J. Amer. Soc. Hort. Sci. 107(2):266–271. 1982.

Evaluation of Injury to Expanded and Expanding Leaves of Peas Exposed to Sulfur Dioxide and Ozone[1]

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Additional index words. necrosis, chlorophyll, dry weight, surface area, *Pisum sativum*

Abstract. **Necrosis, chlorophyll concentration, dry weight and surface area measurements were made to evaluate injury to leaves of *Pisum sativum* L. cv Alsweet (garden pea) grown under controlled environments and exposed to sulfur dioxide, ozone and combinations of sulfur dioxide plus ozone. Injury evaluations were made at low pollutant levels causing slight necrotic injury and high levels causing severe necrotic injury. At low levels, expanded leaves with a trace of necrotic injury had a 10% reduction in chlorophyll concentration but no reductions in dry weight or surface area, while expanding leaves, also with a trace of necrotic injury, had a reduction in chlorophyll concentration accompanied by reductions in dry weight and surface area. At high pollutant levels, expanded leaves with severe necrotic injury had a 70% reduction in chlorophyll concentration and significant reductions in dry weight and surface area, while expanding leaves had a smaller amount of necrotic injury and a smaller reduction in chlorophyll concentration, but reductions in dry weight and surface area similar to those in expanded leaves. Thus, the following measurements are proposed as reliable indicators of injury at pollutant concentrations just above the threshold for injury: chlorophyll concentration for expanded leaves and surface area for expanding leaves. Reliable indicators of injury at higher concentrations causing serious injury to leaves are: necrosis for expanded leaves and chlorophyll concentration, dry weight, and surface area for expanding leaves.**

Quantitative determinations of the extent of injury to leaves have been recognized to be necessary for accurate evaluation of the effects of air pollutant on plants (19). These determinations have been accomplished through several different procedures including measurement of necrotic area, chlorophyll concentration, dry weight and surface area of leaves (19).

Necrosis on leaves commonly has been determined because this is an injury symptom that can be visually estimated (9). However, estimation of the amount of necrosis likely will vary widely between observers and therefore one questions the precision possible in comparing the level of injury obtained between studies in different laboratories.

Chlorophyll concentration of leaves occasionally has been used to evaluate pollutant injury to plants (1, 10, 11, 17). Chlorophyll concentration of leaves has been correlated closely with the amount of necrotic injury to leaves (10), but unlike necrosis determinations, chlorophyll determinations are reported on a comparative basis and thus require careful measurement of chlorophyll concentration in control leaves. Furthermore, chlorophyll concentration is usually presented on the relative basis of amount of chlorophyll per gram of dry weight, and, thus, careful measurement of dry weight also is required.

Dry weight and surface area of leaves have been measured in a few air pollution studies (19), and reductions in dry weight or surface area have been demonstrated with exposures that produced few visible injury symptoms (4, 14). However, dry weight and surface area determinations also require careful measurements of control leaves which must be at exactly the same stage of expansion.

Leaves that are fully expanded have been evaluated most commonly for injury because leaves at this stage of maturity exhibit the greatest amount of necrosis; thus they have been considered to be more sensitive to pollutants than leaves that are still expanding (3, 5, 8). However, expanding leaves may be considered sensitive to pollutants if injury is determined by a method other than necrosis measurement.

Injury to garden pea from sulfur dioxide consisted of small bifacial necrotic areas between the veins when injury was slight (2), and large bifacial necrotic areas extending across the veins when injury was severe (9). Injury from ozone has consisted of flecks scattered over the adaxial surfaces of leaves when injury was slight, and both scattered flecks and large areas of bifacial necrosis when injury was severe (13). Injury to peas from combinations of sulfur dioxide plus ozone at both slight and severe injury levels consisted of necrotic injury similar to the injury produced by ozone alone (7). There have been no injury determinations in peas using measurements of chlorophyll concentration, dry weight or surface area with either sulfur dioxide or ozone alone, or combinations of both pollutants.

[1]Received for publication May 6, 1981. This project was financed in part with Federal funds from the Environmental Protection Agency under grant number R803971, by the Wisconsin Public Service Corporation, Madison Gas and Electric Company, Wisconsin Power and Light Company and by the College of Agriculture and Life Sciences, University of Wisconsin. The contents do not necessarily reflect the views or policies of the Environmental Protection Agency, Department of Horticulture, University of Wisconsin or the above corporations, nor does mention of a trade name for a commercial product constitute endorsement or recommendation for use.

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The authors express appreciation to Janice Kobriger for advice and information pertaining to dry weight lost during extraction of chlorophyll from pea leaves.

This study was undertaken to assess different measurements of pollutant injury to leaves of pea plants. Determinations of amount of necrosis, chlorophyll concentration, dry weight, and surface area were compared for both expanded and expanding leaves, and for plants exposed to sulfur dioxide, ozone and combinations of sulfur dioxide plus ozone.

Materials and Methods

'Alsweet' pea was used for these studies employing plant culture and pollutant exposure procedures as detailed previously (12). Plants were 13 days old with 2 fully expanded leaves and one expanding leaf at the time of exposure.

Plants were exposed to a range of pollutant levels to obtain plants for study which exhibited slight (1–10% necrosis) and severe (60–90% necrosis) injury to the youngest expanded leaf (leaf 2 at fourth node above the cotyledons). Exposures were undertaken with 4 sulfur dioxide concentrations ranging from 0.42 to 1.72 μl/liter, 4 ozone concentrations ranging from 0.13 to 0.27 μl/liter, and combination exposures of sulfur dioxide at 0.11 μl/liter plus a range of ozone concentrations from 0.06 to 0.13 μl/liter, and ozone at 0.13 μl/liter plus a range of sulfur dioxide concentrations from 0.11 to 1.23 μl/liter. Exposure periods were for 2, 4 and 8 hr at the different pollutant concentrations. Exposures were conducted over the middle of the light period between the 5th and 7th, 4th and 8th, and 2nd and 10th hr of the 16 hr light period for the 2, 4 and 8 hr exposures, respectively. There were 6 single plant replicates for each pollutant level, involving 3 separate exposures of 2 plants each, except for low levels of sulfur dioxide plus ozone which had 12 single plant replicates involving 2 separate exposures of 6 plants each.

During the exposures, control plants were moved from the growing room to a control chamber in the exposure room. Immediately following the exposures both pollutant exposed and control plants were returned to the growing room.

Plants were harvested and examined for injury 7 days after exposure. Injury measurements (necrosis, chlorophyll concentration, dry weight and surface area) were taken on both the expanded leaf (leaf 2) and an expanding leaf (leaf 3 at fifth node above cotyledonary node). All 4 measurements were made for each leaf. The amount of necrosis was established by determining the area that was necrotic for one leaflet of each leaf. Only one leaflet was measured because the pattern and amount of injury were essentially the same on each of the two leaflets of each leaf. For the measurements, leaflets were removed from petioles and placed on graph paper. An outline of the injured portion or portions of the leaflet was made on the graph paper by perforating the injured area with a sharp pencil point, leaving an impression on the graph paper beneath it. The area of the injured portion of the leaflet was determined by counting the number of squares within the delineated section. If the injured portion contained only flecks and not large well defined bleached areas, the number of squares delineated by perforations was multiplied by the faction of the surface that was visually estimated to be covered by flecks. The total surface area of the leaflet was determined by scanning the entire outlined paper replica with a Hayashi-Denko Model AAM5 automatic area meter. Percent necrosis was then calculated by dividing the necrotic area by the total surface area for each leaflet.

Chlorophyll concentration for leaves from both exposed and control plants was determined by the alcohol extraction method described by Knudson et al. (10). Chlorophyll was extracted for 3 days with a total of 50 ml of ethanol for each group of 2 leaflets. The light absorption of chlorophyll at 649 nm and at 665nm was measured with a spectrophotometer and the chlorophyll concentration for each leaf was expressed as mg of total chlorophyll per g of dry weight.

Dry weight of leaves was obtained by drying them for 1 day at 70°C following alcohol extraction. After removal from the oven, leaves were held in a desiccator for 1 day before weighting.

Surface area of leaves was obtained with an automatic area meter. One leaflet of the two leaflets of each leaf was measured with the meter and the area of the entire leaf was determined by doubling the area of the single leaflet. Measurement of only one leaflet was adequate because the two leaflets were always essentially the same size and shape.

Chlorophyll concentration, dry weight and surface area of leaves on pollutant exposed plants were expressed as a percent of the level of control plants. Data were subjected to regression analysis to determine correlations between the different types of leaf injury (18).

Results and Discussion

Pollutant levels. The concentrations and exposure periods of pollutants selected for slight injury to expanded leaves (low levels of pollutants) were sulfur dioxide at 1.23 μl/liter for 8 hr, ozone at 0.17 μl/liter for 8 hr, and sulfur dioxide at 0.11 μl/liter for 4 hr plus ozone at 0.11 μl/liter for 4 hr. Concentrations and exposure periods that were selected for severe injury (high levels of pollutants) were sulfur dioxide at 1.72 μl/liter for 8 hr, ozone at 0.27 μl/liter for 8 hr, and sulfur dioxide at 1.23 μl/liter for 8 hr plus ozone at 0.13 μl/liter for 8 hr.

Necrosis. Slight injury treatments had between a trace and 8% necrosis, while severe injury treatments had between 7–89% necrosis in the selected exposures (Tables 1 and 2). The amount of necrosis was similar on both expanded and expanding leaves exposed to low levels of sulfur dioxide and ozone alone and to low levels of sulfur dioxide plus ozone (Table 1). The necrosis was much greater on expanded than expanding leaves exposed to high levels of sulfur dioxide and sulfur dioxide plus ozone (Table 2), but only slightly greater on expanded than expanding leaves exposed to high levels of ozone.

Necrotic injury from sulfur dioxide exposures consisted of bleached necrotic tissue extending from the adaxial surface through the leaf to the abaxial surface (bifacial injury) (Fig. 1-A). Necrosis from ozone exposures was composed of small flecks on the adaxial leaf surface (Fig. 1-B). Necrosis from combinations of sulfur dioxide plus ozone was quite similar to necrosis from exposures to ozone alone (Fig. 1-C). Thus, necrotic injury from pollutants in these exposures was similar to injury observed by other researchers (2, 6, 9, 13, 16).

Chlorophyll concentration. Chlorophyll concentration was reduced by 10–20% in nearly all leaves exposed to low levels of pollutants with the exception of expanding leaves exposed to sulfur dioxide (Table 1). Chlorophyll reductions were large (22–77% of controls) in nearly all leaves exposed to high levels of pollutants except in expanding leaves exposed to sulfur dioxide plus ozone (Table 2). The chlorophyll reductions were greater in expanded leaves compared to expanding leaves of plants exposed to sulfur dioxide and combinations of sulfur dioxide plus ozone, but were only slightly larger in expanded compared to expanding leaves exposed to ozone.

Dry weight. Dry weight of expanded leaves was not reduced for plants exposed to low levels of pollutants (Table 1). Dry weight of expanding leaves was reduced by approximately 20% in plants exposed to sulfur dioxide and the combination of sulfur dioxide plus ozone, but not in expanding leaves of plants exposed to ozone. Dry weight was reduced by 14–36% in all leaves exposed

Table 1. Injury to leaves of peas exposed to low levels of pollutants[z].

Leaf	SO_2 (1.23 µl/liter for 8 hr)		O_3 (0.17 µl/liter for 8 hr)		SO_2 (0.11 µl/liter for 4 hr) plus O_3 (0.11 µl/liter for 4 hr)	
			Necrosis (%)			
Expanded	2 ± 4	y	5 ± 5	NS	6 ± 8	y
Expanding	trace		8 ± 10		trace	
			Chlorophyll concn (% of controls)[x]			
Expanded	91 ± 13	NS	**90 ± 8**[w]	NS	**84 ± 7**	NS
Expanding	105 ± 9		**81 ± 12**		**88 ± 13**	
			Dry weight (% of controls)[x]			
Expanded	99 ± 12	NS	103 ± 21	NS	103 ± 27	NS
Expanding	**82 ± 41**		101 ± 27		**83 ± 18**	
			Surface area (% of controls)[x]			
Expanded	93 ± 19	NS	104 ± 14	*	101 ± 19	NS
Expanding	**75 ± 23**		80 ± 12		**86 ± 23**	

[z]Avg. ± SD for 6 single plant replicates for SO_2 and O_3 alone, and 12 single plant replicates for SO_2 plus O_3.
[y]No statistical calculations made.
[x]Avg. for control plants, expanded and expanding leaves, respectively; chlorophyll concentration of 18 and 21 mg/g dry weight, dry weight of 25 and 29 mg, surface area of 13 and 19 cm^2.
[w]Boldface values are significantly different from levels of control plants at 5% level.
*Injury to expanded vs. expanding leaves is significantly different at 5% level.

to high levels of pollutants (Table 2). The dry weight reductions were less in expanded than expanding leaves with all pollutants.

Surface area. Surface area was reduced slightly for expanded leaves exposed to low levels of sulfur dioxide, but was unchanged for expanded leaves exposed to low levels of ozone or sulfur dioxide plus ozone (Table 1). Surface area reductions of 14–25% occurred for expanding leaves exposed to low levels of all pollutants which were consistently greater reductions than for expanded leaves. Surface area was reduced by 16–34% for leaves exposed to high levels of pollutants (Table 2). The surface area reductions at the high levels were approximately the same for expanded and expanding leaves exposed to sulfur dioxide and sulfur dioxide plus ozone, but less for expanded than expanding leaves exposed to ozone.

Injury correlations. Correlation coefficients between injury measurements from leaves are shown in Table 3. Coefficients are reported only for expanded leaves because coefficients from expanding leaves were similar to those for expanded leaves.

Injury recorded as necrosis and injury recorded as a chlorophyll concentration reduction were highly correlated for all pollutants (Table 3). However, the ratio between the amount of necrosis and chlorophyll reduction differed between pollutants as indicated by the slopes from the individual regression equations. The ratio was approximately 1:1 for plants exposed to sulfur dioxide as shown by a linear equation slope of –0.894 (Fig. 2). The ratio was noticeably less than 1:1 for plants exposed to ozone (linear equation slope of –0.692) (Fig. 3). In these plants, leaves still had 20% of their chlorophyll remaining even though nearly 100% of the surface area was determined to be necrotic. The deviation in the ratio from 1:1 was most apparent for leaves exposed to combinations of

Table 2. Injury to leaves of peas exposed to high levels of pollutants[z].

Leaf	SO_2 (1.72 µl/liter for 8 hr)		O_3 (0.27 µl/liter for 8 hr)		SO_2 (1.23 µl/liter for 4 hr) plus O_3 (0.13 µl/liter for 4 hr)	
			Necrosis (%)			
Expanded	**67 ± 10**[y]	*	**89 ± 11**	*	**87 ± 14**	*
Expanding	15 ± 16		**74 ± 10**		7 ± 8	
			Chlorophyll concn (% of controls)[x]			
Expanded	**23 ± 14**	*	**29 ± 20**	*	**27 ± 20**	*
Expanding	**74 ± 16**		**44 ± 14**		**92 ± 7**	
			Dry weight (% of controls)[x]			
Expanded	**71 ± 15**	NS	79 ± 14	NS	86 ± 20	NS
Expanding	**64 ± 18**		70 ± 12		**68 ± 27**	
			Surface area (% of controls)[x]			
Expanded	**69 ± 11**	NS	**81 ± 17**	NS	84 ± 32	NS
Expanding	**69 ± 10**		**66 ± 13**		83 ± 29	

[z]Avg. ± SD for 6 single plant replicates.
[y]Boldface values are significantly different from levels of control plants at 5% level.
[x]Avg. for control plants, expanded and expanding leaves, respectively; chlorophyll concn of 19 and 20 mg/g dry wt, dry weight of 24 and 31 mg, surface area of 12 and 18 cm^2.
*Injury to expanded vs. expanding leaves is significantly different at 5% level.

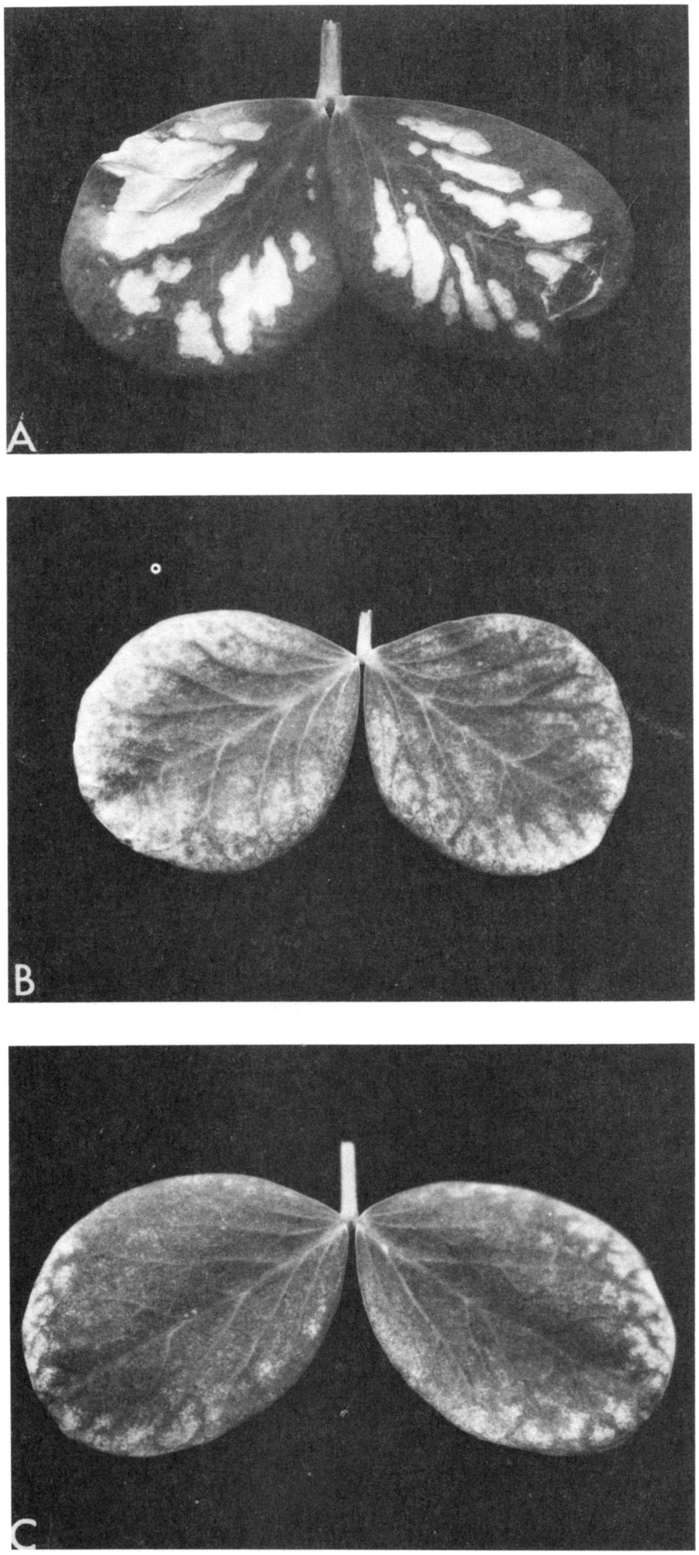

Fig. 1. Necrosis on leaves of peas with exposure to (A) sulfur dioxide, (B) ozone, and (C) sulfur dioxide plus ozone in combination.

sulfur dioxide plus ozone (Fig. 4). In these leaves there was nearly 30% of the chlorophyll remaining even through 100% of the surface area was determined to be necrotic. Furthermore, the regression equation for the sulfur dioxide plus ozone exposures was quadratic, not linear as for the sulfur dioxide and ozone alone exposures.

Table 3. Correlation coefficients for injury to expanded leaves[z].

Comparison	Necrosis	Chlorophyll concn	Dry wt
Chlorophyll concn			
SO_2	–0.84**		
O_3	–0.91**		
$SO_2 + O_3$	–0.79**		
Dry wt			
SO_2	–0.46**	0.29*	
O_3	–0.26*	0.25	
$SO_2 + O_3$	–0.21	–0.15	
Surface area			
SO_2	–0.45**	0.30*	0.60**
O_3	–0.46**	0.48**	0.59**
$SO_2 + O_3$	–0.12	0.16	0.71**

[z]One leaf from each of 50 plants.
*,** Significant correlation between parameters at 5 (*) and 1% (**) levels.

Injury recorded as dry wight reductions and injury recorded as surface area reductions were also highly correlated for all pollutants (Table 3). This high correlation was related to the close relationship between leaf dry weight and surface area.

Injury recorded as necrosis had a low correlation ($r < 0.5$) with injury recorded as dry weight reductions or surface area reductions (Table 3). Similarly, injury recorded as chlorophyll concentration reductions had a low correlation with injury recorded as dry weight reductions or surface area reductions.

Evaluation of measurements as indicators of leaf injury. The four measurement parameters: necrosis, chlorophyll concentration, dry weight, and surface area varied in sensitivity as indicators of injury to expanded vs. expanding leaves, and to plants exposed to low vs. high levels of pollutants.

1. Expanded leaves — low levels of injury. Chlorophyll con-

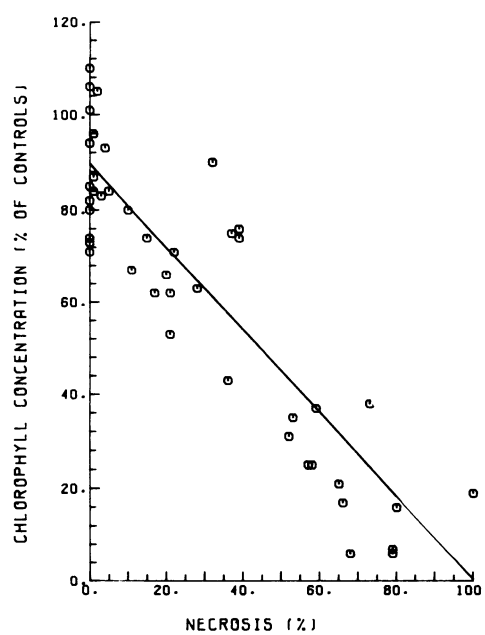

Fig. 2. Necrosis vs. chlorophyll concentration for leaves of peas exposed to sulfur dioxide. Chlorophyll concentration = 89.811 – 0.894 x % necrosis, correlation coefficient = –0.836, standard error of the estimate = 17.299, observations = 49.

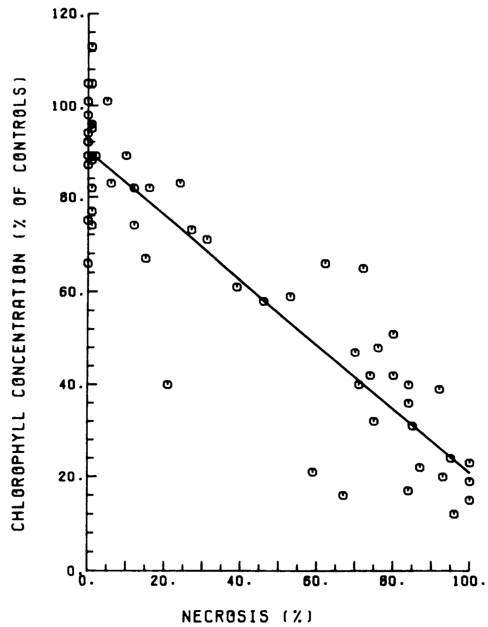

Fig. 3. Necrosis vs. chlorophyll concentration for leaves of peas exposed to ozone. Chlorophyll concentration = 89.993 – 0.692 x % necrosis, correlation coefficient = –0.909, standard error of the estimate = 12.227, observations = 57.

centration was the most sensitive indicator of injury to expanded leaves on plants exposed to low levels of pollutants. Chlorophyll concentrations were consistently reduced with the different pollutants: sulfur dioxide, ozone, and sulfur dioxide plus ozone. The chlorophyll concentration reductions exhibited low variability between exposed plants in the same treatment, which facilitated statistical analysis of the results of different treatments. Previously Rabe and Kreeb (15) had shown that chlorophyll concentration was a good indicator of injury with low levels of pollutants, but these workers did not differentiate between leaves with different degrees of expansion.

A potential disadvantage of the chlorophyll concentration measurements was the removal of other materials in addition to chlorophyll from the leaves during the alcohol extraction. This resulted in a lower dry weight in extracted than unextracted leaves, and, thus, potentially inaccurate determinations of chlorophyll concentrations expressed on a dry weight basis. However, it was determined that the loss in dry weight was approximately 20–25% in leaves regardless of the injury level.

The other parameters: necrosis, dry weight and surface area measurements were not as satisfactory indicators of injury as chlorophyll concentration for expanded leaves exposed to low levels of pollutants. Necrosis was found on some, but not all leaves, with the amount of necrosis varying greatly between leaves. Dry weight and surface area were not reduced on the expanded leaves, and thus these measurements could not be used to indicate plant injury.

2. Expanding leaves — low levels of injury. Surface area was the most sensitive indicator of injury to expanding leaves exposed to low levels of pollutants for this parameter was consistently reduced with all pollutants. Necrosis was not as satisfactory an indicator of injury as surface area because there was little necrosis on most leaves. The other two parameters: chlorophyll concentration and dry weight were not as satisfactory because they showed reductions for some, but not all pollutants.

3. Expanded leaves — high levels of injury. Necrosis was a reliable indicator of injury to expanded leaves exposed to high levels of pollutants. A large amount of necrosis occurred on leaves with all pollutants. Chlorophyll, dry weight and surface area reductions consistently occurred on expanded leaves exposed to high levels of pollutants, however, measurements of these injuries were not as easy to obtain as measurements of necrosis.

4. Expanding leaves — high levels of injury. Chlorophyll concentration, dry weight, and surface area measurements were all reliable indicators of injury to expanding leaves exposed to high levels of pollutants. Large reductions in all three parameters were found in plants exposed to the different pollutants and the amount of variability among exposed plants was about the same for all 3 measurements. Necrosis measurements were not as satisfactory indicators of injury as the other measurements because the amount of necrosis varied greatly with different pollutants.

Conclusions

All 4 parameters: necrosis, chlorophyll concentration, dry weight and surface area, can measure injury to leaves. However, different parameters are reliable indicators of injury under specific conditions: chlorophyll concentration for expanded leaves and surface area for expanding leaves exposed to low levels of pollutants, all measurements for expanded leaves and chlorophyll concentration, dry weight or surface area for expanding leaves exposed to high levels of pollutants.

Necrosis and chlorophyll concentration reduction measurements resulted in equivalent amounts of injury with sulfur dioxide exposures and thus should be interchangeable for injury determinations; whereas necrosis and chlorophyll concentration reductions were not equivalent with ozone and combinations of sulfur dioxide plus ozone.

The time, labor and instrumentation vary for each measurement. Thus, the measurement used will vary with the resources of each researcher. Therefore, for each air pollution experiment individual researchers need to weight these characteristics carefully to determine the best measurement of leaf injury to fulfill their requirements.

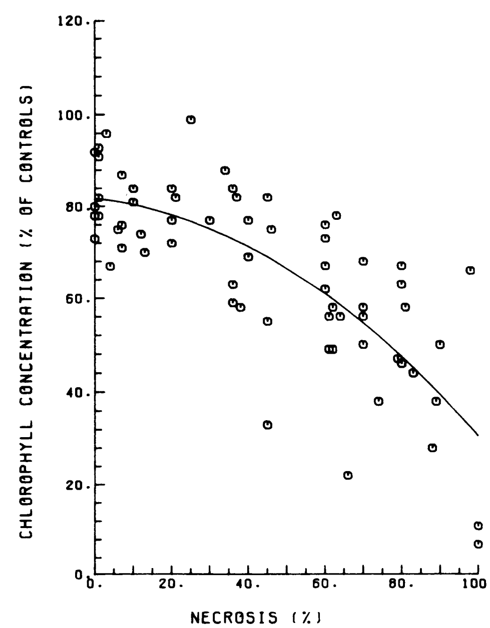

Fig. 4. Necrosis vs. chlorophyll concentration for leaves of peas exposed to sulfur dioxide plus ozone. Chlorophyll concentration = 81.806 – 0.103 x% necrosis – 0.00409 x % necrosis^2, correlation coefficient = –0.793, standard error of the estimate = 12.945, observations = 64.

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J. Amer. Soc. Hort. Sci. 107(2):271–274. 1982.

The Water Relations of Well-watered, Mycorrhizal, and Non-mycorrhizal Onion Plants[1]

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Additional index words. Allium cepa, Glomus etunicatus

***Abstract*. The water relations of mycorrhizal onions (*Allium cepa* L.) were compared with those of non-mycorrhizal controls grown under low and high soil phosphorus conditions. Mycorrhizal plants had higher leaf water potentials, higher transpiration rates, higher hydraulic conductivities and lower leaf resistances than did non-mycorrhizal plants grown in low soil phosphorus conditions. When controls were grown under high soil phosphorus conditions, all 4 parameters were not different from those of mycorrhizal plants. The magnitude of the effect of mycorrhizal fungi on the water relations of the host may, in part, be a function of phosphorus nutrition. The differences in leaf water potentials, transpiration rates and leaf resistances are considered to be the result of the differences found in hydraulic conductivities.**

Vesicular-arbuscular (VA) mycorrhizal fungi have been shown to improve plant growth by augmenting the phosphorus nutrition of the host plants (8, 12, 13, 17). In addition to changes in growth, changes in the water relations of mycorrhizal plants have been reported (16). In the first report of this type, Safir, Boyer and Gerdemann (14) showed an increase of about 60% in hydraulic conductivity to liquid water flow in soybeans when they were infected with the mycorrhizal fungus *Glomus mosseae*. Later, these same authors (15) demonstrated that the differences in hydraulic conductivity between mycorrhizal and non-mycorrhizal soybeans were eliminated after addition of a complete nutrient solution to the soil.

Levy and Krikun (7) reported differences in the water relations of mycorrhizal and non-mycorrhizal citrus plants upon recovery from a single episode of water stress. Upon rewatering, after 4 days of water withholding, the mycorrhizal plants appeared to recover more quickly than the non-mycorrhizal controls to a condition of higher stomatal conductance and higher photosynthetic

[1]Received for publication June 12, 1981. Michigan Agricultural Experiment Station Journal Article Number 9826.

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