Involvement of an Inhibitory Compound in Induced Resistance of Maize to *Helminthosporium carbonum*

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ABSTRACT

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In maize, resistance to *Helminthosporium carbonum* race 1 is induced by prior inoculation with race 2. This induced resistance was consistently associated with the production of a compound(s), which reversibly inhibited conidial germination and germ tube elongation. It also prevented growth of phytopathogenic bacteria in a defined medium. The inhibitor was produced and diffused into liquid on the surface of the leaf in all maize lines tested and in response to inoculation with other fungi. When this inhibitory diffusate was added to the conidial inoculum, lesions did not develop on leaves of susceptible genotypes. The host-specific toxin (HC-toxin) produced only by race 1 prevented the synthesis or release of the inhibitor but did not affect its activity in germination bioassays or its ability to prevent lesion development. The results suggest that the inhibitor has a role in induced resistance.

Additional keyword: cross-protection.

In maize (*Zea mays* L.), resistance to the fungus *Helminthosporium carbonum* Ullstrup (syn. *Bipolaris zeicola* (G.L. Stout) Shoemaker) race 1 is determined by the dominant allele at the *hm* locus (18). The fungus stops growing at the site of penetration and produces small necrotic flecks on the leaf (27). Resistance to *H. carbonum* is associated with insensitivity to the host-specific toxin (HC-toxin) that is produced only by pathogenic race 1 isolates (23,24). Conversely, in susceptible genotypes, which are sensitive to HC-toxin, race 1 grows rapidly and extensively and forms large, expanding necrotic lesions.

In many plant species, inoculation of a susceptible host with a nonpathogen can induce resistance or protection against a normally pathogenic organism (12,22). Our earlier results (5) demonstrated this phenomenon in maize, when inoculation with the nonpathogenic *H. carbonum* race 2 induced resistance in susceptible leaves against a challenge inoculation with race 1. Fewer appressoria were produced by the challenge race 1 conidia, and their subsequent penetration into the leaf was considerably reduced compared to development of race 1 on leaves that were not preinoculated with race 2. This inhibitory effect was nullified, however, when HC-toxin was supplied with the challenge inoculum, resulting in continued growth of race 1 and a susceptible host reaction. The mechanism of containment of the fungus is unknown, but these data suggest that inhibitory compounds are released into the infection court, where they affect pathogenic processes before the host is penetrated.

The involvement of compounds secreted by the host tissue in plant resistance is considered an important component of disease resistance (28). Although some of these compounds have been identified (e.g., phenolic compounds and organic acids) (8,28), in most instances the material responsible for toxicity to the pathogens has not been characterized (4,14,25). Clearly, these compounds are fungistatic, because they inhibit conidial germination, germ tube elongation, or formation of infection structures. Previous investigators (6,10) have not obtained evidence for induced resistance or for production of inhibitory compounds in the interaction between *H. carbonum* and maize leaves.

Our objectives in this investigation were to determine the biochemical basis for the induced resistance observed previously (5) and to establish the involvement of HC-toxin in the phenomenon. We present evidence for an inhibitory compound(s) in diffusates from susceptible leaf tissue inoculated with *H. carbonum* race 2. The compound has a wide spectrum of activity; it reversibly inhibits conidial germination and germ tube elongation and prevents growth of phytopathogenic bacteria in a defined medium. HC-toxin prevents the synthesis of the inhibitor by inoculated leaves but does not abolish its fungistatic activity.

MATERIALS AND METHODS

Fungi, hosts, and inoculations. The inbred lines of maize used in this study were A632, B73, and Mo17, which are resistant to *H. carbonum*, and the differential isogenic hybrids, K61 × Pr (susceptible to *H. carbonum*) and K61 × Pr1 (resistant). Growth, maintenance, and inoculation of plants as well as growth of the fungal cultures and preparation of HC-toxin were as described elsewhere (5). *Colletotrichum graminicola* and *H. maydis* race 0 were grown on oatmeal agar and potato-dextrose agar (26) at 26 and 21 C, respectively.

Collection of diffusates. Leaves of the susceptible genotype were inoculated with water suspensions of *H. carbonum* race 2 conidia in microhumidity chambers (5). After 12–15 hr of incubation, the inoculation fluids were removed with a syringe from the individual wells and replaced with 25 μl of water. After another 10–12 hr of incubation, the liquid in the wells, designated wash fluid, was removed. The inoculation fluids and wash fluids (diffusates) were collected and pooled separately, centrifuged at 10,000 *g* for about 10 min to remove conidia and fragments of germ tubes, and stored at 4 C until they were used to determine their effects on pathogenic processes or on conidial germination. In some experiments, diffusates were collected 24 hr after inoculation with race 2. The pH of the diffusates was between 4.5 and 5, well within the pH range (3.5–10) over which conidia of *H. carbonum* germinated.

Bioassay of diffusates. Conidia from 2- to 3-wk-old fungal cultures were harvested in 0.01 (v/v) aqueous Tween 20 and adjusted to 2–4 × 10^3 conidia/ml. Conidia were centrifuged at 1,000 *g* for 3–5 min, and the supernatant was removed and replaced with diffusates. Twenty-five-microliter droplets (50–100 conidia/droplet) were placed in petri dishes (10 × 1.5 cm) (four droplets per dish) lined with moist filter paper to prevent evaporation. Conidial germination was evaluated microscopically. A conidium was scored as germinated if the length of the germ tube exceeded the width of the conidium.

The effects of the diffusates on appressorium formation and penetration by race 1 were determined by inoculating leaves with conidia in the presence of the diffusates. Leaf disks corresponding to the area under the wells of the microhumidity chambers were cleared and stained as previously described (5) and examined microscopically.

Effect of the diffusates on bacterial growth. Two phytopathogenic bacteria, *Corynebacterium michiganense* and *Erwinia amylovora*, obtained from Dr. John F. Tuite (Purdue University), were grown on sucrose-nutrient agar (sucrose, 50.0 g; Difco beef extract, 3 g; Difco beef peptone, 5 g; per liter of H_2O; pH 7.3). Fifty milliliters of 0.2× modified Fries (MF) broth (minus ammonium tartrate) (sucrose, 10.0 g; NH_4NO_3, 1.0 g; KH_2PO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; NaCl, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.13 g; yeast extract, 1.0 g; per liter of H_2O) in individual 125-ml flasks was inoculated with a loopful of each bacterium, and flasks were incubated on a shaker at 24 C for 48 hr. Ten microliters of these bacterial suspensions (1–2 × 10^6 cfu) were added to sterile 1-dram vials, along with 480 μl of either sterile water or filter-sterilized (0.45 filter; Millipore Corp., New Bedford, MA) diffusates and 10 μl of 10× MF broth (0.2× final concentration). The vials were incubated at room temperature (24–27 C) on a shaker. Bacterial growth, as measured by an increase in turbidity of the suspensions, was quantified on a Beckman DU40 spectrophotometer at 600 nm. The spectrophotometer was calibrated with uninoculated medium.

RESULTS

Evidence for an inhibitor in diffusates from leaves infected with *H. carbonum*. Diffusates harvested from susceptible leaves inoculated with conidia of race 2 contained compounds that inhibited lesion formation by race 1. Inoculation fluids were removed from the individual wells of the microhumidity chamber after 15 hr and replaced with water for an additional 10 hr (wash fluids). Uninfected leaves were inoculated with conidia of race 1 alone (control) or with the diffusates (undiluted inoculation fluids or wash fluids) from race 2-inoculated tissue. Lesions on leaves inoculated with race 1 plus inoculation fluids were fewer and much smaller than those formed by race 1 alone (Fig. 1). Lesions were strikingly absent in tissue inoculated with race 1 conidia in the presence of the wash fluid. Microscopic examination of the tissue inoculated with race 1 in the presence of inoculation fluid showed that, although 91% of the conidia germinated, very few formed appressoria (6%) or penetrated (5%). On the other hand, in the presence of wash fluid, less than 3% of the race 1 conidia germinated, and these did not develop appressoria or penetrate the leaf. The greater activity of the wash fluids was apparently due to a higher concentration of inhibitory material in those solutions.

Inhibitory activity in diffusates was first detected 12 hr after inoculation with race 2. Diffusates were collected at 3-hr intervals, and the effect of each of those diffusates on the formation of lesions on susceptible leaves by race 1 was determined (Fig. 2). Lesion size was substantially reduced when leaves were inoculated

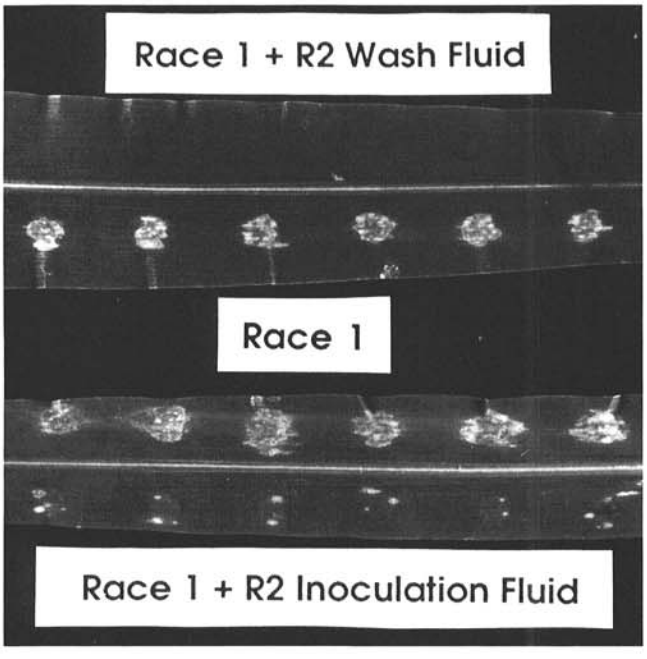

Fig. 1. Inhibitory effect of inoculation and wash fluids (diffusates) from leaves of the susceptible hybrid K61 × Pr inoculated with *Helminthosporium carbonum* race 2. Leaves in microhumidity chambers were inoculated with conidia of race 2 for 15 hr. Then the inoculation fluids were collected and replaced with water for another 10 hr before those wash fluids were collected. Leaves of K61 × Pr were inoculated with conidia of race 1 alone or in the presence of those fluids and incubated for about 24 hr. Photo was taken 4 days after inoculation.

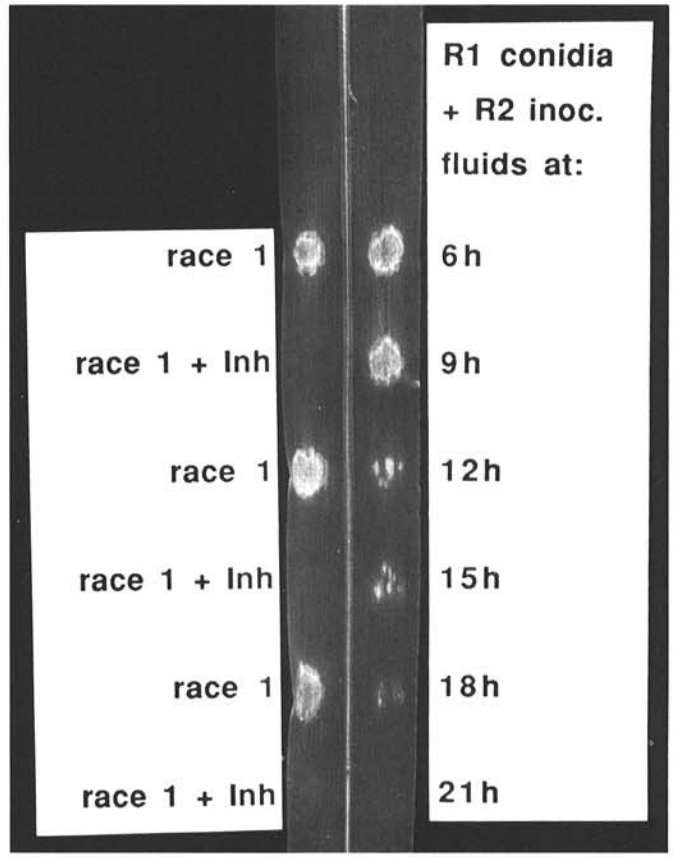

Fig. 2. Inhibitory effect of diffusates on the development of lesions incited by race 1. Diffusates were collected from leaves of the susceptible hybrid K61 × Pr at 6, 9, 12, 15, 18, or 21 hr after inoculation with *Helminthosporium carbonum* race 2. Leaves were inoculated on the right side of the midrib with conidia of race 1 in the presence of the diffusates and on the left side with race 1 alone or race 1 plus inhibitor (diffusates collected at 24 hr after inoculation). Photo was taken 4 days after inoculation.

with race 1 plus diffusates collected at 12, 15, or 18 hr, whereas no lesions at all developed when leaves were inoculated with race 1 plus diffusates collected at 21 hr after inoculation. In germination bioassays, more than 90% of race 1 conidia germinated in diffusates collected up to 18 hr, but none germinated in diffusates collected at 21 hr. The concentration of inhibitor in diffusates appeared to increase between 12 and 18 hr; germ tube lengths were 90% of controls in 12-hr diffusates and only 26% in 18-hr diffusates. Thus, the reduction in lesion size in the presence of inoculation fluids is due to effects on germ tube growth and development; the prevention of lesion formation by the wash fluids is due to inhibition of conidial germination.

In bioassays, the diffusates collected 24 hr after inoculation with race 2 prevented germination of race 1 conidia even after 24 hr of incubation. Full inhibitory activity (0% germination) was retained when the diffusates were diluted 1:2. Slightly higher dilutions (1:4) decreased activity; conidia germinated comparable to controls (about 80%) but germ tube elongation was inhibited by up to 75%. Tenfold dilutions actually stimulated the rate of germination and germ tube growth by 25–30%. Fluids collected from uninoculated leaves and those from leaves pricked with a fine needle did not affect germination or germ tube growth of race 1 conidia in vitro, suggesting that production of the inhibitor is not a general wound response but is induced by fungal invasion.

The fungistatic activity of the inhibitory compound in diffusates was rapid and reversible. Conidia of race 1 were incubated in water for 4 hr (Fig. 3A), then the water was removed and replaced with either an equal volume of diffusates (Fig. 3B) or water (Fig. 3C) for an additional 10 hr. Germ tubes of race 1 conidia in diffusates stopped elongating, and some of the germ tube tips were deformed (Fig. 3B). Conidia were rinsed three times with water and incubated for an additional 10 hr (24 hr cumulative time) (Fig. 3D). Germ tube lengths increased rapidly and were similar to those of conidia incubated in water alone for 14 hr (Fig. 3C). In a similar experiment, conidia incubated in inhibitor for 24 hr (0% germination) were then rinsed three times with water, and 94% of the conidia germinated within 8 hr.

Effect of HC-toxin on production and activity of inhibitor. When added with the inoculum, HC-toxin prevented the production of the inhibitory compound(s). Susceptible leaves, with no pretreatment, were inoculated with conidia of the toxin-producing race 1 or race 2 plus HC-toxin (5 μg/ml), and diffusates were assayed for inhibitory activity with race 1 conidia in vitro. Within 8 hr, at least 84% of the conidia germinated in the diffusates collected from both treatments, although germ tube elongation was inhibited by 50% in fluids from tissue inoculated with race 2 conidia plus HC-toxin.

To determine if the inhibitor was present in diffusates from challenged tissue, we inoculated susceptible leaves with race 2 for 12 hr and then challenged with race 1, race 1 plus HC-toxin (5 μg/ml), or HC-toxin alone for an additional 12 hr. Conidia did not germinate in diffusates collected from challenge inoculations with race 1 alone, but they germinated at least 98% in diffusates collected from treatments or inoculations supplemented with HC-toxin, suggesting that HC-toxin prevents the expression of induced resistance by preventing the production of the inhibitory compound(s).

The addition of HC-toxin to the challenge inoculum abolished the expression of induced resistance (5), but the toxin did not prevent the effect of the inhibitor on spore germination or germ tube growth in vitro. Conidia of race 1 were incubated with diffusates alone, HC-toxin (25 μg/ml) alone, or HC-toxin plus diffusates. Within 8 hr, more than 80% of the conidia germinated in HC-toxin, whereas conidia failed to germinate in the diffusates with or without HC-toxin. Furthermore, when the inhibitor and HC-toxin were applied together with the race 1 conidial inoculum on susceptible leaves, lesions failed to develop (Fig. 4). Thus, the toxin cannot prevent or overcome the effects of the inhibitor on the host leaf surface or in vitro.

Continued production of the inhibitory compound(s) from race 2 lesions was prevented by HC-toxin. Microhumidity chambers were reattached directly over 3-day-old lesions on susceptible leaves, and the lesions were then treated with water (control)

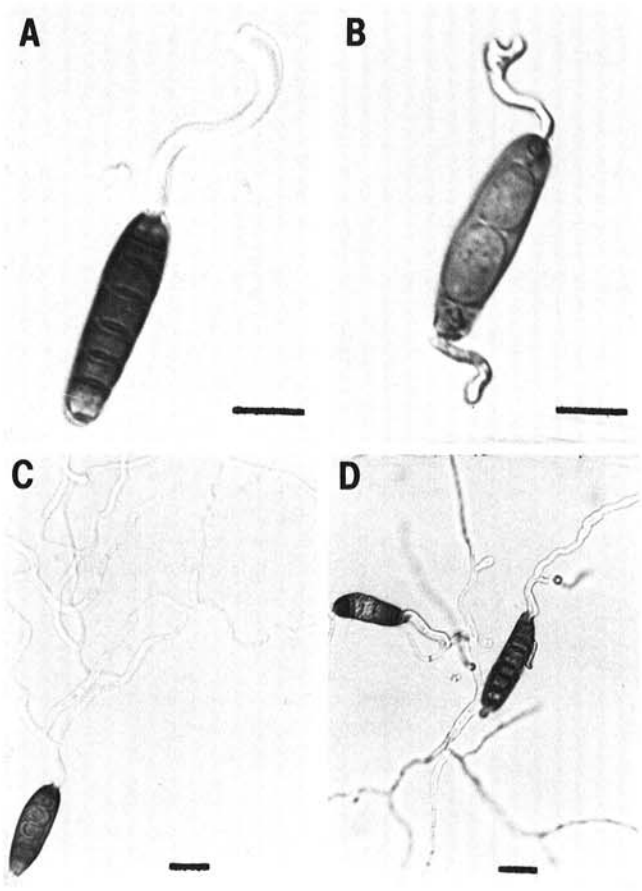

Fig. 3. Effect of diffusates, collected from leaves of the susceptible hybrid K61 × Pr inoculated with *Helminthosporium carbonum* race 2, on germ tube growth of race 1 conidia. The diffusates were collected 24 hr postinoculation. Conidia of race 1 were **A,** incubated in water for 4 hr; **B,** in water for 4 hr and then in the diffusates for an additional 10 hr; **C,** in water for 14 hr; or **D,** in water for 4 hr, in diffusates for 10 hr, and in water for another 10 hr. Bright field optics. Bars equal 20 μm.

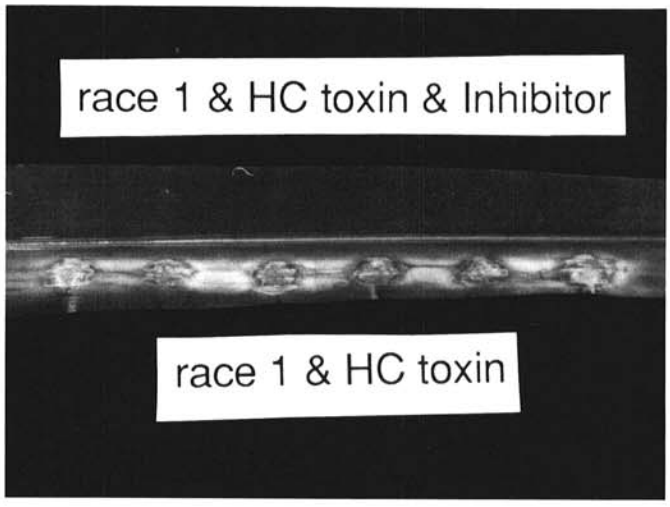

Fig. 4. Effect of HC-toxin on the activity of the inhibitor in diffusates collected from leaves of the susceptible hybrid K61 × Pr inoculated with conidia of *Helminthosporium carbonum* race 2. The diffusates were collected 24 hr after inoculation. Leaves were inoculated with conidia of race 1 supplemented with HC-toxin (5 μg/ml) in the presence (top) or absence (bottom) of the inhibitor. Photo was taken 4 days after inoculation.

or inoculated with race 1 or with race 1 plus HC-toxin (5 μg/ml). As previously observed (5), only challenge race 1 conidia supplemented with HC-toxin produced significantly enlarged but finite-sized lesions compared with those of controls (water or race 1 conidia alone). Diffusates collected from these treatments were bioassayed, and fluids from the 3-day race 2/race 1 treatment as well as from lesions treated with water prevented germination of race 1 conidia. In contrast, 91% of the race 1 conidia germinated within 6 hr in diffusates collected from the 3-day race 2/race 1 plus HC-toxin treatment.

Effect of the diffusates on other organisms. The inhibitory material in the diffusates has a broad spectrum of activity, affecting other species of fungi as well as bacteria. Conidia of *H. carbonum* race 2, *H. victoriae* F. Meehan & Murphy, *H. turcicum* Pass. race 1, *H. maydis* race 0, *C. graminicola*, and an *Alternaria* sp. did not germinate even after 24 hr of incubation in diffusates. The growth of two phytopathogenic bacteria, *Corynebacterium michiganense* and *Erwinia amylovora*, in a defined liquid culture medium also was prevented during 20 hr of incubation in the diffusates (Table 1).

Induction of inhibitor production by other fungi and in other maize lines. Other fungi also induced production of the inhibitor. This was established when susceptible leaves were inoculated with *H. victoriae, H. turcicum* race 1, or an *Alternaria* sp. for 12 or 18 hr and then challenged with race 1 for 12 hr. As demonstrated previously (5), all three fungi prevented infection of susceptible leaves by race 1 but only when the interval between inoculations was 18 hr. Germination of race 1 conidia in diffusates from 12-hr treatment with the inducers demonstrated a general absence of inhibitor activity, although diffusates from 12-hr inoculation with *H. victoriae* inhibited germ tube elongation compared with conidia germinating in water. Conidia incubated in the diffusates from 18-hr pretreatment with either *H. victoriae* or *H. turcicum* did not germinate within 6 hr but germinated at least 90% after 12 hr. However, germ tube elongation was substantially reduced (14–30% of controls) in diffusates from all pretreatments.

Other genotypes of maize produced the inhibitor in response to attempted infection by *H. carbonum*. The resistant isogenic hybrid, K61 × Pr1, along with resistant inbred lines A632, B73, and Mo17 were inoculated with race 1 or race 2. Leaf diffusates were collected and bioassayed. Conidia incubated in diffusates from all genotypes inoculated with either race did not germinate.

Preliminary characterization of the inhibitory compound. Results of experiments to isolate the inhibitor indicated that it is a low molecular weight, negatively charged compound. Inhibitory activity was removed when the diffusates were treated with activated charcoal or with anion exchange resin (Bio-Rad AG 2-X) and when they were dialyzed against water at 4 C for 32 hr in tubing with an 8,000 MW cutoff. Concentration of the solutions in vacuo resulted in the loss of activity in germination bioassays. Diffusates heated at 80 C for 30 min in a sealed container retained activity, indicating that the active component(s) is heat stable. However, the activity was lost when the gas phase above the solution in the heated vial was removed with a syringe, suggesting that the compound, or some component required for activity, is volatile. Attempts to recover activity by microdistillation of the diffusates or by dissolving the volatile materials collected from the head space were unsuccessful.

TABLE 1. Effect of inhibitory diffusates from susceptible maize leaves inoculated with race 2 of *Helminthosporium carbonum* on growth of *Corynebacterium michaganense* and *Erwinia amylovora*

Organism	Treatment[a]	Absorbance at 600 nm	
		10 hr	20 hr
C. michaganense	Control	0.15 ± 0.01[b]	0.22 ± 0.01
	Inhibitor	0.00 ± 0.00	0.00 ± 0.00
E. amylovora	Control	0.26 ± 0.02	0.60 ± 0.02
	Inhibitor	0.00 ± 0.00	0.00 ± 0.00

[a] Each bacterial suspension (10 μl containing 1–2 × 10^6 cfu) was added to 0.2× modified Fries medium in sterile water (control) or filter-sterilized inhibitor.

[b] Values, corrected for zero time, are the means ± standard error of the three replicates.

DISCUSSION

Our experiments establish that resistance to invasion by race 1 of *H. carbonum* is induced when susceptible leaves are first inoculated with the nonpathogenic race 2. Apparently, an inhibitory substance produced at the host-pathogen interface suppresses the formation of appressoria and consequently inhibits the ingress of the pathogen through the leaf. Evidence that this inhibitor has a role in inducing resistance is as follows: Diffusates collected from susceptible leaves inoculated with race 2 or race 1 challenge inoculations (e.g., race 1 following *H. carbonum* race 2, *H. victoriae, H. turcicum,* or *Alternaria*) inhibited conidial germination and germ tube elongation in vitro and reduced the size of lesions formed by race 1 on susceptible leaves. In contrast, no inhibitory activity was recovered in diffusates collected from susceptible leaves inoculated with race 1 alone, with challenge race 1 supplemented with HC-toxin, or in leaf diffusates from uninoculated or wounded tissue. The inhibitor always was associated with treatments that resulted in a resistant phenotype, and it appears that activities of the fungus are responsible for its induction.

The time required for expression of induced resistance (5) is related to the appearance of inhibitory activity in diffusates. Diffusates from susceptible leaves inoculated with race 2 for 12–15 hr inhibited germ tube elongation of race 1 conidia in vitro, and germ tube emergence was completely inhibited in fluids collected at 21 hr. Similarly, on the host, inhibition was first observed at about 15 hr as a decrease in the number of appressoria formed by challenge race 1 on tissue protected by race 2 (5). Appressorium formation and penetration were completely inhibited in diffusates collected 24 hr after inoculation with race 2. There is no evidence that *H. carbonum* has deleterious effects on susceptible cells until after the fungus is established (6). Thus, intact, functional cells apparently produce the inhibitor, which diffuses into the intercellular spaces and onto the leaf surface. These observations on inhibitory activity of diffusates and their effects on events of pathogenesis provide a persuasive argument for the role of the inhibitor in the protection of susceptible tissue against infection by *H. carbonum* race 1.

The association of chemical compounds with the resistant reaction of plants to pathogens is well documented (8,15,28). Compounds such as the phytoalexins, glyceollin and phaseollin in soybean and French bean, respectively, accumulate to fungitoxic levels in resistant hosts after infection by the pathogen or treatment with abiotic agents (8). In maize, necrotic lesions on leaves infected with *C. graminicola* leach toxic, water-soluble phenols, including *p*-coumaric and ferulic acids, into water droplets on the leaf surface (20). In addition, a number of uncharacterized toxic compounds are involved in the differential host response to pathogens (4,14,21,25). Plants that have been wounded or invaded by pathogens and pests also emit volatile compounds that have very effective antimicrobial activities (15,30). Naturally occurring aldehydes and alcohols of intermediate chain length are among the most prevalent biologically active compounds released, but none of those tested (including hexanal, 2-hexenal, 1-octanol, octanal, 1-nonanol, nonanal, and 2-nonenal, at concentrations up to 10^{-4} M) nor ethylene (at 100 μl/L) affected germination of conidia of *H. carbonum* (data not shown).

A role in induced resistance has been documented for very few compounds. Berard et al (2,3) obtained evidence for a cultivar-specific protection factor in the diffusates from incompatible interactions between bean and *C. lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. Phytoalexins accumulate at the site of penetration in systemically protected tissue challenged with a pathogenic race of *C. lindemuthianum* (7). The inhibitor detected in the diffusates from maize inoculated with *H. carbonum* appears to be a low molecular weight, negatively charged compound, since activity is lost when the diffusates are dialyzed or treated with

anion exchange resins. The inhibitory activity of the diffusates may be due to a single component or a mixture of compounds. It rapidly prevents or suppresses morphological development of conidial germ tubes. These effects, like the activity of the self-inhibitors of rust fungi (1,16), are quickly and easily reversed by rinsing with water, suggesting a noncovalent binding or weak interaction with a sensitive site in the conidia.

The failure of previous investigators (6,10) to detect induced resistance or the production of an inhibitor in maize inoculated with *H. carbonum* can be explained by our observations. The protective effects induced by interaction between conidia of race 2 and host leaf cells were confined to a limited area, since at least two to four conidia/mm^2 of the inoculated leaf surface were required to induce resistance. The microhumidity chambers used in our experiments maintained an aqueous solution on the leaf surface and, thus, permitted the concentration of materials produced from numerous sites of interaction (about 50/well) between fungal and host cells. Nicholson et al (19) recently demonstrated that 3-deoxyanthocyanidin phytoalexins in sorghum leaves infected with *C. graminicola* accumulated rapidly in an extremely limited area (about 2,000 μm^2) around the infection site. Thus, a distance of only a few cells separating the inducer and challenge inoculations may be sufficient to circumvent the resistance mechanism(s).

Although the early events of pathogenesis (i.e., germination, appressorium formation, and penetration) by race 1 and race 2 are similar (6,17), further growth by race 2 is reduced significantly, whereas race 1 continues to colonize leaf tissue. Continued growth by race 1 is presumably due to the production of HC-toxin, which prevents the synthesis of the inhibitor. Similarly, when pear leaves were inoculated with *A. alternata*, a defense mechanism that resulted in smaller lesions was induced by germination fluids of the pathogen that were devoid of the host-specific toxin, AK-toxin, produced by this pathogen (9,11). This resistance was suppressed by AK-toxin released by conidia of *A. alternata* prior to host invasion.

The inability of challenge race 1 to colonize tissue protected by race 2 may be due to the lack of sufficient quantities of HC-toxin. Although the ratio of fungal mass to HC-toxin is unknown, the critical event of resistance (i.e., production of the inhibitor) is probably expressed before race 1 accumulates in amounts necessary to produce an adequate amount of HC-toxin. The addition of HC-toxin to challenge inoculum enabled race 1 to overcome the mechanism of induced resistance in susceptible tissue and allowed further growth and colonization by the pathogen. If an inhibitor is responsible for protection, then it is reasonable to suggest that the increased growth of race 1 is due to the primary action of HC-toxin on the activity of the inhibitor. However, it is readily apparent from in vitro germination assays and from inoculations in vivo that race 1 or HC-toxin has no effect on activity of the inhibitor once it is present in the diffusates. Instead, secondary consequences of HC-toxin action may affect the synthesis or release of the inhibitor.

The ability of other fungi to induce resistance and production of inhibitor is significant and suggests the operation of a singular, nonspecific mechanism of induction (29). Hayami et al (9) demonstrated that resistance in pear tissues to the pathogen, *A. alternata*, was induced by fluids from germinating spores of various nonpathogenic fungi. In other host-pathogen interactions, phytoalexins are also thought to be induced by constituents in culture filtrates or by preparations from fungal cell walls (8). We did not test whether the inducing fungi released unique components into germination (inoculation) fluids. Alternatively, production of inhibitor and expression of induced resistance might be induced by perturbations resulting from the formation of infection structures, attempted penetration, or subcuticular growth common to the fungi and not by simple, acute wounding of the tissue (13,29).

The effect of diffusates on the growth of phytopathogenic bacteria as well as other pathogenic and nonpathogenic fungi demonstrates the general biostatic activity. The presence of inhibitory activity in diffusates from other maize genotypes suggests that the production of inhibitor is a common response to attempted pathogen invasion and may serve as a general defense mechanism.

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