventilated diffusion porometer to measure separately the stomatal resistance of each surface of the first trifoliolate leaf of soybean plants and found the value of $r_s$ for the upper leaf surface to be double that of the lower leaf surface.

The diffusion pathway of the ozone into the cells will be treated as taking the same pathway as that of CO$_2$. Mansfield (1973) pointed out that a similar consideration for pollutant intake as those for the CO$_2$ transfer model could be recommended, even though there might be limitations imposed by lack of knowledge of the exact sites of pollution damage. Other differences in the diffusion processes occur because of the highly oxidative and unstable properties of ozone. At the leaf surface, extensive pubescence, glandular hairs, or reactable surface deposits decompose the unstable ozone readily. Inside the parenchyma cells, where the dissolved CO$_2$ is converted to hexose products via the carbon assimilation process, the concentration of ozone in the cell suspension is limited by its solubility which is about 1/3 that of CO$_2$. The ozone concentration remains low as a result of
its decomposition to $O_2$ after the oxidation reactions with cell substances. The greater the amount of ozone which reacts inside the cells, the greater will be the amount which diffuses into the leaf. Damage of a leaf results when the oxidations of cell substances result in biological inactivation of these substances so that they become unavailable for vital processes in the cells. Ozone reacts much more quickly with organic matter in water than in air. The instability and loss of ozone in air was neglected in the following analysis.

The diffusion from leaf surface to cell wall was treated as being analogous to that of $CO_2$. The resistances to ozone diffusion will be estimated according to

$$r_{O_3} = r_{CO_2} \left( \frac{D_{CO_2}}{D_{O_3}} \right)^3$$

with

$$\frac{D_{CO_2}}{D_{O_3}} = 1.044.$$

The pathway of ozone is outlined in Figure 18. There are three important resistances involved, namely (i) the boundary layer resistance, $r_a$, (ii) the resistance to a reaction at the leaf surface, $r_e$, and (iii) the stomatal resistance, $r_s$. $[O_3]_a$, $[O_3]_{surf}$, $[O_3]_e$ and $[O_3]_s$ are the concentrations of ozone in the ambient air, on the leaf surface, at the reactive sites of the leaf surface, and at the cell wall of the parenchyma cells, respectively. $[O_3]_{surf}$ is the
Figure 18. Schematic diagram of the electrical analogue of ozone exchange between leaf and ambient air.
concentration at the leaf surface before ozone reacts with the reactive sites at the surface. $J_1$ represents the total flux into the leaf and is the sum of $J_2$, a flux to the reactive sites of the leaf surface and $J_3$, a flux absorbed into the leaf structure. The data on the ozone exchange will be discussed in three sections, namely the loss in the chamber, the loss on the leaf surface which yields $J_2$ and the rate of diffusion into the leaf $J_3$.

**Ozone Losses in the Chamber**

Measurements were made of the rate of ozone loss as it passed the leaf chamber (Figure 19). The decomposition rate of ozone upon impact with the surfaces of chamber reached a constant level within the first half an hour. The decomposition rates were about 20 percent at all concentrations used. The breakdown rate of ozone decreased slightly with increasing concentration. The ozone loss was measured during each experiment to obtain the desired level of ozone concentration in the ambient air. These measurements showed the same rate of $O_3$ decomposition as measured without leaves in the chamber indicating that no changes occurred in the reaction rate of ozone with the plexiglass surfaces. The ozone concentrations specified for the experiments were the inflow concentrations measured at a point just ahead of the leaf chamber entrance. Because the decomposition process occurred inside the chamber, the leaves were actually
Figure 19. Ozone outflow concentration after passage through the leaf chamber as a function of inflow concentration.
exposed to lower concentrations.

In the presence of a leaf, an assumption is made that the reactions of ozone with the chamber wall and the leaf would take place simultaneously.

Ozone Losses at the Leaf Surfaces

The rates of ozone loss at the leaf surfaces for an influx concentration of 30 pphm O₃ are shown in Table 14.

The decrease in rate of loss with time suggests that there was a finite number of sites on the leaf surface to react with ozone. The reaction might be absorption or adsorption. After the available sites were occupied, the rate of ozone loss was constant or became zero in the case of young leaves. When stomates are closed, no ozone can enter the leaf so that $J_3$ approaches zero and $J_1 = J_2$ which gives

$$J_1 = J_2 = \frac{[O_3]_a - [O_3]_e}{r_a + r_e}$$

(36)

The reading obtained from the ozone analyzer gives the value of ozone concentration in pphm. Taking the reading before and after putting a leaf in the chamber gives the ozone concentration difference, $\Delta [O_3]$, which is used to compute the rate of absorption by the leaf as follows:
\[
J_{O_3} = \frac{\Delta[O_3] \times F}{S} \times 1.944 \times 10^{-5} \mu g O_3/cm^2/s
\] (37)

where \( F \) and \( S \) are the flow rate and the leaf surface area as defined by Equation (17). The conversion factor of \( 1.944 \times 10^{-5} \) is for the change from pphm ozone to \( \mu g O_3/cm^3 \). In the experiments where leaves with closed stomates were used, \( \Delta[O_3] \) will be the amount of ozone absorbed on the leaf surface. Such experiments were conducted to obtain \( O_3 \) losses at the leaf surfaces (Table 14).

<table>
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<tr>
<th>Cultivars</th>
<th>Age</th>
<th>Exposure time</th>
<th>Rate of loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>hr</td>
<td>( 10^5 \mu g O_3/cm^2/s )</td>
</tr>
<tr>
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<td>14</td>
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<td>0.8</td>
</tr>
<tr>
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<td></td>
<td>2.0</td>
<td>0.5</td>
</tr>
<tr>
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<td>0</td>
</tr>
<tr>
<td></td>
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<td>0</td>
</tr>
<tr>
<td>Hood</td>
<td>14</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
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<td>3.0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>0</td>
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<td>1.0</td>
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<td>1.6</td>
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</table>
The decreasing rate of loss on the surface when \([O_3]_a\) is constant indicates an increase in concentration at the reaction sites, \([O_3]_e\), i.e., there was an accumulation of ozone at these sites. The greatest losses occurred with the 20-day old leaves of the Hood cultivar. These losses were not very large. The highest decomposition rate of ozone at the leaf surface was \(4.2 \times 10^{-5} \mu g O_3/cm^2/s\). When exposed to 30 pphm ozone the corresponding leaf sample of the 20-day old Hood cultivar was shown (Table 15) to absorb \(O_3\) at the rate of \(18.2 \times 10^{-5} \mu g O_3/cm^2/s\) at the end of the first hour. The rate included losses at the leaf surface. This left a net absorption rate by the leaf of \(14 \times 10^{-5} \mu g O_3/cm^2/s\). Thus the absorption by the leaf was 3.3 times greater than the rate decomposed at the leaf surface.

In comparison, the total uptake increased to \(30.7 \times 10^{-5} \mu g O_3/cm^2/s\) at the end of the first hour for the leaf exposed to 58 pphm, which gave the net absorption rate into the leaf of \(24.5 \times 10^{-5} \mu g O_3/cm^2/s\). At this high concentration, the rate into the leaf was about 6 times greater than the rate of loss at the leaf surface. Furthermore, when the exposure period was prolonged, the decomposition rate became less. Using the same comparison method as described above, for the rate at the end of the fourth hour, at 30 pphm the leaf would have a net uptake rate 9.3 times the rate of loss at the surface and at 58 pphm, it would be 10.8 times greater than the rate of loss at the leaf surface. It can be seen, then, that the dilution of ozone at the leaf
surface due to decomposition does not substantially reduce the flux absorbed into the leaf when the ambient concentration is high and the duration of exposure long. For the 20-day old Hood cultivar exposed to 58 pphm $O_3$, the flux absorbed by the leaf had decreased from $30.7 \times 10^{-5} \mu g/cm^2/s$ at the end of the first hour to $18.8 \times 10^{-5} \mu g O_3/cm^2/s$ at the end of the fourth hour due to ozone-induced increase in stomatal resistance. Damage occurred to the leaf, while the rate absorbed into the leaf was still more than 10 times greater than the rate of decomposition at the surface. Clearly, the absorption at the leaf surface does not provide much protection to plants exposed to high ozone concentration.

**Ozone Absorption into the Leaf Structures**

For a condition where both leaf surface loss ($J_2$) and absorption of ozone into the leaf ($J_3$) occur, the diffusion process can be described as follows:

\[ J_1 = J_2 + J_3 \]  \hspace{1cm} (38)

\[ J_1 = \frac{[O_3]_a - [O_3]_{surf}}{r_a} \]  \hspace{1cm} (39)

and

\[ J_2 = \frac{[O_3]_{surf} - [O_3]_e}{r_e} \]  \hspace{1cm} (40)
therefore

\[ J_3 = \frac{[O_3]_{\text{surf}} - [O_3]_s}{r_s} \]  \hspace{1cm} (41)

\[ J_1 = \left[ \frac{[O_3]_{\text{surf}} - [O_3]_e}{r_e} \right] + \left[ \frac{[O_3]_{\text{surf}} - [O_3]_s}{r_s} \right] \]  \hspace{1cm} (42)

\( J_1 \) was obtained from ozone analyzer measurements by using Equation 37 and the resistance to ozone diffusion were derived from known values for CO\(_2\) diffusion. Calculated values of \( J_1 \), \( r_a \) and \( r_s \) for ozone are shown in Table 15.

The ozone flux and the inverse values of stomatal resistances --which changed during the 4 hour exposure period-- showed a linear relationship. The linear correlation equations and the correlation coefficients obtained by the least square method are tabulated below. The parameters are:

\[ Y = J_{O_3} \times 10^{-5} \mu g \ O_3/cm^2/s, \]

and

\[ x = 1/r_s(O_3). \]
Table 15. Calculated resistances to ozone diffusion, measured fluxes of ozone into the leaves and calculated ozone concentrations at leaf surfaces and cell surfaces inside the leaves.

<table>
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<th>Resistances</th>
<th>Flux</th>
<th>Concentration</th>
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<td>$r_a$ sec/cm</td>
<td>$r_a + r_s$ sec/cm</td>
<td>$r_m$ sec/cm</td>
<td>$J_1 \times 10^5$ g/cm$^2$/s</td>
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Table 15, Continued.

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<th>O₃ concentration</th>
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1 Denotes the statistical significance at the .01 level.
2 Denotes the statistical significance at the .05 level.
In these linear correlation equations $Y = a + bx$, the intercept "a" is defined according to Equations (41) and (39) as:

$$a = J_2 = \left[ \frac{[O_3]_{\text{surf}} - [O_3]_e}{r_e} \right] \times 1.944 \times 10^{-5} \ \mu g \ O_3/cm^2/s \quad (43)$$

where $[O_3]_{\text{surf}}$, $[O_3]_e$ are in pphm and $r_e$ in sec/cm. The value "a" or $J_2$ is the reaction rate of ozone with the reactive sites at the leaf surface. High positive "a" values occurred mostly in the leaves at 30 pphm. At ozone concentration above 30 pphm, the values "a" became negative. The physical interpretation of this finding is that a reverse flux of ozone occurred. Absorption (or adsorption) of ozone resulted in formation of an ozone layer at the reactive sites of the leaf surface. When the stomates closed, the high concentration at the leaf surface could then cause a reverse diffusion of ozone into the ambient air as suggested by the negative sign.

The greater loss in the 20-day old leaves seemed to reassure the results obtained from the direct measurement of ozone loss. The greater magnitude than the direct measurement may in part be explained by the fact that the interpolated values "a" were obtained under conditions where the leaves were actively functioning with stomates opening. The higher loss might be due to decomposition of ozone in the layer of water vapor. Higher loss rates could also be caused by greater production in excretion products from the
leaves. As for 14-day old Dare leaf, the greatest loss at the surface of $8.43 \times 10^{-5} \mu g/cm^2/s$ was as high as of the net absorption rate into the corresponding leaf sample exposed to 30 pphm $O_3$. In the 14-day old Hood leaf, the loss rate of $8.49 \times 10^{-5} \mu g/cm^2/s$ was 4/5 of the net absorption rate. While the direct measurement of ozone loss rate at the surface showed the ratio to be about 1:3 at 30 pphm $O_3$ for 20-day old Hood leaf. It is possible, then, that at low ozone concentration of 30 pphm (or about 25 pphm in the ambient air) the absorption rate at the leaf surface does reduce the absorption rate going into the leaf and provides some protection to the leaf interior. However, at higher concentration, saturation of ozone absorption at the leaf surface took place which made the loss rate smaller, particularly in comparison with the higher absorption rate into the leaf as ozone concentration in the ambient air increased. In such a case, the advantage is very small.

The parameter "$b$" describes the concentration difference, $[O_3]_{surf} - [O_3]_s$, as shown in Equation (41). The "$b$" values were plotted versus the applied ozone concentrations (Figure 20). The diagram shows that as the applied ozone concentrations increased, the concentration difference of ozone, $[O_3]_{surf} - [O_3]_s$, decreased in a curvilinear manner, except for 20-day old Hood. From Table 15, it could be shown that $[O_3]_{surf}$ increased as a straight line when the applied ozone concentration was raised from 25 to 58 pphm. In fact,
Figure 20. Changes in ozone concentration differences, $[O_3]_{\text{surf}} - [O_3]_{\text{i}}$, between leaf surfaces and mesophyll-intercellular space interface as a function of inflow ozone concentrations.
the ratio was about 1:1 for the change in concentration of ozone in the ambient air to the change in concentration at the leaf surface $[O_3]_{surf}$. Therefore, the decrease in $\left([O_3]_{surf} - [O_3]_s\right)$ indicated that it was $[O_3]_s$ which did not increase in a direct proportion to the increase in applied ozone concentration but that $[O_3]_s$ was higher due to ozone accumulation at the mesophyll-intercellular space interface. This should be expected as the diffusion of ozone from cell surface into the cell is limited by its solubility. A point of interest in Figure 20 is the linear relationship found for the 20-day old leaves of the Hood cultivar. This suggests that $[O_3]_s$ did not accumulate in the substomal cavities of these plants. Whether ozone diffuses into the cells more readily or is decomposed more when reacting with cell surfaces is not known at present.

Figures 21 and 22 show the decreases of ozone uptake rates ($J_1$) as a function of exposure time whereas Figure 23 shows how the stomatal resistance to $O_3$ changed during the same period. These three figures best depict the linear correlation previously described that the decrease in $J_1$ was closely related to the increase in $r_s$. At the low ozone concentration of 25 pphm, the stomatal resistances were not affected by the presence of ozone. The flux, as a result, remained constant. Figures 24 and 25 show the cumulative uptake of ozone by the leaves per unit surface area. In general, the younger leaves, where the relative changes in stomatal resistance were
Figure 21. Rates of ozone uptake by the leaves of the Dare cultivar during four hour exposures to several ozone concentrations.
Figure 22. Rates of ozone uptake by the leaves of the Hood cultivar during four hour exposures to several ozone concentrations.
Figure 23. Stomatal resistances to O₃ diffusion as a function of time during four hours of exposure to several ozone concentrations.
Figure 24. Cumulative ozone uptake by the leaves of the Dare cultivar during four hour exposures to several ozone concentrations.
Figure 25. Cumulative ozone uptake by the leaves of the Hood cultivar during four hour exposures to several ozone concentrations.
greater absorbed less ozone per unit surface area than the older leaves.

A further attempt was made to determine the levels of \([O_3]_{\text{surf}}\) and \([O_3]_s\) in order to clarify the distribution of ozone on the inside of the leaf. The main interest was to find out if the initiation of visible damage at 40 pphm and higher ozone concentrations was associated with higher concentrations inside the leaf. It was assumed that at high ozone concentrations the loss on the surface was negligible after the reactive sites on the leaf surface became saturated as previously discussed. This assumption might not apply to the concentration of 30 pphm which showed a high rate of loss probably because the reactive sites were not yet saturated with ozone. But at this concentration, the plants did not show any injury. The following relationships were assumed to apply:

\[
J_1 = \frac{[O_3]_a - [O_3]_s}{r_a + r_s}
\]  

(44)

or

\[
(J_1)(r_a + r_s) = [O_3]_a - [O_3]_s
\]

(45)

and from Equation (39)

\[
(J_1)(r_a) = [O_3]_a - [O_3]_{\text{surf}}
\]

(46)
Knowing $J_1$, $r_a$, $r_s$, and $[O_3]_a$ which is the average concentration of the influx and efflux air, $[O_3]_{surf}$ and $[O_3]_s$ could be determined. Ozone concentrations shown in Table 15 are presented in units of pphm in order to be comparable to the applied ozone concentrations.

The negative values of $[O_3]_s$ for 20-day old Dare suggest that concentrations were not sufficiently high to overcome the stomatal resistance. Little ozone could enter the stomates to accumulate on the cell surfaces of the mesophyll cells. However, it must be taken into consideration that the stomatal resistance,

$$r_s = \Delta x D$$

where $D$ is the diffusion coefficient and $\Delta x$ is the effective length of the diffusion paths. Therefore, $r_s$ is an integral resistance for the entire leaf. Ozone might have reached cells close to the stomates but was attenuated before it could reach other cells. Eventually, as the exposure time progressed and more ozone diffused into the leaf, $[O_3]_s$ gradually increased. Likewise, ozone might not diffuse through to all chloroplasts, but it could react with those close to cell plasmalemma and oxidize the membranes of some chloroplasts.

The $O_3$ concentrations in the stomatal cavity $[O_3]_s$ were similar for young and old leaves of the same cultivar at concentrations below 40 pphm. Pronounced differences occurred at higher concentrations, however. Here the ozone concentrations in the stomatal cavity were much higher in the younger leaves than in the older. These differences could not have resulted from the higher diffusion resistances
of the old leaves because values of these resistances, as shown in Table 15 were not much different within the same cultivar.

A possible explanation might be that the surfaces of the cells in the substomatal cavities of the older leaves have more exposed area where ozone could react with and/or more cell solute medium to dissolve ozone so that ozone is continuously broken down and the concentration remains low, whereas the cell surface of younger leaves are less reactive allowing the concentration to increase more. The lower concentration of $[O_3]_s$ caused the flux into the leaf to be greater. The 20-day old leaves showed more damage when the uptake flux of ozone into the leaf was higher in comparison with the 14-day old leaves. In the experiment with the Dare cultivar exposed to 58 ppm O$_3$, the resistance in the older leaf became so great and yet the flux into the leaf remained high and the intensity of leaf injury was severe.

An attempt was also made to calculate the values of mesophyll resistance to ozone diffusion. Even though it was pointed out by Mansfield (1973) that the exact reaction site inside the cell is not known at present, the assumption was made here that ozone concentration inside the cells is zero ($[O_3]_{cell} = 0$), i.e., they were assumed to be an infinite sink for ozone. To justify this assumption, Bennett et al. (1973) showed that Henry's Law may be applied to computing
the concentration in a solvent of a dissolved gas provided that it does not appreciably combine with the solvent molecules. They proposed a partition coefficient, $K$, which relates the concentration of dissolved pollutant absorbed into the cell to the concentration of gaseous pollutant at the mesophyll-intercellular space interface, i.e.,

$$[O_3]_{\text{cell}} = K[O_3]_s.$$ The coefficient $K$ is a function of the absorbing medium, the diffusion rate, and pollutant fate in the medium. It can be estimated from the solubility of gaseous pollutants in water or be appraised experimentally. A rough estimation will be made here.

Table 15 shows that the highest concentration found in $[O_3]_s$ was about 26 pphm (for 14-day old Dare leaf exposed to 58 pphm ozone) which was about $50.5 \times 10^{-5}$ μg O₃ per cm³ of air. Had the cell solution behaved like H₂O, the solubility of ozone would be 12 μmoles O₃ or about 576 μg O₃ per cm³ of water (at 20°C). The solubility in the real composition of cell solute could be less due to dissolving of other ions. Yet, roughly speaking, the solubility in water was five orders of magnitude greater than the maximum concentration of ozone in the mesophyll-intercellular space interface. As a first approximation, it could thus be assumed that saturation of ozone in the cell did not take place, and the above assumption, $[O_3]_{\text{cell}} = 0$ would hold. Then the values of mesophyll resistance to ozone diffusion could be calculated by
\[ r_m = \frac{[O_3]^s - [O_3]^c}{J_1} \]

or

\[ r_m = \frac{[O_3]^s}{J_1} \quad \text{when} \quad [O_3]^c = 0 \] (47)

The results shown in Table 15 indicate that the calculated \( r_m \) were much higher in the 14-day old leaves than in the 20-day old ones. This might suggest that there was more ozone dissolved into the cells of the latter.

**General Discussion**

Two major theories have been offered to explain ozone damage to plants (Nobel and Wang, 1973). The first theory suggests that ozone affects membrane components, especially the oxidation of unsaturated bonds of lipids. These changes in turn change membrane permeability. The second theory suggests that the damage also occurs by oxidation of sulfhydryl groups in proteins. The result is that membranes are unable to repair the damage. These sulfhydryl-containing proteins are also involved in photophosphorylation.

The earliest cytologically observable effect of ozonation has been shown to be the granulation of the chloroplast stroma. The second phase of ozone damage includes the rupture of cellular membranes (Thomson et al., 1966). It is generally accepted that once the
plasmalemma is ruptured, cells are irreversibly damaged. Nobel and Wang (1973) using isolated pea chloroplasts, found that ozone can increase the permeability of the limiting membranes surrounding the chloroplasts. In studies carried out with whole plants, it was found that ozone treatment had increased the solute permeability of subsequently isolated chloroplasts, although $O_3$ could interact with the cell walls, plasmalemmas, and parts of the cytoplasm before reaching the chloroplasts in a mesophyll cell. This produces the possibility for escape of intermediates formed during the photosynthetic process into the suspending media.

Ozone bubbled into a suspension of isolated chloroplasts was found to inhibit electron transport in both photosystems (Coulson and Heath, 1974). Studies with cytoplasmic and chloroplastic enzymes in soybeans indicated that ozonation resulted in a loss of both nitrite reductase activity and chlorophyll content of treated tissues. Both these compounds were localized in the chloroplast. That both these parameters declined, even at low-level ozone exposure of 44 pphm for 4 hours, implies that chloroplast lesions occurred. A reduction in nitrate reductase activity which is localized in the cytoplasm occurred only at exposures of 80 pphm for 4 hours. This decline in nitrate reductase activity likely reflects the phenomenon of cell disruption (Leffler and Cherry, 1974). The present study also showed that exposure to concentrations higher than 40 pphm for 4 hours,
decreased rate of photosynthesis due to chloroplasts damage per se and not only due to closure of stomates. The increase of the compensation points at these exposures also confirmed ozone-induced damage to the photosynthetic capacity. At the ozone concentration of 58 pphm for 4 hours, leaves showed severe necrosis two days after the treatment was terminated which suggests that cell damage had occurred which went beyond the capacity of the cell to recover.

From the above observations and several similar studies a general concept of ozone damage has evolved. At low concentrations, ozone does not appear to penetrate cell membranes readily. Thus, only the first encountered outer plasmalemma is oxidized. Because of the low concentrations, the membrane will not be entirely disrupted, but only sections of unsaturated lipid or -SH groups in the membrane are oxidized. This allows a temporary and passive increase in the efflux rate of K⁺, which returns to normal shortly after cessation of exposure (Heath, Chimiklis and Frederick, 1974). Because the leakage rate is low it will generally take a long time for the subsequent electrolytic or osmotic imbalance to become large enough for any significant alternation in metabolism to occur. The recovery rate may keep up by a continuous repair of the membrane.

More substantial damage occurs when the concentration of O₃ is raised high enough for it to penetrate the cytoplasm so that the membranes of chloroplasts can be attacked. Leakage of this membrane
means loss of photosynthate intermediates and a reduction in the ability of chloroplasts for CO$_2$ carboxylation.

Once the CO$_2$ accumulates in the air spaces of the leaf, its primary impact is on the stomata. Stomates are known to close in response to high CO$_2$ concentrations in the substomatal cavities. Howell and Kremer (1972) studied the effect of ozonation on soybean cotyledon leaves. When exposed to ozone at 50 pphm, some reduction in stomatal size was noted after 5 minutes of exposure. After 25 minutes of treatment the stomates were closed more than half. These stomata remained closed for at least 96 hours. After 2 hours of ozonation, injury occurred in the upper one or two layers of palisade cells immediately underneath the stomata. After investigating many species of plants including pinto beans and tobacco, Hill and Littlefield (1969) concluded that ozone concentrations from 40-90 pphm caused a reduction in CO$_2$ assimilation ranging from 40 to 70 percent of the control within 30-90 minutes. Ozone treatment generally resulted in a rapid reduction in the average width of the stomatal opening. When oats were exposed to 60 pphm O$_3$, over half of the stomates were closed completely after 65 minutes of exposure. But about 15 minutes after the treatment was discontinued, the stomata apparently began to reopen. When the same ozone treatment was repeated on the same plants on the following day, the reductions in apparent photosynthesis and stomatal closure were more rapid than before and no injury
developed. Apparently, closure of stomata taking place before exposure to ozone protected these plants from further ozone injury.

In this study, stomates were not observed but measurements of Tr and Pn and calculations of resistances yielded results showing stomatal closure to occur during ozone fumigation period. Recovery a day after exposure indicated that when the parenchyma cells were not severely damaged, the guard cells regained turgidity and the stomates reopened. Engle and Gabelman (1966) indicated that guard cells showed differential response to ozone. Tolerant cultivars of onion and corn showed rapid stomatal closure in the presence of ozone. As for soybeans in this study, the guard cells themselves might be effected by ozone. Full turgidity of cells, especially guard cells is essential and is sustained by the presence of abundant ions and metabolic products which govern the osmotic potential.

Evans and Ting (1973) used $^{86}$Rb ions to simulate $K^+$ absorption and translocation of $K^+$ ions. Leaf discs of pinto beans exposed to 50 pphm O$_3$ for 0.5 to 1 hour, lost nearly three times more $^{86}$Rb than the control discs. Mudd (1965) found that ozone oxidized NADH and Tomlinson and Rich (1968) showed that ozonation lowered the ATP content of pinto bean leaves. Bean leaves could be made resistant to ozone by allowing them to take up $10^{-3}$ M ATP through their petioles before ozonation. These results seem to support but not necessarily prove the possibilities that: (i) the energy required for the active
uptake of $K^+$ by the cells or guard-cells in order to maintain turgor pressure can be restricted by ozone damage and (ii) the leaky membranes allow the escape of internal solutes including $K^+$ more easily. If this is so, then the stomatal closure associated with ozone exposure can be attributed to a direct effect rather than the indirect $CO_2$ mediated mechanism or the combination of the two. Hill and Littlefield (1969) stated that during ozone fumigation, stomatal closure, reduced apparent photosynthesis and reduced transpiration occurred simultaneously, indicating that suppression of transpiration and apparent photosynthesis resulted from the stomatal closure. This explanation appears to best fit their results as well as those from this experiment. They considered it unlikely that the initial effect of ozone was on $CO_2$ utilization resulting in a rapid increase in the $CO_2$ content of the guard cells with subsequent stomatal closing. They based this conclusion on the observation that in a number of fumigations at lower concentrations, a definite but relatively small reduction in stomatal opening occurred without measurable effect on apparent photosynthesis or transpiration. They believed that a relatively large reduction in apparent photosynthesis is required to produce a small reduction in stomatal opening.

In this experiment, the increase in stomatal resistance occurred during the initial stages of exposure and the decrease in $Tr$ was shown to be greater than the decrease in $Pn$. The mesophyll
resistance and CO$_2$ compensation point remained intact during this period. When the ozone concentration was increased to 40 pphm, the increase in stomatal resistance became substantial and the damage to $Pn$ was about as great as that to $Tr$. Both mesophyll resistance and CO$_2$ compensation point increased significantly. The observed increase in $\Gamma$ suggested that the closure of the stomates could have been in part mediated by CO$_2$ accumulation in the substomatal cavity.

The consequences of ozone-induced closure of stomata and leaf damages can be described on a purely hypothetical basis as follows:

Initial ozone exposure at low concentrations has little effect on stomatal opening. Ozone that diffuses into the leaf airspaces reacts with outer cell membranes, increasing the membrane permeability. Subsequent ion leakage upsets the ion balance of the cell milieu and finally affects its water potential. The capacity of cells to repair the membranes as exposure progresses depends on exposure duration, original width of the stomatal opening, and the leaf sensitivity at this stage of growth. Damage becomes apparent when the recovery rate is exceeded.

Higher ozone concentrations would result in further diffusion of ozone into the cytoplasts. Chloroplasts are most readily attacked by ozone. When the photosynthetic capacity of cells is impaired, the rate of CO$_2$ assimilation decreases, as might be indicated by increase in CO$_2$ compensation point. It is likely that the increasing CO$_2$ content
of the substomal cavity then causes substantial closure of stomates. The protection by closure of stomates might come too late because the cells are already severely damaged by the first large amount of intruding ozone, particularly when the biochemical conditions within plant tissues are favorable for ozone injury. As the injury progresses, the plasmalemma cannot maintain its function and ions and substrates move out of the cell. It can easily be visualized that the alterations observed in metabolic studies and electron microscopic examinations must be due to secondary effects following the osmotic and ionic imbalances caused by leaky plasmalemma.

The role of stomates in leaves exposed to air pollutants is analogous to the role of stomata in plants subjected to water stress. The closure of stomates can help reduce the loss of water from leaves. This is only a temporary relief from the stress. Duration and extent of stress are factors affecting the extent of injury. The changes in rates of transpiration and photosynthesis may only be the end result of a long series of changes in enzymatic and biochemical processes in a cell. Could it be that the first effect of ozone is the same as water stress, i.e., the leaky plasmalemma prevents cells from sustaining their cell water potential? Then subsequent secondary and tertiary effects are enhanced in such a way that the high oxidizing property of ozone causes more severe damage to the cells.
The stomatal closure did in part restrict the uptake of ozone by the leaves. This closure did not give an explanation for the finding that the leaves of the tolerant cultivar (Hood), absorbed more ozone and yet showed less damages as measured by changes in \( \Gamma \). The difference in ozone uptake was demonstrated to be caused by different sink strengths between the plant cultivars.

In summary, the damage in plants is dependent upon three major factors: (i) the concentration of the oxidant, (ii) the duration of exposure and (iii) the external (environmental) conditions and internal (metabolism and genetic) conditions which predispose plants to be susceptible to injury. Clearly, the closing operation of stomates can play a role only by limiting the amount of ozone which is absorbed into the leaf structure and is therefore unable to totally protect the plant from injury if the exposure is prolonged and the conditions of the plants are favorable to injury. This is especially so when the closure of stomates does not take place before plants are exposed to air pollutants but happens as a result after cells are already badly damaged.
SUMMARY AND CONCLUSIONS

It was proposed that knowledge of the interaction between diffusion of gaseous pollutants into the leaf and other factors such as sensitivity of leaf, leaf age, and extent of leaf damage would produce a better understanding of the mode of action of such toxicants.

The approach used in the investigation of these effects was the simultaneous measurement of rates of transpiration (Tr) and rates of net photosynthesis (Pn), along with rates of ozone uptake into the leaf. Two soybean cultivars were used. These were, Dare, a sensitive variety, and Hood, a resistant variety. Measurements were made at leaf ages of 14 days representing the expanding phase and 20 days, representing the fully maturing phase. Five ozone concentrations ranging from 25 to 58 pphm were tested during a 4 hour exposure period. Transpiration and photosynthesis rates were measured 24 hours after cessation of ozone fumigation. Measurements were also made for CO$_2$ compensation point before and after 4 hour ozone exposure.

Ozone was observed to cause a decrease in both transpiration and photosynthesis. The decrease in transpiration was greater in the young leaves and greatest in the young leaves of the resistant cultivar, Hood. The initial decrease did not occur immediately. The time of exposure necessary for the initial decrease to occur was a function of
ozone concentration. The higher the concentration, the sooner the initiation. Once the decrease developed, it took place rapidly in the first few hours followed by a period of more gradual decline. The first measurable effect of ozone exposure was stomatal closure. This effect was slight at low ozone concentration ($\leq 30$ pphm), as shown by the small increase in stomatal resistance ($r_s$). The increase in $r_s$ was greater in the 14-day old leaves than in the 20-day old leaves for both cultivars. The mesophyll resistance remained constant, and there was no change in $CO_2$ compensation point during 4 hour exposures at low concentrations. After allowing 24 hours for recovery, the leaves exposed to the low ozone concentrations ($\leq 30$ pphm) showed no sign of visible injury. Apparently, damages which occurred during the exposure period were not permanent and the recovery ability of plants managed to compensate for the injury.

Table 16 shows that at ozone concentrations above 40 pphm, the decrease in $Pn$ was nearly as great as that in $Tr$. The greatest decrease in $Pn$ occurred in the 20-day old leaves of the sensitive (Dare) cultivar whereas the leaves at the same age of the tolerant (Hood) cultivar showed the least decrease. The stomatal resistance increased most in the youngest leaves showing that the stomates of the young leaves were more responsive to exposure. The 20-day old leaves of the resistant cultivar showed the smallest increase in $r_s$. Mesophyll resistance as well as $CO_2$ compensation point $\Gamma$ began to
Table 16. A summary on the ozone-induced changes in transpiration rates, rates of photosynthesis, leaf resistances as compare to control, and CO2 compensation points at the end of four hour exposure periods to ozone concentrations of 40 and 58 pphm. Rates of Tr and Pn, 24 hours after terminating exposure were expressed as percent of the rate at the start of the exposure period.

<table>
<thead>
<tr>
<th>Plant cultivar</th>
<th>Age</th>
<th>Ozone conc.</th>
<th>Decrease in</th>
<th>Increase in</th>
<th>Increase in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>days</td>
<td>pphm</td>
<td>Tr</td>
<td>Pn</td>
<td>Tr</td>
</tr>
<tr>
<td>Dare</td>
<td>14</td>
<td>40</td>
<td>56</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>60</td>
<td>43</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>40</td>
<td>47</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>60</td>
<td>55</td>
<td>22</td>
</tr>
<tr>
<td>Hood</td>
<td>14</td>
<td>40</td>
<td>58</td>
<td>39</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>63</td>
<td>46</td>
<td>82</td>
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<td>84</td>
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<td></td>
<td></td>
<td>58</td>
<td>43</td>
<td>38</td>
<td>82</td>
</tr>
</tbody>
</table>
increase at concentrations above 40 pphm. The greatest increases in both $r_m$ and $\Gamma$ occurred in the 20-day old leaves of the sensitive (Dare) cultivar. Measurement of $T_r$ and $P_n$ 24 hours after terminating exposures revealed a relationship between the recovery of $P_n$ and the increase in $\Gamma$. The greater the increase in $\Gamma$ after ozone fumigation, the more intense necrosis was apparent on the leaves. The rates of $T_r$ and $P_n$ were expressed in percent of the rates measured at the start of the exposure period in the same plants.

The increase in the level of $\Gamma$ also suggested the possibility that $CO_2$ accumulated in the intercellular spaces of the leaves. The closure of the stomates could have been caused by high concentrations of $CO_2$ inside the leaves. This observation led to the suggestion that, at advanced stages of ozone damage, the closure of stomates may be the result of both direct damage from the ozone on the guard cells and indirect effect from accumulation of $CO_2$ in the stomatal cavity.

At low ozone concentration ($\leq$ 30 pphm), ozone-induced closure of stomates did, in fact, limit the amount of ozone absorbed by the leaf. However, the stomates closed only to a small degree. This closure did not prevent a substantial reduction in ozone uptake by the leaves. The amount absorbed was almost the same for both leaf ages and both cultivars. When leaves were exposed to ozone concentrations above 40 pphm, the difference in ozone absorption became obvious. A summary of ozone absorption is shown in Table 17. The
Table 17. A summary of leaf resistances, ozone fluxes being absorbed by the leaves and calculated concentrations of ozone at the cell-air interfaces inside the leaves at the end of four hour exposure periods.

<table>
<thead>
<tr>
<th>Age conc.</th>
<th>(rs + ra)O₃</th>
<th>Ozone flux after 4 hrs exposure</th>
<th>[O₃]s</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>pphm</td>
<td>sec/cm</td>
<td>10⁵ µg/cm²/s</td>
</tr>
<tr>
<td>Dare</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>1.93</td>
<td>14.93</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.01</td>
<td>13.90</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>3.06</td>
<td>14.18</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5.10</td>
<td>6.84</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>6.34</td>
<td>7.76</td>
</tr>
<tr>
<td>20</td>
<td>25</td>
<td>2.48</td>
<td>12.94</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>3.26</td>
<td>12.54</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>3.48</td>
<td>13.87</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>5.07</td>
<td>9.19</td>
</tr>
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<td></td>
<td>58</td>
<td>7.56</td>
<td>14.12</td>
</tr>
<tr>
<td>Hood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>1.76</td>
<td>17.80</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2.73</td>
<td>14.95</td>
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<tr>
<td></td>
<td>34</td>
<td>3.96</td>
<td>5.52</td>
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<td></td>
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<td>4.55</td>
<td>7.98</td>
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<td>58</td>
<td>4.24</td>
<td>12.67</td>
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<td>20</td>
<td>30</td>
<td>2.36</td>
<td>16.53</td>
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<td>2.77</td>
<td>16.05</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>3.60</td>
<td>13.58</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>3.97</td>
<td>18.78</td>
</tr>
</tbody>
</table>
14-day old leaves usually absorbed less ozone than the 20-day old ones. At certain ozone concentrations, the resistances \( r_a + r_s \) were essentially identical and differences in the ozone diffusion into the leaves could not be explained on this basis. Remarkably, leaves of the tolerant (Hood) cultivar absorbed more ozone than the sensitive (Dare) cultivar at both plant ages. The concentrations of ozone at the mesophyll-intercellular space interfaces were computed by applying the resistance model of CO\(_2\) diffusion to the diffusion of ozone. The results showed that differential sink strengths between leaf ages caused the differences in ozone uptake. The 14-day old leaves of both cultivars showed an accumulation of ozone inside the leaves and absorbed less ozone than the older leaves. How the ozone concentration inside the mature leaves remained low was not known for certain. The most attractive explanation appears to be that the internal cells are more accessible to ozone attack in the older leaves because of more complete development. More necrosis apparent on these mature leaves suggested that more reactions occur between ozone and cell components. Thus more ozone would be continuously broken down inside the leaf.
Specific Conclusions

1. At ozone concentration below 30 pphm, none of the leaves shows visible injury. The direct effect of ozone on increasing the stomatal resistance was small. The closure of stomata did not eliminate the rate of ozone diffusion into the leaves.

2. At ozone concentration greater than 40 pphm, stomatal resistance increased, as well as mesophyll resistance and CO₂ compensation point. The accumulation of CO₂ inside the leaves might also have contributed to the closure of stomates apart from caused by ozone effects on the guard cells.

3. Ozone accumulated inside the intercellular spaces of the leaves. Fourteen-day old leaves absorbed less ozone per unit leaf surface area than the 20-day old leaves, due to higher ozone concentrations inside the leaves.

4. The degree of leaf injury was not proportional to the amount of ozone absorbed.
BIBLIOGRAPHY


