Effects of Sulfur Dioxide on Expansion of Lesions Caused by *Corynebacterium nebraskense* in Maize and by *Xanthomonas phaseoli* var. *sojensis* in Soybean

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ABSTRACT

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In order to assess the effects of air pollution on plant disease development, we investigated the effects of SO_2 on lesion development by two bacterial pathogens. Maize or soybean plants were exposed to sulfur dioxide (SO_2) at 524 $\mu g\ m^{-3}$ or 262 $\mu g\ m^{-3}$ before, after, or before and after inoculation with *Corynebacterium nebraskense* or *Xanthomonas phaseoli* var. *sojensis*, respectively. Lesion development was inhibited in both cases, regardless of when the exposures occurred. The time of exposure, however, altered the subsequent effect on lesion size. Dry weight and sulfur content of host tissue were not altered by the joint effects of the pollutant and the pathogens.

The possible importance of air pollutant-pathogen interactions and the justification for their study have been discussed extensively (4–10). Nevertheless, understanding of the manner in which air pollutants modify the biotic environment of a plant remains very limited. This is particularly true of the effects of air pollutants on the epidemiology of plant diseases, an area in which the effects of pollutants on plants might be less obvious.

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Effects of sulfur dioxide (SO_2) on the development of bacterial plant diseases have not been reported, although it is known that they are affected by ozone (7,8,11), hydrogen fluoride (9), and acidic precipitation (10).

This study was undertaken to determine the effects of SO_2 on two diseases caused by bacteria and to begin to estimate possible effects of the pollutant on their epidemiology. We selected leaf freckles and wilt (Goss' wilt) which is caused in maize by *Corynebacterium nebraskense* (Schuster, Hoff, Mandel, Lazar, 1972) and is important in certain areas of the corn belt (1), and bacterial pustule which is caused in soybean by *Xanthomonas phaseoli* (E. F. Smith)

Dowson var. *sojensis* (Hedges) Starr and Burkholder and is a common disease in warm, moist areas where soybeans are grown (3).

MATERIALS AND METHODS

Maize. Seeds of Asgrow RX-94 (Asgrow Seed Co., Kalamazoo, MI 49001) maize (*Zea mays* L.) were sown in sterile soil, sand, and peat mixture (2:1:1, v/v) and grown in a greenhouse at 22 C with supplemental lighting from high-pressure sodium vapor lamps to provide a photoperiod of 16 hr/day. Two weeks after planting, pots were randomly assigned to treatments, thinned to two uniform plants each, and placed in different chambers receiving 0 or 524 $\mu g\ m^{-3}$ (0.2 ppm) SO_2. After 5 days of continuous exposure, plants were removed from the chambers for inoculation.

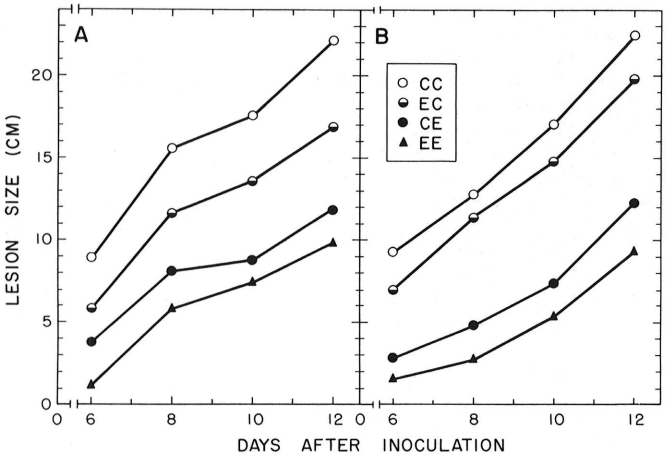

Fig. 1. Effects of SO_2 on expansion of lesions caused by *Corynebacterium nebraskense* in maize leaves. Data represented by A and B are from an initial run and a repetition of the same experiment. Treatments CC, CE, EC, and EE refer to exposures to either a control atmosphere (C) or a SO_2-supplemented atmosphere (E) before or after inoculation.

Inoculations were performed by uniformly wounding the second leaf of the plant with closely grouped needles embedded in a cork (2) and applying 0.5 ml of a suspension containing 10^7 cells of *C. nebraskense* per milliliter (grown for 48 hr on nutrient agar at 27 C) with an atomizer. Uninoculated control plants that were wounded and atomized with water were maintained. After inoculation, one half of the plants that had received SO_2 along with one half of the control plants, were returned to the SO_2 atmosphere for 2 days. The remaining plants were returned to the control atmosphere. This resulted in four exposure treatments with regard to the time of inoculation: ie, control → control; control → exposed; exposed → control; and exposed → exposed. Each of the exposure treatments consisted of both inoculated and uninoculated plants, a total of eight treatments with 30 replicate plants in each treatment. The experiment was repeated once.

After disease symptoms became apparent, lesion lengths were measured every other day. At the end of the experiment, aerial plant parts were collected for determinations of total S and dry mass.

Soybean. Seeds of soybean (*Glycine max* (L.) Merrill 'Hodgson') were grown under conditions similar to those described above except that supplemental lighting was supplied by multivapor lamps. A similar experimental design was employed except that SO_2 was supplied at 262 $\mu g\ m^{-3}$ (0.1 ppm) for 5 days before and 5 days after inoculation. Inoculations were made by pressure spraying (8) 0.5 ml of a suspension containing 10^7 cells of *X. phaseoli* var. *sojensis* per milliliter on the underside of each of two unifoliolate leaves per plant. Appropriate controls were maintained as in the maize experiments. There were 36 replicate leaves in each treatment and the experiment was repeated once.

After the onset of disease symptom expression, measurements of lesion diameters were made approximately every 24–48 hr. At the end of the experiment, all leaves present during the exposures were

TABLE 1. Effect of 5-day preinoculation and 2-day postinoculation exposures to 524 $\mu g\ m^{-3}$ SO_2 on length of lesions caused on maize leaves by *Corynebacterium nebraskense*

		Lesion length (cm) after exposure before inoculation to			
		$-SO_2$	$+SO_2$	$-SO_2$	$+SO_2$
		and exposure after inoculation to			
Exp. no.	Days after inoculation	$-SO_2$	$-SO_2$	$+SO_2$	$+SO_2$
I	6	8.9 a[z]	5.8 b	3.7 b	1.2 c
	8	15.5 a	11.6 b	8.4 bc	5.8 c
	10	17.7 a	13.6 b	8.8 c	7.5 c
	12	22.1 a	16.7 b	11.8 c	9.7 c
II	6	9.3 a	6.9 b	2.8 c	1.6 c
	8	12.8 a	11.3 a	4.8 b	2.8 b
	10	17.1 a	14.8 a	7.4 b	5.4 b
	12	22.4 a	19.8 a	12.3 b	9.4 b

[z] Means of 30 plants. Means in the same row followed by different letters are significantly different based on Tukey's HSD ($P = 0.05$).

TABLE 2. Percent S content of maize plants exposed to 524 $\mu g\ m^{-3}$ SO_2 before, after, or both before and after inoculation with *Corynebacterium nebraskense*

	Percent S following exposure before inoculation to							
	$-SO_2$		$+SO_2$		$-SO_2$		$+SO_2$	
	and exposure after inoculation to							
	$-SO_2$		$-SO_2$		$+SO_2$		$+SO_2$	
Exp. no.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
I[a]	0.158[c]	0.156	0.180	0.154	0.174	0.149	0.180	0.180
II[b]	0.199	0.172	0.275	0.220	0.257	0.219	0.366	0.251

[a] Mean-square estimates of contributions to total S content in experiment I: Preinoculation exposure = 0.00204, postinoculation exposure = 0.00075, nonadditivity= 0.00019, error = 0.00033.

[b] Mean-square estimates of contributions to total S content in experiment II: Preinoculation exposure = 0.0432, postinoculation exposure = 0.0323, nonadditivity = 0.00017, error = 0.0070.

[c] Each value is the mean of measurements from nine plants.

harvested for measurements of area, dry mass, and total S.

Data analysis. Data were analyzed by analysis of variance and the various main-factor sums of squares were partitioned to obtain single degree of freedom estimates. The soybean data were divided into two groups that had common days of lesion measurement. This allowed parts of each experiment to be analyzed together, providing better estimates of error variances. Data could not be pooled due to significant repetition × treatment interactions.

RESULTS

Maize. *Lesion size and development.* Maize plants were not visibly injured by the SO_2 exposures. Lesion sizes and rates of development indicated significant effects of SO_2 (Table 1). It is apparent from Fig. 1 that the 2-day postinoculation exposure was more effective in inhibiting lesion length than was the 5-day preinoculation exposure. The effects of preinoculation and postinoculation exposure were highly significant and additive; that is, the interaction of the two exposures was not significant. A significant interaction of time of exposure × time after inoculation was found which indicates that the lesion development curves (Fig. 1) are not parallel and suggests SO_2-induced reductions in the rate of increase in the disease. The main effects of SO_2 treatment indicate that the means of the treatments are different and, therefore, we reject the hypothesis of equal intercepts, implying that SO_2 increases the time from inoculation to the onset of symptoms.

Sulfur content. In both the initial experiments and their repetition, the effect of preinoculation exposure on total S concentrations was significant. The 2-day postinoculation exposure caused a significant increase in total S in the second repetition (Table 2). The effects of preinoculation and postinoculation exposure were additive indicating there is no effect of one exposure on the uptake of S by the plant during successive exposure.

Dry mass. No significant effects of SO_2 or disease on dry mass of aboveground portions of the plant were found.

Soybean. *Lesion size and development.* SO_2 treatment did not induce visible air pollution symptoms but resulted in significantly smaller bacterial pustule lesions (Table 3). We performed analysis of variance for lesion diameter data in two parts, using days of measurement common to both repetitions of the experiment in each case. For the earlier time (2, 3, and 5 days postinoculation), both preinoculation and postinoculation exposure caused significant effects, but neither time of measurement nor the interaction of SO_2 and time of measurement were significant. This indicated that early in the development of the disease the rate of development is about the same, but the lesion diameters are smaller in SO_2-treated plants than in unexposed controls. Later in the experiment (5 and 8 days after inoculation) the rates of lesion development became different as indicated by a significant SO_2 × time of measurement interaction. The result is that by the end of the experiment, preinoculation and postinoculation exposure contributed equally to the inhibition of bacterial pustule lesions, and the lesions on SO_2-treated plants had developed at a reduced rate compared to those on control plants (Fig. 2).

Sulfur content. Significant increases of total S caused by preinoculation and postinoculation SO_2 exposure were found in both repetitions (Table 4). In addition, a significant nonadditivity was found in the second experiment due to the large effect of postinoculation exposure. This indicates a much greater uptake of S after inoculation compared to preinoculation exposure uptake (Table 5).

Leaf area and dry mass. In the first repetition, leaf area and dry mass of inoculated plants were reduced by preinoculation, but not by postinoculation SO_2 exposure (Table 4). In the second experiment, the nonadditivity terms were significant, indicating a significant preinoculation exposure × postinoculation exposure interaction (Table 5).

DISCUSSION

Air pollutants are known to affect the incidence and severity of plant disease (5). This study shows that they affect the rate at which lesions develop and, in some cases, the lag time from inoculation to the onset of symptom development. In the case of bacterial diseases, this is also the time period during which secondary inoculum is produced.

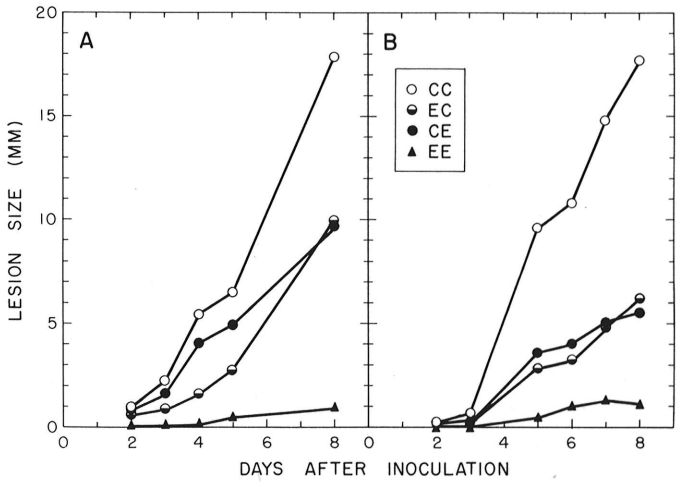

Fig. 2. Effects of SO_2 on lesion expansion by *Xanthomonas phaseoli* var. *sojensis*. A and B are repetitions of the same experiment. Treatments CC, CE, EC, and EE refer to exposures to either a control atmosphere (C) or a SO_2-supplemented atmosphere (E) before or after inoculation.

TABLE 3. Effect of 5-day preinoculation and postinoculation exposures to 262 $\mu g\ m^{-3}$ SO_2 on diameter of lesions on soybean leaves caused by *Xanthomonas phaseoli* var. *sojensis*

		Lesion diameter (mm) after exposure before inoculation to			
		$-SO_2$	$+SO_2$	$-SO_2$	$+SO_2$
		and exposure after inoculation to			
Exp. no.	Days after inoculation	$-SO_2$	$-SO_2$	$+SO_2$	$+SO_2$
I	2	0.88 a[z]	0.64 a	0.88 a	0.08 b
	3	2.22 a	0.86 bc	1.61 ab	0.05 c
	4	5.38 a	1.61 c	4.00 b	0.14 d
	5	6.55 a	2.69 c	4.88 b	0.41 d
	8	17.86 a	9.77 b	9.83 b	0.94 c
II	2	0.22 a	0.00 b	0.16 b	0.00 b
	3	0.66 a	0.08 c	0.25 b	0.00 c
	5	9.50 a	2.88 b	3.63 b	0.41 b
	6	10.88 a	3.25 b	4.00 b	1.02 b
	7	14.83 a	4.86 bc	5.02 b	1.30 c
	8	17.66 a	5.50 b	6.16 b	1.05 c

[z] Means of 36 measurements. Means in the same row followed by different letters are significantly different based on Tukey's HSD ($P = 0.05$).

TABLE 4. Total S content, leaf area and dry mass of soybean plants exposed to SO_2 before, after, or before and after inoculation with *Xanthomonas phaseoli* var. *sojensis*

	Plants were exposed before inoculation to							
	$-SO_2$		$-SO_2$		$+SO_2$		$+SO_2$	
	and exposed after inoculation to							
	$-SO_2$		$+SO_2$		$-SO_2$		$+SO_2$	
Exp. no.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.
I								
Total S (%)	0.140 d[z]	0.142 d	0.226 bc	0.142 d	0.206 b	0.197 c	0.288 a	0.264 a
Leaf area (cm^2)	197.5 ab	214.5 a	203.7 ab	214.5 a	182.7 b	189.1 ab	181.0 b	165.8 b
Dry mass (g)	0.57 ab	0.64 a	0.62 ab	0.61 ab	0.53 b	0.56 ab	0.55 ab	0.53 b
II								
Total S (%)	0.238 c	0.249 c	0.367 c	0.451 b	0.294 d	0.297 d	0.477 b	0.537 a
Leaf area (cm^2)	248.0 a	285.1 a	219.6 a	232.4 a	236.3 a	262.4 a	244.4 a	282.1 a
Dry mass (g)	0.515 a	0.604 a	0.608 a	0.545 a	0.526 a	0.505 a	0.596 a	0.662 a

[z] Means in the same row followed by different letter are significantly different based on Tukey's HSD ($P = 0.05$). Each value is the mean of measurements from nine plants.

TABLE 5. Partitioned sums of squares for time-of-exposure effects on total S content, leaf area, and dry mass of soybean plants exposed to 262 $\mu g\ m^{-3}$ SO_2 before, after, or before and after inoculation with *Xanthomonas phaseoli* var. *sojensis*

		Mean-square estimates		
Exp. no.	Source of variation	S content (%)	Leaf area (cm^2)	Dry mass (g)
I	Preinoc. exposure	0.0963**[a]	14007.47**	0.073**
	Postinoc. exposure	0.0735**	397.99	0.00002
	Nonadditivity	0.0016	1092.62*	0.00067
	Error	0.0004	370.00	0.004
II	Preinoc. exposure	0.1013**	1810.01	0.0025
	Postinoc. exposure	0.6369**	3198.40	0.6136**
	Nonadditivity	0.0098**	13372.66*	0.3325**
	Error	0.0004	2598.65	0.0197

[a] * Significant at $P = 0.05$ and ** significant at $P = 0.01$.

Differences in the lag period and rate of lesion development could have important epidemiological consequences over a growing season. If symptoms develop more slowly (and in this case, secondary inoculum is available at a later date), the epidemic could be shifted both in time of occurrence and in rate of development. The decrease in the rate of lesion development in bacterial pustules might result in a reduced rate of disease development and a concomitant reduction in disease severity at the end of the epidemic. Further research, especially in the field, is needed to substantiate these findings and to evaluate these effects during a growing season.

The presence of significant nonadditivity terms or trend toward nonadditivity (as indicated by large nonadditivity contributions) indicate that the effect of SO_2 on disease development may be affected by the time when exposure takes place in relation to inoculation. This is especially true for the postinoculation exposure of maize plants infected with *C. nebraskense*. Lesion expansion by the bacterium was inhibited by the 2-day postinoculation exposure more effectively than by the 5-day preinoculation exposure or the combined 7-day exposure. Whether these differences are due to a particularly sensitive stage during infection and colonization or to ephemeral toxic products of the SO_2 stress is unknown.

The total S data indicated large variability in S concentration, and that S is not taken up as readily in maize as in soybean. The nonadditivity in the soybean S data reflect the fact that much more S was taken up in the postinoculation exposure than in the preinoculation exposure.

Examination of dry mass and leaf area data indicated significant reductions caused by SO_2 for soybean, but not for maize (dry mass only). However, the interaction terms for SO_2 and the pathogens were not significant so we concluded that the combination of the two stresses did not affect the variables of plant growth that we measured.

The effect of SO_2 on the epidemiology of plant diseases is virtually unknown, but initial indications are that the result may be a decrease in the rate of disease development. Further investigations both in controlled environment chambers and in the field are needed to assess the importance of these effects on yield and quality.

LITERATURE CITED

1. Calub, A. G., Schuster, M. L., Compton, W. A., and Gardner, C. O. 1974. Improved technique for evaluating resistance of corn to *Corynebacterium nebraskense*. Crop Sci. 14:716-718.
2. Calub, A. G., Schuster, M. L., Gardner, C. O., and Compton W. A. 1974. Effect of plant age and inoculum concentration on leaf freckles and wilt of corn. Crop Sci. 14:398-401.
3. Kennedy, B. W., and Tachibana, H. 1973. Bacterial diseases. Pages 491–504 in: B. E. Caldwell, ed. Soybeans: Improvement, Production, and Uses. American Society of Agronomy, Madison, WI.
4. Heagle, A. S. 1973. Interactions between air pollutants and plant parasites. Annu. Rev. Phytopathol. 11:365-388.
5. Laurence, J. A. 1981. Effects of air pollutants on plant-pathogen interactions. Z. Pflanzenkrankh. PflanzenSchutz 88: (In press).
6. Laurence, J. A. 1979. Response of maize and wheat to sulfur dioxide. Plant Dis. Rep. 63:468-471.
7. Laurence, J. A., and Wood, F. A. 1978. Effects of ozone on infection of soybean *Pseudomonas glycinea*. Phytopathology 68:441-445.
8. Laurence, J. A., and Wood, F. A. 1978. Effects of ozone on infection of wild strawberry by *Xanthomonas fragariae*. Phytopathology 68:689-692.
9. McCune, D. C., Weinstein, L. H., Mancini, J. F., and Van Leuken, P. 1973. Effects of hydrogen fluoride on plant pathogen interactions. Pages A146–A149 in: Proc. 3rd Clean Air Congr., 8–12 October 1973, Dusseldorf. Verein Deutsher Ingenieure Verlag.
10. Shriner, D. S. 1978. Effects of simulated acid rain on host-parasite interactions in plant disease. Phytopathology 68:213-218.
11. Temple, P. J., and Bisessar, S. 1978. Response of white bean to bacterial blight, ozone, and antioxidant protection in the field. Phytopathology 69:101-103.