Histopathological Relationship of Fusarium and Thielaviopsis with Beans

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ABSTRACT

Fusarium solani f. sp. *phaseoli* and *Thielaviopsis basicola* were utilized in a comparative histopathological study of four bean lines: *Phaseolus coccineus* 'Scarlet Runner'; *P. vulgaris* (P.I. 203958, also called N203); N203 × (*P. vulgaris*^{2} × *P. coccineus*)—F6 (2051-02); and *P. vulgaris* 'Red Kidney'. Red Kidney is susceptible to both pathogens, but the other bean lines show various degrees of resistance. The mode of ingress and pattern of development of the pathogens were different, but the early response of the bean lines to either pathogen was essentially similar. Certain differences in host response became evident as infection progressed. There was a rapid accumulation of brown materials in resistant lines in advance of the area invaded by the fungi. Infection of Scarlet Runner at or near soil level by either pathogen resulted in hypertrophy of the cortical cells internal to the lesion, and subsequent formation of a periderm. A protective periderm also developed in N203 and 2051-02, but in these lines, cell division generally began in the endodermal layer. Since the development of hyphae was restricted in resistant lines regardless of the presence or absence of this barrier, periderm formation apparently is of secondary significance as a factor in the resistance of beans to these pathogens. Phytopathology 60:821-824.

Several fungi may be involved in the bean root rot complex (4, 5, 8, 13), but three species predominate: *Fusarium solani* f. sp. *phaseoli*; *Thielaviopsis basicola*; and *Rhizoctonia solani*. *Fusarium* causes extensive damage to the bean crop, and the development of commercially acceptable varieties resistant to this fungus is a major aspect of the current breeding programs. *F. solani* f. sp. *phaseoli* is pathogenic to all commercial varieties of *Phaseolus vulgaris* and several other species of bean (1, 2). In spite of this wide host range, there is substantial variability in the degree of susceptibility of different bean lines. A basically similar situation exists with *T. basicola,* both in regard to host range and host response (11).

Some histopathological work with *Fusarium,* particularly with susceptible bean varieties, already has been reported (2, 3, 5). Huber (7) compared the response of resistant and susceptible bean plants to *Fusarium.* He attributed resistance to a nonspecific wound response characterized by intense enzymatic activity around the infection site and rapid deposition of wound substances. Huber (7) noted the formation of a wound periderm in resistant plants, but found only scattered cell divisions in susceptible ones. Because Huber's inoculation procedure included wounding, it was not possible to distinguish wound response from pathological response.

Most early histo-pathological work with *T. basicola* was done on tobacco (12, 16). Conant (6), after comparing the response of resistant and susceptible tobacco varieties, concluded that the rapidity of initiation and continuation of cork formation below the lesion indicated resistance in tobacco. Jewett (9), however, found no correlation between wound periderm formation and resistance of tobacco to *T. basicola.* Christou (4) made a detailed study of the histopathological relationship of *T. basicola* with susceptible beans, but no similar study with resistant beans has been reported.

In order to provide a basis for investigating the nature of resistance in bean to *F. solani* f. sp. *phaseoli* and *T. basicola,* comparative histological studies were made of plants infected with each pathogen. These results are reported here.

MATERIALS AND METHODS.—The bean lines used in our study were *Phaseolus coccineus* L. 'Scarlet Runner'; *P. vulgaris* L. (P.I. 203958, also called N203); *P. vulgaris* L. 'Red Kidney'; and N203 × (*P. vulgaris*^{2} × *P. coccineus*)—F6 (2051-02). Red Kidney is susceptible to both pathogens, whereas the other bean lines show various degrees of resistance.

The isolate of *Fusarium solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans. was obtained from infected, field-grown Red Kidney bean. The fungus was cultured in the laboratory on potato-dextrose agar (PDA). Inoculum was prepared from 7- to 14-day-old cultures by washing macroconidia from PDA slants. The spore suspensions were adjusted so that 2 to 2.5 million spores were applied in 100 ml water to each pot.

Two isolates of *Thielaviopsis basicola* (Berk. & Br.) Ferr. were used, a tobacco isolate and isolate RS6. The latter was isolated from infected Red Kidney bean and was more pathogenic to Scarlet Runner than was the tobacco isolate. *T. basicola* was cultured in potato-dextrose broth under conditions similar to those used for *Fusarium.* Inoculum was prepared from 12- to 14-day-old cultures by blending the mycelial mat with distilled water. The suspension was adjusted so that 2 to 3 million endoconidia were applied in 100 ml water to each pot.

Twelve seeds, three from each line, were sown in steam-sterilized soil in 5- or 6-inch clay pots and kept in a greenhouse. Inoculum was applied at time of sowing or soon after germination. Specimens were harvested at various intervals during a 6-week period.

For studying the initial stages of invasion, seeds were surface-disinfested in 10% Sunny Sol (5.25% sodium hypochlorite) and germinated on moist filter paper in

sterilized moist chambers in the laboratory. Seedlings with 1- to 1.5-inch radicles were dipped in a spore suspension of either pathogen, and were kept separate from those used as controls.

Specimens were harvested daily for 7 days, fixed in a 2:1 mixture of absolute alcohol and glacial acetic acid, and stored in 70% ethanol until examined. Epidermal strips were cleared in lactophenol and stained in 0.1% acid fuchsin or 0.1% cotton blue in lactophenol prior to examination.

Materials harvested from greenhouse tests were fixed in FAA, dehydrated by passage through the tertiary butyl alcohol series (10), and embedded in tissuemat. Serial microtome sections (10-20 μ) were made of both healthy and infected tissues. Phloxine:light green and thionin:orange G (15) were the main stain combinations used. Phloroglucinol and Sudan IV were used to ascertain the presence of lignin and suberin, respectively (10).

RESULTS.—*Infection by F. solani f. sp. phaseoli.*—The mode of ingress and pattern of early development of *F. solani* f. sp. *phaseoli* were similar in all bean lines, and agreed with previous descriptions (3, 5). Macroconidia germinated, and each produced one or two germ tubes that formed a hyphal network on the surface of the hypocotyl. Dense agglomerations of hyphae were formed, some of which were located above the stomata. From these agglomerations, hyphae grew into the stomatal cavity en masse or produced branches on the surface, some of which gained ingress individually or in small groups. Although ingress was principally through the stomata, entry also was effected by direct penetration and through wounds.

Following penetration, the pathogen produced a vigorous and abundant mycelial mat in the substomatal cavity from which groups of hyphae developed, and rapidly progressed intercellularly in all directions. The hyphae showed a distinct gregarious tendency, especially when developing radially. Invasion along the longitudinal axis of the plant was more rapid than in other directions. Intercellular invasion was predominant, with intracellular development occurring principally in cells that were discolored and apparently in a phase of degeneration. Intercellular development was so profuse that cortical cells often were separated from each other.

The pathogen produced chlamydospores both within and between cells in necrotic tissues. No macroconidia were observed on plants grown in the greenhouse.

Infection by T. basicola.—The mode of ingress and pattern of early development of *T. basicola* were similar in all bean lines, and agreed with that described by Christou (4). Endoconidia germinated and produced a network of hyphae on the surface of the hypocotyl. Ingress was principally by direct penetration of epidermal cells. No appressoria were formed, but the hyphae frequently enlarged at points of penetration. A few stomatal penetrations were also observed.

Following penetration, there was a profuse development of fingerlike, constricted hyphae intracellularly. From the initially infected cell, hyphae advanced into adjacent cells by thin penetration pegs, each of which produced either a spearlike or knoblike structure in the newly invaded cell. Growth continued from the base or apex of this structure, and gave rise to a mass of intracellular hyphae that nearly filled the invaded cell.

Within 2 weeks, numerous chlamydospores were formed both intercellularly and intracellularly. Clusters of chlamydospores and conidiophores bearing endoconidia were also produced on the surface of necrotic tissues.

Comparative response of bean lines to Fusarium and Thielaviopsis.—In general, fewer and smaller lesions were produced on resistant lines, particularly by *T. basicola*. Although the mode of ingress and pattern of development of *Fusarium* and *Thielaviopsis* were different, the early response of the bean lines to either pathogen basically was similar. As infection progressed, certain differences in the host reaction became evident, and these differences were more marked when *T. basicola* was the infecting organism.

In Red Kidney (susceptible), hyphae of either pathogen continued to develop rather profusely, with the result that large lesions were formed quickly (Fig. 1-D). In resistant lines, hyphal development was rather restricted, with a consequent reduction in the size of lesions formed. Long, thin reddish streaks were produced by *Fusarium,* and small dark spots generally were produced by *Thielaviopsis* on resistant lines. Within 2 weeks, lesions were restricted in resistant lines, but in susceptible lines, the fungus continued to develop prolifically and even invaded pericycle fibers. In addition to this differential rate of hyphal spread among resistant and susceptible lines, hyphae were less abundant in resistant lines (Fig. 1-B) than in Red Kidney (Fig. 1-A). Furthermore, reproductive structures were less numerous in bean lines with high levels of resistance.

Initially, hyphae of both fungi were observed in or between cells in advance of brown necrotic areas; later, brown substances accumulated in cells beyond the point of hyphal penetration. These materials gave the cells a granular appearance, and accumulated more readily in resistant lines, especially Scarlet Runner. At or near soil level, cellular hypertrophy occurred in the cortex of Scarlet Runner in areas internal to lesions, and

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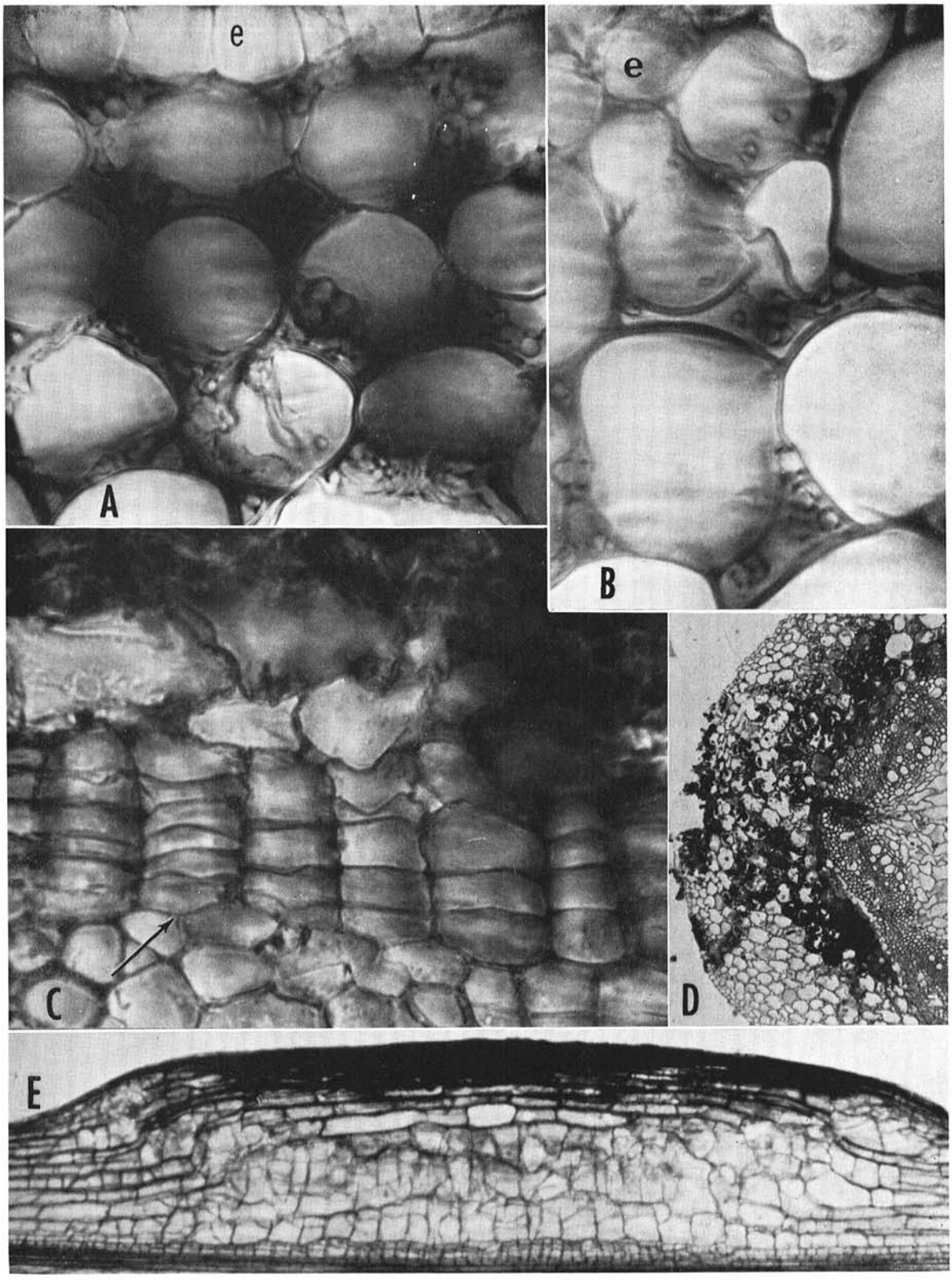
Fig. 1. A) Transverse section through a lesion on hypocotyl of Red Kidney showing inter- and intracellular hyphae of *Fusarium solani* f. sp. *phaseoli*. Note the greater quantity of hyphae in this susceptible line in comparison to 1-B; e = epidermis (×450). **B)** Transverse section through a lesion on hypocotyl of Scarlet Runner showing inter- and intracellular hyphae of *F. solani* f. sp. *phaseoli*; e = epidermis (×450). **C)** Transverse section through hypocotyl of 2051-02 infected by *Thielaviopsis basicola* showing periderm (arrow) formed from the endodermal layer (×550). **D)** Transverse section through lesion on hypocotyl of Red Kidney 2 weeks after inoculation with *T. basicola* (×45). **E)** Longitudinal section through lesion on hypocotyl of Scarlet Runner infected by *T. basicola* showing pustulelike bulge caused by hypertrophy of the cortical cells internal to the lesion.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caused a pustulelike enlargement (Fig. 1-E). The hypertrophied cells later divided, and within 2 to 3 weeks, a complete lignified periderm was produced. No marked hypertrophy occurred on parts which were deeper in the soil, but a protective periderm sometimes was formed. Generally, lesions in such locations tended to be larger than those located at or near soil level. The *Thielaviopsis* isolate from tobacco either failed to infect Scarlet Runner or produced pinpoint lesions in which hyphae progressed to a depth of only two to three cells into the cortex. A protective periderm also developed in 2051-02 and N203. In these lines, however, no cortical hypertrophy was evident, and cell division generally began in the endodermal layer (Fig. 1-C). No complete periderm was formed in Red Kidney, but a few cell divisions were observed in the endodermal layer, particularly when the pathogen was *T. basicola.*

In all cases in which a periderm was observed, uninvaded cells with brown accumulations separated the lignified area from cells that were invaded by hyphae. This indicated a lack of physical contact between the hyphae and the periderm.

In general, infection by *T. basicola* resulted in greater accumulation of brown substances in the uninvaded cells of resistant lines, more marked cellular hypertrophy in Scarlet Runner, and a periderm that was formed slightly earlier than when the pathogen was *F. solani* f. sp. *phaseoli.* Except for these differences, the basic response of a particular bean line to infection by either pathogen was similar.

DISCUSSION.—Undoubtedly, a substantial amount of variability occurs in the host response to *F. solani* f. sp. *phaseoli* and *T. basicola.* This is evident mainly as a reduction in the number and size of the lesions produced on resistant bean lines when compared with a susceptible variety Red Kidney. Since penetration occurred in all lines, it appears that the pathogen-host relationships were compatible up to that stage. The resistance mechanism of the host appears to have been activated sometime after penetration, with the result that growth and spread of hyphae within tissues of resistant lines were restricted.

There was a definite correlation between the rapidity of periderm formation and resistance. However, the periderm is not considered a major factor in the resistance of beans to these pathogens for the following reasons: (i) The periderm is not always associated with lesions, and development of hyphae is restricted in resistant lines even in the absence of periderm; (ii) when a periderm was observed, adversely affected but uninvaded cells were present between the invaded region and the lignified area of the periderm, suggesting that hyphae were effectively restricted before they were confronted by the physical barrier; and (iii) compared to the rate of invasion of Red Kidney, the periderm was formed too late, particularly in the lines with intermediate resistance, to be of much significance in limiting the development of the pathogens. It has been pointed out that in lines with intermediate resistance the periderm developed mainly in the endodermal layer, and even in the susceptible line, *F. solani* f. sp. *phaseoli* was rarely observed beyond this point. It seems clear, therefore, that if the periderm is at all effective as a barrier to the pathogens, it is of secondary rather than primary significance in the resistance of beans to *F. solani* f. sp. *phaseoli* and *T. basicola.* Instead, resistance appears to be associated with some chemical product of the pathogen-host interaction (14), which quickly and effectively suppresses the spread of either pathogen in resistant Scarlet Runner. This apparently occurs at a somewhat slower rate in the lines with intermediate resistance (N203 and 2051-02), and is less effective in the susceptible Red Kidney.

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