Growth and Lesion Development of *Xanthomonas campestris* pv. *phaseoli* on Leaves of Red Kidney Bean Plants Exposed to Hydrogen Fluoride

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ABSTRACT

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Low concentrations of atmospheric hydrogen fluoride (HF) (1 or 3 $\mu gF \cdot m^{-3}$) applied continuously for 5 days after inoculation of red kidney bean leaves with *Xanthomonas campestris* pv. *phaseoli* resulted in longer latent periods and smaller initial lesion size. When HF was supplied continuously for 5 days prior to inoculation or in intermittent exposures of 12 $hr \cdot day^{-1}$ for up to 4 days after inoculation, no measurable differences in lesion characteristics were found. Growth of the bacterium in the resident phase on leaf surfaces was slowed by continuous, but not by intermittent, exposures. Sodium fluoride, when incorporated in medium at a concentration of 0.01 M, inhibited growth of the pathogen.

Air pollutants affect the development of plant disease (2) and the growth of leaf surface microflora and resident pathogens (4,6,7). The mode of action is not well understood, but it could be related to either a direct effect of the pollutant on the pathogen or a modification of the suitability of the host for infection and pathogen growth.

Hydrogen fluoride (HF) is a compound that is highly toxic to vegetation (10). Its emission to the atmosphere is associated with industries such as aluminum smelting and the manufacture of phosphate fertilizer. It is also released during combustion of coal. HF is readily sorbed by leaves and may affect pathogens as they grow inside the leaf or on leaf surfaces.

As part of our ongoing program to assess the effects of several pollutants on the development of plant disease, we studied the effects of fluoride, either as gaseous HF or sodium fluoride (NaF) in solution, on *Xanthomonas campestris* pv. *phaseoli*.

The objectives of our research were: to determine if HF affects the development of lesions caused by *X. campestris* pv. *phaseoli*; to determine the effect of HF on the development of resident populations of *X. campestris* pv. *phaseoli* known to be important in disease development (11); and to determine the effect of fluoride (F^-), when incorporated into a growth medium as NaF, upon the growth of *X. campestris* pv. *phaseoli*. Many organisms are known to be sensitive in vitro to fluoride in concentrations that might occur in leaves exposed to HF (0.001–0.003 M) (9).

MATERIALS AND METHODS

Plant culture. Seeds of red kidney bean (*Phaseolus vulgaris* L. 'California Light Red Kidney') were sown in 10-cm-diameter pots containing a pasteurized mixture of peat, sandy loam, and sand (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 high-intensity discharge (HID) lamps. Fertilizer (20:20:20, NPK) was applied weekly in solution. After 2 wk, seedlings were thinned to one plant per pot to achieve uniformity among plants. At 3 wk after seeding, plants were transferred to controlled environment chambers maintained at 25/20 C and 60/70% relative humidity (light/dark) and a 14-hr photoperiod with an irradiance of 600–700 $\mu E \cdot s^{-1} \cdot m^{-2}$. The 2-hr difference in photoperiod was introduced to achieve optimal growth under either greenhouse or growth chamber conditions.

Pathogen culture and inoculation. *X. campestris* pv. *phaseoli* was grown on rifampin agar medium (11) at 27 C and reisolated from plants frequently to maintain its pathogenicity and virulence. Resident populations were established on the surface of one trifoliolate leaf of each plant by spray application of a suspension of bacteria ($\sim 10^8$ $cfu \cdot ml^{-1}$) in sterile water containing 0.001% Tween-80. Lesions were induced by water-soaking an area of a different leaf with inoculum.

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

Response to continuous exposure. HF gas was generated by volatilizing aqueous HF solutions in a hot air stream, which was then metered into the air inlet of the chamber (5). During the experiments chamber air was sampled continuously by using single tape samplers (Anderson Samplers, Inc., RAC Division, Atlanta, GA 30366) 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 (5). HF injections were modified based on analyses of the tapes.

Continuous exposures at 0, 1, or 3 $\mu gF \cdot m^{-3}$ for 5 days were provided either before or after, or before and after inoculation with the pathogen. The experiment was a 3^2 factorial in a completely randomized design with pre- and postinoculation exposure as main factors. Each experiment was repeated three times. At 0, 5, 10, and 15 days after inoculation, four leaves from each treatment were collected and leaf areas were determined. The population of the organism on each leaf was estimated by washing leaves in sterile water with Tween-80 for 1 hr, serially diluting the washings, and plating them on rifampin agar medium. Growth rates of surface populations of *X. campestris* pv. *phaseoli* were estimated by regressing the log of cfu per square centimeter of leaf area on time after inoculation. An analysis of variance was then performed on the 5- and 10-day growth rates (β_1 of the regression equations) by using pre- and postinoculation HF concentration as main factors. The diameters of lesions were measured daily for 5 days following the latent period (time from inoculation to first appearance of symptoms). At the conclusion of each experiment, the first and second trifoliolate leaves were harvested, dried, and analyzed for F (1).

Response to intermittent exposure. In intermittent exposures, plants were exposed to all possible combinations of two levels of HF for 12 hr on each of 4 consecutive days after inoculation, resulting in 16 treatments. The levels were 0 and 1 $\mu gF \cdot m^{-3}$ in one

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experiment, and 0 and 3.0 $\mu gF \cdot m^{-3}$ in another. The experiment was a 2^4 factorial in a completely randomized design with daily exposure to HF as main factors. Each experiment was repeated three times and analyses were conducted on treatment means, resulting in a blocking factor of experiments.

Initial and final lesion sizes, latent period, and foliar concentration of F were measured for both the 1.0 and 3.0 $\mu gF \cdot m^{-3}$ treatments. An analysis of variance was performed on treatment means for each variable at each level of HF. Treatment sums of squares were partitioned to allow estimation of the effects of total dose (0–4 exposures) and order within a given dose (the temporal order within equal numbers of exposures). Growth of surface populations of the pathogen was measured for the 3.0 $\mu gF \cdot m^{-3}$ treatment only. Growth rates for each treatment were determined by the regression of cfu cm^{-2} of leaf area on time after inoculation. Analyses of variance were performed on 5- and 10-day growth rates with treatment sums of squares partitioned to estimate the effects of dose and order within dose.

Growth of *X. campestris* pv. *phaseoli* in liquid media containing NaF. Liquid medium was prepared by adding NaF to modified Czapek Dox broth (Difco Laboratories, Detroit, MI 48201) to provide six F concentrations: 0.0, 0.001, 0.005, 0.01, 0.05, and 0.1 M NaF. Twelve 250-ml flasks (two flasks per NaF treatment, each containing 50 ml of broth) were inoculated with a bacterial suspension in sterile water ($\sim 10^8$ cfu per flask). The flasks were placed on a shaker at 20 C for 2 days. One milliliter was sampled from each flask 0, 24, and 48 hr after inoculation, serially diluted, plated on rifampin agar medium, and incubated at 27 C for 2–3 days. Colonies were counted to estimate bacterial populations. The experiment was repeated three times. During the second repetition of the experiment, a 5-ml sample was taken from each flask at 0, 24, and 48 hr after inoculation and analyzed for F by using an ion-specific electrode.

RESULTS

Effects of HF on lesion development by *X. campestris* pv. *phaseoli*. With continuous exposure, an increase in the concentration of HF resulted in an increase in the length of the latent period and a decrease in the initial size of lesions, but had no effect on final lesion size in bean plants that were inoculated with the pathogen prior to HF exposure (Table 1). Continuous exposure to HF before inoculation had no effect on the latent period or the lesion size. There were no significant interactions between pre- and postinoculation exposures (Table 2).

With intermittent HF exposures using 1.0 or 3.0 $\mu gF \cdot m^{-3}$ there were no significant effects of HF dose (number of exposures given) or order within dose on the length of the latent period or on the initial or final lesion sizes. There was a significant linear effect of the number of HF exposures on the accumulation of F in leaves of plants exposed to 1.0 $\mu gF \cdot m^{-3}$ and a significant quadratic effect of dose on foliar F accumulation in plants exposed to 3.0 $\mu gF \cdot m^{-3}$ (Table 3).

Effects of HF on growth of *X. campestris* pv. *phaseoli* on leaf surfaces. In continuous exposures of bean plants, an increase in HF concentration both before and after inoculation significantly decreased 5- and 10-day growth rates of the bacterium on leaf surfaces (Tables 4 and 5). Intermittent exposures to 3 $\mu gF \cdot m^{-3}$ did not cause significant effects on the 10-day growth rates of *X.*

TABLE 1. Effect of the concentration of hydrogen fluoride (HF) in postinoculation exposures on the initial size of leaf lesions and the length of the latent period in red kidney bean inoculated with *Xanthomonas campestris* pv. *phaseoli*

HF concentration ($\mu gF \cdot m^{-3}$)	Initial lesion diameter (mm)	Latent period (days)
0	4.49	3.36
1	3.59	3.37
3	3.55	3.59

TABLE 2. Analysis of variance and mean square estimates for effects of continuous exposures to hydrogen fluoride (HF) on characteristics of lesion development caused by *Xanthomonas campestris* pv. *phaseoli* on red kidney bean leaves

		Mean square estimates		
		Lesion size		Latent
Source	df	Initial	Final	period
Experiment	1[a]	1.38*[c]	12.98**	0.97*
Preinoculation exposure	2	0.38	0.42	0.03
Postinoculation exposure	2		0.80	
Linear	(1)	2.06**		0.10*
Quadratic	(1)	1.32*		0.01
Pre- × post-	4	0.22	0.13	0.01
Error	8[b]	0.18	0.80	0.01

[a] For final lesion size, experiment df = 2.
[b] For final lesion size, error df = 16.
[c] Asterisks (* and **) indicate the probability ($P \leq 0.05$ and 0.01, respectively) of a value larger than F.

TABLE 3. Effects of the number of exposures to hydrogen fluoride (HF) on the accumulation of fluoride by leaves of red kidney bean plants

Number of 12-hr exposures	F content ($\mu gF \cdot g^{-1}$ dry weight) of leaves exposed to HF at:	
	1 $\mu gF \cdot m^{-3}$	3 $\mu gF \cdot m^{-3}$
0	6.1	6.6
1	41.8	73.8
2	57.8	102.5
3	79.4	131.8
4	129.4	151.9

TABLE 4. Growth rates of *Xanthomonas campestris* pv. *phaseoli* on leaf surfaces of red kidney bean plants continuously exposed to hydrogen fluoride (HF)

HF concentration ($\mu gF \cdot m^{-3}$)		Growth rate (log cfu·cm^{-2}·day^{-1})	
Preinoculation	Postinoculation	5 days[a]	10 days
0	0	0.038	0.070
0	1	0.029	0.059
0	3	−0.034	0.030
1	0	0.063	0.096
1	1	0.043	0.056
1	3	−0.003	0.039
3	0	0.003	0.051
3	1	−0.028	0.020
3	3	−0.074	0.026

[a] Growth rates are based on change in surface populations during the period following inoculation.

TABLE 5. Analysis of variance and mean square estimates for the effects of continuous exposure to hydrogen fluoride (HF) on the growth of *Xanthomonas campestris* pv. *phaseoli* on leaf surfaces

		Mean square estimates	
Source	df	5-day growth rate[a]	10-day growth rate
Experiment	2	0.012**	0.004**
Preinoculation exposure			
Linear	1	0.007**	0.025**
Quadratic	1	0.020**	0.000
Postinoculation exposure			
Linear	1	0.013**	0.003*
Quadratic	1	0.008**	0.002
Pre- × post-	4		
Pre- × (linear) post-	(2)	0.004*	
Pre- × (quadratic) post-	(2)	0.001	0.001
Error	16	0.001	0.001

[a] Growth rates are based on change in surface populations during the period following inoculation. Asterisks (* and **) indicate the probability ($P \leq 0.05$ and 0.01, respectively) of a value larger than F.

TABLE 6. Effects of sodium fluoride (NaF) in medium on the in vitro growth of *Xanthomonas campestris* pv. *phaseoli*

NaF concentration (M)	Bacterial population (log cfu·ml^{-1}) after:		
	0 hr	24 hr	48 hr
0.0	7.46	9.73	11.95
0.001	7.46	9.56	11.83
0.005	7.46	9.50	11.80
0.010	7.46	9.50	11.52
0.050	7.46	8.51	9.85
0.100	7.46	6.54	7.82

campestris pv. *phaseoli* populations on foliar surfaces. Some differences were noted in the 5-day growth rates, but they rarely occurred. No differences could be attributed to either total dose or order within a given dose of HF.

Effects of NaF on the growth of *X. campestris* pv. *phaseoli* in vitro. Analysis of variance and regression indicated a significant linear effect of the concentration of NaF on both the 24- and 48-hr growth rates of the bacterium. In general, growth of the bacterium in broth containing 0.01 M NaF was not different from the control (Table 6). At the highest concentration (0.1 M), however, there was a marked reduction in growth, which was especially noticeable 48 hr after inoculation.

No detectable changes in concentrations of F in the media were observed between samples taken at 0, 24, and 48 hr after inoculation. If the bacterium sorbed and sequestered F from solution, it was probably in amounts below the detection limits of the analytical technique employed.

DISCUSSION

Although direct effects of pollutants alone, or in combination, have been studied intensively to estimate their impact on agriculture (8), the indirect effects of air pollutants on plant productivity (eg, effects on diseases and insects) have not been a major consideration. The present study was undertaken to estimate the potential importance of the indirect effects of fluoride compounds on productivity of red kidney beans.

In general, intermittent exposures to HF of the type used in these experiments are similar to those proximal to recently constructed industrial facilities that emit HF. Continuous exposures, similar to those used in these studies would probably only occur under conditions of atmospheric stagnation or very stable and persistent winds.

HF does affect some processes that are important in the development of common blight. As with SO_2 (3), continuous postinoculation exposure to HF causes an extension of the latent period and smaller initial lesion sizes. However, there was no effect on the final size of lesions. Apparently, once the pathogen becomes established, development is not affected by the presence of HF in the atmosphere or F in the leaves.

The fact that intermittent exposures did not cause measurable effects on lesion development may be due either to the low dosage of HF supplied or to metabolic recovery during periods of no exposure. It is interesting to note that accumulation of F in leaves was similar at both exposure concentrations. It appears that the concentration of HF in the first intermittent exposure is important because plants exposed to 3 $\mu gF \cdot m^{-3}$ accumulate much more fluoride than do plants exposed to 1 $\mu gF \cdot m^{-3}$ (Table 3). However, subsequent exposures resulted in similar rates of accumulation at both concentrations.

The development of resident populations of *X. campestris* pv. *phaseoli* was delayed in plants continuously exposed to HF, but not in those given intermittent exposures. There were some indications that intermittent exposures reduce growth rates during the first 5 days after inoculation, but not after 10 days. The slower growth rates of the bacterium on the leaf surface could be a result of both direct and indirect effects of HF. When supplied before inoculation, only the higher concentration of HF resulted in slower growth, while both concentrations induced slower growth when the pollutant was supplied after inoculation. It may be that at the higher HF concentration the amount of F sorbed by leaf surfaces prior to inoculation was sufficient to directly inhibit bacterial growth.

Our results differ from those produced with SO_2 (4) which would not be as readily sorbed by the leaf surface, and therefore, would be less likely to cause growth inhibition directly. The in vitro tests with NaF confirm that the bacterium is sensitive to fluoride, but at concentrations greater than would be expected in homogenized leaf tissue. Thus, the results of these studies suggest primarily indirect effects of fluoride on the bacterium. Indirect effects (those that occur from preinoculation exposure) may be the result of either accumulated and concentrated F in the leaf, or HF-induced metabolic changes in the plant.

To evaluate the applicability of these laboratory results, field research is in progress to verify them and determine the response of disease variables to pollutant interactions.

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