Inhibition of Helminthosporium turcicum Spore Germination by Leaf Diffusates from Northern Leaf Blight-Susceptible or -Resistant Corn

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ABSTRACT

Diffusates collected from susceptible or chlorotic-lesion-type resistant corn leaves 1-3 days after inoculation with *Helminthosporium turcicum*, completely inhibited *H. turcicum* spore germination. The pH of diffusates from inoculated leaves ranged from 2.5-2.8. Diffusates from control leaves had pH values of 6.8-7.0. Percentages of spore germination in 3-day diffusates from inoculated and control leaves were similar when pH was adjusted over a range of 2.5-7.0. Percentages of germination were: 0 at pH 2.5; 3-7 at pH 3.0; 46-56 at pH 3.5; and >95 at pH 4.5-7.0. Increased hydrogen ion concentration appears to account for the inhibition of spore germination in diffusates from inoculated leaves of both the susceptible and the resistant corn lines.

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The chlorotic-lesion resistance to northern corn leaf blight incited by *Helminthosporium turcicum* Pass. is conditioned by a single dominant gene, Ht (3). Lim et al. (5) reported the inhibition of *H. turcicum* spore germination by diffusates from the resistant corn leaves 3 days after inoculation. They suggested that resistance is a function of phenolic compounds detected in the diffusates (4). During studies on the infection of susceptible and resistant corn lines by *H. turcicum*, we noted complete inhibition of *H. turcicum* spore germination by diffusates from inoculated susceptible and resistant leaves 1 day after inoculation.

Preliminary observations indicated that the inhibition of spore germination was related to the low pH of diffusates. This paper reports data on the function of hydrogen ion concentration in the inhibition of *H. turcicum* spore germination by corn leaf diffusates.

MATERIALS AND METHODS.—Two pairs of nearly isogenic inbred lines of corn (*Zea mays* L.) were selected for study: (i) susceptible B37 and resistant RB37Ht and (ii) susceptible Oh51A and resistant OH51AHt (6). Plants were grown in the greenhouse in a 2:1 soil-vermiculite mixture, and soluble 20:20:20 fertilizer was applied as part of the watering regime.

H. turcicum was obtained from the American Type Culture Collection, Rockville, Md. (ATCC No. 11536). The genetic stability of the pathogen from one experiment to the next was insured by using lyophilized stock cultures for each experiment. *H. turcicum* spores harvested from 5-day-old cultures on V-8 agar were lyophilized using a technique reported for *Fusarium* (7), but *H. turcicum* spores were pretreated at 4 C for 4 hr and then at −24 C for 1 hr before being placed in liquid nitrogen.

Spores for inoculations were washed from 5-day-old cultures on V-8 agar previously seeded with lyophilized spores, collected on a filter (1.2 μm pores), washed with distilled water, and suspended in distilled water at 50,000 spores/ml. Spores for bioassays were collected as described above, but to avoid dilution of the assay solution, they were washed from the filter with the assay solution directly into the wells of a glass spot plate.

The youngest fully expanded leaf of susceptible or resistant plants with five to six leaves (ca. 1 month old) was inoculated according to procedures of Lim et al. (5). Leaves were cut into 5-6 cm segments, floated on a solution containing 5% sucrose and 20 μg/ml kinetin, and placed in moist chambers. Several drops of spore suspension were placed on the upper leaf surface, and leaves were incubated for 1-3 days at 25 C under 2,152 lx (200 ft-c) continuous light intensity. These sections expressed the typical symptoms of intact plants (2, 3). Drops of distilled water placed on similar leaves served as controls. Samples of drops were collected at daily intervals for 3 days, and pH was determined before bioassays were conducted.

Bioassays were performed in glass spot plate wells. Diffusates were filtered through a 0.22 μm Millipore filter to remove spores or mycelial fragments, and 0.6 ml of a test solution was added to each of three wells. Spores were added to the solutions, and incubated in a moist chamber for 4 hr at 25 C. The average percentage of germination in each test solution was determined by examining 100 spores from each well.

The influence of pH on spore germination was determined by adjusting the pH of 3-day diffusates from inoculated and control leaves and distilled water with 0.1 N HCl or NaOH solutions. Bioassays were performed as described above. Nonbuffered distilled water was used because various buffers for low pH ranges were toxic.

RESULTS AND DISCUSSION.—One- to 3-day diffusates from inoculated susceptible (Oh, B37) or resistant (ROh, RB37) corn leaves completely inhibited *H. turcicum* spore germination during a 4-hr incubation. The pH of these diffusates ranged from 2.5-2.8. In 1- to 3-day control diffusates, 97-99% of

TABLE 1. Influence of pH on germination of *Helminthosporium turcicum* spores after 4-hr incubation at 25 C in corn leaf diffusates from susceptible (Oh, B37) and resistant (ROh, RB37) corn lines 3 days after inoculation

pH and Treatment	Germination (%)[a]			
	Corn lines			
	Oh	ROh	B37	RB37
2.5:				
Inoculated leaves[b]	0	0	0	0
Noninoculated leaves	0	0	0	0
Distilled water (control)	0			
3.0:				
Inoculated leaves	5	5	4	3
Noninoculated leaves	4	6	7	6
Distilled water (control)	7			
3.5:				
Inoculated leaves	50	47	53	47
Noninoculated leaves	46	48	56	51
Distilled water (control)	48			
4.5-7.0:				
Inoculated leaves	>95	>95	>95	>95
Noninoculated leaves	>95	>95	>95	>95
Distilled water (control)	>95			

[a] Mean of three experiments.
[b] Nonadjusted pH range was 2.5-2.8.

the spores germinated. The pH of the control diffusates ranged from 6.8-7.0 during the 3-day test period.

Percentages of spore germination in diffusates from inoculated (3-day) and noninoculated leaves and in distilled water over the adjusted pH range of 2.5-7.0 were similar (Table 1). In all three test solutions, no spores germinated at pH 2.5; only 3-7% germinated at pH 3.0; but 46-56% germinated at pH 3.5. At pH 4.5-7.0, germination exceeded 95% in diffusates from inoculated and noninoculated leaves and in distilled water.

Increased hydrogen ion concentration (pH 2.5-2.8) appears to be responsible for the complete inhibition of *H. turcicum* spore germination in diffusates from inoculated leaves. Diffusates from inoculated leaves were no more inhibitory than those from noninoculated leaves or distilled water when pH was adjusted over a range of 2.5-7.0. Large increases in organic acid content occur in wheat leaves infected with *Puccinia graminis tritici* (1). A similar accumulation of organic acids might account for the low pH observed in diffusates from *H. turcicum*-infected corn.

Possible differences in experimental conditions also might explain in part the differences between our results and those of Lim et al. (4, 5). They also used the B37 and RB37Ht corn lines we used in our study. Our inoculum was standardized at 50,000 spores/ml, and the youngest mature leaf of 1-month-old plants was selected for inoculation; Lim et al. (4, 5) did not specify their inoculum concentration or the age of plant material. If the age of plant material used in the two studies differed greatly, this could explain the lack of agreement in our data. However, the leaf material used in our studies exhibited typical symptoms of the chlorotic-lesion-type resistance (2, 3) when intact 1-month-old plants were inoculated. This fact, along with our failure to detect any difference in the inhibition of spore germination by diffusates from inoculated susceptible or resistant lines, indicates that the chlorotic-lesion resistance to northern corn leaf blight is unrelated to quantities of phytoalexin-like compounds that may be detected in diffusates from inoculated resistant leaves.

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