

Acute Foliar Injury of Eastern White Pine Induced by Sulfur Dioxide and Ozone

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ABSTRACT

Ramets of eastern white pine (*Pinus strobus*) were exposed to both SO₂ and O₃ in a controlled-environment chamber. Current needles between 4 and 5 weeks old were acutely injured by SO₂ at dosages ranging from 15 ± 5 parts/hundred million (pphm) for 2 hr to concentrations as low as 5 ± 1.5 pphm for 1 hr. These results demonstrated that certain strains of eastern white pine trees are extremely sensitive to low levels of SO₂. New needles

on different branchlets of the same tree could be independently injured by either O₃ or SO₂ at approximately 25 ± 5 pphm for 2 hr. Sulfur dioxide was considerably more phytotoxic than O₃. Macroscopic differences in development of injury on new needles induced by both gases are discussed. No histological differences of diagnostic significance were noted between the two kinds of injury. Phytopathology 60:994-999.

The gross effects of low concentrations of atmospheric O₃ on new needles of eastern white pine (*Pinus strobus* L.) were first reported by Berry (1) and later by Berry & Ripperton (3). Recently, a more detailed study of O₃ injury on white pine was reported by Costonis (6). During the course of this latter study, another type of injury was frequently noted on new needles of eastern white pine in the field similar in appearance to O₃ injury. This new injury, however, occurred when recorded O₃ concentrations were below the known injury threshold of the ozone-sensitive trees under study. Because the injury was so similar to the syndrome induced by O₃ and occurred on trees selected for their known sensitivity to O₃, it seemed probable that another atmospheric pollutant was the incitant. The further possibility that certain trees might be sensitive to more than one air pollutant was raised. The nonozone-induced injury was most often observed on sensitive trees close to powerplants where the chief gaseous pollutant was SO₂. A series of experiments were conducted to establish whether low concentrations of SO₂ caused the acute foliar injury observed, and to determine whether some individual trees were independently sensitive to both O₃ and SO₂. In addition, macroscopic and histologic development of the acute injury induced by these gases were compared.

MATERIALS AND METHODS.—Eastern white pine ramets (scions grafted on nursery-run 2-year-old rootstocks) known from preliminary testing to be sensitive to air pollutants were employed in all experiments. The use of these vegetatively reproduced eastern white pines (ramets) provided genetic homogeneity in all experiments. Ramets were potted in 1-gal metal pots containing forest topsoil. All ramets were maintained in a greenhouse where the temp varied from 18 C at night to around 32 C during the daylight hr. Pots containing the ramets were watered to soil saturation each day. Completely randomized block experimental designs were employed in all experiments. Branchlets were randomly selected and serially tagged before treatment. Ten fascicles/branchlet were marked on the

basal scales with a drop of artist's oil paint to insure that the same fascicles could be observed each time data were taken. Treatments were evaluated immediately after fumigation and twice each day for the next 7 days. A fascicle was considered injured if any one needle/fascicle was injured. Subsequent readings were taken at selected intervals thereafter. Selected branchlets on all trees were covered with polyethylene bags immediately before fumigation. Preliminary tests revealed that neither O₃ nor SO₂ could penetrate the bags. Thus, branchlets on the same trees were used as controls on the effects of fumigation. The bags were removed immediately after fumigation.

Sulfur dioxide (Matheson Company, 1% SO₂ in 99% N₂) was introduced into the chamber directly from a pressurized cylinder. Concentrations were monitored with a Davis Series 11-7000 SO₂ analyzer (Davis Instruments, Newark, New Jersey) and manually controlled by means of a flowmeter and needle valve. Accuracies were maintained at approximately ± 5 pphm of the desired concentrations in the ranges of 8-25 pphm. In the range of 5.0 pphm, deviations of ± 1.5 pphm of the concentration desired were obtained. Air samples were also manually collected and analyzed colorimetrically for SO₂ by a modification of the method described by West & Gaeke (17). Concentrations of SO₂ determined by the colorimetric method and the Davis instrument generally agreed closely (within 2 pphm). All of the SO₂ concentrations reported in this paper are the continuous readings obtained from the Davis instrument. All fumigations were conducted in a fumigation chamber described by Berry (2).

Ozone was generated by passing filtered tank oxygen through a modified Krona-type silent discharge unit (Vita-Aire Process Co., Milwaukee, Wisconsin) and added as needed to recirculating ambient air in the chamber. The concentration was manually controlled by means of a flowmeter and needle valve. Concentrations were maintained within ± 1 pphm of that desired. Ozone was continuously monitored with a Mast ozone meter 724-2 (Mast Development Co., Daven-

port, Iowa) during fumigations. Air samples were also manually collected and analyzed colorimetrically for ozone by a method employing neutral-buffered KI as described by Byers & Saltzman (4). Concentrations reported in this paper were determined by the Mast instrument and averaged 82.6% of those determined colorimetrically.

Observations of severity of acute foliar symptoms were quantified by the use of the following numerical ratings: 1, no visible symptoms; 2, minute silvery flecks, in groups of 1-2 mm extent, radiating from the stomata, seen best under magnification ($\times 60$); 3, yellow lesion up to 3 mm long accompanied by a sinking of the stomatal face with internal resin secretion; 4, pink lesion 3-5 mm long, easily seen with the naked eye; 5, brown lesion 3-6 mm long; 6, necrosis of up to 1 cm of needle tip; 7, tip necrosis involving 1-2 cm; 8, tip necrosis involving 2-3 cm.

Samples of injured foliage were photographed in color and black and white. Additional samples were saved as herbarium specimens. Sections of fresh needles depicting all stages of injury, 10-25 μ thick, were cut with a freezing microtome, mounted in a drop of clear 20% glycerine on a microslide, and covered with a coverslip. Sections were examined at magnifications of $\times 100$ to 1,000.

Four separate experiments employing four different concentration and time combinations of SO_2 were conducted on four different groups of sensitive ramets. New foliage was between 3 to 5 weeks of age during any given treatment. In the first experiment, eight sensitive ramets were fumigated with 15 ± 5 ppm SO_2 for 2.0 hr. Ten fascicles on three branchlets/tree were marked as previously described. Two branchlets/tree were treated, while one branchlet/tree, protected with a polyethylene bag during the fumigation period, served as a check. In the second experiment, 10 sensitive ramets, each with three branchlets available for treatment, were selected in the same manner previously described. Two branchlets/tree were enclosed in polyethylene bags just prior to fumigation. The one exposed branchlet/ramet was then fumigated with 10 ± 3 ppm SO_2 for 2.5 hr.

The same ramets were then fumigated approximately 24 hr later with 8 ± 3 ppm SO_2 for 2.0 hr. For this fumigation, one of the previously protected branchlets on each of the ramets was exposed and the remainder was bagged. Thus, each tree finally bore one branchlet that had been fumigated with 10 ± 3 ppm SO_2 for 2.5 hr, one that had been fumigated with 8 ± 3 ppm SO_2 for 2.0 hr, and one that had been protected with a polyethylene bag during both treatments. In the third experiment, six sensitive ramets and four resistant ramets were simultaneously fumigated with 8 ± 3 ppm SO_2 for 2.0 hr.

In the fourth experiment, seven sensitive ramets, each with four branchlets available for treatment, were selected. Each ramet was treated in the following way: three branchlets/tree were enclosed in polyethylene bags just prior to fumigation; one exposed branchlet/tree was then fumigated with 5.0 ± 1.5 ppm SO_2 for 1.0 hr; these treated branchlets were then immediately

covered with polyethylene bags and one bagged branchlet was exposed and then fumigated with 5.0 ± 1.5 ppm SO_2 for 2.0 hr. Thus, at the end of the experiment each of the ramets bore one branchlet that had been fumigated with 5.0 ± 1.5 ppm SO_2 for 1 hr; one with 5.0 ± 1.5 ppm SO_2 for 2.0 hr; one with 5.0 ± 1.5 ppm SO_2 for 3.0 hr; and one branchlet that had been protected with a polyethylene bag during all fumigations. In addition to the seven sensitive trees, one resistant ramet was treated simultaneously and in the same manner as the sensitive ramets.

Three air pollutant-sensitive ramets, each with five branchlets available for treatment, were selected when the foliage was about 4 weeks old in order to study the effects of SO_2 and O_3 on new foliage of the same ramets. Three branchlets/tree were enclosed in polyethylene bags just before treatment. Two branchlets/ramet were fumigated with 30 ± 1 ppm of O_3 for 2 hr. This time-concentration combination of O_3 was employed to ensure that acute injury would develop in order to compare the resulting O_3 and SO_2 treatments on different fascicles of the same ramets. Two of the bagged branchlets on each of the same ramets were fumigated approximately 24 hr later with 25 ± 5 ppm SO_2 for 2.0 hr. Thus, each tree finally bore two branchlets that had been fumigated with SO_2 , two that had been fumigated with O_3 , and one control branchlet that had been protected with a polyethylene bag during both fumigations. All bags were removed from the branchlets after the second fumigation, and the plants were returned to the greenhouse.

RESULTS.—Effect of low concentrations of SO_2 and O_3 on new needles of sensitive ramets.—It is apparent from Table 1 that the new needles on all the fumigated sensitive ramets were acutely injured by all of the treatments. Symptoms were most severe on ramets fumigated for longer time periods. For example, 5.0

TABLE 1. Acute foliar injury on new needles of eastern white pine sensitive to low levels of sulfur dioxide^a

Treatment	Concentration	Duration	Lesion severity rating ^b		Fascicles injured ^c
			avg	%	
1	15.0 \pm 5.0	2.0	6.0	100	
2	10.0 \pm 3.0	2.5	7.8	100	
3	10.0 \pm 3.0	1.0	5.0	100	
4	8.0 \pm 3.0	2.0	4.5	97.3	
5	5.0 \pm 1.5	3.0	8.0	100	
6	5.0 \pm 1.5	2.0	5.1	100	
7	5.0 \pm 1.5	1.0	3.1	100	
All controls ^d	0.0	0.0	1.0	0	

^a All ramets fumigated under natural sunlight (average 0.21 cal/cm² per min) during treatment. Temperatures ranged between 26-31 C; relative humidity, 80 \pm 10%.

^b Injury rated from 1 (healthy needle) to 8 (tip necrosis involving up to 3 cm). Final injury severity ratings were made 7 days after each treatment.

^c Per cent based on 60 fascicles in treatment 1; 100 in treatment 2; 70 in treatment 3; 20 in treatment 4; and 70 fascicles in treatments 5-7.

^d Ten fascicles on one branchlet/ramet fumigated in all treatments were protected by a polyethylene bag only during the treatment. The bag was removed immediately after the treatment.

pphm SO_2 for 3.0 hr caused as much injury as 10 pphm SO_2 for 2.5 hr. It is significant that 5.0 ± 1.5 pphm of SO_2 for 1 hr was sufficient to cause acute injury on new sensitive needles. None of the fascicles in any of the treatments were ever injured in the bags. In addition, five ramets selected for their known tolerance to SO_2 were simultaneously treated with the sensitive plants during all treatments and were not visibly injured.

Description of acute SO_2 injury on new needles of sensitive eastern white pine.—Injury caused by low levels of SO_2 has never been described in detail. The initial macroscopic symptom of acute SO_2 injury on new needles appears 4-6 hr after treatment as a slightly sunken, resin-impregnated area 2-5 mm long on the stomatal-bearing face of the needles (Fig. 1-A, left; B, arrow). It usually occurs from 2 to 10 mm above the basal scales and is barely perceptible to the naked

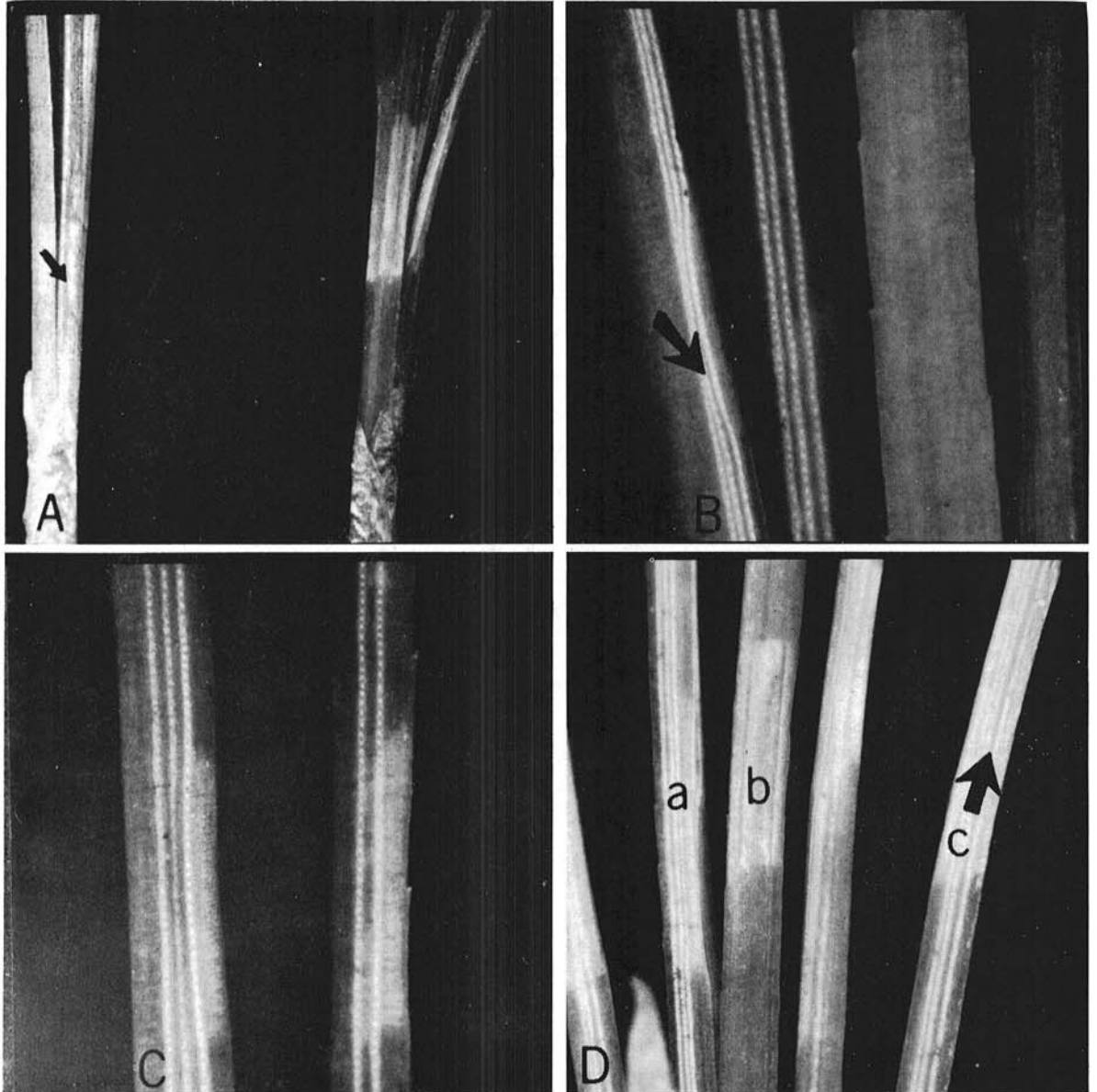


Fig. 1. Comparison of injury induced by SO_2 and O_3 on current needles of eastern white pine. Fumigation treatment: **A**) (left), **B**, **C**, **D**) 25 ± 5 pphm SO_2 for 2.0 hr; **A**) (right) and **C**) (right), 30 ± 1 pphm O_3 for 2.0 hr. In **A**) (left), note collapse of needle tissue with no banding about 6 mm from basal scales (arrow). In **A**) (right), note banding effect in affected needle tissue. Photograph taken about 48 hr after treatment ($\times 48$). **B**) Closeup of collapsed area of stomatal face of needle in the semimature tissue (arrow). Photograph taken about 24 hr after treatment ($\times 200$). **C**) Similarity of injury induced by SO_2 (left) and O_3 (right) on comparable needles of eastern white pine. Photograph taken about 48 hr after treatment ($\times 200$). **D**) Different types of lesions caused by SO_2 on new needles: collapsed area on stomatal face of needle (a), band on abaxial face (b), and tip necrosis progressing distally (arrow) in needle.

eye. It is best seen with a stereoscopic microscope ($\times 60-120$) and viewed on a black background. Within 10-24 hr after fumigation the lesion becomes readily apparent, even to the naked eye (Fig. 1-C, left). A critical examination of the lesion at this stage reveals a pink spot on the stomatal face of the needle in the injured area and a collapsed resin-soaked area on the abaxial needle face (Fig. 1-D, b). Necrosis of the needle tissue progresses distally from the point of attack (Fig. 1-D, c). The injured tissue changes from an olivaceous green to light brown and then becomes a conspicuous bright orange-brown about 2 weeks after exposure to the gas. All needles in a fascicle are usually not equally affected by a given exposure to SO_2 , nor is the injury always uniform from fascicle to fascicle or from tree to tree. It is common to find all stages of lesion development on a given fascicle (Fig. 1-D, a, b, c).

Histological examination of a lesion beginning to develop revealed a partial or complete collapse of the mesophyll cells near the stomata (Fig. 2-A, E). Within 24-48 hr, lesions may progress from the incipient stage to yellowish-pink bands. This is clearly seen histologically as groups of dead mesophyll cells accompanied by a copious internal resin secretion (Fig. 2-E). A vascular discoloration often accompanies death of the mesophyll cells (Fig. 2-E).

A comparison of the type injury caused by SO_2 and O_3 on new needles from the same ramet was made. Differences in development of injury induced by the two gases is clearest within the first 24 hr after treatment. The SO_2 -induced lesion begins as a slight yellowing and is characterized by a collapsing of the semimature tissue (12) accompanied by an internal resin secretion on the stomatal face of the needle. Death of the needle may occur as early as 5 days after treatment. Often all five needles in a fascicle are equally affected.

By way of contrast, O_3 -induced lesions develop as minute silvery flecks on the stomatal face of the needle. Semimature tissue is the most severely affected, but immature and mature tissues are often also affected to a lesser degree. Usually tip necrosis takes between 1 and 2 weeks to develop, with only one or two needles in a fascicle being affected. Ozone causes a bleaching of treated needles.

After about the first 72 hr of lesion development, it is very difficult to distinguish between lesions induced

by either gas. Sulfur dioxide and O_3 injuries are similar in that death of the tissue progresses distally; the mesophyll cells adjacent to the stomata are the first to show visible injury while the endodermis and stele are the last tissues to be affected. New, rapidly elongating needles from about 1 up to 6 weeks of age are most sensitive to the gases. Susceptibility to both gases appears to be genetically controlled.

DISCUSSION.—The results of this study demonstrated that concentrations of SO_2 as low as 5.0pphm for periods of time as short as 1.0 hr can cause acute injury to new needles of certain sensitive strains of eastern white pine under artificial conditions. This finding conflicts with reports of previous investigators (7, 8, 9, 10) who indicated that threshold concentrations in the magnitude of 25pphm of SO_2 for several hr were required to induce acute foliar injury on eastern white pine growing in forests near smelters at Sudbury, Ontario, and at Trail, British Columbia.

This study has also demonstrated that certain strains of eastern white pine trees may be injured by either of the two pollutants, SO_2 or O_3 . The induction of acute foliar injury on needles of different branchlets of the same tree permitted a critical comparison of injury by the two toxicants in the same genetic system. The pattern of lesion development is similar and could easily be confused. Differentiation between the two kinds of injury is possible only when observations are made on a daily basis immediately after occurrences of toxic levels of the gas in question. Histopathologic techniques, coupled with records of ambient levels of the gases, will be necessary to establish which gas induced the injury.

Also from recent studies in Ontario, Linzon (11, 12) has described "semimature-tissue needle blight (SNB)", and has compared the symptoms of SNB with those of SO_2 injury. He concluded that they are distinct disorders (10, 13), although he was unable to identify the SNB causal agent. It should be pointed out, however, that Linzon has neither monitored SO_2 in forested areas remote from SO_2 sources where SNB was studied nor fumigated SNB-sensitive ramets with SO_2 artificially. This work is needed to determine whether or not SO_2 alone or with some cofactor(s), such as O_3 , is involved in this disease.

It is important to note that in the 3-year period reported by Linzon (12, 14) only seven outbreaks of

TABLE 2. Injury induced on current year's foliage of eastern white pine sensitive to both ozone and sulfur dioxide^a

Treatment	Concentration	Duration	Avg exposed length of new needles	New lesion		Fascicles injured ^e
				From tip of needle	Severity rating ^b	
	<i>pphm</i>	<i>hr</i>	<i>mm</i>	<i>mm</i>	<i>avg</i>	<i>%</i>
SO_2	25 ± 5	2.0	22.0	20.0	7.2	100
O_3	30 ± 1	2.0	21.0	15.0	5.1	100
Control ^d	0	0.0	18.0	0.0	1.0	0

^a All ramets fumigated under natural sunlight (average 0.21 cal/cm^2 per min) during treatment. Temperatures ranged between $26-31 \text{ C}$; relative humidity, $80 \pm 10\%$.

^b Lesions rated from 1 (healthy) to 8 (tip necrosis up to 3 cm).

^c Percentage based on 60 fascicles/treatment.

^d Thirty fascicles/treatment were protected with a polyethylene bag.

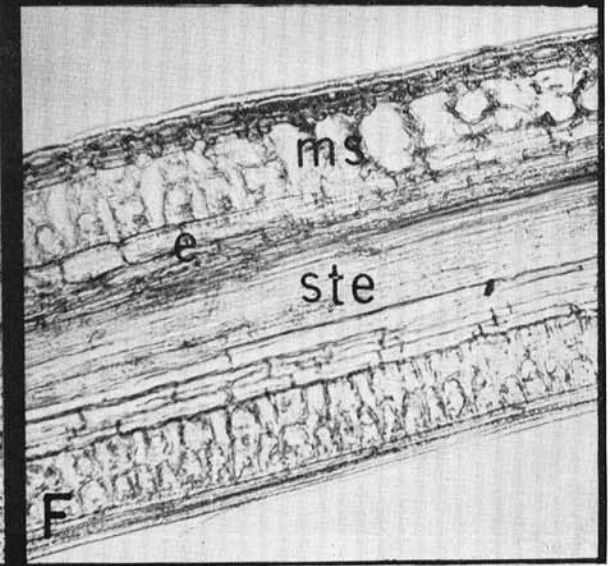
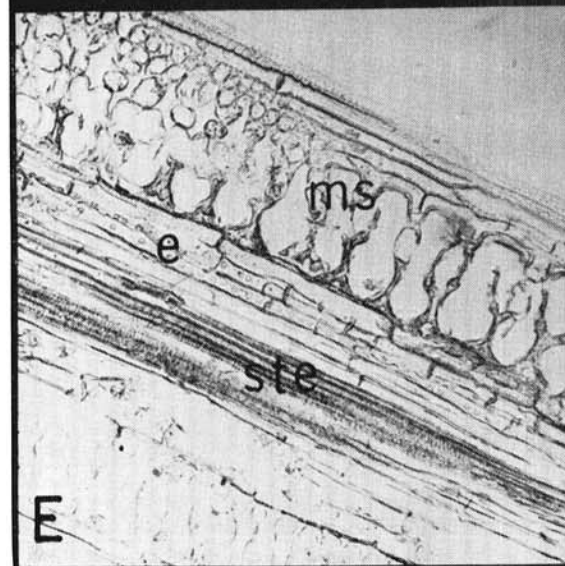
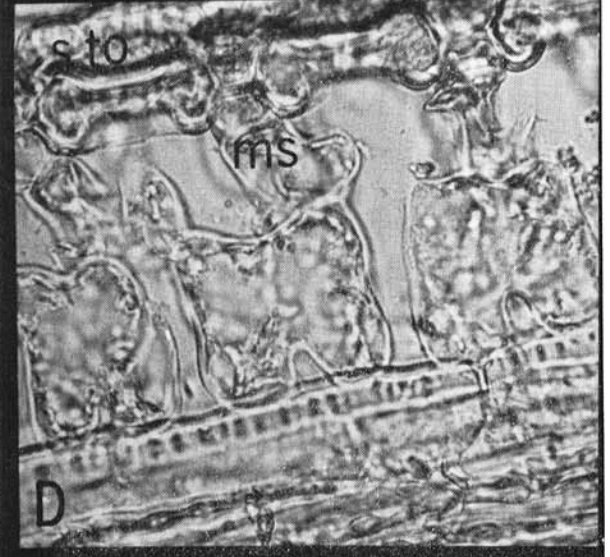
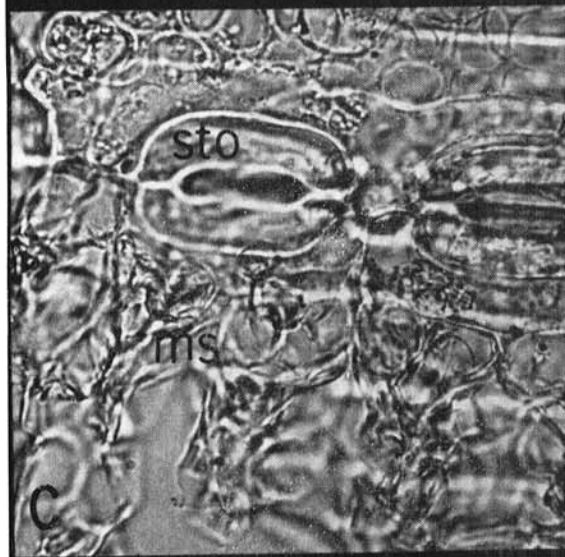
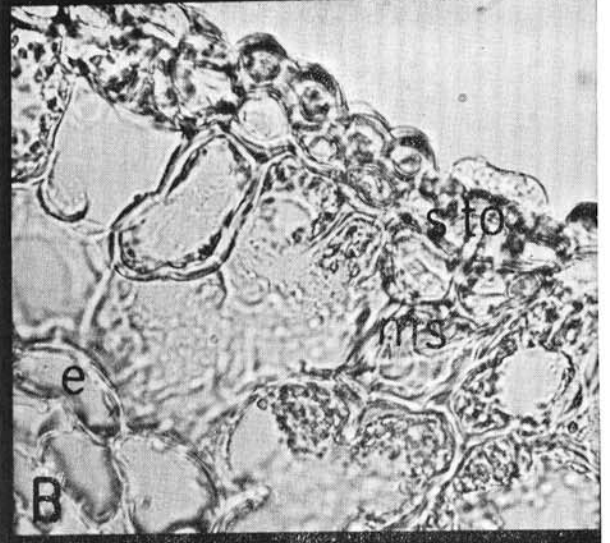
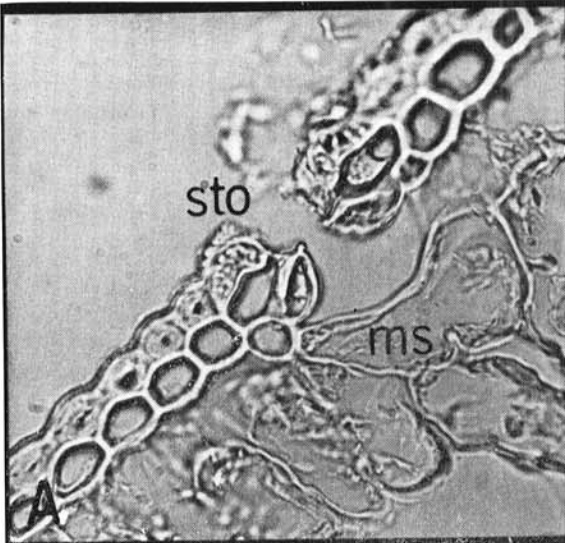


Fig. 2. Histologic comparison of acute injury induced independently by SO₂ and O₃ on comparable current needles of eastern white pine. Fumigation treatments: **A, C, E**) 25 ± 5 ppm SO₂ for 2 hr; **B, D, F**) 30 ± 1 ppm O₃ for 2 hr. In **A, B, C, D**), note injury to mesophyll cells (ms) adjacent to the stomata (sto) (×400). In **E, F**) 48 hr after treatment, note the complete collapse of the affected mesophyll cell and injury in the endodermis (e) adjacent to stele (ste) (×200).

SNB occurred. It seems reasonable to assume that even at a distance of 175 miles, SO₂ from the giant Sudbury smelters might have reached the threshold levels reported in this paper and caused the acute foliar injury. For example, Cole & Katz (5) recorded in the predominantly "semirural" area of Port Burwell, Ontario, a maximum 1-hr value of 5.0 ppm SO₂ on 5 July 1961, although the average value for the summer was only 0.47 ppm. Thus, it is possible for transient occurrences of SO₂ to occur in "rural" areas. In addition to the major smelters, other local sources of SO₂ pollution may exist.

In August 1967, prior to the research reported in this paper, the author visited the area in Ontario where Linzon had conducted his SNB studies. The SNB-sensitive trees examined appeared to be O₃-tolerant. Ozone-tolerant trees usually bear long, dark-green new needles which only occasionally display spotting and are retained up to 27 months. In subsequent fumigation studies, SNB-sensitive ramets (obtained from Linzon) were not injured by O₃ in artificial exposures at concentrations below 40 ppm for 2.0 hr (Mast determination), but O₃-sensitive eastern white pines were severely injured by this treatment.

Therefore it is postulated that SO₂ at concentrations previously considered to be nonphytotoxic to some strains of eastern white pine may indeed be a cause of SNB. The absence of these extremely SO₂-sensitive trees near smelters in the Sudbury region may be due to the fact that they were killed soon after the smelters began to operate. Perhaps only the trees that possessed tolerance to SO₂ were able to survive the frequent and severe fumigations close to the source. Many workers (7, 9, 10, 16) have concluded that SO₂ in concentrations high enough to injure foliage of sensitive trees should not be expected in forest areas remote from a source of pollution. This may be a valid conclusion, but it is not yet supported by SO₂ analyses.

Sulfur dioxide and O₃ can exist simultaneously in the atmosphere, as pointed out by Menser & Heggestad (15). They also demonstrated the synergistic effects of O₃ and SO₂ in the leaf tissue of tobacco. This finding, as well as minimum time-concentration thresholds at which SO₂ will injure sensitive eastern white pine, is currently under investigation.

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