

36-kD protein by pp60<sup>v-src</sup> occurs exclusively in the membrane (29).

Finally, in addition to being transformation-sensitive, the extent of myristylation of the 36-kD protein in CEF cells may be far less than that recently reported for pp60<sup>v-src</sup> protein kinase (31). Buss and Sefton (31) suggested a near stoichiometric modification of pp60<sup>v-src</sup> with myristate that may not differentially influence the structure, function, or location of pp60<sup>v-src</sup>. The partial acylation of the 36-kD protein may have a more regulatory role in this regard.

#### References and Notes

1. J. M. Bishop and H. Varmus, in *RNA Tumor Viruses*, R. Weiss, N. Teich, H. Varmus, J. Coffin, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), pp. 999-1108.
2. M. S. Collett, A. J. Purchio, R. L. Erikson, *Nature (London)* **285**, 167 (1980).
3. T. Hunter and J. A. Cooper, *Adv. Cyclic Nucleotide Protein Phos. Res.* **17**, 443 (1984).
4. K. Radke and G. S. Martin, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5212 (1979).
5. E. Erikson and R. L. Erikson, *Cell* **21**, 829 (1980).
6. J. A. Cooper, *J. Biol. Chem.* **258**, 1108 (1983).
7. V. Gerke and K. Weber, *EMBO J.* **3**, 227 (1984).
8. K. L. Gould, J. A. Cooper, T. Hunter, *J. Cell Biol.* **98**, 487 (1984).
9. M. E. Greenberg, R. Brackenbury, G. M. Edelman, *ibid.*, p. 473.
10. B. M. Sefton, I. S. Trowbridge, J. A. Cooper, E. M. Scolnick, *Cell* **31**, 465 (1982).
11. E. A. Garber, J. G. Krueger, H. Hanafusa, A. R. Goldberg, *Nature (London)* **302**, 161 (1983).
12. F. R. Cross, E. A. Garber, D. Pellman, H. Hanafusa, *Mol. Cell Biol.* **4**, 1834 (1984).
13. J. E. Buss, M. P. Kamps, B. M. Sefton, *ibid.*, p. 2697.
14. A. M. Schultz, L. E. Henderson, S. Oroszlan, *Science* **227**, 427 (1985).
15. M. F. G. Schmidt and M. J. Schlesinger, *Cell* **17**, 813 (1979).
16. —, *J. Biol. Chem.* **255**, 3334 (1980).
17. M. J. Schlesinger, A. I. Magee, M. F. G. Schmidt, *ibid.*, p. 10021.
18. M. B. Omary and I. S. Trowbridge, *ibid.* **256**, 4715 (1981).
19. S. Decker, *Biochem. Biophys. Res. Commun.* **109**, 434 (1982).
20. M. A. Bolanowski, B. J. Earles, W.-J. Lennarz, *J. Biol. Chem.* **259**, 4934 (1984).
21. M. F. G. Schmidt, *Curr. Top. Microbiol. Immunol.* **102**, 101 (1983).
22. S. A. Carr, K. Biemann, S. Shoji, D. C. Parmelee, K. Titani, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6128 (1982).
23. A. Aitken *et al.*, *FEBS Lett.* **150**, 314 (1982).
24. L. E. Henderson, H. C. Krutzsch, S. Oroszlan, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 339 (1983).
25. A. F. Voronova, J. E. Buss, T. Patschinsky, T. Hunter, B. M. Sefton, *Mol. Cell Biol.* **4**, 2705 (1984).
26. A. I. Magee and S. A. Courtneidge [EMBO J. **4**, 1137 (1985)] recently reported that neither palmitate nor myristate could be detected in a 36-kD protein which was immunoprecipitated from Rat-1 cell lysates. It should be noted that intense labeling in the region of a 36-kD protein was shown in their whole cell lysates labeled with [<sup>3</sup>H]myristate from normal and RSV-transformed CEF. Their labeling patterns were nearly identical to those reported here. Furthermore, the absence of fatty acid acylation of a 36-kD protein from Rat-1 cell lysates that was immunoprecipitated with their rabbit antiserum to chicken p36 was unaccompanied by any indication that 36-kD protein was, in fact, immunoprecipitated.
27. M. E. Greenberg and G. M. Edelman, *Cell* **33**, 767 (1983).
28. E. A. Nigg, J. A. Cooper, T. Hunter, *J. Cell Biol.* **96**, 1601 (1983).
29. S. Courtneidge, R. Ralston, K. Alitalo, J. M. Bishop, *Mol. Cell Biol.* **3**, 340 (1983).
30. E. Erikson, H. G. Tomasiewicz, R. L. Erikson, *ibid.* **4**, 77 (1984).
31. J. E. Buss and B. M. Sefton, *J. Virol.* **53**, 7 (1985).
32. J. S. Brugge and R. L. Erikson, *Nature (London)* **269**, 346 (1977).

33. U. K. Laemmli, *ibid.* **227**, 680 (1970).
34. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974).
35. E. N. Olson, L. Glaser, J. P. Merlie, *J. Biol. Chem.* **259**, 5364 (1984).
36. We have noted some slight variations in pattern intensity, but not total incorporation, with [<sup>3</sup>H]myristate labeling, as with [<sup>35</sup>S]methionine, when a comparison was made between cells at 41°C and 36°C. Quantitation by liquid scintillation of whole cell lysates, TCA precipitates, and the isolated 36-kD protein supports the observa-

tions made here that the myristate reduction in 36-kD protein is not due to a generalized alteration in myristate metabolism or a reduction in 36-kD biosynthesis.

37. Supported by grant CA-35378 (J.A.G.) and indirectly by grant CA-15823 from the National Institutes of Health. We thank S. Decker (Rockefeller University) for a generous supply of antiserum to the 36-kD protein and E. Erikson for help and encouragement.

7 June 1985; accepted 10 July 1985

## Ambient Levels of Ozone Reduce Net Photosynthesis in Tree and Crop Species

**Abstract.** Experiments were conducted to measure the photosynthetic response of three crop and four tree species to realistic concentrations of ozone and (for tree species only) simulated acidic rain. The ozone concentrations were representative of those found in clean ambient air, in mildly to moderately polluted air such as occurs in much of the United States during the summer, and in more heavily polluted air. However, the highest concentrations of ozone used were lower than those found regularly in the Los Angeles area. The mean pH of the simulated acid rain treatments ranged from more alkaline to much more acidic than the mean pH of precipitation in the United States. Exposure to any increase in ozone reduced net photosynthesis in all species tested. In contrast, acidic rain had no negative effect on photosynthesis in tree species, and no interaction between ozone and acidic rain was observed. Ozone-induced reductions in photosynthesis were related to declines in growth or yield. Species with higher stomatal conductances and thus higher potential for pollutant uptake exhibited greater negative responses to similar ozone treatments. Since exposure to ozone concentrations typical of levels of the pollutant observed in the eastern half of the United States reduced the rates of net photosynthesis of all species tested, reductions in net photosynthesis may be occurring over much of the eastern United States.

PETER B. REICH\*

ROBERT G. AMUNDSON  
Boyce Thompson Institute,  
Cornell University, Tower Road,  
Ithaca, New York 14853

\*Present address: Department of Forestry, University of Wisconsin, Madison 53706.

The question of how atmospheric pollution affects vegetation in North America is receiving much attention in the scientific and political communities. Forest decline has been observed on extreme sites such as mountaintops (1), and there is some evidence for decline in forest growth elsewhere (2, 3). However, the nature and magnitude of effects of atmospheric pollution on American forests are relatively unknown, although it is generally believed that the situation in the United States is not yet as dire as that in Central Europe (4, 5).

The agents responsible for the observed dieback of forest trees in the United States or for potential decline in forest growth have not been identified, but many candidates have been proposed. In addition to natural agents such as insects, drought, and disease, atmospheric pollution has been considered a prime factor (4, 5). Acidic rain is the

form of pollution currently receiving the most attention. Rainfall that is considered unnaturally acidic has been monitored across much of the United States, and its widespread occurrence has led to the suggestion of potentially large-scale impacts. However, in spite of considerable data indicating that acidic rain might be responsible for damage to lakes and aquatic systems (6), similar effects on terrestrial systems have not yet been documented.

In contrast, ozone (O<sub>3</sub>) is considered to cause the greatest amount of damage to vegetation of any gaseous pollutant (7). In much of North America during the summer, vegetation is exposed frequently, if not daily, to low or moderate concentrations of O<sub>3</sub> of anthropogenic origin. For instance, mean daily 7-hour (1000 to 1700 hours) concentrations of O<sub>3</sub> in major agricultural areas of the United States are estimated to be between 0.04 and 0.07 ppm during the growing season (8). Such concentrations have reduced yields of crops and growth of tree species without visibly injuring foliage (8-10). It is still not clear how O<sub>3</sub> causes such effects or why certain species or cultivars are more sensitive than others to this pollutant.

The net photosynthetic assimilation of

CO<sub>2</sub> ( $P_n$ ) is closely related to crop yield (11), and for all plants  $P_n$  largely determines the dry matter accumulation. Thus, effects of air pollutant stress on  $P_n$  should be directly related to effects on growth or yield. In fact, alteration of  $P_n$  could be the primary way in which gaseous pollutants affect plant growth. Reduced  $P_n$  as a result of exposure to low concentrations of O<sub>3</sub> (<0.15 ppm) has been reported for pine (12, 13), bean (14), and poplar (15); however, few other studies have assessed the response of  $P_n$  to low O<sub>3</sub> concentrations.

During the past several years in this laboratory we monitored the  $P_n$  response to O<sub>3</sub> of four tree and three crop species (Table 1). Either individual leaf or whole plant  $P_n$  was measured for each species in response to treatment with three or four concentrations of O<sub>3</sub>. In addition, the leaf diffusive conductance and water use efficiency (grams of water transpired per milligram of CO<sub>2</sub> uptake) of the plants were measured. Ten studies were conducted with the seven species. In four studies (soybean, wheat, clover, and red oak) we used open-top chambers (16) in the field, and in six studies (soybean, two white pine, hybrid poplar, sugar maple, and red oak) we used controlled-environment fumigation chambers (15). In each study, O<sub>3</sub> fumigations were applied during the same hours on the same days in all O<sub>3</sub> treatments. Thus, differences in concentration and dose (concentration multiplied by the number

of hours of exposure) between treatments within a study were exactly related. However, since different exposure regimes were used in the different experiments (for example, hours of fumigation per day, weeks of fumigation per experiment), comparisons between species will be made on the basis of dose-response. No "zero" O<sub>3</sub> treatments were used in any studies; instead, the lowest treatment level was in the range of background O<sub>3</sub> concentrations in clean ambient air (0.01 to 0.04 ppm).

To analyze the impact of long-term O<sub>3</sub> pollution on  $P_n$ , one must realize that  $P_n$  rates in all species change as leaves age (15). Therefore, for example, plants (or leaves) that have received O<sub>3</sub> treatments for 25 days and are 60 days old should not be compared directly to those that have received identical treatments for 25 days but are 45 days old. In our studies, comparisons between treatments were made between leaves (or plants) of similar ages that were simultaneously exposed to O<sub>3</sub> but that received different doses of O<sub>3</sub>. Also, for measurements of individual leaf  $P_n$ , dose was calculated for each leaf used and not per plant.

Detailed descriptions of the plant culture, exposure techniques, and experimental methods used will be presented elsewhere (15). We generated the O<sub>3</sub> used in the fumigations by exposing O<sub>2</sub> to an ultraviolet light source (laboratory) or to electrical discharge (field). We used Teflon lines to sample the chamber at-

mospheres; O<sub>3</sub> concentrations were measured with chemiluminescent O<sub>3</sub> monitors (model 8410E, Monitor Laboratories, San Diego, California) which were calibrated with an Environmental Protection Agency standard O<sub>3</sub> monitor (model 1003-PC, Dasibi Environmental Corporation, Glendale, California) dedicated for calibration purposes only. The amount of O<sub>3</sub> "lost" while chamber air was passing through the Teflon sampling lines was measured and treatment concentrations were adjusted for such losses.

Three or four O<sub>3</sub> treatments were used in each experiment (Table 1). Mean O<sub>3</sub> exposure concentrations were within the range of 0.02 to 0.14 ppm, and the maximum doses were less than 30 ppm-hour for the tree species and 15 ppm-hour for the crop species. By comparison, mean daytime concentrations of O<sub>3</sub> during the summer are 0.01 to 0.04 ppm in clean ambient air, 0.04 to 0.07 ppm in major agricultural areas of the central United States, and 0.10 to 0.15 ppm in southern California (8, 17). The dose of O<sub>3</sub> in most of the eastern United States during a 10-week period in the summer ranges between 30 to 45 ppm-hour. Thus, the experimental treatments met our objective of exposing plants to O<sub>3</sub> concentrations ranging from those found in clean air to those comparable to and slightly higher than currently found in rural areas.

We used several methods to measure

Table 1. Experiments conducted to measure net photosynthesis ( $P_n$ ) in response to frequent exposure to O<sub>3</sub>. Three species were also exposed to simulated acidic rain (see the last footnote). All laboratory studies were conducted with 15- or 16-hour photoperiods, day:night temperatures of 25°:20°C (except 22:17°C for sugar maple), and day:night relative humidities of 50:60 ± 5 percent.

Species	Mean O <sub>3</sub> concentration (ppm)	O <sub>3</sub> exposure			Type of P <sub>n</sub> measurement; technique used†
		Hours per day	Days per week	No. of weeks*	
<i>Laboratory studies</i>					
<i>Acer saccharum</i> ‡ (sugar maple)	0.03, 0.06, 0.09, 0.12	7.0	5	7	Leaf; mass balance
<i>Glycine max</i> cv. Hodgson (soybean)	0.01, 0.05, 0.09, 0.13	6.8	7	3	Leaf, whole plant; mass balance
<i>Pinus strobus</i> ‡ (eastern white pine)	0.02, 0.06, 0.10, 0.14	7.0	3	12	Whole plant; mass balance
<i>Pinus strobus</i>	0.02, 0.10, 0.14	7.0	5	12	Whole plant; mass balance
<i>Populus deltoides</i> <i>X trichocarpa</i> (hybrid poplar)	0.025, 0.085, 0.125	5.5	6–7	6	Leaf; mass balance
<i>Quercus rubra</i> ‡ (northern red oak)	0.02, 0.07, 0.12	7.0	5	10	Leaf; mass balance
<i>Field studies</i>					
<i>Glycine max</i> cv. Hodgson	0.017, 0.035, 0.060, 0.084, 0.122	7.0	7	3	Leaf; CO <sub>2</sub> depletion
<i>Quercus rubra</i>	0.023, 0.048, 0.068	7.0	7	9	Leaf; mass balance
<i>Trifolium repens</i> cv. Arlington (red clover)	0.019, 0.045, 0.070, 0.090	10.0	6	3	Leaf; <sup>14</sup> CO <sub>2</sub> fixation
<i>Triticum aestivum</i> cv. Voha (winter wheat)	0.027, 0.054, 0.076, 0.096	7.0	7	3	Head, whole plant; mass balance

\*Maximum exposure for foliage used in measurements of  $P_n$ .

†Except for the <sup>14</sup>C method, all measures of  $P_n$  were nondestructive and were made on intact foliage or plants. ‡All plants within each O<sub>3</sub> level were also exposed to acidic rain treatments: pH 5.6, 4.0, and 3.0 for sugar maple; pH 5.0, 4.0, and 3.0 for red oak; and pH 5.6, 4.0, 3.5, and 3.0 for white pine.

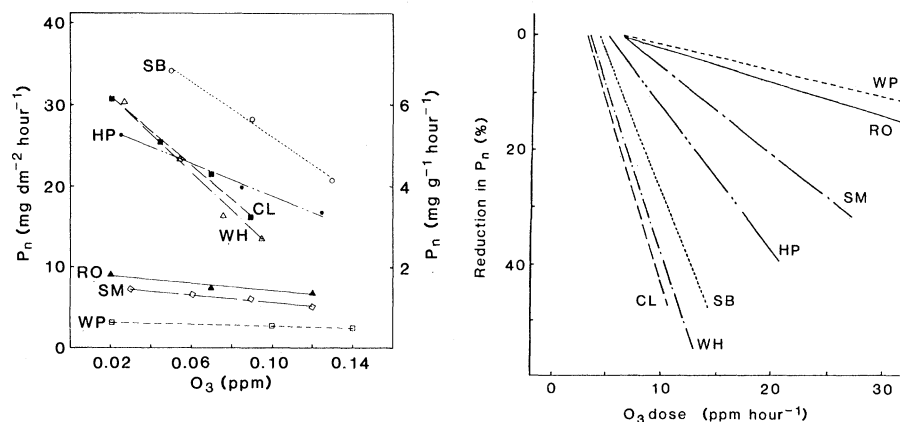


Fig. 1 (left). Net photosynthesis ( $P_n$ ) of seven species in response to mean  $\text{O}_3$  exposure concentration. The plants of each species were exposed to  $\text{O}_3$  for differing lengths of time and thus received different doses (see Fig. 2). Species abbreviations: CL, red clover; WH, wheat; SB, soybean; HP, hybrid poplar; SM, sugar maple; RO, northern red oak; and WP, eastern white pine. All  $P_n$  values are reported on a leaf area basis (milligrams per square decimeter per hour) except WP and WH, which are reported on a dry weight basis (milligrams per gram per hour). Each data point is, on average, a mean value of over ten determinations.  $P_n$  had a significant ( $P < 0.05$ ,  $t$  test) linear response to concentration in all species. Fig. 2 (right). Percent reduction in  $P_n$  (below the  $P_n$  of plants exposed to background doses of  $\text{O}_3$ ) for seven species in relation to increased dose of  $\text{O}_3$ . Symbols for species are explained in Fig. 1.

$P_n$ . The primary method was the continuous monitoring of  $\text{CO}_2$  mass balance. Intact, individual leaves of maple, oak, poplar, and soybean, and whole white pine seedlings, were placed inside portable thermoelectrically cooled and heated cuvettes (15). In the field,  $P_n$  was measured when the air temperature was 22° to 26°C. In each experiment, all  $\text{O}_3$  treatments were monitored for  $P_n$  under similar conditions of temperature, irradiance, and relative humidity. The air temperature controller in the cuvette was set to equal the ambient air temperature in either the growth chamber (see Table 1) or the field (open-top chamber). Air was pulled through the cuvette at a controlled flow rate (between 1 and 3 liter  $\text{min}^{-1}$ ), and the difference in the  $\text{CO}_2$  concentra-

tion between the airstream entering and the airstream leaving the cuvette was continuously monitored with an infrared  $\text{CO}_2$  analyzer (ANARAD model AR-6000). During measurements of  $P_n$ , the  $\text{CO}_2$  concentration in the cuvette was 320 to 330 ppm. Irradiance in the cuvette was generally about 700 and  $>1300 \mu\text{mol m}^{-2} \text{ sec}^{-1}$ , respectively, for measurements of  $P_n$  in the growth chamber and in the field (open-top chamber). Irradiance levels were greater than 80 percent of saturation for all species. Continuously stirred tank reactors modified from the design of Rogers *et al.* (18) were used to measure the  $\text{CO}_2$  mass balance of whole soybean plants, and cylindrical cuvettes were used in the field to measure the  $\text{CO}_2$  mass balance of a canopy of winter

wheat plants. With both systems,  $\text{CO}_2$  exchange was measured as described above. We measured photosynthesis in clover leaves by using a  $^{14}\text{CO}_2$  exposure technique (19). Stomatal conductance of water vapor was measured with a steady-state porometer (model LI-1600, LI-COR, Lincoln, Nebraska).

All data were subjected to analysis of variance. Treatment sums of squares (and degrees of freedom) were partitioned with the use of orthogonal contrasts; linear, quadratic, and, where applicable, cubic effects of the pollutants were tested by regression analysis. Once these effects had been determined, exposure-response equations (for both dose and concentration) were developed. Inasmuch as the number of  $\text{O}_3$  treatment levels was three to four, the analyses could only test for and contrast between linear, quadratic, and sometimes cubic responses.

In all seven species, long-term exposure to  $\text{O}_3$  caused a linear reduction in  $P_n$  in relation to  $\text{O}_3$  concentration (Fig. 1). In no case did the foliage show the "classic" symptoms of visible injury associated with acute exposure to  $\text{O}_3$  (20). Because of the differences in exposure regimes (hours per day and days per experiment) between experiments, it may be inappropriate as well as difficult to directly compare the responses of the species on the basis of a mean  $\text{O}_3$  exposure concentration. However, when the responses are compared on the basis of a unit dose of  $\text{O}_3$ , the results are more easily interpreted. The crop species (clover, soybean, and wheat) and hybrid poplar had high inherent rates of  $P_n$  (Fig. 1) and experienced much greater declines in  $P_n$  per unit dose of  $\text{O}_3$  than the three other tree species (Fig. 2). For instance,  $P_n$  was reduced by 50 percent in clover and wheat after these species had received a dose of 10 ppm-hour, whereas in white pine a reduction of 10 percent was observed after plants received a much higher dose (30 ppm-hour). In comparison, the dose (between 1000 and 1700 hours daily) from exposure to ambient  $\text{O}_3$  for 15 weeks in major agricultural regions of the United States (but not California) would average between 35 and 50 ppm-hour (8). Doses would be higher in parts of California.

Within species, reductions in growth were related to reductions in  $P_n$  (Fig. 3A). In addition, the relative (percentage) decline in  $P_n$  per unit dose of  $\text{O}_3$  of the species was apparently related to the respective relative decrease in growth per unit dose of  $\text{O}_3$  (Fig. 3B). Decreased  $P_n$  is probably the main cause of reduced growth in plants exposed to relatively

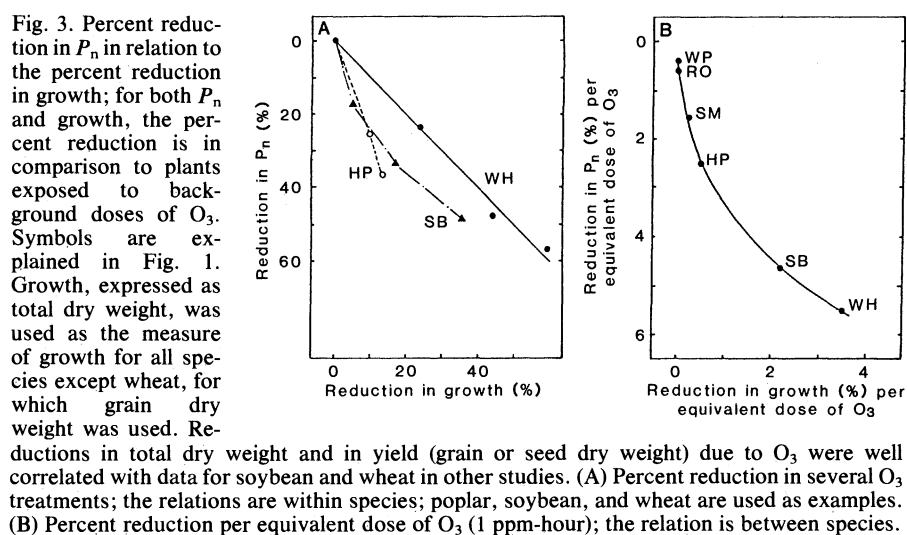


Fig. 3. Percent reduction in  $P_n$  in relation to the percent reduction in growth; for both  $P_n$  and growth, the percent reduction is in comparison to plants exposed to background doses of  $\text{O}_3$ . Symbols are explained in Fig. 1. Growth, expressed as total dry weight, was used as the measure of growth for all species except wheat, for which grain dry weight was used. Reductions in total dry weight and in yield (grain or seed dry weight) due to  $\text{O}_3$  were well correlated with data for soybean and wheat in other studies. (A) Percent reduction in several  $\text{O}_3$  treatments; the relations are within species; poplar, soybean, and wheat are used as examples. (B) Percent reduction per equivalent dose of  $\text{O}_3$  (1 ppm-hour); the relation is between species.

low concentrations of  $O_3$  (concentrations that do not cause symptoms of visible injury).

Furthermore, the apparent sensitivities of the species were well related to their inherent leaf conductances: there was a positive relation between the light-saturated leaf conductance of the plant under nonpolluted conditions and the average percentage reduction in  $P_n$  per unit dose of  $O_3$  (Fig. 4). Inasmuch as the uptake of  $O_3$  at low concentrations should be determined primarily by leaf conductance (21), the large inherent differences in conductance between the species would correspond to large differences in internal  $O_3$  dose, even at similar  $O_3$  concentrations. Therefore, in spite of species differences in response to apparently similar internal doses (see soybean versus poplar, Fig. 4), the internal dose a plant receives will, in general, be the most important factor determining the extent of impact of  $O_3$  pollution, and the internal dose is usually largely determined by leaf conductance. Clearly, other factors can also affect pollutant uptake by influencing stomatal function (22). For example, relative humidity, light, temperature, or drought can affect leaf conductance and thus the internal dose of  $O_3$ , thereby influencing the impact of a given  $O_3$  concentration on  $P_n$  or growth, or both. Drought-stressed soybean plants had lower conductances than similar but well-watered plants (23), and the relative effect of  $O_3$  on growth was less in the water-stressed treatment (24).

There are several ways by which  $O_3$  could affect  $P_n$  and leaf conductance. It could directly affect both processes, or it could directly affect one of them and indirectly influence the other. Since declining leaf conductance was not observed in pine, oak, or maple as a result of  $O_3$  exposure (25),  $P_n$  in these species much have been directly affected by  $O_3$ . Even for species in which leaf conductance was affected by  $O_3$ , examination of leaf conductance,  $P_n$ , and water use efficiency data indicates that  $P_n$  did not decrease as a result of stomatal closure but that, on the contrary, increased internal  $CO_2$  concentration, due to reduced  $P_n$ , was probably responsible for the decline in leaf conductance (15, 23, 26).

Low concentrations of  $O_3$  may affect  $P_n$  through several mechanisms. For instance,  $O_3$  reduced the quantum yield of poplar leaves at low light levels as well as  $P_n$  at saturated light levels (15) and reduced the content of RUBP carboxylase in alfalfa (27). Long-term exposure to  $O_3$  can also accelerate the leaf aging process. Such an acceleration appears to

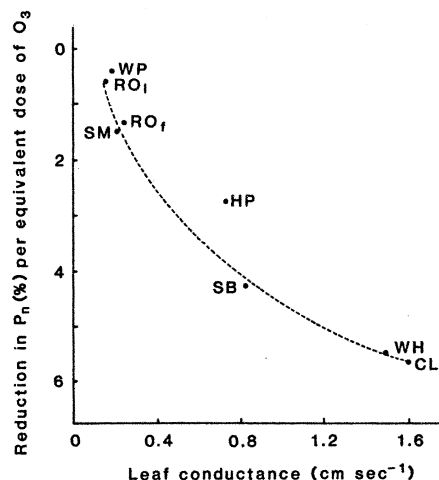


Fig. 4. Percent reduction in  $P_n$  per equivalent dose of  $O_3$  (1 ppm-hour) in relation to mean light-saturated leaf conductance (centimeters per second) of unpolluted plants. The relation is between species. Symbols are explained in Fig. 1; ROl, laboratory red oak; ROf, field red oak.

account, at least partially, for the way that  $O_3$  affects hybrid poplar (15, 28). Accelerated aging is a general response to many stresses, and the specific aging mechanism or mechanisms influenced by  $O_3$  may be as difficult to characterize as the overall processes of aging and senescence have been.

In contrast to the results with  $O_3$ , intermittent exposures for up to 10 weeks to simulated rain (acidic rain) with pH as low as 3.0 had no direct negative effects on  $P_n$  in sugar maple, red oak, or white pine seedlings. Seedlings were treated twice weekly with 1.25 cm of simulated rain (pH between 5.6 and 3.0). The rain solutions were acidified with a mixture of sulfuric and nitric acids (2:1 on a weight basis). Droplet size, deposition rates, and rainfall amounts were typical of ambient rainfall in the eastern United States. The acidity of the rain treatments ranged from more basic to much more acidic than the mean acidity of rainfall in the United States. For red oak and sugar maple, acidic rain treatments had no detectable effect on  $P_n$  or plant growth. In white pine,  $P_n$  and growth increased linearly with the increasing acidity of the acidic rain in seedlings grown in forest soils of low nitrogen availability, and there was no effect in soils with high nitrogen availability. There was no interaction between acidic rain and  $O_3$  treatment for any of the species: acidic rain did not alter the effect of  $O_3$  on  $P_n$ , and  $O_3$  exposure had no influence on the response to acidic rain. Acidic rain treatments did, however, affect mycorrhizal infection in oak and pine seedlings (29).

Frequent exposure of a variety of plant species to relatively low  $O_3$  concentrations caused reductions in  $P_n$  that were linear with respect to both  $O_3$  concentration and dose, and the reductions in  $P_n$  resulted in similar declines in growth or yield. Thus, for exposures of equal duration, an increase in  $O_3$  concentration from 0.01 to 0.05 ppm will have the same effect on  $P_n$  and growth as an increase from 0.06 to 0.10 ppm. Similarly, an increase in dose of  $O_3$  from 4 to 10 ppm-hour should be equivalent in its effect on  $P_n$  to an increase from 14 to 20 ppm-hour (30). Therefore, because the  $O_3$  concentrations and doses used in this study ranged from background (clean air) to moderately high levels, these data suggest that any increase in  $O_3$  concentration above those found in clean air will cause a decrease in  $P_n$ . Moreover, these results indicate that many plant species in the eastern United States probably are experiencing some decrease in  $P_n$  and growth as a result of current ambient  $O_3$  pollution. Field studies at various other sites corroborate this point (8, 10, 31).

In addition, our data suggest that response to  $O_3$  (as a general trend) is determined largely by the inherent gas exchange of the species in question. Fast-growing plants with high  $P_n$  (such as most crops) also have higher  $O_3$  uptake rates than plants (such as trees) with lower conductances; therefore, the annual measurable impact of  $O_3$  is greater for crop than for forest ecosystems. However, trees probably experience annual reductions in growth due to  $O_3$ , which may be compounded over time. If the growth of trees was reduced by even 1 to 2 percent annually, that would result in much larger reductions over one or two decades. Also, reduced vigor in trees could render them more susceptible to insects, diseases, or abiotic stresses such as cold or drought.

A distinction should be made between forests at high versus low elevations. Forests at high elevations experience a far more naturally stressed environment than other forests, and in North America dieback has been reported only for these areas. High-elevation forests may be responding to a different, or larger, set of stresses than other forests. The combination of natural stresses (for example, low fertility, extreme temperatures, lack of soil, high winds, low availability of water) with both  $O_3$  and acidic deposition may be responsible for forest dieback at high elevations. In other forests, reduction in growth has been tentatively observed for only a few species or areas, and it is possible that these forests are

responding primarily to long-term O<sub>3</sub> pollution. The situation is complex, however, since all forest ecosystems are composed of unique combinations of biotic, geologic, edaphic, and climatic factors.

The results of this study do not conclusively answer the question of how present levels of atmospheric pollution are affecting vegetation. In general, improved monitoring of ambient O<sub>3</sub> concentrations in agricultural and forested regions throughout the country must occur before good estimates of the extent of O<sub>3</sub>-induced reductions in P<sub>n</sub> and growth can be made. In addition, O<sub>3</sub> concentrations may reach peak values in ambient air at potentially critical times, such as during periods of leaf expansion, flowering, or seed maturation. Furthermore, many questions about the long-term effects of acidic rain on soils, plants, or soil microorganisms remain unanswered. The results of this and other studies do, however, indicate that O<sub>3</sub> is an important pollutant affecting vegetation. Researchers working toward identifying whether North American forests or agro-ecosystems are suffering a general decline and understanding the causes of such a decline must assess the impact of O<sub>3</sub> pollution as well as that of acidic deposition.

#### References and Notes

1. H. W. Vogelmann, *Nat. Hist.* **91**, 8 (1982).
2. T. G. Siccama, M. Bliss, H. W. Vogelmann, *Bull. Torrey Bot. Club* **109**, 162 (1982).
3. L. J. Puckett, *J. Environ. Qual.* **11**, 376 (1982).
4. A. H. Johnson and T. G. Siccama, *Environ. Sci. Technol.* **17**, 294A (1983).
5. G. H. Tomlinson II, *ibid.*, p. 246A.
6. C. L. Schofield, *Ambio* **5**, 228 (1976).
7. *Ozone and Other Photochemical Oxidants* (National Academy of Sciences, Washington, D.C., 1977).
8. W. W. Heck et al., *J. Air Pollut. Control Assoc.* **32**, 353 (1982).
9. P. B. Reich and R. G. Amundson, *Environ. Pollut.* **34**, 345 (1984).
10. S. F. Duchelle, J. M. Skelly, B. I. Chevone, *Water, Air, Soil Pollut.* **18**, 363 (1982).
11. I. Zelitch, *BioScience* **32**, 796 (1982).
12. P. R. Miller, J. R. Parmeter, Jr., B. H. Flick, C. W. Martinez, *J. Air Pollut. Control Assoc.* **19**, 435 (1969).
13. Y. Yang, J. M. Skelly, B. I. Chevone, J. B. Birch, *Environ. Sci. Technol.* **17**, 371 (1983).
14. D. P. Ormrod, V. J. Black, M. H. Unsworth, *Nature (London)* **291**, 585 (1981).
15. For example, see P. B. Reich, *Plant Physiol.* **73**, 291 (1983).
16. R. H. Mandl, L. H. Weinstein, D. C. McCune, M. Keveny, *J. Environ. Qual.* **2**, 371 (1973).
17. *SAROAD: Storage and Retrieval of Aerometric Data-Information* (EPA Publication 450/4-79-005, Environmental Protection Agency, Office of Air Quality Planning and Standards, Washington, D.C., 1979).
18. H. H. Rogers, et al., *J. Air Pollut. Control Assoc.* **27**, 1192 (1977).
19. L. D. Incoll and W. H. Wright, *Special Bulletin, Soils XXX/100* (Connecticut Agricultural Experiment Station, New Haven, 1969).
20. J. S. Jacobson and A. C. Hill, Eds., *Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas* (Air Pollution Control Association, Pittsburgh, 1970).
21. D. T. Tingey and G. E. Taylor, in *Effects of Gaseous Air Pollution in Agriculture and Horticulture*, M. H. Unsworth and D. P. Ormrod, Eds. (Butterworths, London, 1982), pp. 113-138.

22. S. G. McLaughlin and G. E. Taylor, *Science* **211**, 167 (1981).
23. P. B. Reich, A. W. Schoettle, R. G. Amundson, *Physiol. Plant.* **63**, 58 (1984).
24. R. G. Amundson, R. M. Raba, A. W. Schoettle, P. B. Reich, *J. Environ. Qual.*, in press.
25. P. B. Reich and P. G. Amundson, unpublished results.
26. P. B. Reich and J. P. Lassoie, *Plant, Cell Environ.* **7**, 661 (1984).
27. E. J. Pell and N. S. Pearson, *Plant Physiol.* **73**, 185 (1983).
28. P. B. Reich, J. P. Lassoie, R. G. Amundson, *Can. J. Bot.* **62**, 2835 (1984).
29. P. B. Reich, A. W. Schoettle, H. F. Stroo, J. Troiano, R. G. Amundson, *ibid.*, in press; P. B. Reich and R. G. Amundson, unpublished data.

30. Ozone concentration and dose were correlated for a given species in these studies; not enough information is available to permit one to use dose to predict P<sub>n</sub> in all cases. To our knowledge, no one has yet tested whether the same dose applied in the form of several low concentrations for differing exposure periods (for example, 0.08 ppm for 3 hours per day versus 0.03 ppm for 8 hours per day) would have the same effects or not. However, our data comparing different species suggest that, at relatively low concentrations, dose should be an effective measure of O<sub>3</sub> exposure.
31. R. K. Howell, E. J. Koch, L. P. Rose, Jr., *Agron. J.* **71**, 285 (1979).

12 December 1984; accepted 5 July 1985

## Functional Relation Between HTLV-II x and Adenovirus E1A Proteins in Transcriptional Activation

**Abstract.** *The mechanism of cellular transformation by the human T-cell leukemia viruses (HTLV) is thought to involve a novel gene known as the x gene. This gene is essential for HTLV replication and acts by enhancing transcription from the HTLV long terminal repeat. The HTLV x gene product may also cause aberrant transcription of normal cellular genes, resulting in transformation of the infected cells. Although there is no evidence as yet for such a mechanism, it was shown that the HTLV-II x gene product can activate transcription from adenovirus E1A-dependent early promoters and therefore has the potential to activate cellular genes. It was also shown that the adenovirus and herpes pseudorabies immediate early proteins activate expression from the HTLV-I and HTLV-II long terminal repeats, though at lower levels than with the x gene product. These findings indicate possible common mechanisms of action for transcription-regulatory genes of distinct viruses.*

IRVIN S. Y. CHEN

ALAN J. CANN

NEIL P. SHAH

RICHARD B. GAYNOR

*Division of Hematology-Oncology, Department of Medicine, and Jonsson Comprehensive Cancer Center, UCLA School of Medicine, Los Angeles, California 90024*

The human T-cell leukemia viruses type I (HTLV-I) and type II (HTLV-II) are associated with specific human T-cell malignancies (1). HTLV-I is the apparent etiologic agent of adult T-cell leukemia, a highly malignant leukemia/lymphoma endemic to parts of Japan, the Caribbean, and Africa (1). HTLV-II has been associated with a single case of T-cell variant hairy-cell leukemia (1-4). Both types of HTLV transform normal human cord or peripheral blood T lymphocytes in vitro, as defined by the continued proliferation of the T cells in the absence of exogenous interleukin-2 (5). The mechanism of cellular transformation by HTLV is unknown. HTLV is unlike acutely transforming retroviruses in that no viral oncogene is present in the HTLV sequence (3, 4), and it is unlike the nonacute leukemogenic retroviruses in that it does not appear to induce malignancies by association with cellular oncogenes (6). Therefore, HTLV appears to transform cells by a mechanism

different from that of other retroviruses.

Both HTLV and bovine leukemia virus (BLV), an oncogenic virus of cattle sharing many biological and molecular features with HTLV (7), have a gene termed *x* or *lor* (3, 4) that is not present in the genomes of other animal retroviruses. This gene, which is hypothesized to be the transforming gene of HTLV and BLV, encodes a protein of 40 kD and 37 kD in HTLV-I- and HTLV-II-infected cells, respectively (8). The *x* gene is essential for HTLV replication and acts to enhance transcription by the HTLV long terminal repeat (LTR) (9). Mutants of HTLV that are defective for *x* gene functions transcribe only very low levels of HTLV RNA. The *x* gene product has a nuclear subcellular localization consistent with its proposed function in transcriptional activation (10).

Certain DNA viruses have gene products that act to enhance transcription by some viral promoters (11). For example, a gene product expressed by the E1A gene of adenovirus early after viral infection facilitates transcription by six unique adenovirus promoters (11), several endogenous cellular genes (12), and newly transfected genes (13). In addition to those genes transcribed by RNA polymerase II, the E1A proteins can also activate genes transcribed by RNA polymerase III (14). Adenovirus mutants lacking the E1A gene can transcribe