Effects of Concentration of Sulfur Dioxide and Other Characteristics of Exposure on the Development of Lesions Caused by *Xanthomonas phaseoli* in Red Kidney Bean

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ABSTRACT

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Sulfur dioxide (SO_2) supplied at constant (square wave) or variable (triangular wave) concentrations affected development of lesions caused by *Xanthomonas phaseoli* by increasing the latent period (time for symptom expression) and suppressing lesion expansion. Similar effects were found with both patterns of exposure. Timing of exposures relative to inoculation was important and generally was most effective when the exposure and inoculation periods were close together. The time between repeated triangular wave SO_2 exposures also affected lesion development and indicated a decay of inhibition if exposures were separated by >1 day.

Qualitative effects of air pollutants on plant diseases have been recently reviewed (1). This paper describes a series of experiments designed to quantify the effects of sulfur dioxide (SO_2) on the development of *Xanthomonas phaseoli* (E. F. Smith) Dows. lesions in red kidney bean. The ultimate goal is to estimate the potential for pollutant-induced alterations in the epidemiology of common blight of bean.

SO_2 occurs at low levels for extended periods of time in urban and industrial areas and in high concentration near point sources. SO_2 can alter the incidence and severity of plant diseases caused by fungi, viruses, and bacteria (1–4). The extent to which these alterations affect disease severity in the field is not clear, but observations made near various sources of pollution suggest that severity can be increased or decreased (1).

Common blight is an important disease in bean production areas of the world (8). Although it can usually be controlled by growing pathogen-free seed, losses can reach 50% or more. The development of bacterial blight has been described and quantified by Weller and Saettler (9,10), and effects of ozone on the disease have been reported (7). It is clear that air pollutants could affect disease development during any of several phases of the disease cycle.

The experiments reported here were designed to answer three questions: does SO_2 affect the development of lesions; do exposures at variable concentrations cause different effects than those induced by exposures at constant concentrations; and are effects of multiple exposures to SO_2 independent, or does a single exposure affect the response of a plant to a subsequent one.

MATERIALS AND METHODS

Plant materials and culture. Seeds of red kidney bean (*Phaseolus vulgaris* L. 'California Light Red Kidney' and 'Red Kloud') were obtained from a commercial source (Agway Bean Plant, Geneva, NY 14456) and sown in 10-cm-diameter pots in a pasteurized peat, sandy loam, sand (1:2:1, v/v) mixture. After 1 wk, seedlings were thinned to one uniform plant per pot. Plants were grown in a greenhouse maintained at 25/20 C (day/night) with a 16-hr photoperiod provided by multi-vapor HID lamps. Fertilizer (20:20:20, NPK) was applied in solution weekly to maintain adequate plant nutrition. Plants were watered twice daily. Experiments began 2 wk after seeding.

Pathogen culture and inoculation methods. *X. phaseoli* was obtained from A. W. Saettler (USDA-AR, Michigan State University, E. Lansing 48824) and grown on rifampin agar medium (9,10) at 27 C. The bacterium was reisolated frequently to maintain pathogenicity and virulence.

Inoculations were made by pressure spraying (3) unifoliolate leaves with 0.75 ml of a solution containing approximately 10^9 colony-forming units (CFU) per milliliter. At the time of inoculation, several typical specimens of inoculum were collected by spraying solution into a test tube, and the concentration of inoculum was determined by dilution plating.

Exposure to SO_2. Plants were maintained and exposed in controlled-environment chambers supplied with SO_2 at 0, 260, or 520 $\mu g/m^3$ in filtered air. Chambers were maintained at 25/20 C, 70/80% RH, and 600–700/0 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (day/night). SO_2 was monitored with pulsed fluorescence SO_2 analyzers (Series 43, Thermoelectron Corporation, Hopkinton, MA 01748), which were calibrated and checked by using permeation tubes and standard gas.

Variable (triangular wave) concentrations of SO_2 were achieved by metering gas through a motorized micrometer valve. Concentrations were controlled by using a Hewlett-Packard 1000 computer (Hewlett-Packard Corp., Cupertino, CA 95014) which queried the SO_2 analyzer, compared the monitored value to the desired concentration, and actuated the motorized valve causing an increase or decrease in SO_2 flow as determined by the control algorithm. Two peak concentrations were used: one increased linearly from 0 to 1,300 $\mu g/m^3$ SO_2 and fell to 0 in 3 hr and the other increased from 0 to 2,600 $\mu g/m^3$ SO_2 and fell to 0 in 3 hr. These peak concentrations result in 3-hr averages that are either one-half of or equal to those currently allowed by the National Ambient Air Quality Standard for SO_2, respectively.

Experimental design and statistical analysis. *Repeated exposures to constant (square wave) concentrations.* Seven plants per treatment were exposed to SO_2 6 hr each day for 5 days before, after, or both before and after inoculation with *X. phaseoli.* At the conclusion of the exposure sequence, the plants were returned to the greenhouse, and measurements of each lesion (water-soaked area) were made daily for 5 days with the unaided eye under high-intensity reflected light. The experiment was repeated three times. An analysis of variance was performed using experiments, cultivars, preinoculation exposure, and postinoculation exposure

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as main factors.

Repeated exposures to triangular wave concentrations. Five plants per treatment were inoculated and then exposed in all possible combinations on 4 successive days after inoculation. This resulted in 16 treatment combinations for each of two cultivars at each of two peak concentrations. At the conclusion of the exposures, the plants were returned to the greenhouse and measurements were made as described above.

An analysis of variance was performed and treatment sums of squares were partitioned to allow estimation of the effects of total dose (zero to four exposures) and order within a given dose (the temporal effects within equal numbers of exposures). High and low peak concentration experiments were analyzed separately.

RESULTS

Regardless of concentration, visible symptoms of SO_2 injury did not develop. Symptoms of common blight were first apparent as small, lightened areas at the point of inoculation, but these rapidly progressed to water-soaked areas that expanded radially. Some chlorosis was observed at the margins of lesions regardless of treatment. Lesions were similar in physical appearance from treatment to treatment. Red Kloud plants were generally more susceptible to *X. phaseoli* than were those of California Light Red Kidney, regardless of SO_2 treatment.

Square wave exposures. Four variables of disease development were measured: percentage of plants infected, initial lesion size, final lesion size, and latent period (the time between inoculation and first visible water-soaking). SO_2 treatment did not affect the percentage of plants infected or the initial lesion size, but was related to changes in both final lesion size and latent period.

Postinoculation exposure to SO_2, when averaged over preinoculation levels, resulted in smaller lesions and longer latent periods. The magnitude of these effects tended to increase with the level of exposure after inoculation (Table 1).

When considered as a main factor, preinoculation exposure to SO_2 did not result in significant alterations of disease development, probably due to the relatively large effect of postinoculation exposure. There was a significant preinoculation × postinoculation interaction: with SO_2 at 0 or 260 $\mu g/m^3$ preinoculation, lesion diameter generally decreased with increasing postinoculation exposure to SO_2, but this trend was reversed when plants were exposed to 520 $\mu g/m^3$ prior to inoculation (Fig. 1). The contributions to total variance of experimental factors for these experiments is presented in Table 1.

Multivariate analyses of variance were used to determine if combinations of the measured response variables were associated and important in describing the effects of SO_2 on disease development. These analyses substantiated the univariate analyses and showed these variables to be independent.

Triangular wave exposures. *Initial lesion diameter.* There was a significant linear effect of number of exposures at both peak concentrations on the initial size (diameter at first appearance of symptoms) of common blight lesions; as number of exposures increased, the initial lesion size decreased (Figs. 2 and 3, Table 2).

Final lesion diameter. The size of lesions at the end of the experiment was influenced by both cultivar and number of exposures peaking at an SO_2 concentration of 2,600 $\mu g/m^3$ (Fig. 4),

TABLE 1. Analysis of variance for effects of square-wave SO_2 concentration exposures on development of common blight in red kidney bean

		Mean squares		
Source	df	Initial size	Final size	Latent period
Experiment	2	36.73**[a]	34.08**	1.06
Cultivar (cv)	1	7.80**	17.82**	0.57
Preinoculation exposure (pre)	2	0.45	0.45	0.02
Postinoculation exposure (post)	2	3.47**	3.08**	2.68**
Linear	1	6.87**	5.65**	5.19**
Quadratic	1	0.07	0.51	0.17
Cv × pre	2	0.11	0.62	0.28
Cv × post	2	0.80	0.86	0.57
Pre × post	4	0.53	2.22**	0.66
Cv × pre × post	4	0.25	0.19	0.19
Error	34	0.46	0.49	0.46

[a] Asterisks indicate the probability ($P < 0.01$) of a value larger than F.

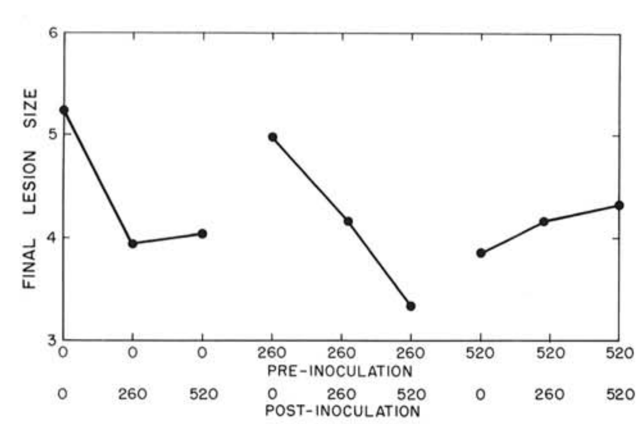

Fig. 1. Effects of increasing postinoculation exposure concentration at each of three preinoculation SO_2 concentrations on final lesion size of *X. phaseoli* on red kidney bean.

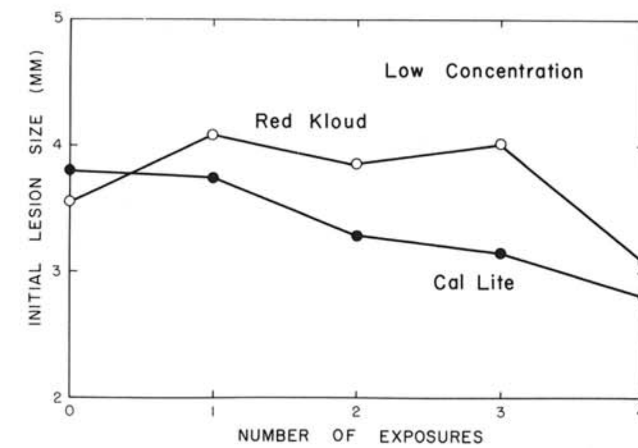

Fig. 2. Effect of the number of daily exposures to an SO_2 concentration peak of 1,300 $\mu g/m^3$ SO_2 on initial lesion size of *Xanthomonas phaseoli* in red kidney bean.

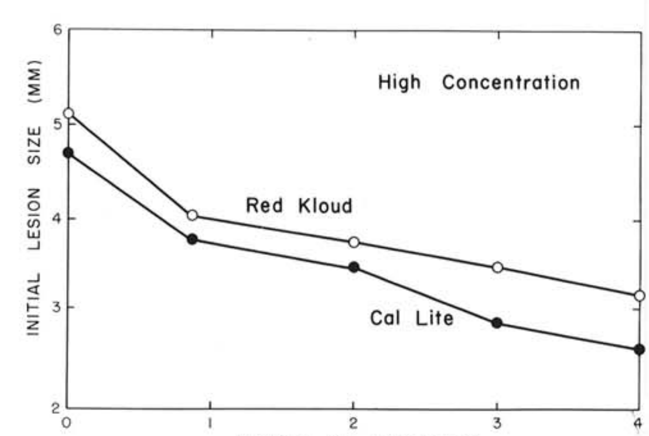

Fig. 3. Effect of the number of daily exposures to an SO_2 concentration peak of 2,600 $\mu g/m^3$ SO_2 on initial lesion size of *Xanthomonas phaseoli* in red kidney bean.

TABLE 2. Mean squares for major effects of triangular-wave concentrations of SO_2 on initial and final lesion size in common blight of red kidney bean

Source	df	Initial lesion size[a]		Final lesion size		Latent Period	
		Low conc.	High conc.	Low conc.	High conc.	Low conc.	High conc.
Cultivar	1	4.32**[b]	4.09*[b]	21.94**	10.08**	4.04**	15.76**
Linear (total dose)	1	1.94*	21.14**	0.12	28.69**	5.91**	3.31
Quadratic (total dose)	1	0.40	1.34	0.30	0.17	0.15	0.03
Order W/I 1 exposure[c]	3	1.25	0.26	1.77*	0.46	0.08	0.02
Order W/I 2 exposures[c]	5	1.51*	1.41*	1.89*	1.45*	0.52	1.10
Order W/I exposures[c]	3	1.73*	0.35	2.92**	0.88	0.77	0.90
Error	35	0.45	0.56	0.57	0.42	0.43	1.08

[a] Low concentration corresponds to a peak of 1,300 $\mu g/m^3$ and high concentration corresponds to a peak of 2,600 $\mu g/m^3$. Low and high concentration experiments were analyzed separately.
[b] Asterisks: *, $P = 0.05$; **, $P = 0.01$.
[c] Values for low concentrations are cultivar × order within (W/I) exposures.

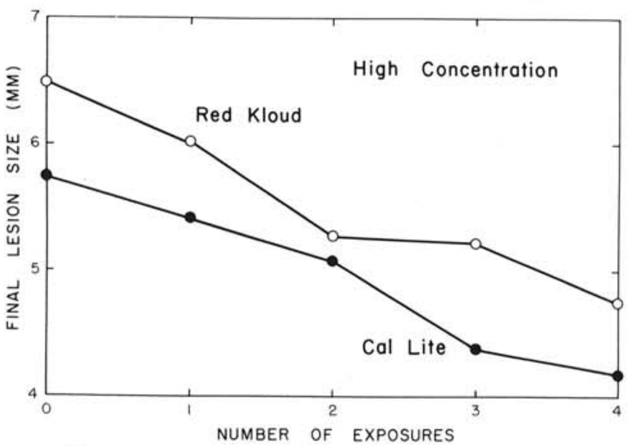

Fig. 4. Effects of the number of daily exposures to an SO_2 concentration peak of 2,600 $\mu g/m^3$ on final lesion size of *Xanthomonas phaseoli* in red kidney bean.

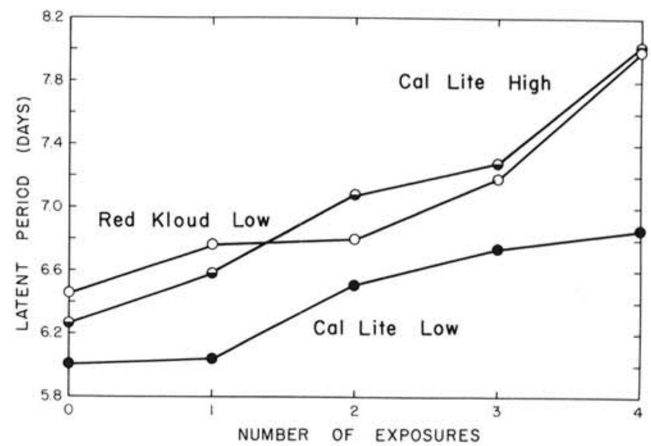

Fig. 5. Effects of the number of daily exposures to SO_2 concentration peaks of 1,300 or 2,600 $\mu g/m^3$ on the latent period of *Xanthomonas phaseoli* in red kidney bean. Low and high refer to peaks of 1,300 and 2,600 $\mu g/m^3$, respectively.

but it was influenced only by cultivar in the exposures at the lower concentration (Table 2). Regardless of cultivar, lesions on plants exposed four times to the higher concentration were only about three-fourths of the size of those on control plants.

Latent period. The latent period was affected by both cultivar and number of exposures at both concentrations. As the number of exposures increased, the latent period increased (Fig. 5, Table 2). The latent period for Red Kloud was affected by SO_2 at peaks of 2,600 $\mu g/m^3$, but control plants had an abnormally long latent period. These plants were not used in further analyses.

Order within recurring exposures. As part of the experimental design, it was possible to evaluate the importance of when the components of the total dose were applied. At the lower concentration, it appears that exposures during the last 2 days affected the initial and final lesion size more than those on the first 2 days for California Light Red Kidney, while the reverse was true for Red Kloud ($P < 0.05$). At the higher concentration, there were no differences between cultivars regarding the importance of the time between exposures. Only those treatments that included two exposures showed differences in effects due to order. Exposure on the first day was important in reducing both initial and final lesion size ($P < 0.05$). As the time between the first and second exposures increased, the inhibitory effect decreased (Table 3). Order within dose did not affect latent period.

TABLE 3. Effect of the time spacing of two exposures to a peak SO_2 concentration of 2,600 $\mu g/m^3$ on initial and final size of lesions caused by *Xanthomonas phaseoli*

Exposure on day	Initial lesion size (mm)	Final lesion size (mm)
3 and 4	4.2 a[z]	6.1 a
2 and 4	3.9 ab	5.4 ab
2 and 3	3.5 ab	5.3 ab
1 and 4	3.3 ab	5.1 ab
1 and 3	3.6 ab	4.7 b
1 and 2	2.5 b	4.4 b

[z] Numbers followed by different letters are significantly different ($P = 0.05$) based on Tukey's test for comparison of means.

DISCUSSION

Concentrations of phytotoxic air pollutants in ambient air are expected to increase substantially during the rest of this century (6). Along with these increases, increased crop losses, greater effects on natural systems, and modifications of host-parasite relationships can be anticipated. It is evident that SO_2 can affect processes that have epidemiological importance in common blight as well as in other plant diseases (1–3). The results of these studies indicate that concentrations of SO_2 occurring in distinctly different patterns, and at levels that do not cause foliar symptoms on plants, may induce modifications in the host that alter the host-parasitic relationship.

Both square-wave and triangular wave exposures to SO_2 caused similar effects (smaller lesions and longer latent periods) even though the total number of exposures and peak concentrations differed substantially. Thus, it might be expected that the effects of occasional fumigations by emissions of SO_2 from large point sources in rural areas would be similar in magnitude to those that occur around urban or regional sources of SO_2.

Results from recurrent triangular wave exposures to SO_2 indicate that effects are cumulative. As the number of exposures increased, lesion size decreased and latent period increased. Whether these effects tend to level off with greater numbers of exposures was not indicated by our results over a limited time period. It appears, however, that recovery (decreased inhibition of lesion development) occurs as exposures are separated in time. Furthermore, exposures occurring shortly after inoculation are more effective in inhibiting lesion growth than those which are temporally removed from inoculation. The results of these studies suggest strongly that the inhibition of lesion development is related to metabolic changes in the plant induced by SO_2. McLaughlin et al (5) found photosynthesis of kidney beans to be depressed by similar exposures (four daily exposures to peaks of either 0.5 or 2 ppm for 3 or 1 hr, respectively) and suggested a biochemical basis for the effect. Little residual effect was noted, indicating that recovery had occurred and no cumulative effect of repeated exposures was found.

The evaluation of the effects of SO_2 on disease development, and the joint action of pollutant and pathogen on crop yield, await field verification, but results of these and other studies suggest important effects of air pollutants on the normal development of pathogen and pest populations.

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