Effects of Sulfur Dioxide Exposure on the Development of Common Blight in Field-Grown Red Kidney Beans

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ABSTRACT

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Field-grown red kidney beans *Phaseolus vulgaris* 'California Light Red Kidney' were exposed, using an open air fumigation system, to concentrations of SO_2 that ranged from 0 to 1.00 ppm (2,620 $\mu g\ m^{-3}$ at standard temperature and pressure) for 3 hr two or three times per week between 10 July and 6 September 1982. Plants were inoculated at the beginning of the exposure period by spray application of a suspension of *Xanthomonas campestris* pv. *phaseoli* in sterile water. Lesions were counted at 2- to 3-day intervals throughout the exposure period. Increasing cumulative SO_2 concentration resulted in a significant decrease in the rate of lesion appearance. The rate of this response remained constant over the course of the exposure period. Increasing cumulative SO_2 concentration also led to a reduction in yield of noninfected plants; however, there was no apparent reduction in yield of infected plants. Exposure to SO_2 effectively inhibited disease development but also reduced yield.

Common blight is an important disease in bean production areas of the world, particularly where dispersal of the pathogen is not controlled by irrigation (8). Internally infected seed is the main source of primary inoculum and control of the disease is based primarily on a rigorous seed certification program. The bacteria colonize the developing seedling and may continue to multiply on symptomless leaves until a threshold population is attained and lesions appear. Bacteria from lesions are splashed by rain to other parts of the plant, initiating the secondary disease cycle.

Sulfur dioxide (SO_2) occurs at low levels for extended periods of time in urban and industrial areas and in high concentration near point sources (1,9). Although emissions of SO_2 in the United States have decreased by approximately 17% between 1975 and 1982, ambient concentrations in agricultural areas may in fact be increasing due to a recent shift in the use of high sulfur fuels from urban areas to more rural locations (9). Future levels of SO_2 could continue to rise due to conversion from oil to coal fuel sources.

SO_2 is known to affect the incidence and severity of plant disease in laboratory and greenhouse experiments; however, the effect of SO_2 on the epidemiology of these diseases, particularly under field conditions, is virtually unknown (3). It has been reported that exposure to SO_2 suppresses expansion of lesions caused by *Corynebacterium nebraskense* (= *C. michiganese* pv. *nebraskense*), *Xanthomonas phaseoli* var. *sojensis* (= *X. campestris* pv. *glycines*) and *X. campestris* pv. *phaseoli*, on maize, soybean, and kidney bean, respectively, under conditions of controlled environment (2,4,6). An increase in the incubation period was also observed on bean plants inoculated with *X. campestris* pv. *phaseoli*, in response to SO_2 exposure (6). Because such effects on lesion development could have important epidemiological consequences over a growing season, it was desirable to substantiate these findings by evaluating the effects of SO_2 on disease development in the field.

The experiment reported here was specifically designed to determine the effect of SO_2 exposure on the development of common blight, caused by *X. campestris* pv. *phaseoli* in field-grown red kidney beans.

MATERIALS AND METHODS

Red kidney bean seeds were obtained from a commercial source (Agway Bean Plant, Geneva, NY) and planted in 75-cm rows according to recommendations for commercial dry bean production in New York (7).

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SO_2 was introduced through an open-air fumigation system. Concentrated gas was delivered from the source tank to a mixing box with two attached blowers. Diluted gas was then forced out through three polyethylene tubes approximately 20 cm in diameter, situated between rows of beans. Holes punched in the sides of these tubes allowed the pollutant to be dispensed into the field, and different levels of SO_2 were achieved by varying the number of holes per meter of tube (5).

Four SO_2 delivery systems were used, with high, low, and zero SO_2 treatments (48, 24, and 0 holes per meter of tube, respectively) randomly assigned among three 6.1-m sections along the length of each set of tubes. Each 6.1-m section was separated from the next by a 1-m buffer zone of nonperforated tubing. Because of wind-induced variation in the level of SO_2 in the plots, 12, rather than three different pollutant concentrations, ranging from 0 to 1.00 ppm, were obtained.

Plants were exposed to the pollutant for 3 hr during the day, for 2 or 3 days each week throughout the season (10 July to 6 September), provided that the average wind speed did not exceed approximately 5.0 m s^{-1} and bean foliage was dry. A total of 14 exposures was given and all exposures were 3 hr in duration. During each exposure, air was sequentially sampled directly above the canopy at each of four predetermined positions within each plot. Air from each sampling point was continuously drawn through 6.3-mm inside-diameter polyethylene tubing. During each exposure, sampled air was then diverted to a Thermo Electron pulsed fluorescence SO_2 analyzer (Series 43, Thermo Electron Corporation, Hopkinton, MA), for 5 min each hour for 3 hr, resulting in a total sampling time of 20 min each hr for each plot (or 1 hr per plot per 3-hr exposure). One analyzer was used for each of the four blocks and all instrumentation was subjected to frequent span checks and calibrations (when necessary) to ensure accuracy of measurements.

TABLE 1. Foliar injury to red kidney bean plants exposed to SO_2, recorded on 16 July 1982 for each of 11 individual plots

Plot	Cumulative SO_2 concentration (ppm)	Leaf area injured (%)
1	0.18	0
2	0.37	<5
3	0.22	0
4	0.33	<5
5	0.28	0
6	0.49	30
7	0.06	0
8	0.46	<1
9	0.24	0
10	0.75	5
11	0.11	0

TABLE 2. Atmospheric concentrations of SO_2 in plots of field-grown red kidney beans[a]

	SO_2 Concentration (ppm)		Observations (no.)	
Plot	Mean	Standard deviation	Exceeding 1.00 ppm	Total
1	0.088	0.131	0	211
2	0.184	0.171	1	292
3	0.040	0.067	0	282
4	0.237	0.213	1	201
5	0.056	0.051	0	284
6	0.241	0.280	6	272
7	0.033	0.058	0	208
8	0.188	0.185	2	284
9	0.210	0.273	9	206
10	0.270	0.255	7	289
11	0.031	0.034	0	282

[a] Plants exposed to SO_2 for 3 hr each day, 2 or 3 days each week for a total of 14 3-hr exposures. Air sampled sequentially from four locations within each plot for 5 min each, for a total of 20 min during each hour of exposure.

Three separate inoculations were made in July by spray application of plants still in the seedling stage with a suspension of rifampin-resistant *X. campestris* pv. *phaseoli* in sterile water (10). The first inoculation was made on 8 July by watersoaking one entire leaf in the center of each plot with a suspension of the bacterium containing approximately 4.5×10^9 colony-forming units (cfu) per milliliter. These inoculated leaves served as a source of secondary inoculum and primary locus of disease. As of 24 July, symptoms were visible on inoculated leaves and, in fact, in some cases the disease had advanced so far that the leaf abscised. Leaf prints on rifampin-agar medium confirmed the presence of epiphytic populations of the bacterium on symptomless leaves adjacent to the inoculated ones; however, there were still no symptoms on leaves that were not inoculated. Therefore, a second leaf in the center of each plot was inoculated on 24 July using the same method and bacterial concentration. After another week, it became clear that the disease was developing too slowly to result in significant epidemics by the end of the season. Therefore a third inoculation was made on 30 July in an attempt to ensure epidemic development. Instead of inoculating a single leaf, for the third inoculation, all plants along a 40-45-cm section of row in the center of each plot were misted with a bacterial suspension containing approximately 9×10^8 cfu per milliliter. Individual common blight lesions were counted on all leaves within each plot, and the total number of lesions within each plot was recorded at 2- to 3-day intervals throughout the remainder of the growing season. The percent leaf area affected was not recorded. Plots were also surveyed for SO_2 injury after three exposures to the pollutant.

Beans from infected and noninfected plants within each plot were harvested separately at the end of the season. Pods were stripped from the stems and sorted into three groups: mature, immature (green), or aborted pods. Mature pods were then placed in a drying oven (70 C) for 24 hr before seeds were removed and weighed. Dry mass of seeds and number of mature, immature, and aborted pods per plant were recorded for each plot.

RESULTS

Injury to foliage due to feeding by Mexican bean beetles (*Epilachna varivestis*) was observed during the experiment, but damage was negligible in most plots (less than 5% total leaf area removed). However, plants in one plot were significantly defoliated by the insects (25% total leaf area removed) and data from this plot were omitted from the analysis.

Foliar injury due to SO_2 was also observed, primarily during the first several exposures, when plants were small and leaves were closer to exit holes in the plenum where SO_2 was more concentrated. An assessment of SO_2 injury was made on 16 July after three exposures to the pollutant. Plants in six of the plots showed no injury, 5% or less of the total leaf area was injured in four of the plots, and 30% of the leaf area was injured in one plot (Table 1). Within individual plots injury was most severe on foliage located directly over the exit holes in the plenum and increased with the distance from the blower, also corresponding with the prevailing wind direction. Severely injured leaves senesced rapidly and dropped off the plants. No significant injury was observed after the first three exposures as the plants grew up and away from the plenum.

Concentrations of the pollutant in air were difficult to control because of wind drift. Concentrations of SO_2 varied considerably between replicate plots of the same treatment but also within individual plots (Table 2). When treatment plots within each block were ranked according to the average SO_2 concentration received during a given 3-hr exposure, the ordering of plots was not always consistent from day to day because of changes in wind speed and direction.

Because of the variability in pollutant concentrations from one exposure to the next, a constant concentration could not be used to characterize the pollutant burden within an individual plot over the course of the experiment. Therefore, a cumulative measure of pollutant concentration was used to establish the relationship between exposure to the pollutant and development of the disease.

A cumulative measurement of pollutant burden was used because the frequency of peak concentrations of SO_2 exceeding 1.00 ppm was quite low and not believed to have much influence.

The number of lesions in each plot was cumulative because sampling was not destructive and leaf loss after appearance of symptoms was negligible. To determine the relationship between SO_2 and development of the disease, cumulative pollutant concentrations were calculated so that the resulting data points, consisting of lesion counts on a given date (dependent variable) and the corresponding cumulative pollutant concentration received up to that date (independent variable), matched in time. Within each plot, pollutant concentration measurement errors were assumed to be multiplicative; therefore, a geometric rather than arithmetic mean was considered to be the best estimate of the actual concentration in the plot during a given exposure. The pollutant concentrations were then made cumulative by summing the geometric means for each consecutive exposure period up to the appropriate date (Table 3). It should be noted that throughout this paper, the pollutant burden has been expressed in terms of concentration, rather than dose (concentration × duration of exposure). However, because all exposures were 3 hr in duration, total or cumulative pollutant dose differs from total or cumulative pollutant concentration by a constant factor, and therefore, these two measures of pollutant burden are virtually interchangeable with respect to their relationship to disease development.

It is characteristic of many growth processes that the rate of increase at any time is proportional to the size already attained and can be described by the following differential equation:

$$dy/dx = by,$$

where y is the size, x is time, and b is the constant relative rate increase. The closed form solution is

$$y = A \exp b(x-x_0)$$

where A is the size at the initial time (x_0) This expression can then be linearized by taking natural logarithms, resulting in an equation of the following form:

$$\log_e y = \log_e A + b(x-x_0).$$

Because time is an intrinsic factor in both cumulative pollutant concentration and lesion number, this particular linear model was used to describe the relationship between the two variables, where x is the cumulative geometric mean of the pollutant concentration in each plot and y is the cumulative number of lesions (+ 1, so that the logarithm is finite). An additive error term was included and was assumed to be normally distributed, with mean 0 and variance σ^2. The regression lines obtained from this analysis are shown in Figure 1, and the corresponding prediction equations and coefficients of determination are provided in Table 4. The rate of lesion appearance was highest in those plots receiving low concentrations of SO_2 and decreased proportionately with increasing cumulative SO_2 concentration.

The same data set was then divided into subsets by date. For each subset, the same model was used, regressing the natural logarithm of the number of lesions (+ 1) on cumulative SO_2 concentration. The resulting regression lines, for each of the last four dates appear in Figure 2. The estimated slopes are negative, indicating that increasing cumulative SO_2 concentration results in

TABLE 3. Number of common blight lesions and cumulative geometric means of atmospheric SO_2 concentration (ppm) in plots of field-grown red kidney bean plants

	Cumulative geometric mean SO_2 concentration (ppm)					Lesions (no.)				
	Date					Date				
Plot	7/19	7/29	8/10	8/14	8/26	7/19	7/29	8/10	8/14	8/26
1	0.23	0.27	0.43	0.55	0.75	0	1	8	9	52
2	0.52	0.62	1.31	1.73	1.97	0	0	2	7	32
3	0.29	0.35	0.49	0.60	0.62	0	0	1	3	38
4	0.62	0.71	1.40	1.74	2.37	0	0	1	1	39
5	0.47	0.50	0.62	0.73	0.80	0	1	5	5	31
6	0.85	0.96	1.69	2.01	2.35	0	0	3	2	6
7	0.08	0.10	0.21	0.27	0.38	0	0	6	3	21
8	0.66	0.78	1.33	1.59	1.87	0	1	4	3	35
9	0.33	0.46	1.28	1.69	1.96	0	1	1	0	5
10	1.09	1.28	2.09	2.58	2.90	0	1	0	0	14
11	0.21	0.25	0.33	0.37	0.41	0	2	4	6	26

TABLE 4. Prediction equations and coefficients of determination for linear regressions of $\log_e$ (lesions + 1) on the cumulative geometric mean SO_2 concentration, for each of 11 individual plots[a]

Plot	Prediction equation	r^2
1	$Y = -0.48 + 3.25\ X$	0.89
2	$Y = -0.29 + 0.91\ X$	0.85
3	$Y = -1.31 + 4.77\ X$	0.91
4	$Y = -0.51 + 0.94\ X$	0.84
5	$Y = -1.13 + 3.36\ X$	0.77
6	$Y = -0.14 + 0.32\ X$	0.81
7	$Y = -0.04 + 4.23\ X$	0.87
8	$Y = -0.34 + 0.93\ X$	0.67
9	$Y = -0.10 + 0.41\ X$	0.25
10	$Y = -0.45 + 0.42\ X$	0.51
11	$Y = -0.67 + 5.14\ X$	0.93

[a] Y = $\log_e$ (lesions + 1) and X = cumulative geometric mean SO_2 concentration in ppm.

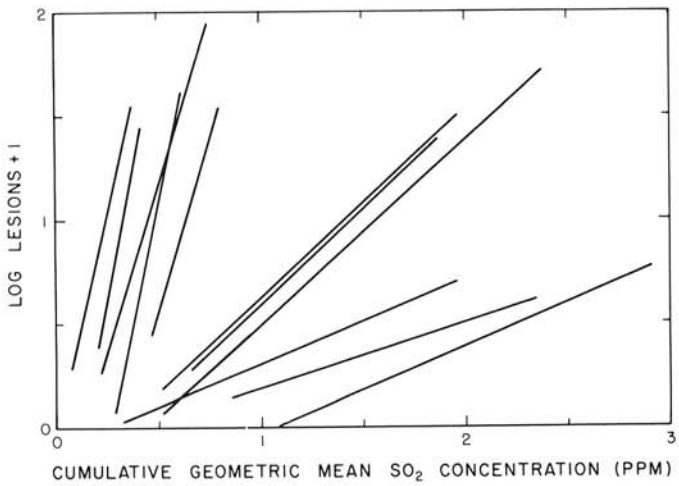

Fig. 1. Regressions of $\log_e$ (lesions + 1) on cumulative geometric mean SO_2 concentration (ppm). Each line represents data from a single plot.

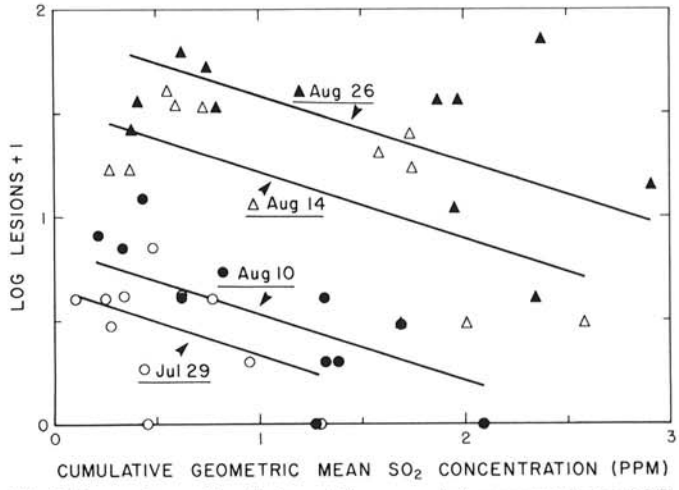

Fig. 2. Regressions of $\log_e$ (lesions + 1) on cumulative geometric mean SO_2 concentration. Data are the same as for Fig. 1, except that they are grouped by date instead of individual plot. Note equality of slopes.

TABLE 5. Yield of kidney bean plants exposed to SO_2, expressed in terms of dry mass of seeds (g) per plant[a]

	Noninfected		Infected		
Plot	Yield per plant (g)	Plants (no.)	Yield per Plant (g)	Plants (no.)	Lesions (maximum no.)
1	14.52	284	12.70	13	82
2	13.88	315	15.84	10	47
3	14.59	319	19.46	12	61
4	13.30	303	14.88	10	73
5	15.61	323	12.49	10	32
6	12.60	314	11.08	10	6
7	13.82	337	15.71	6	28
8	12.54	307	10.18	10	35
9	11.83	360	8.16	8	10
10	11.93	361	9.68	7	16
11	15.37	342	14.33	10	35

[a] Infected plants inoculated with *Xanthomonas campestris* pv. *phaseoli* at the beginning of the growing season and showed visible symptoms of common blight during the season. Noninfected plants showed no symptoms of common blight.

a reduced rate of lesion appearance. This relationship between lesion appearance and cumulative SO_2 concentration was consistent for each date except 19 July. Before this time there was no apparent effect of cumulative SO_2 concentration on disease due to low pollutant concentration or low levels of disease development or both.

Yield, expressed in terms of dry mass of seeds per plant, was not significantly different between infected and noninfected plants within each plot (Table 5). However, the number of lesions per infected plant was small, and they did not appear until late in the season, which may account for the apparent lack of difference between infected and noninfected plants.

Cumulative SO_2 concentration, on the other hand, did have a significant effect on yield but only in those plants without visible symptoms of common blight. Yield of noninfected plants decreased significantly with increasing cumulative pollutant concentration ($y = 15.4 - 1.17x$, $r^2 = 0.65$, where y = dry mass of seeds per plant in grams, and x = cumulative geometric mean SO_2 concentration, in ppm). A similar trend was not observed among infected plants. This discrepancy may be due to a large difference in sample size, as the number of infected plants in the total population of each plot was low. There was no relationship between cumulative SO_2 concentration and the numbers of immature or aborted pods per plant.

DISCUSSION

Exposure to SO_2 can result in a suppression of common blight development under field conditions. Although concentrations of SO_2 that resulted in extensive foliar injury are probably considerably higher than current ambient SO_2 concentrations, the remaining levels used in the study are well within the range of concentrations found in the vicinity of sources of the pollutant (9).

There is no evidence of an additive effect of exposure to SO_2 and common blight in terms of yield loss when disease severity is relatively low. However, under conditions favorable to the development of severe common blight epidemics, the interaction between the pathogen and SO_2 may be important. The presence of the pollutant may actually provide some protection against loss from disease due to the inhibitory effect on disease development. This relationship requires further examination and quantification, particularly at higher levels of disease severity and under a more controlled pollutant regime.

Air pollutants, however, with the possible exception of ozone, rarely occur alone. For this reason, future research on pollutant-pathogen interactions should focus on the effects of combinations of pollutants that are known to occur concurrently or consecutively. The experiment reported here was conducted in the presence of ambient ozone (O_3) at an average daily concentration of approximately 0.04 ppm (0900–1600 EST). No attempt was made to modify the level or to protect the plants from possible oxidant injury. Had a range of ozone concentrations been included in addition to levels of SO_2, the complexity of the experiment would have been greatly increased. However, it would have provided considerably more detailed information about the possible interactive effects of these two gases on disease development. Other pollutant mixtures of interest might include SO_2 and HF, HF and ozone, or SO_2 and nitrogen dioxide (NO_2).

Future research should also consider effects of pollutants on other pathosystems and interactions with other pests and management practices. An effort should be made to seek out and characterize those modifications in host-pathogen interactions that have the greatest potential impact on current disease management strategies near sources of air pollutants.

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