Histochemical Studies of Yellow Poplar Infected with Fusarium solani

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Published with the approval of the Director of the South Carolina Agricultural Experiment Station as Technical Paper No. 1110. Portion of a Ph.D. thesis submitted to Clemson University by the senior author.

Accepted for publication 17 October 1973.

ABSTRACT

Canker development initiated by *Fusarium solani* was studied histochemically on 1-yr-old yellow poplar stems 2 to 84 days, 365 days, and 547 days after inoculation. The pathogen did not degrade lignin, but did degrade pectin and cellulose to a limited extent, and completely degraded stored starch in infected areas of the stems. Cessation of lateral growth of the fungus was associated with lignification of a surrounding barrier of cells in the cortex, phloem, and rays of the bark. In the presence or absence of the pathogen, a dark-brown pigment (presumably an oxidized, conjugated phenolic compound) was produced in cells adjacent to wounds. Cankers produced by the pathogen on stems inoculated in the fall or spring were healing 18 mo after inoculation; however, the pathogen could be isolated from the tissues beneath those healing cankers.

Phytopathology 64:414-418

Additional key words: Liriodendron tulipifera, Fusarium canker.

Lipscomb (11) reported canker development 2 wk after inoculation with *Fusarium solani* (Mart.) App. and Wr. emend Synd. and Hans. on 1-yr-old yellow poplar (*Liriodendron tulipifera* L.) seedlings. Beneath the bark, brown discoloration was evident in the xylem 2 cm above and below the canker and 2.5 mm into the xylem. In infected segments of the stems, pith ray cells were discolored and contained gum-like materials, granules, and hyphae. In discolored xylem elements, gum deposits and tyloses were abundant, especially near the point of inoculation. Gum deposits appeared as droplets on the walls of some cells, and occluded the cavities of others. Hyphae were observed in xylem parenchyma, ray parenchyma, fiber-tracheids, and vessels. Some hyphae extended through pits of cells, but no infection pegs were seen. Anastomosed hyphae and swollen intercalary hyphal cells were present.

Vascular discoloration is a generally recognized feature of vascular infection and has been attributed to an increase in concn of oxidized phenolic compounds (1, 5, 16). Beckman (1) suggested that these compounds may function as a defense mechanism by combining with gels to form gum plugs or by inhibiting growth of the pathogen by inactivation of enzymes. He also suggested that oxidized phenolic compounds may induce protective overgrowth.

Discoloration of yellow poplar wood infected with *F. solani* has been attributed to accumulation of gums and associated materials (11). In sugar maple wood infected with *Polyporus glomeratus* Peck., the contents of the parenchyma cells killed in advance of the hyphae were transformed into dark-brown wound gum which seemed to inhibit the further development of decay (6).

Shigo (14) found that tissues of northern hardwoods formed after wounding were seldom discolored or decayed. Lignin formation may act as a barrier to further spreading of the pathogen (10).

Discoloration may occur in wounded wood in the absence of microorganisms (12, 15). Jorgensen (9) hypothesized that the compounds in this type of discolored wood are formed as a part of a natural defense reaction against fungus penetration. In the absence of *F. solani,* no discoloration was noted in wounded yellow poplar stems (11).

This research focused on histological and histochemical studies of canker development induced by *Fusarium solani* in the stem of yellow poplar.

MATERIALS AND METHODS.—The main stems of 84 one-yr-old yellow poplar seedlings in the greenhouse were swabbed with 95% ethyl alcohol. One ml of *F. solani* spore suspension (6.75×10^3 spores per ml) was injected immediately into the wood with a hypodermic syringe. Six control trees were wounded to the xylem and six were wounded to the phloem only and injected with sterile distilled water. All wounds were covered with cotton and masking tape. Sunlight was supplemented by 40-W fluorescent tubes to afford 16 h of light each day in the greenhouse. No attempt was made to critically control temp.

At 2-day intervals for 84 days, two inoculated stem sections were removed and immediately fixed in FAA. Each section included the developing canker and a small portion of healthy tissue above and below the canker. Wounded control stem sections were fixed in FAA at the end of the 84-day period. From each 2-day sample, one stem was sectioned transversely and the other longitudinally at 30 μ. Controls were sectioned in the same manner 84 days after wounding.

Concurrently with the above, 12 seedlings were inoculated in both the field and greenhouse in November and 12 more in both the field and greenhouse in May. Trees in the greenhouse were kept under light 16 h per day. Three hundred sixty-five days after the spring inoculations, and 547 days after the fall inoculations, segments containing the points of inoculation were fixed in FAA. Half the stem segments of each group were sectioned transversely, and half longitudinally. All stem sections were stained for cellulose (7), pectin (7, 13), lignin (7, 8), starch (7), insoluble polysaccharide (7), callose (7), aldehyde (7), and tannin (8).

RESULTS.—*Histological changes accompanying canker development.*—Cankers formed on all stems inoculated with *F. solani.* The first evidence of canker formation was a brown discoloration of tissues around

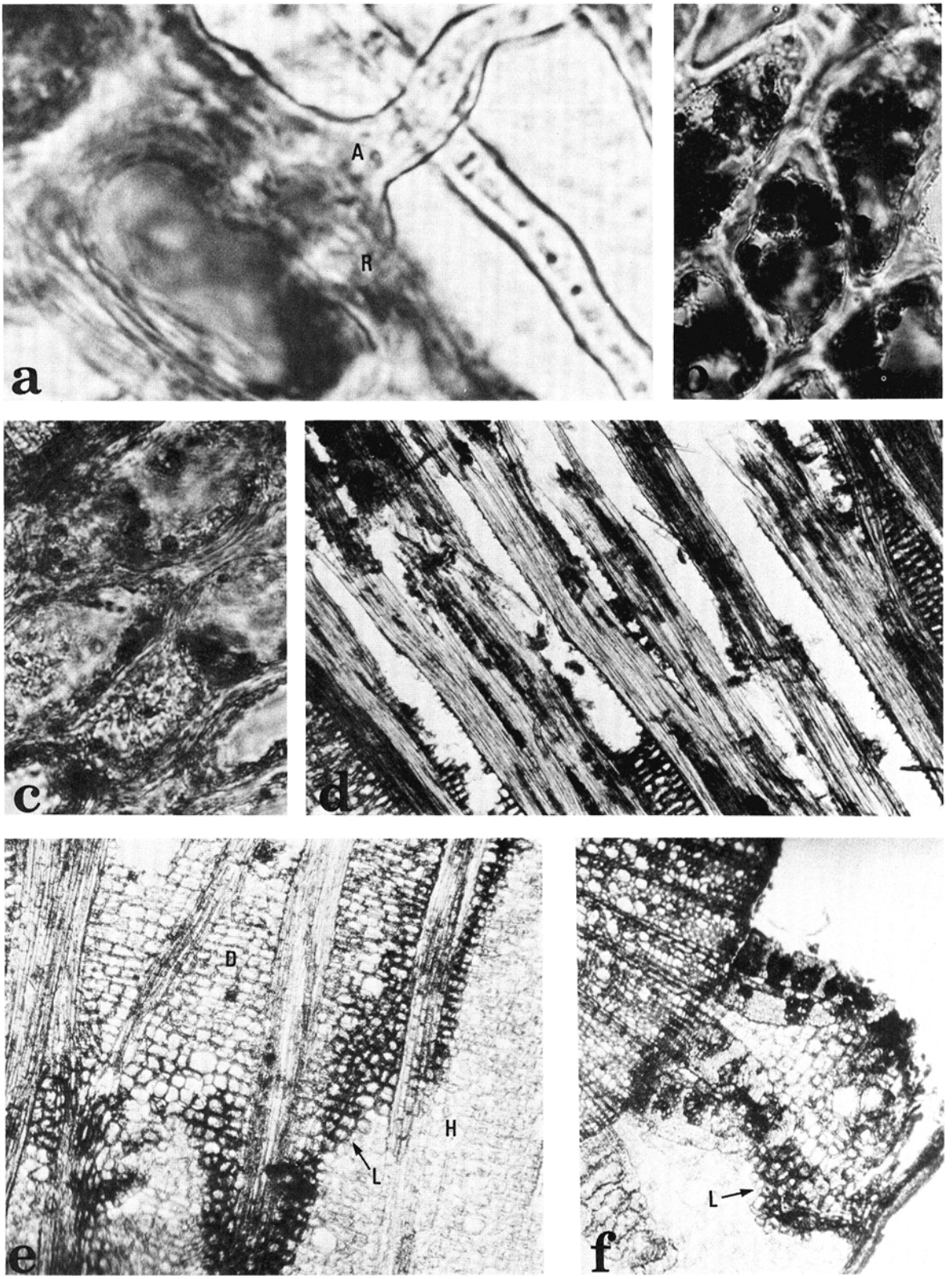

Fig. 1-(a to f). **a)** Appressorium-like structure (A) on ray cell wall (R) at the end of branch hyphae (× 376). **b)** Healthy pith ray tissue showing abundant starch. **c)** Diseased pith ray tissue showing starch depleted (× 376). **d)** Longitudinal section of canker with degradation of all tissues except fibers 24 days after inoculation (× 28). **e)** Longitudinal section with irregular band of lignified cells (L) in the bark separating diseased tissue (D) from healthy tissue (H) (× 282). **f)** Cross section with band of lignified cells (L) from the cork to the vascular cambium (× 94).

the point of inoculation. Discoloration was present by the 4th day in both walls and cytoplasm of cells in the cortex, phloem, pith rays, and the distal portion of xylem rays. After 8 days, the area of discoloration had extended to the primary xylem. In cross section this area was wedge-shaped and limited on each side by pith rays. The brown material did not stain for callose, tannin, or aldehyde. After 84 days the area of discolored and infected tissue in the wood was still delineated by the two pith rays beneath the extremes of the bark canker. Vertically, however, the area of discoloration in the wood extended some distance above and below the exterior canker margins. The brown substance did not stop the progressive ramification of *F. solani* in the wood but did delimit the fungus in the bark tissues.

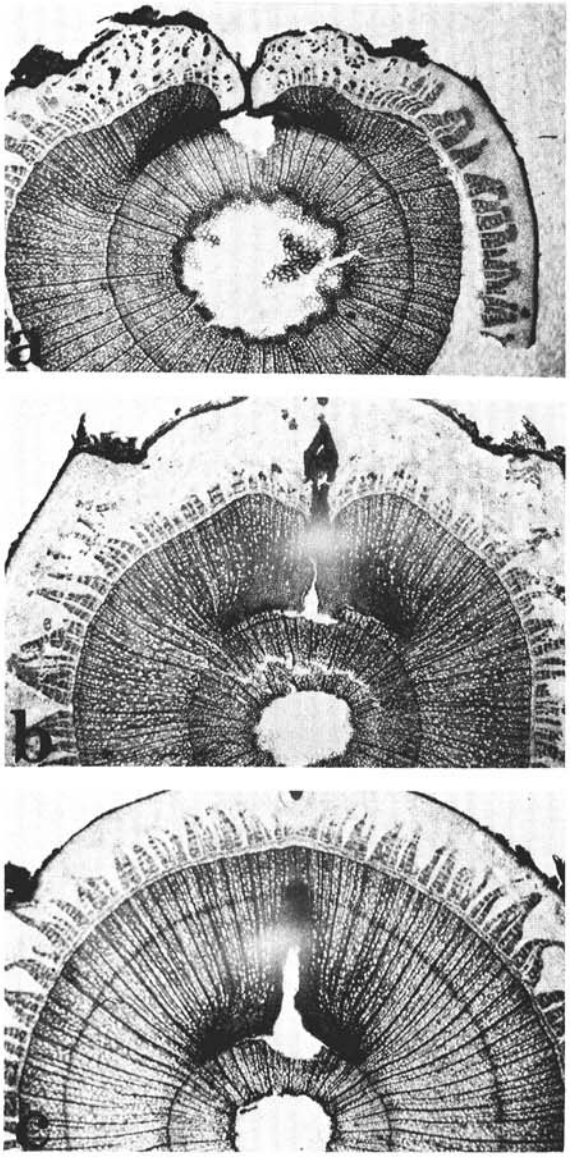

Fig. 2-(a to c). Differential stages of canker healing. **a)** Cross section of stem canker of seedling grown in greenhouse (18 mo after inoculation). **b)** Cross section of stem canker of seedling grown in field (12 mo after inoculation). **c)** Cross section of stem canker of seedling grown in field (18 mo after inoculation).

In all sections within the canker, hyphae were observed in and between cells external to the vascular cambium and within vessels and tracheids in the discolored area of the stem. Often hyphae, parallel with the long axis of the xylem elements, were branched toward ray cells, some of which contained hyphae. On the ray cell walls the branches flattened out and resembled appressoria, although no infection peg mechanism was noted (Fig. 1-a). In 50% of the stems with 28- to 84-day-old infections, the pith was invaded by the fungus and in 48% of these stems the fungus had grown through the pith and infected xylem on the side of the stems opposite the canker. In a very few instances, a canker was produced on the stem opposite the point of inoculation. In all sections studied, only five spores were observed: three microconidia and two macroconidia. These were located in the infected cortex and pith regions.

Histochemical changes during canker development.—Four days after inoculation, the reserve starch, which was abundant in healthy tissue, had disappeared from the infected cortex, ray, and phloem cells. After 8 days, the starch was partially depleted in the ray cells of the infected xylem; and after 24 days, all reserve starch of infected areas was depleted (Fig. 1-b, c).

Eight days after inoculation, the pectic material of the walls of all infected bark cells except fibers had been degraded and the cellulose of these cells partially degraded. After 24 days, the cellulose was completely gone from these cells also (Fig. 1-d). Eighty-four days after inoculation, cellulose and pectic materials in cell walls of the infected wood remained unaffected.

The walls of some cortex and phloem cells at the extremes of the infected and discolored bark tissues had become slightly lignified 10 days after inoculation. By the 32nd day, the canker was completely delineated in the bark by a group of cortex, phloem, and ray cells with highly lignified walls. In cross-sectioned stems, these cells formed a line between healthy and diseased tissue from the vascular cambium to the cork of the stem. Walls of these cells stained negatively for cellulose and the cells contained no cytoplasm. In longitudinal section, this band of lignified cells formed an irregular line from the outer edge of the canker inward to the vascular cambium (Fig. 1-e, f). The lignified zone of cells blocked all vertical and lateral progression of hyphae in the bark tissue, although hyphae had penetrated to the pith.

The zone of lignified cells in the bark increased in thickness until the 54th day following inoculation. The maximum thickness observed was seven cells. Between the 54th and 84th day, several layers of cork-like cells developed beneath this zone; and by the 84th day, a slight overgrowth of the canker by the action of the vascular cambium was apparent.

Walls of healthy xylem ray cells were highly lignified and contained well-defined, half-bordered pits. Within infected wood, walls of the ray cells near the canker were eroded, especially at the pits; whereas the pits of the ray cells closest to healthy wood appeared to be plugged by the production of new wall components.

Histology of advanced canker.—Sections of 365- and 547-day-old cankers of stems inoculated in the

greenhouse and field were essentially alike. Cankers in both cases had partially healed by the production of new cambium, xylem, phloem, and callus tissues by the cambium surrounding the cankers. Healing was more advanced on the field-grown trees. New xylem tissue which had been produced immediately external to cankers consisted of small, highly lignified, isodiametric cells which contained no cellulose. In cross section this zone of cells had not covered the xylem involved in the original canker. External to the xylem of the original canker, normal xylem tissue had been produced, although the cells were distorted lengthwise. Just external to the vascular cambium which had been produced over the cankers, normal phloem had formed beneath a zone of cortex parenchyma which contained many scattered groups of sclerids. Normal-appearing cork layers developed beyond the cortex and the surface of the stems were remnants of necrotic bark tissue of the cankers (Fig. 2-a). Since the seedlings were not allowed to become dormant, no annual ring was formed in stems that were kept under 16 h of light in the greenhouse.

In field-grown trees the zone of callus tissue, as well as the functional xylem and phloem external to it, were more extensive than in trees kept in the greenhouse. The second annual ring had been produced. Remnants of the necrotic bark tissue of the canker were apparent on the outer surface of the stem (Fig. 2-b, c).

In stems sectioned 365 or 547 days after inoculation, there was no infection of wood which was produced after inoculation. Viable *F. solani* was confined to wood which was in existence when the stems were inoculated, although the cankers were successfully healing.

In control stems wounded to the phloem or to the wood, a zone of thick-walled cells which stained positively for lignin developed around the wound to the wood. These cells developed from existing cells in the cortex, phloem, and pith rays. Cells between the wound and this zone of cells contained the dark-brown substance in the walls and cytoplasm, but it was much less intense than in stems with cankers. Xylem ray cells and some vessels and tracheids nearest the wound also contained the dark-brown discoloration. There was little or no degradation of starch, pectin, or cellulose in cells surrounding the wound. Cambium at the wound in both sets of controls was killed, but the cambium near the wound had produced new cells which were partially covering the wound. Lignin of xylem cells and fibers was unaffected by wounding.

DISCUSSION.—The hyphae infected healthy cells of all tissue types, growing from cell to cell through pits by way of simple hyphal constriction in some cases or apparent appressorial formation in others. Pith cells became infected, but hyphal progression was slow in the pith, perhaps because of naturally occurring, scattered groups of highly lignified cells.

Evidence suggests that the fungus derived its nourishment from cellulose, pectin, and starch contained in the bark canker proper; and that, in the wood, only starch in living cells was utilized by the fungus. The fungus was apparently incapable of degrading lignin.

Although *F. solani* has been reported to sporulate on the margins of old cankers on cottonwood (2), no sporulation of this type was observed on yellow poplar. Only five spores were observed on small cankers of young stems; these were produced in no consistent area of infected stems.

The brown-pigmented substance produced upon wounding and infection may have been oxidized phenolic compounds (1), which are fungistatic and conducive to protective overgrowth. They may also be precursors of lignin-like compounds (10), since extensive lignification was observed in the stem tissues following the production of these substances.

The lignified barrier of cells which developed from existing cells in the bark, appeared to be impervious to penetration by the pathogen because lateral progression of the fungus was halted in the bark. Callus tissue which was produced over infected xylem appeared impermeable to infection, possibly because of the nonpitted, highly lignified nature of the cell walls. Normal xylem tissue produced after infection of the existing xylem, also seemed resistant to infection by the fungus. This new xylem tissue appeared to be structurally the same as the older xylem, but did resist infection from within. In infected trees in nature, either the resistance of the new wood breaks down with age, or the resistant nature of the new wood in older trees did not exist or was not as great as that of young seedlings.

Production of the brown-pigmented substance by bark and wood cells surrounding a wound, production of a lignified barrier of cells around a wound, and production of lignified callus tissue over a wound appeared to be reactions to wounding, not infection; although these reactions were more extensive in yellow poplar stems infected with *F. solani* than in the control stems. The apparent retardation of progress of the pathogen in the infected portion of the stems 18 mo after inoculation was presumably due to the resistant reactions of the stem tissues. It is not known whether this was juvenile resistance that would result in cessation of growth of *F. solani* and ultimate elimination of the pathogen from the stems, or whether these barriers eventually would break down, resulting in extensive infection of the trees.

Other investigators (3, 4) have reported that cankers on inoculated yellow poplar in the field healed when the stems were inoculated in the spring, but did not heal when the stems were inoculated in the fall. This was not found to be the case in this study; the cankers healed when the stems were inoculated in either spring or fall. The only apparent difference between the two studies is that in the former, older naturally occurring trees in the field were inoculated; whereas in this study, the inoculated trees were 1-yr-old seedlings either potted in the greenhouse, or planted in the field. The younger seedlings may have possessed juvenile vigor which enabled them to combat fall and winter infection more readily than older trees.

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