Vascular Response of Cotton to Infection by Fusarium oxysporum f. sp. vasinfectum

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ABSTRACT

Occlusion of xylem vessels followed by toxin formation in the cotton plant are major mechanisms of resistance to *Fusarium oxysporum* f. sp. *vasinfectum*. Tracheal fluids of *Fusarium*-infected stems were toxic to *Fusarium* or *Verticillium albo-atrum* only if the fluids came from occluded vessels. Resistance to *Fusarium* increased with host age.

One resistant and one susceptible line of cotton had the same occlusion rate, but the resistant line produced more xylem vessels. It is suggested the higher capacity conductive system compensated for the relatively slow vascular occlusion rate. Phytopathology 60:121-123.

Plants may respond to infection by the formation of chemical or physical barriers, or both. Increasing evidence suggests that formation of physical rather than chemical barriers plays a primary role in resistance to systemic invasion of the vascular system by *Fusarium* (1). This is true immediately after vascular invasion. The formation of gels or gums that plug xylem vessels restricts the movement of water as well as the pathogen.

Upland cotton, *Gossypium hirsutum* L., is susceptible to *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. Vascular occlusion may be responsible for restricting the systemic invasion of *Fusarium* in this relationship (1). My experience indicated that resistance to *Fusarium* increases as the age of the cotton plant increases. I performed experiments to further characterize the rate and nature of the responses of cotton to invasion of the vascular system by *F. oxysporum* f. sp. *vasinfectum*. Plant age and variety were factors considered when studying these responses. Varietal resistance is expressed whether roots or stems are inoculated, so resistance exists in both (3). Therefore, an evaluation of host responses after stem inoculation to reveal mechanisms of resistance seems valid, even though initial infection normally occurs in the roots.

MATERIALS AND METHODS.—The Seabrook variety of Sea Island (SBSI) cotton, *G. barbadense* L., was used as a resistant host, and the upland variety Stardel (*G. hirsutum*) as a susceptible host. Mo. 63-277 is an upland line bred and selected in southeast Missouri for resistance to *Verticillium albo-atrum* Reinke & Berth. (microsclerotial form). It also exhibits resistance to *F. oxysporum*. Plants were grown in the greenhouse in steamed soil under continuous supplemental fluorescent light.

The isolate of *F. oxysporum* used was obtained from infected cotton in southeast Missouri. The fungus was cultured for 3 to 4 days in a Czapek's broth shake culture at 25°C. Conidia were obtained by filtration through cheesecloth. Nearly all were microconidia. Conidial numbers were measured with an hemacytometer.

Inoculations were made by stem puncture at three sites on the hypocotyl 1.5-3.0 cm above the soil line. The inoculum contained 3×10^6 conidia/ml. Control plants were inoculated with sterile distilled water.

SBSI and Stardel were planted on 3 dates to give plants that were 3, 5, and 7 weeks old at inoculation. Seven days after inoculation, the stems were severed 1 cm below the cotyledonary node and placed in basic fuchsin for 5 min (6). The stems were sectioned by hand at the second node, and the number of functional xylem vessels (stained red) was recorded.

In another test, Mo. 63-277 was also included, and the three varieties were inoculated when 3 weeks old. Functional vessels were counted at 1, 2, 3, and 4 days after inoculation. Treatments were arranged in a randomized block design, with three plants comprising each of three replicates. The average value of three stems was used for each replicate. The test was repeated.

Vascular fluids were obtained by root pressure or an ethanol flush method. SBSI and Stardel were inoculated when 3 weeks old. Stems were severed 1 cm below the cotyledonary node, and a glass tube with a rubber adapter was attached to the stump. Root pressure forced fluid into the tube.

Vascular fluid was taken from each of 10 plants and combined 5 hr after the stem was severed. Collections were made 1, 2, 3, and 4 days after inoculation.

The hypocotyls from which fluid was collected by root pressure were cut at the site of inoculation. These sections were 3.5 to 5.5 cm long. Each stem piece was cut in half, and 1 ml of 95% ethanol was pulled through the xylem by vacuum. The extracts also were combined. The ethanol extracts and vascular fluids obtained by root pressure were evaporated to dryness. One-half ml of chloroform and 0.2 ml of 0.01 M K_2HPO_4 were added to each residue. The chloroform evaporated, leaving 0.2 ml of a suspended residue at an approximate concentration found in each hypocotyl (0.02 ml). A wire loop, 3 mm in diam, was used to transfer one part of the residue to a glass slide in a petri dish moist chamber. To this were added two parts (loopsful) of a germination medium that contained NH_4NO_3, 1 g; $MgSO_4$, 0.5 g; KH_2PO_4, 3.5 g; sucrose, 1.0 g; and distilled water, 1,000 ml. Conidia were washed three times by centrifugation from sterile, glass-distilled water, then

concentrated by centrifugation and added to the germination medium prior to mixing with the residues. The percentage germination on the glass slides was determined after 6 hr at 24°C for *Fusarium* and 10 hr at 22°C for *Verticillium*. One hundred conidia were examined in each of three replicates.

RESULTS.—*Effect of plant age.*—Notes on wilt development were taken 7 days after inoculation. When 3-week-old plants were inoculated, Stardel showed considerable wilting, while SBSI had only occasional wilted leaves. When 5-week-old plants were inoculated, only occasional chlorosis and darkened leaf veins occurred on both varieties. When 7-week-old plants were inoculated, symptoms were rare, and only in the form of darkened leaf veins.

Functional vessels in infected stems were expressed as a percentage of those in healthy stems. Only 12% of the vessels were functional in wilted Stardel plants that were 3 weeks old when inoculated (Table 1). Fifty-four percent were functional in SBSI of the same age. The percentage of functional vessels was greater in plants that were 5 or 7 weeks old when inoculated.

Rate of xylem dysfunction.—In the second test, the rate of vessel occlusion for Stardel and Mo. 63-277 was nearly the same, and gradually decreased to 65% 4 days after infection. The decrease was most rapid in SBSI, and 2 days after infection, only 40% of the vessels were functional as compared to 82-97% for the other varieties. Functional vessels in SBSI increased to 70% on the 3rd day, and decreased to 50% on the 4th day. The difference between SBSI and the other varieties was statistically significant only on the 2nd day of infection (Duncan's Multiple Range Test, 5%).

On the 4th day after inoculation (25 days after planting), the average number of vessels per noninoculated stem was: Mo. 63-277, 163; SBSI, 127; and Stardel, 131. The value for Mo. 63-277 was significantly higher than for the other two varieties. Even though the rates of dysfunction were comparable for Mo. 63-277 and Stardel, the rate of water movement may not have been equally effected because of the larger capacity of the vascular system of Mo. 63-277.

Germination in vascular fluids.—The germinations (77-90%) of conidia of *Fusarium* (microconidia) and *Verticillium* were not inhibited in the vascular fluids obtained by extraction or root pressure from noninoculated plants. Neither was germination inhibited in vascular fluids that were obtained by root pressure from infected plants. Inhibition occurred only in fluids from inoculated plants that were obtained by flushing the vascular system with 95% ethanol. This inhibition was apparent 4 days after the inoculation of SBSI. The germination in this fluid was less than 1% for *Fusarium* and 7% for *Verticillium*. No inhibition was apparent in Stardel during the 4 days, nor in SBSI during the first 3 days after infection.

TABLE 1. Percentage of functional xylem vessels compared to noninoculated cotton plants 7 days after stem inoculation with *Fusarium oxysporum* f. sp. *vasinfectum*

Variety	Age of host when inoculated (weeks)		
	3	5	7
	%	%	%
Seabrook Sea Island	54	73	64
Stardel	12	90	90

DISCUSSION.—The response of the vascular system within 24 hr after infection was occlusion by gels. The occlusion rate was more rapid in resistant SBSI than in less resistant Stardel and Mo. 63-277. Toxin formation was not evident until the 4th day after inoculation in SBSI. The toxin was obtained only by ethanol treatment, which may have dehydrated the gels and allowed toxins to pass through the xylem. This suggests that the toxins were localized in the infected vessels. It is not known whether the toxins were produced by the host or fungus. Movement of the toxins must have been restricted, or they would have been detected in the fluids collected by root pressure.

The response of the resistant cotton vascular system is rapid occlusion, followed by toxin formation in the occluded cells. The localization by occlusion agrees with the results of Beckman (1). He found that *F. oxysporum* f. sp. *vasinfectum* grew slowly in cotton. My results suggest that toxin also contributes toward localization by inhibiting normal growth of the pathogen. Bell (2) has shown that a nonspecific toxin, gossypol, is formed in cotton in response to *Verticillium*. These results show that cotton can resist vascular invasion by an occlusion-toxin mechanism.

But there is a lack of correlation between vascular response and observed resistance in Mo. 63-277 if the occlusion mechanism is operative. Occlusion occurred at the same rate in Mo. 63-277 and Stardel for the first 4 days after infection of the vessels, even though Mo. 63-277 is more resistant than Stardel to *F. oxysporum*. But 4 days after infection, or 25 days after planting, the rate of formation of xylem tissue increased more rapidly in Mo. 63-277 than in Stardel or SBSI. This increase also was evident in healthy stems; it was not a response to the fungus. The more extensive vascular system may have compensated for the slow rate of occlusion, because functional vessels were still available in sufficient quantities after the fungus had finally been walled off.

The oldest xylem tissue, i.e., distal to the cambium, developed occlusions and darkened first. In advanced pathogenesis, a single broken ring of functional xylem remained adjacent to the cambium. If the oldest cells in the xylem tissue are the first to respond to infection, then the occlusion theory would partially account for increased resistance in older plants. Polygalacturonase is produced in greater quantities by *Fusarium* in the presence of polygalacturonic acid (4). Gossypol also was produced at a higher rate in older, irritated vascular tissues of cotton (2). The quantity and quality of pectins in older tissue may have affected the rate of depolymerization and plug formation.

Resistance of older plants to *Fusarium* is not always evident in the field because of susceptibility to *Meloidogyne incognita*. Cotton suffers greater damage when infected with both *M. incognita* and *F. oxysporum* as

compared to infection by either pathogen alone. Smith & Dick (5) could not determine the inheritance of resistance to *Fusarium* until nematodes had been reduced by fumigation. If gumosis, tylose, and toxin formation are mechanisms of resistance, then the host that is resistant to *F. oxysporum* should respond in one of two ways after infection by both pathogens: (i) by complete occlusion of the vascular system as if it had received multiple infections of a high inoculum level; or (ii) by less occlusion or at least a slow rate of response to *Fusarium* (thus systemic invasion) because of physiological weakening from nematode injury. Studies in this area should add to our knowledge of the mechanisms of resistance to vascular wilts in cotton.

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