The Localization of Fusarial Infections in the Vascular Tissue of Single-Dominant-Gene Resistant Tomatoes

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ABSTRACT

Results indicate that the sealing off of vascular infections by rapid tylose development is the basis for the single-dominant-gene type of resistance of tomato to Fusarium wilt. Extensive secondary distribution of the pathogen in the susceptible host occurs because tylose development, although initiated normally, is retarded about 2 days after inoculation. Complete occlusion in many infected xylem vessels is delayed 7 days or more after inoculation.

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Additional key words: *Lycopersicon*, *Fusarium*, genetics, resistance mechanisms, tyloses.

In a previous paper (9), it was reported that the buildup of *Fusarium* propagules was comparable in infected vessels of tomato plants that were near isogenic except for resistance and susceptibliity to wilt caused by the race 1 biotype of the fungus. Initial distribution of the parasite after inoculation was also comparable in the two isolines. The only discernible difference was that secondary distribution was negligible in the resistant isoline in contrast to that in the susceptible isoline in which most of the xylem vessels in all of the bundles gradually became infected.

The objective of these experiments was to determine the basis for the observed difference in distribution of the parasite in these two isolines. The results with these plant isolines were of particular interest because the resistance exhibited was known to be controlled by a single-dominant-gene (7, 12). An advance in our understanding here would represent an additional step in the process of mapping genes by which resistance mechanisms as a whole are controlled.

MATERIALS AND METHODS.—Improved Pearson (IP) and Improved Pearson VF-11 (VF-11) are near-isogenic lines (isolines) of tomato (*Lycopersicon esculentum* Mill.) that were developed by Cannon & Hanna (7) and are, respectively, susceptible and resistant to Fusarium wilt (10,11). These plants and the parasite, *Fusarium oxysporum* (Schlecht.) emend. Snyd. & Hans. f. sp. *lycopersici* (Sacc.) Snyd. & Hans. race 1, were grown according to methods outlined previously (9). Inoculations with mixed microspore and tracer-particle suspensions into washed, severed tap roots, and incubation in environment chambers at 28 ± 1 C and 2,000 to 2,500 ft-c of light for 15 hr day were also carried out according to methods already described (9). This method of inoculation was used because the highly colored tracer particles provided the means for detection of inoculum trapping sites (on perforation plates and end walls) within minutes of the time of inoculation, and because, with these two isolines at least, differences in secondary distribution from the hypocotyl tissue, if any, were likely to be most obvious (9). At daily intervals through the 4th and again on the 7th day, six plants of each isoline were uprooted and washed free of soil. All lateral roots were removed. The fresh tap roots were sectioned longitudinally (5), placed on a microscope slide, and stained with 1% cotton blue in lactophenol. Inoculum trapping sites (2,5) were located by the accumulation of inert red vinyl tracer particles, and these sites were examined microscopically. Hyphal penetration of trapping membranes, growth of hyphae in length, and sporulation beyond trapping membranes were recorded for each trapping site at daily intervals. The presence of tyloses and occlusion of xylem vessels by tyloses were also recorded for each trapping site. These experiments were repeated 3 times. Observations of special interest were photographed using type A Kodachrome II film. Black and white prints were prepared as described (6).

RESULTS AND DISCUSSION.—The trapping of spores and tracer particles on perforation plates and end walls of functional xylem vessels (Fig. 1-D) was readily detected by the bright red luminescence of the tracer particles when exposed to incident light (4). A total of 353 such trapping sites in 90 VF-11 plants and 345 sites in 90 IP plants were observed and recorded. Possible host response could be meaningfully observed in only three or four sites/plant because the vascular system in tomato is very tortuous, and because tylose development, at least, is not continuous within a given xylem vessel. Therefore, although many more trapping sites were present in each plant, it was decided to record data only for those xylem vessels that could be observed for a distance of ca. 500 μ or more above the trapping site.

The rate of hyphal elongation at such sites was found to be comparable in the susceptible IP and resistant VF-11 plants. Average hyphal lengths were 35 μ in both isolines after 1 day of incubation, 140 and 190 μ, respectively, after 2 days, and 340 and 375 μ, respectively, after 3 days of incubation. Reliable observations after 3 days were impossible because the developing tyloses obscured hyphal growth. These results confirm those reported earlier (2,9) that there is essentially no difference in the growth or multiplication of *Fusarium* in resistant and susceptible hosts.

There was also no apparent difference in the time or degree of penetration of perforation plates and end walls in resistant and susceptible hosts, nor was there a difference in the time of sporulation from hyphae that had penetrated membranes at trapping sites. Hyphal penetration of trapping site membranes occurred by 2 days after inoculation in both isolines, and sporulation on these hyphae was first observed 3 days after inoculation in both isolines. Thus there was no apparent difference in the potential of the parasite for secondary distribution within the vascular systems of resistant and susceptible hosts.

Previous results (9) have shown, however, that ultimate distribution of the parasite was very extensive in the susceptible host, although it remained very limited in the resistant host. The only remaining alternatives that could account for these results seemed to be (i) that infected xylem vessels were more quickly or effectively obstructed by host response in the resistant than in the susceptible host, or (ii) that vascular obstructions were more readily reduced in the susceptible than in the resistant host. Our data indicate (i) to be the case.

No host responses that could obstruct infected xylem vessels were observed 1 day after inoculation. After 2 days, however, tyloses had been initiated in both plant isolines (Fig. 1, 2). The xylem vessels in the resistant host were often occluded well beyond the sites of parasite trapping in the resistant VF-11 isoline, but this was rare in the susceptible IP isoline. Three days after inoculation vascular occlusion by tyloses had essentially reached the observable maximum (ca. 60%) in VF-11, but had not done so even 7 days after inoculation in IP plants. Actual occlusion in the resistant isoline was probably 100% of the infected xylem vessels even as early as 3 days after inoculation. Since tyloses do not form along the

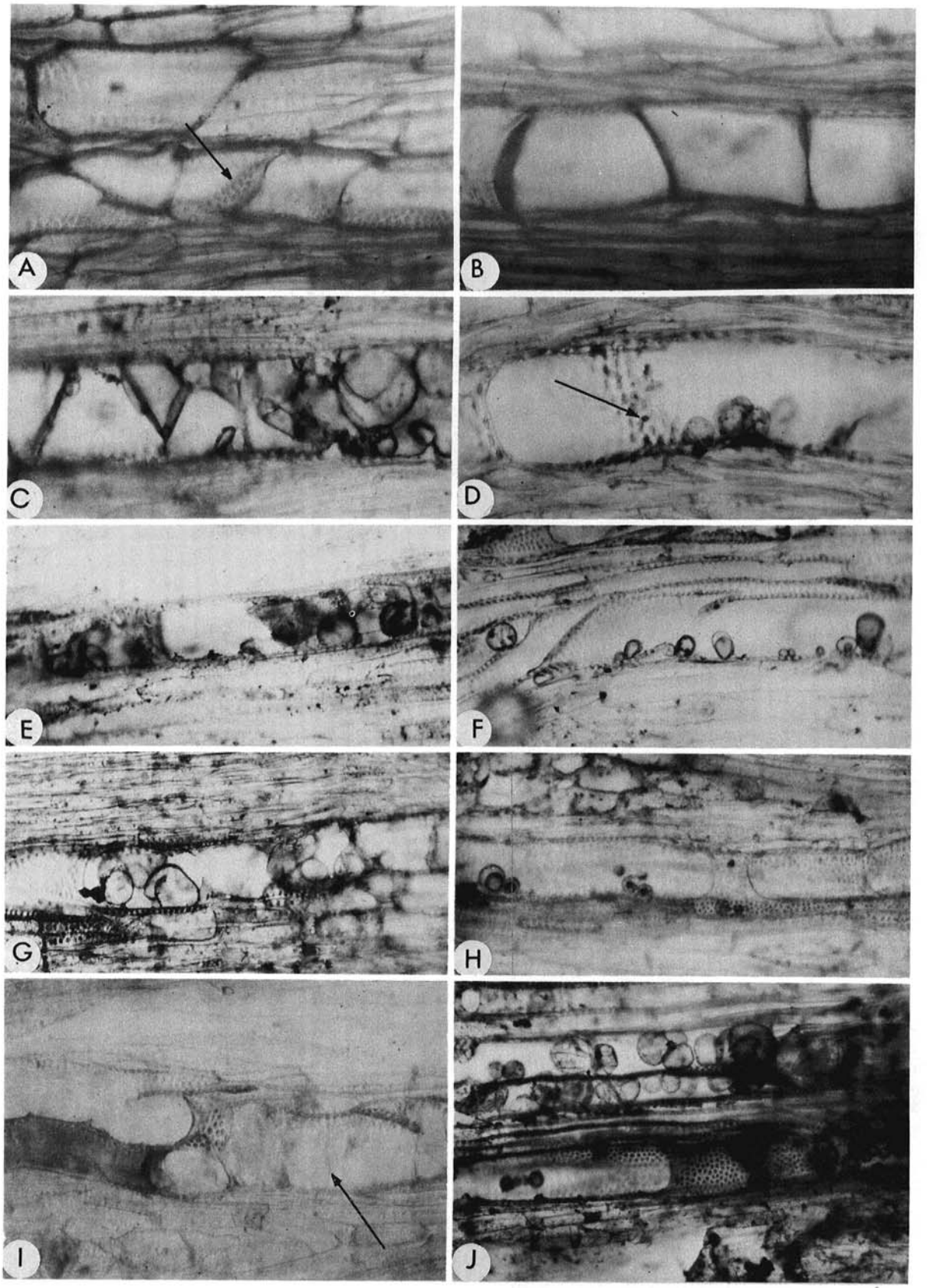
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Fig. 1. A,B) Photomicrographs of longitudinal sections of vascular tissue of Improved Pearson VF-11 (VF-11) and Improved Pearson (IP) tomato isolines showing **A)** reticulate thickening of end walls of xylem vessel elements (arrow) and **B)** the simple unthickened membranes that constitute perforation plates. Tracer particles (arrow) may be seen trapped on a reticulate end wall in (D). No differences in structures or their occurrence were noted in the vascular elements of VF-11 and IP isolines. **A,B,C,D,E,F, G,H,I,J)** Photomicrographs of longitudinal sections of vascular tissue of VF-11 (left column) and IP (right column) **A,B)** 1 day; **C,D)** 2 days; **E,F)** 3 days; **G,H)** 5 days; **I,J)** 21 days after inoculation with *Fusarium oxysporum* f. sp. *lycopersici* showing no tyloses in either isoline 1 day after inoculation, complete occlusion 2 days after inoculation and thereafter in VF-11 (left column), and retarded tylose development in IP until final occlusion at 21 days (right column). All sections except that shown in (I) were stained with cotton blue in lactophenol to show tylose formations. The section shown in (I) was left unstained so that discoloration at the trapping site (left) could be seen. Several tyloses may be seen, faintly outlined, to the right of the trapping site (arrow).

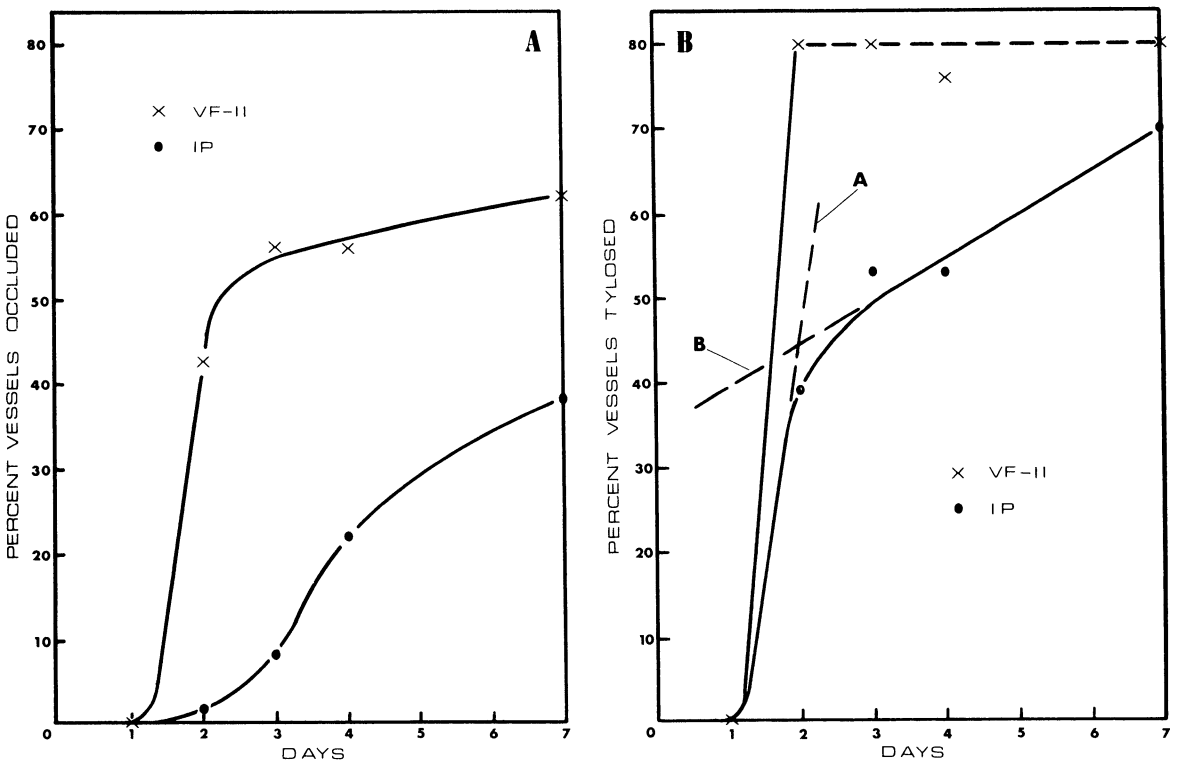

Fig. 2. A) Per cent xylem vessels occluded by tyloses at or near visible trapping sites at different times after inoculation. In the resistant Improved Pearson VF-11 (VF-11) isoline, complete occlusion had occurred at some point in essentially all infected xylem vessels, to the 60% observable maximum, 3 days after inoculation. In the susceptible Improved Pearson (IP) isoline, this percentage of occlusion in infected xylem vessels was not achieved even 7 days after inoculation. **B)** Per cent of xylem vessels with visible trapping sites in which tyloses had been initiated (occluding or not) in relation to time in days after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*. In the resistant VF-11 isoline, the rate of tylose initiation was rapid, and all infected vessels, to the 80% observable limit, contained tyloses 2 days after inoculation. The situation in the susceptible IP isoline was somewhat more complex. A projection of the flat of the curve as drawn between days 1 and 2 (line A) suggests that initiation of tyloses in the susceptible IP isoline was at first comparable to that in the resistant VF-11 isoline. A projection of the flat of the curve for tylose initiation in IP between 2, 3, 4, and 7 days (line B) suggests that a second, inhibitory factor comes into play in the Fusarium-infected susceptible isoline on or soon after the 2nd day. Enlargement of tylose initials in the susceptible IP isoline was also visibly retarded during this period, thus accounting for the long delay in occlusion shown in Fig. 1.

entire length of xylem vessels, but only intermittently (1), the tortuous shape of the xylem vessels, the random cutting of longitudinal sections in relation to these vessels, and the resulting inability to observe vessels for a distance of more that 1,000 μ precluded the observation of this 100% occlusion. In any case, there seems little doubt that rapid occlusion of infected xylem vessels above the trapping sites results in a rapid sealing off of these infected vessels in the resistant isoline (Fig. 1,2-A).

This sealing-off process seems to be a very general type of resistance mechanism in higher plants, since it has been found to function successfully in many plant species (1,2,8,13), and against many soil-borne

organisms that can grow and multiply in the vascular environment (3). Most of these organisms, though they can grow parasitically in vascular elements, are successfully confined and therefore do not become pathogenic.

The basis for delayed occlusion and the specific susceptibility of isoline IP to *F. oxysporum* f. sp. *lycopersici* are suggested in Fig. 2-B. Tylose initiation was rapid in the resistant VF-11 isoline, and tyloses were found in the observable maximum of xylem vessels (in this case 80%) 2 days after inoculation. Tylose initiation in IP appears to have been comparable to that in VF-11 during the period 1 to 2 days after infection (line A), but further development of tyloses appears to have been severely retarded in the IP isoline after 2 days (line B). These results suggest either that the potential for tylose formation was equivalent in the IP and VF-11 isolines, but that *Fusarium* produced a growth-inhibiting substance in IP that it did not in VF-11; or that the vascular parenchyma cells of IP were more susceptible than were cells of VF-11 to the action of a metabolite produced equally well in both hosts by *Fusarium*.

Whatever the ultimate cause, there is an intriguing and significant difference between the two host-parasite interactions that becomes apparent 2 to 3 days after inoculation, and that presumably is dependent upon the single-dominant-gene for resistance. This host-parasite system, then, provides an excellent tool for closing the gap in our knowledge of gene function and the molecular basis for one type of resistance to vascular diseases.

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