The Joint Action of Hydrogen Fluoride and Sulfur Dioxide on the Development of Common Blight of Red Kidney Bean

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ABSTRACT

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Three-week-old *Phaseolus vulgaris* 'California Light Red Kidney' plants were exposed to fluoride as HF at 0, 1, or 3 $\mu g \cdot m^{-3}$ in filtered air, SO_2 at 0, 260, or 780 $\mu g \cdot m^{-3}$ in filtered air, or all combinations of the two gases. Exposures were conducted continuously (HF) or for 6 hr daily (SO_2) for 5 days before, after, or before and after inoculation with *Xanthomonas campestris* pv. *phaseoli*. Diameters of lesions were measured when first visible and again 10 days after inoculation. Leaf-surface populations of the pathogen were established and assayed at 5-day intervals for 15 days. Preinoculation and postinoculation exposure to SO_2 caused significantly smaller lesions and longer latent periods. Postinoculation exposure to HF resulted in significantly longer latent periods. Interactions between the two gases occurred only when the exposures were concurrent. In general, the joint action of the two gases resulted in smaller lesions and longer latent periods. The pollutants did not significantly alter the growth of the pathogen in the resident phase.

Many authors have speculated on the importance of indirect effects of air pollutants on the productivity of crop plants (1,2,4,16–18). Of particular interest are pollutant-mediated alterations in normal host-parasite or host-predator interactions (1,2,4). While the economics of the potential effects are unknown, they could be considerable since alterations of disease and insect development could increase the need for control measures or result in changes in crop quality. In addition, pollutants may affect control measures directly (16). Most of the information on the effects of pollutants on host-pathogen relations is based on single-pollutant experiments. There are few data on the interactive effects of three stresses imposed at the same time.

We have reported the effects of SO_2 and HF, separately, on the development of common blight of red kidney bean, caused by *Xanthomonas campestris* pv. *phaseoli* (5–7). In growth chamber experiments, exposure to pulses of SO_2, supplied either as square-waves or triangular-waves, increased the latent period and slowed lesion development but had no effect on the development of leaf-surface populations of the pathogen. Exposure of plants to HF in growth chambers resulted in smaller lesions compared to those produced on control plants, and the development of leaf-surface populations of the pathogen was slowed significantly. In the field, increasing concentrations of SO_2 also delayed the development of common blight, and significantly lessened yield of healthy, but not infected, bean plants (15). Experiments conducted in the field with extremely low doses of HF did not show significant reductions in disease development due to the pollutant (K. L. Reynolds, *unpublished*). There is a need to evaluate the importance of these interactions as part of a crop production system, which includes multiple stresses, such as mixtures of pollutants. The purposes of the research reported here were to assess potential effects of the joint action of HF and SO_2 on development of common blight; to evaluate effects of the two pollutants on the development of leaf surface populations of the pathogen; and to determine whether concentrations of the two gases that occur in the field near a source such as a primary aluminum smelter are likely to cause effects on a sensitive plant/pathogen system, such as the combination of red kidney bean and *X. campestris* pv. *phaseoli*.

MATERIALS AND METHODS

Plant culture. Seeds of red kidney bean (*Phaseolus vulgaris* L. 'California Light Red Kidney') were sown in 10-cm-diameter pots in a pasteurized peat, sandy loam, and sand mixture (1:1:1; w/w). Plants were grown in a greenhouse maintained at 25/20 C (day/night) with a 16-hr photoperiod provided by multi-vapor HID lamps. Liquid fertilizer (20:20:20 NPK) was applied weekly. After 2 wk, seedlings were thinned to one plant per pot. One week later, plants were randomly assigned to controlled environment chambers maintained at 25/20 C and 60/70% relative humidity (light/dark). A 14-hr photoperiod with an intensity of 600–700 $\mu mol \cdot s^{-1} \cdot m^{-2}$ was provided to achieve optimal growth in the chambers.

Pathogen culture and inoculation. *X. c.* pv. *phaseoli* was grown on rifampin agar medium (19) at 27 C and reisolated from infected plants frequently to maintain virulence and pathogenicity. Lesions were induced by water-soaking a spot on the first trifoliolate leaf of each plant with a suspension of about 10^8 colony-forming units (cfu) per milliliter of sterile water. Tween-80 at a concentration of 0.001% was included as a wetting agent. Resident populations of the bacterium were established by gently spraying a different leaf of the plant with a similar suspension of the bacterium in sterile water.

Naturally occurring populations of *X. c.* pv. *phaseoli* were not detected on leaf surfaces of control plants, nor were lesions found on plants inoculated only with sterile water.

Generation and monitoring of pollutants. Gaseous HF was generated by volatilizing aqueous solutions of HF in a hot air stream, which was subsequently metered into the air inlet of the chamber (10). Chamber air was sampled continuously during the experiments by using single-tape samplers (Anderson Samplers, Inc., RAC Division, Atlanta, GA) with NaOH-impregnated filter paper tapes. After sampling, spots representing 4-hr collections were cut from the tapes and eluted, and the concentration of F was determined by using an ion-specific electrode (10).

Sulfur dioxide was supplied from a tank of 20% SO_2 in nitrogen. The gas was metered through a micrometer valve into the inlet air of the chambers. Concentrations of SO_2 were monitored with a pulsed fluorescence analyzer (Thermoelectron Corporation,

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Hopkinton, MA) that was calibrated against certified permeation tubes and verified by using certified span gas.

Design, sampling, and measurement. The experiment was designed as a complete 3^4 factorial in which the four factors were HF before inoculation, SO_2 before inoculation, HF after inoculation, and SO_2 after inoculation; the three levels of fluoride as HF were 0, 1, or 3 $\mu g \cdot m^{-3}$ in filtered air and the three levels of SO_2 were 0, 260, or 780 $\mu g \cdot m^{-3}$; four plants were allocated to each of the treatments; and the experiment was replicated (at 20-day intervals) three times (i.e., in three blocks). The duration of the exposure period before or after inoculation was 5 days. During each period, HF was supplied continuously but SO_2 was supplied only for 6 hr each day.

Plants were observed daily for lesion development; the day when a lesion first appeared, the size of the initial lesion, and the size of the lesion 10 days after inoculation were recorded for every plant. Pollutant-induced foliar injury did not occur.

At 0, 5, 10, and 15 days after inoculation, leaves were collected from four plants of 27 of the 81 treatments for use in estimating resident populations of the pathogen. After measurement of area, the leaves were washed for 1 hr in sterile water containing 0.005% Tween-80. The washings were serially diluted, plated on rifampin agar medium, incubated at 27 C for 2–3 days, and the colonies were counted. Due to the size of the experiment, only one-third of the treatments could be sampled for resident populations in any one replication of the experiment. Consequently, the experimental design was modified for the estimate of resident populations. A 3^4 factorial was used with four plants per treatment, but it was only partially replicated; the three blocks of the experiment resulted in one complete replicate. Therefore, the analysis had some effects confounded.

An analysis of variance was performed and, where main effects or interactions were of interest, the sums of squares were partitioned to provide information concerning the shape of the response curve or surface.

RESULTS

Estimation of resident populations. The development of populations of *X. c.* pv. *phaseoli* in resident phase was not affected by SO_2, HF, or their combination. Plots of population growth indicated that the growth curves were diverging at 15 days after inoculation, but did not depart from a common model.

Measurement of lesion development. The main effect of experiment (block) was significant, indicating that blocking by time was appropriate, and the experiment by treatment interaction term was used as experimental error.

Exposure of plants to SO_2 resulted in smaller lesions and longer latent periods regardless of whether exposure occurred before or after inoculation with the pathogen. Exposure of plants to SO_2 before inoculation resulted in longer latent periods, and slightly smaller lesions (Tables 1 and 2). Response to postinoculation exposure was curvilinear (Tables 1 and 2) with SO_2 concentration. The preinoculation by postinoculation interaction was not significant. Latent period increased linearly with increasing SO_2 concentration, whereas effects on both initial and final lesion size were curvilinear. The results of lesion size measurements indicate a slight stimulation in lesion development at a concentration of SO_2 of 260 $\mu g \cdot m^{-3}$ in filtered air. The magnitude of the stimulation is approximately one-half of the magnitude of the inhibition observed in plants given postinoculation exposure to SO_2.

HF exposures also resulted in longer latent periods in plants exposed after inoculation, but effects on lesion size were not detected (Tables 1 and 2). The preinoculation by postinoculation interaction was not significant.

Significant interactive effects of the two gases on lesion development and latent period occurred only when the pollutants occurred together and the action of one gas was not altered by previous or subsequent exposure to the other (Tables 1 and 3). When plants were exposed before inoculation, the size of lesions decreased and latent period increased with increasing concentrations of both gases except fluoride (as HF) at 1 $\mu g \cdot m^{-3}$ in filtered air where increasing SO_2 concentrations resulted in a slight increase in lesion size. The major inhibitory effect, however, was at the highest concentration of both gases (Fig. 1). Postinoculation exposure resulted in similar interactions between the two gases (Fig. 2). In general, when they occur together, the highest concentration of both gases caused a significant decrease in lesion size and an increase in the latent period. A slight increase in the size of lesions was found as the HF concentration was increased with SO_2 at 260 $\mu g \cdot m^{-3}$ in filtered air.

TABLE 1. Summary of analysis of variance of lesion variables in plants exposed to HF and/or SO_2, before or after inoculation with *Xanthomonas campestris* pv. *phaseoli*

		Mean square estimates for:		
Source	df	Initial size	Final size	Latent period
Experiment	2	17.65**[a]	35.54**	3.51**
Preinoc SO_2[b]	2	3.12**	2.95**	3.13**
Linear (L)	1	0.95	2.47	4.91*
Quadratic (Q)	1	5.28**	3.34*	1.34
Postinoc SO_2	2	3.52**	10.68**	7.99**
Linear	1	2.24	10.09**	13.72**
Quadratic	1	4.79**	11.27**	2.25
Preinoc HF	2	0.40	0.18	2.10
Linear	1	0.02	0.37	2.91
Quadratic	1	0.78	0.00	1.30
Postinoc HF	2	0.80	0.53	9.89**
Linear	1	0.51	1.08	10.23**
Quadratic	1	1.09	0.07	9.55**
Pre- HF × Pre- SO_2	4	2.45**	4.00**	1.84
L × L	1	0.72	4.73*	1.39
L × Q	1	4.48**	4.08**	1.32
Q × L	1	3.93*	6.76**	4.13*
Q × Q	1	0.66	0.41	0.50
Post- HF × Post- SO_2	4	1.25	2.14*	6.75**
L × L	1	0.05	0.32	4.23*
L × Q	1	1.82	5.87**	21.96**
Q × L	1	2.31	1.36	0.09
Q × Q	1	0.83	1.15	0.74
Pre- HF × Post- HF	4	0.51	0.59	1.17
Pre- SO_2 × Post- SO_2	4	0.38	0.30	1.06
Pre- HF × Post- SO_2	4	0.83	0.98	0.75
Pre- SO_2 × Post- HF	4	1.14	0.84	0.34
Error	208	0.62	0.80	0.91

[a]Asterisks (* and **) indicate the probability ($P < 0.05$ and 0.01, respectively) of a value larger than F.
[b]Preinoc and Postinoc refer to the timing of exposure with relation to inoculation.

TABLE 2. Effects of HF or SO_2 on development of lesions caused by *X. campestris* pv. *phaseoli* in red kidney bean

		Lesion variables[a]		
Pollutant	Level ($\mu g \cdot m^{-3}$)	Initial size (mm)	Final size (mm)	Latent period (days)
HF preinoc[b]	0	3.0	4.0	5.5
	1	3.0	4.0	6.0
	3	3.0	4.0	6.0
HF postinoc	0	3.0	4.0	5.0
	1	3.5	4.0	6.0
	3	3.0	4.0	5.0
SO_2 preinoc	0	3.0	4.0	5.5
	260	3.5	4.5	6.0
	780	3.0	4.0	6.0
SO_2 postinoc	0	3.5	4.5	5.0
	260	3.0	3.5	6.0
	780	3.0	4.0	6.0

[a]Values are treatment means rounded to the nearest 0.5.
[b]Preinoc and postinoc refer to the timing of exposure in relation to inoculation.

DISCUSSION

The co-occurrence of pollutants in the atmosphere has generated considerable concern in the past few years, because plants might respond differently to mixtures of gases than they do to single pollutants (9,11,14). Studies that have been done have focused on combinations of ozone and SO_2. However, significant concentrations of ozone and SO_2 are not likely to co-occur in rural North America. SO_2 is generally associated with large point sources that also release large quantities of oxides of nitrogen, which remove ozone from the air (9,14). Gases that do co-occur are those that are released from the same source, or from nearby sources, and that do not interact in the atmosphere. An example of such a co-occurrence is HF and SO_2, released from primary aluminum smelters.

Relatively few studies have examined the joint action of pollutants on disease development (3,17,18), and these have not considered the question of co-occurrence versus sequential occurrences of the gases. The results of this study show that exposures to HF and SO_2 affect the development of common blight in red kidney bean. When the two gases occur together, either before or after inoculation, the delay in disease development is greater than might be expected from the sum of the two main effects. The results presented confirm earlier findings that exposure to SO_2 and HF result in smaller lesions and longer latent periods in disease caused by *X. c.* pv. *phaseoli*. In our previous studies (5–7), we did not find an effect of preinoculation exposure to either pollutant on disease development. In this study, modifications in disease development from preinoculation exposure were found, but are due, in the case of SO_2, to the slight stimulation of lesion

TABLE 3. Effects of HF and SO_2 applied alone and concurrently on lesion development by *X. campestris* pv. *phaseoli* in red kidney bean

Concentration ($\mu g \cdot m^{-3}$)		Lesion variables[a]					
		Preinoculation exposure			Postinoculation exposure		
HF	SO_2	Latent period (days)	Initial size (mm)	Final size (mm)	Latent period (days)	Initial size (mm)	Final size (mm)
0	0	5.0	3.0	4.0	4.5	3.5	5.0
0	260	6.0	3.5	4.5	6.0	3.0	3.5
0	780	5.5	3.0	4.0	6.0	3.5	4.0
1	0	5.5	3.0	4.0	5.5	3.5	4.5
1	260	6.0	3.5	4.0	6.5	3.0	4.0
1	780	6.0	3.5	4.5	6.0	3.0	4.0
3	0	5.5	3.5	4.5	6.0	3.0	4.5
3	260	6.0	3.5	4.0	5.5	3.0	5.0
3	780	6.0	3.0	3.5	6.0	3.0	3.5

[a] Values are treatment means rounded to the nearest 0.5.

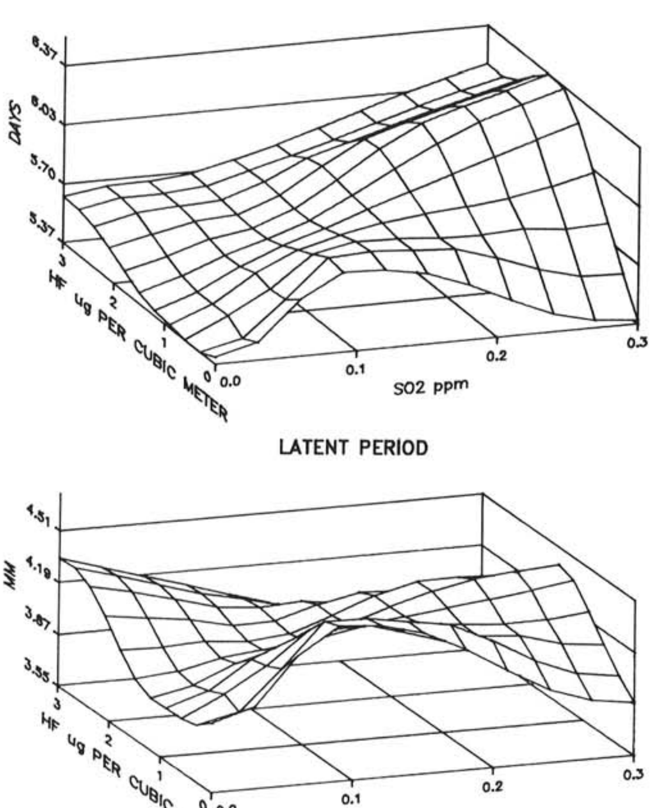

Fig. 1. The effects of preinoculation exposure of red kidney bean to HF and SO_2 on latent period (upper) and lesion size (lower) of *Xanthomonas campestris* pv. *phaseoli* measured 10 days after inoculation.

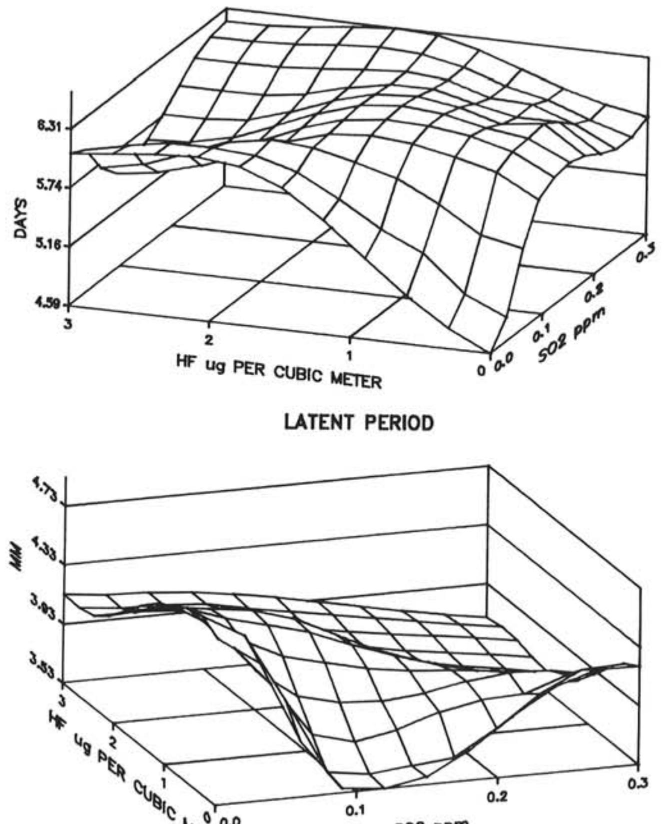

Fig. 2. The effects of postinoculation exposure of red kidney bean to HF and SO_2 on latent period (upper) and lesion size (lower) of *Xanthomonas campestris* pv. *phaseoli* measured 10 days after exposure.

size. There was little difference between the control and either of the preinoculation treatments. Preinoculation exposure to SO_2 caused an increase in latent period, an effect not previously observed. We did not observe effects of HF or SO_2 on resident populations of the pathogen. In our earlier studies (5,6), however, we did measure a reduced growth rate of *X. c.* pv. *phaseoli* on surfaces of leaves exposed to HF, but not on those exposed to SO_2. We conclude that the difference between this experiment and the previous study is due to the partial replication (and greater variability) of this experiment due to the large number of treatments involved in the study.

McCune (11) has reviewed the literature concerning the joint action of HF and SO_2 with respect to foliar injury and accumulation of F. He reported that cases of nonadditivity of response due to joint action of the gases occur, but not often. In particular, the two pollutants have not been shown to interact with respect to *P. vulgaris*, either when the exposures occurred consecutively or concurrently. Our results, obtained by using disease development as the response variable, are different. The difference may be due to variation in plant material, growing conditions, or exposure concentrations and durations, but it may also be due to the nature of the response observed. In many experiments, it has been found that plants apparently respond to pollutants at very low levels when the response is measured by disease development or insect feeding preference (1,2,4) or when exposure regimes simulating field conditions are used (8,12,13). Thus, plants may be more sensitive to pollutants than generally accepted, and significant effects on agricultural productivity could occur at pollutant levels generally considered to be safe (i.e., below the threshold for visible injury or the level at which direct effects on productivity have been reported).

This study was conducted under controlled conditions and used pollutant regimes not routinely encountered in the field. However, the concentrations of pollutants used were realistic, and the duration and frequency of exposures, particularly for SO_2, were not far removed from what might be experienced in some agricultural areas. The results demonstrate that modifications of plant-pathogen relationships by pollutants may occur, and furthermore, that such effects, even though they are limited in this particular case, represent a sensitivity of the plant (through host-parasite relations) to pollutants at levels much lower than generally accepted for the plants themselves. These interactions should be considered as an indicator of the overall sensitivity of plants in the establishment of air quality standards.

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