Induced Resistance to Bean Anthracnose at a Distance From the Site of the Inducing Interaction

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ABSTRACT

Resistance induced in *Phaseolus vulgaris by a* varietal nonpathogenic race of *Colletotrichum lindemuthianum* against a varietal pathogenic race is exhibited by cells distant from the inducing interaction. Induced resistance is expressed after the varietal pathogenic race has penetrated, and is microscopically indistinguishable from the resistant reaction to a varietal nonpathogenic race. Phytopathology 61:1110-1112.

Rahe et al. (6) recently demonstrated induced resistance in *Phaseolus vulgaris* L. 'Perry Marrow' to bean anthracnose. Etiolated Perry Marrow seedlings are susceptible to attack by the gamma race of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Schribner and resistant to the beta race. Susceptibility is characterized by the development of large, dark brown, spreading lesions and resistance by minute brown flecks usually involving only a few epidermal cells penetrated by the fungus (5). When etiolated plants were inoculated with conidia of the beta race (inducing inoculation) followed by the gamma race 24 hr later (challenge inoculation), few lesions typical of the susceptible interaction developed; when the lag period was increased to 48 hr, no lesions developed; i.e., the plants were completely protected from the gamma race. Since the conidia of the two races are morphologically indistinguishable, there was no way, using Rahe's techniques, of determining whether resistance was induced in cells distant from the fungus used for induction and whether it was expressed before or after penetration by the varietal pathogenic race. Using improved techniques, we investigated these possibilities.

Uniform etiolated plants were obtained for use as follows: Perry Marrow seeds weighing 0.40-0.45 g were surface-sterilized with 1% sodium hypochlorite and imbibed in CO_2-saturated water for 12 hr at 26-28 C in a manner similar to that described by Barton (1). The seed coats were removed and the seeds planted 6 cm deep in autoclaved 50 ml Erlenmeyer flasks containing 4.5 g vermiculite and 27 ml water. The flasks were kept in a light-tight incubator at 21-22 C for 9 days. Plants 8-10 cm tall (measured from the top of the flask to the top of the hypocotyl hook) were used in the experiments.

The hypocotyls were marked with India ink at 0.5 cm intervals, starting at the cotyledonary node. The marks permitted location of physiologically similar areas of the hypocotyls for inoculation. Only regions which had completed elongation were used (4 cm or more below the cotyledons).

Inocula were prepared from 9- to 10-day-old cultures of the beta and gamma races of *C. lindemuthianum* grown on bean juice agar in the dark at 22-24 C. The cultures were flooded with water, the conidia dislodged with a glass rod, and the resulting suspensions filtered through several layers of cheesecloth. Spore concentrations were adjusted to $1\text{-}2 \times 10^6$ conidia/ml. Inoculum was applied at specific levels of each hypocotyl as 1.5 µliter drops, using a 25-µliter syringe. Five drops were equally spaced around the periphery of the hypocotyl at each level. Sterile water was substituted for suspensions of conidia in control treatments.

Induced resistance treatments consisted of inoculations with conidia of the beta race at the 4-, 5-, 6-, 7-, 8-, and 9-cm levels followed by inoculations with the gamma race at the 4.5-, 6.5-, and 8.5-cm levels 0, 18, or 36 hr later. Controls were inoculated with the beta race with water applied at the challenge sites (induction control), or water was applied at the induction sites at 0 hr, followed by inoculation with the gamma race at the challenge sites 0, 18, or 36 hr later (challenge control). Each treatment included three replicates.

Following inoculation, the plants were kept in indi-

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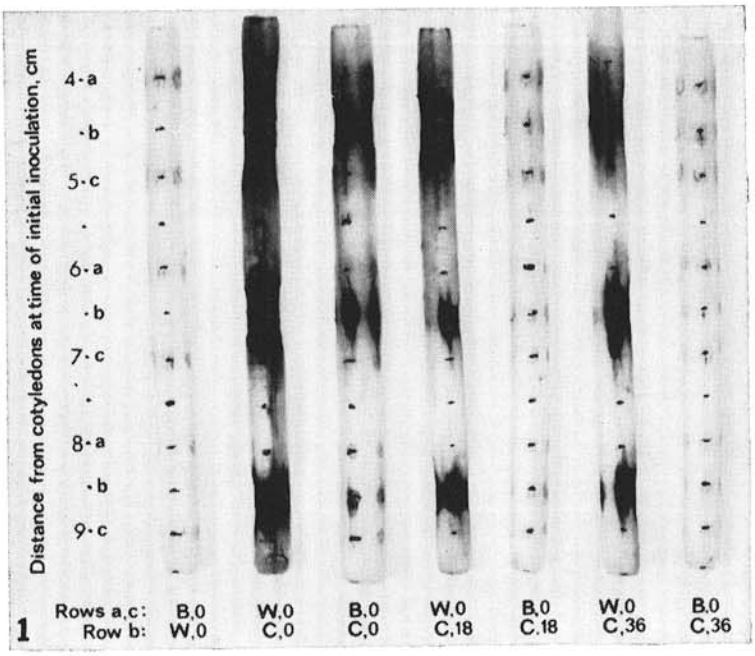
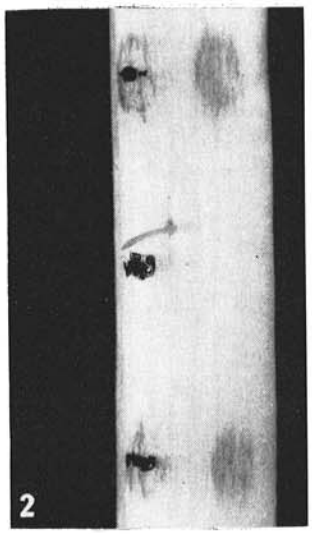
Fig. 1-8. **1)** Protection of Perry Marrow hypocotyl tissue against the gamma race of *Colletotrichum lindemuthianum* 0.5 cm from the sites of resistant reactions to the beta race. (B) beta race (C) gamma race; (W) water; numbers are hours between inoculation with beta spores or treatment with water and inoculation with gamma spores. **2-8)** Details of the 6- to 7-cm regions of hypocotyls inoculated as follows: **2)** beta spores at 6 and 7 cm, water at 6.5 cm, all at 0 hr; **3)** water at 6 and 7 cm, gamma spores at 6.5 cm, all at 0 hr; **4)** water at 6 and 7 cm at 0 hr, gamma spores at 6.5 cm at 18 hr; **5)** water at 6 and 7 cm at 0 hr, gamma spores at 6.5 cm at 36 hr; **6)** beta spores at 6 and 7 cm, gamma spores at 6.5 cm, all at 0 hr; **7)** beta spores at 6 and 7 cm at 0 hr, gamma spores at 6.5 cm at 18 hr; **8)** beta spores at 6 and 7 cm at 0 hr, gamma spores at 6.5 cm at 36 hr.


Distance from cotyledons at time of initial inoculation, cm
4-a
-b
5-c
6-a
-b
7-c
8-a
-b
9-c
Rows a,c: B,0 W,0 B,0 W,0 B,0 W,0 B,0
Row b: W,0 C,0 C,0 C,18 C,18 C,36 C,36

1



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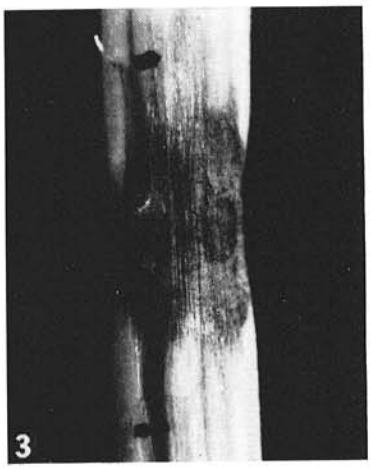

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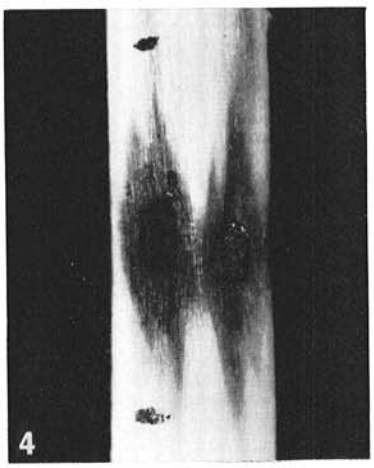

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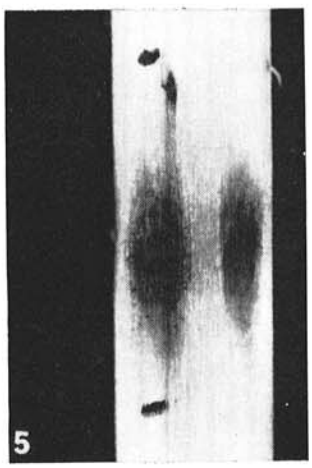

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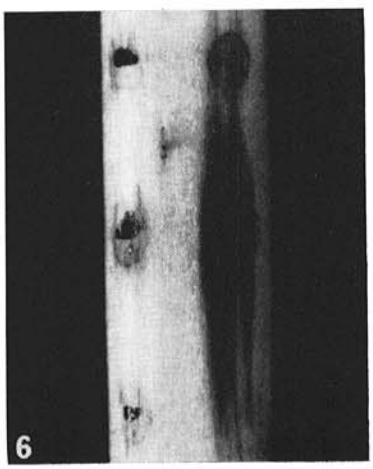

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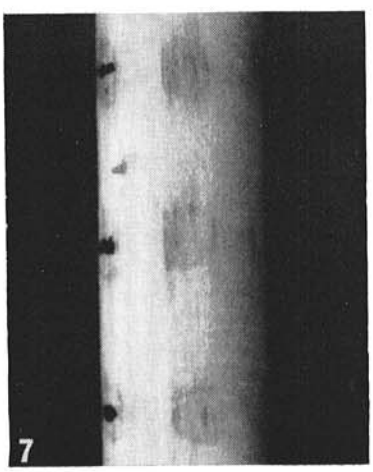

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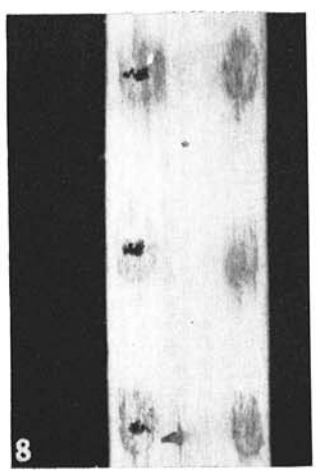

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vidual high-humidity chambers in a light-tight incubator at 21-22 C. Each chamber consisted of a glass cylinder 40 cm tall × 6 cm (outside dimension) lined with seed germination paper and fitted with a rubber stopper base. The top was covered with cheesecloth. The germination paper was kept saturated with water by means of a reservoir of water at the bottom of the chamber surrounding the flask. The high humidity inside of the chamber prevented the inoculum drops from drying, thus providing a uniform environment for the host-parasite interactions. The droplets retained their original positions throughout the incubation period. The plants were kept in the incubator for 5-6 days after the final inoculations.

Hypocotyl sections representative of each of the seven treatments are included in Fig. 1. The induction control resulted in dense flecking at the sites of inoculation with beta race (Fig. 2). The 0-, 18-, and 36-hr challenge control treatments resulted in development of dark, spreading lesions typical of the susceptible interaction (Fig. 3, 4, 5). Complete protection occurred in all three 1-cm regions when the challenge followed the inducing inoculation by 18 or 36 hr (Fig. 7, 8). The 0-hr challenge resulted in development of restricted lesions (Fig. 6), and the extent of restriction increased with increasing age of the tissue (4-5 cm < 6-7 cm < 8-9 cm).

The challenge sites in the protected plants were identical in appearance with the induction sites (Fig. 7, 8). Microscopic examination of epidermal strips containing the induction and challenge sites also revealed them to be indistinguishable. Both beta and gamma race spores germinated, formed appressoria, penetrated the epidermal cells, and formed primary mycelium extending into several epidermal cells. Development of mycelia of both races had ceased at this stage, and cells collapsed. The normal extensive proliferation of the gamma race mycelium typical of the susceptible interaction did not occur.

Induced resistance at a distance was also observed when the varieties Topcrop, Harvester, and Wade were tested. Unlike Perry Marrow, these varieties are susceptible to the beta race and resistant to the gamma race. Thus, the phenomenon operates in representatives of both types of beta-gamma differential varieties.

In the resistant (inducing) interactions, the fungus stimulates the plant cells to respond hypersensitively; the penetrated cells granulate and brown, and the fungus is contained. These experiments indicate that this response alters cells at a considerable distance from those directly involved in the inducing interaction. When the protected cells are inoculated with a pathogenic race, the spores germinate, form appressoria, and penetrate the epidermal cells. If the lag between inoculation with the inducer and challenge is sufficient, the normal proliferation of the challenge does not occur; the penetrated cells granulate and brown, and the fungus is contained as in a typical resistant interaction.

The induced resistance apparently is not due to a diffusable phytoalexin or spore germination inhibitor on the spores of the challenge as the latter germinated and penetrated the host cells. The effect appears expressed after penetration. Phytoalexins such as pisatin (4), phaseollin (3), and 6-methoxy mellein (2) readily diffuse into water on an inoculated surface, but significant amounts are not translocated into tissues surrounding the sites of inoculation. We therefore suggest that protected cells are primed for the synthesis of inhibitors as a result of the inducing interaction, but rapid synthesis occurs only upon interaction with the challenge.

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