Longevity of Mycelium of Colletotrichum lindemuthianum in Hypocotyl Tissue of Resistant and Susceptible Bean Cultivars

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ABSTRACT

The longevity of mycelium of *Colletotrichum lindemuthianum* in hypocotyl tissue of resistant and susceptible beans (*Phaseolus vulgaris* 'Perry Marrow' and 'Red Kidney', respectively) was investigated. The fungus was isolated from old hypocotyls of susceptible plants and from young and old hypocotyls of resistant plants up to 25 days after inoculation. Microscopic observation of apparently intact mycelium in stained hypocotyl sections supported these results which suggest that, under the conditions of our experiments, even in a physiologically resistant bean hypocotyl where small lesions develop, portions of the mycelium remain viable for extended periods of time after ingress.

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The reaction of physiologic races of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. on different bean (*Phaseolus vulgaris* L.) cultivars has been shown to be controlled by major-gene resistance factors (1, 3). Griffey & Leach (4) showed that hypocotyls of a susceptible bean cultivar became more resistant with age due to anatomical changes which mechanically inhibit extension of the mycelium into the stelar tissue of the hypocotyl. Also, extensive cortical invasion is inhibited by desiccation of the necrotic tissue.

Determining the longevity of an invading pathogen in tissue of resistant and susceptible plants would be helpful toward an understanding of the nature of resistance. Isolations were made from old hypocotyls of a susceptible ('Red Kidney') and young and old hypocotyls of a resistant ('Perry Marrow') bean cultivar to determine how long the mycelium of *C. lindemuthianum* remains viable in the hypocotyl tissue.

MATERIALS AND METHODS.—The *beta* race of *C. lindemuthianum* was used in this study. The bean cultivars used were Perry Marrow, which has major-gene resistance to the *beta* race (1), and Red Kidney, which is susceptible to the *beta* race.

Hypocotyls of resistant and susceptible bean cultivars were obtained from plants grown in a greenhouse until used in an experiment. They were then moved to a growth room with temperature of approximately 20 C and relative humidity of 70-90%. Light was supplied for 12 hr during each 24-hr period by two banks of four 40-W Sylvania fluorescent tubes and two 300-W incandescent bulbs. The highest intensity reaching the plants from these lights was approximately 3,660 lx (340 ft-c) and intensity was considerably less in some areas of the growth room.

Hypocotyl tissue was considered young until the leaves at the first node above the cotyledonary node were expanded to one-half their full size, and old when the leaflets at the second node above the cotyledonary node were fully expanded. The terms "young hypocotyl" and "old hypocotyl" are used throughout the paper to refer to tissue in that defined age category at the time of inoculation.

Inoculations were made by finger-rubbing the hypocotyl with a spore suspension obtained from 10- to 15-day-old cultures of *C. lindemuthianum* grown on sterilized green beans. Following inoculation, the plants were placed in a humidity chamber for 48 hr to insure good ingress.

Five days after inoculation, the hypocotyl was wrapped for 9-10 min with a cheesecloth pad saturated with a 10% commercial Clorox solution in an attempt to avoid late infection from surface inoculum. Isolations were made 0, 1, 5, 10, and 20 days after the Clorox treatment.

At designated times after inoculation, 3-cm hypocotyl

TABLE 1. Recovery of *Colletotrichum lindemuthianum* from old hypocotyls of susceptible ('Red Kidney') and young and old hypocotyls of resistant ('Perry Marrow') bean plants

Days after inoculation	Old susceptible	Percentage recovery[a]	
		Young resistant	Old resistant
5	78	100	30
6	54	80	42
10	46	84	19
15	67	89	33
25	77	95	33

[a]Each percentage entry is based on isolations attempted from 24-36 hypocotyl tissue sections.

sections were obtained by cutting the hypocotyls 1 cm and 4 cm below the cotylendonary node. These were immersed in a 10% Clorox solution for 10 min and then washed with sterile distilled water. Three thin tangential sections 4-8 mm in length were cut from each 3-cm section. Each section was then plated in a glucose-neopeptone-rose bengal medium [glucose 2.8 g, neopeptone 2 g, $MgSO_4 \cdot 7H_2O$ 1.23 g, KH_2PO_4 2.72 g, rose bengal 33 mg, agar 20 g, distilled water 1,000 ml, pH 6 ± 0.1 (6)]. After approximately 1 month the plates were examined for the presence of *C. lindemuthianum*. Isolations were also made from control hypocotyls which received distilled water rather than the spore suspension. These plates were always negative for *C. lindemuthianum*. Isolations were not attempted from young susceptible plants because they collapsed approximately 7 days after inoculation due to disease development.

Microscopic examinations of stained hypocotyl tissue were made to support the results obtained from the isolation experiments. Hypocotyl sections were killed and fixed in an absolute alcohol-glacial acetic acid (1:1 v/v) solution and cleared in 75% lactic acid for 48 hr at 40 C. They were then stained in a lactophenol-aniline blue solution [lactophenol, 100 ml (phenol 20 ml, lactic acid 20 ml, glycerin 40 ml, distilled water 20 ml); glacial acetic acid, 6 ml; 1% aqueous aniline blue, 3 ml]. Thin tangential sections were cut from these stained sections and observed.

RESULTS AND DISCUSSION.—Cultures of *C. lindemuthianum* were obtained consistently through 25 days after inoculation from old susceptible hypocotyls and from young and old resistant hypocotyls (Table 1). The experiments were repeated with similar results in each case. Portions of apparently intact mycelium observed by microscopic observation of stained hypocotyl sections supported these results.

Our results suggest that portions of mycelium of *C. lindemuthianum* remain viable in old hypocotyls of susceptible bean plants and in young and old hypocotyls of resistant bean plants for extended periods of time after ingress. These results tend to support the hypotheses of physiological resistance in beans to *C. lindemuthianum* which have appeared in the literature. Leach (5) proposed that the fungus cannot acquire an adequate food supply in the resistant host. Consequently the fungus dies releasing autolytic products which cause death and discoloration of the host cell or cells in the immediate vicinity of the hypha. The result is a small hypersensitive lesion. Müller (7) detected a toxic principle in a diffusate obtained when bean pods were inoculated with a spore suspension of *Monilinia fructicola* (Wint.) Honey or *Phytophthora infestans* (Mont.) de Bary. The principle was shown to be toxic to *C. lindemuthianum* and other fungi. Bailey & Deverall (2) extracted the phytoalexin phaseollin from bean (*P. vulgaris*) hypocotyls inoculated with *C. lindemuthianum*. This suggests that resistance in bean plants to *C. lindemuthianum* may be due to the production of a post-infectional toxic compound. In either case portions of mycelium of *C. lindemuthianum* could remain viable in physiologically resistant bean tissue for extended periods of time in a latent condition. Bailey & Deverall (2) showed that high concentrations of phaseollin were required to stop in vitro mycelial development of *C. lindemuthianum*, and that the mycelium apparently metabolized some of the phaseollin even in situations where mycelial development had been halted. Skipp & Deverall (8) reported some limited extension of hyphae of *C. lindemuthianum* in necrotic cells of resistant bean (*P. vulgaris*) hypocotyls as long as 10-14 days after inoculation.

The morphological and physiological characteristics of portions of mycelium which remain viable in tissue of physiologically resistant bean hypocotyls should be investigated further. Also, the occurrence of such mycelium in host tissues generally and its possible role in maintaining the pathogen in a crop area should be explored. If viable fungal mycelium in a resistant host is capable of growth after the host dies, the dead plant could provide an excellent substrate for growth leading to possible development and selection of virulent fungal strains.

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