# Distribution and Growth of Fusarium oxysporum f. sp. lycopersici Race 1 or Race 2 within Tomato Plants Resistant or Susceptible to Wilt

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## ABSTRACT

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The distribution and growth rate of *Fusarium oxysporum* f. sp. *lycopersici* race 1 or race 2 in near-isogenic tomato lines Improved Pearson (IP) and Pearson VF-11 (VF) were investigated. Cultivar IP is susceptible to both races; VF is resistant to race 1 but susceptible to race 2. The three susceptible combinations (IP/race 1; IP/race 2; VF/race 2) exhibited similar patterns of pathogen population increases within the taproot/hypocotyl vascular tissue followed by extensive invasion and build-up within epicotyl vascular bundles. In contrast, continual distribution of race 1 within VF did not occur. The extensive invasion and build-up of race 2 within VF epicotyls, compared to the limited invasion of race 1 in VF, suggests that the virulence of race 2 toward VF is related to its capacity to overcome the resistance mechanism that normally localizes infections within the root/hypocotyl region. Antifungal compounds reported to be present within the upper stem do not appear to be effective in limiting the pathogen and preventing symptom development once the localization process is overcome and there is extensive hypocotyl colonization and subsequent pathogen spread, within many vessels, to the epicotyl. The possible contributions of host-produced fungal inhibitors and physical barriers to fungal growth and movement are discussed.

*Additional key words*: resistance mechanisms, vascular wilt diseases.

The nature of host resistance to Fusarium wilt of tomato [which is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans., race 1] has been studied intensely in recent years using as hosts the two near-isogenic lines, Improved Pearson (IP) and Pearson VF-11 (VF). The two isolines differ principally in the single gene for resistance carried by VF. Two principal mechanisms have been postulated to explain resistance of tomato plants carrying the I gene for resistance to race 1. Mace and Veech (4) suggested that the presence of a fungal inhibitor found in highest concentration above the cotyledonary node is the paramount resistance factor. On the other hand, Beckman et al. (1) and Elgersma et al. (2) concluded that the main distinction between the two isolines was the rapid development of tyloses within the taproot/hypocotyl region of the resistant isoline that provided a physical barrier to continued distribution of race 1 upward into the stem.

The contradictory results and conclusions may be associated with differences in experimental procedures. Mace and Veech (4) severed the stem at the cotyledonary node, placed the cutting in spore suspension and then examined the epicotyl tissue for pathogen increase or decrease. Beckman et al. (1) and Elgersma et al. (2) employed taproot or root dip inoculations and examined the taproot and hypocotyl region to determine the extent of pathogen distribution and population changes.

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The present study was undertaken to resolve some of the differences detected in the earlier studies. We used the experimental design of Elgersma et al. (2) but included tissue above the cotyledonary node. In addition we used *F. oxysporum* f. sp. *lycopersici* race 2 which was not included in the previous studies. Specifically, we used tomato isolines VF-11 (resistant to race 1 but susceptible to race 2) and IP (susceptible to both races) to compare fungal distribution and rate of growth within hypocotyl and epicotyl vascular bundles of three susceptible combinations (IP/race 1; IP/race 2; VF/race 2) and one resistant combination (VF/race 1).

## MATERIALS AND METHODS

**Host.**—Seed of IP and VF were sown in Jiffy-mix (W. R. Grace & Co., Cambridge, MA 02140) and transplanted at the two-leaf stage into 10-cm-diameter standard plastic pots containing a mixture of soil:peat:pearlite (1:1:1, v/v). The plants were grown in a controlled-environment chamber at 26-28 C under Sylvania cool-white lights at 27,000 lx programmed to provide a 15-hr photoperiod. The plants were fertilized weekly by applying 16-32-16 fertilizer (17 mg P; 16 mg K; 19 mg N) to each pot.

**Pathogen.**—*Fusarium oxysporum* f. sp. *lycopersici* races 1 and 2 (obtained from M. E. Mace, National Cotton Pathology Laboratory, College Station, TX 77840) were grown on rehydrated Difco potato-dextrose agar (PDA). Microconidia for inoculum were harvested

from 2-wk-old cultures incubated at 30 C. The microconidia were filtered through one layer of lens paper, and the spore concentration was adjusted using a haemocytometer.

**Inoculation.**—Plants were inoculated in the five- to six-leaf stage. The plants were uprooted and the root system washed free of soil. The taproots were severed with a razor blade where they were approximately 2 mm in diameter (approximately 7 cm below the cotyledonary node). The roots of the plants were submerged in a spore suspension of $10^6$ microconidia/ml of distilled water and spore uptake was allowed to occur for 30 min while the plants were transpiring under greenhouse conditions. The plants were then re-potted and returned to the controlled-environment chamber.

The severed taproot technique was selected because initial spore transport along vessels averages less than 1 cm and only a few spores reach the hypocotyl and epicotyl (2). Such limited spore distribution is what one would expect in the early stages of natural infections. The sampling techniques described below were designed to measure fungal population and colonization changes within the hypocotyl region and continued fungal distribution and subsequent population changes within the epicotyl.

**Detection of Fusarium propagules and vascular discoloration in the upper plant axis.**—A preliminary experiment was undertaken to determine the extent of distribution of race 1 and race 2 within the upper axis of both IP and VF. Ten plants of each host/pathogen combination were sampled 30 days after inoculation by making free-hand sections at each internode and placing them on sterile microscope slides. The total number of vascular bundles exhibiting discoloration was recorded. The sections then were incubated for 24 hr at 27 C in an inverted position in sterile, glass petri dishes to allow for mycelial growth within infected vascular bundles. The sections then were stained with 1% cotton blue in lactophenol, and the total number of vascular bundles from which hyphae had grown was recorded.

**Determining Fusarium population growth curves.**—*Fusarium* propagules in vascular tissues were determined at various intervals after inoculation to establish population growth curves in the two isolines. The plants were uprooted, and the root systems were washed free of soil. Leaves and lateral roots were removed and the stem surfaces were sterilized by dipping them into 70% ethanol and flaming them. Hypocotyl and epicotyl segments 1 cm in length were taken 3 cm below and 5 cm above the cotyledonary nodes, respectively. Then the cortex was aseptically removed from the segments. The segments were sliced as thinly as possible with a sterile razor blade and homogenized in 3 ml of sterile distilled water for 2 min using a VirTis (Model 60) homogenizer at 40,000 RPM. Samples of the homogenized plant material were pipetted into sterile plastic petri dishes and molten (46 C) PDA was added. The medium contained 40 μg/ml streptomycin sulfate to minimize bacterial growth. Colony counts were made after 2 and again after 3 days of incubation at 27 C.

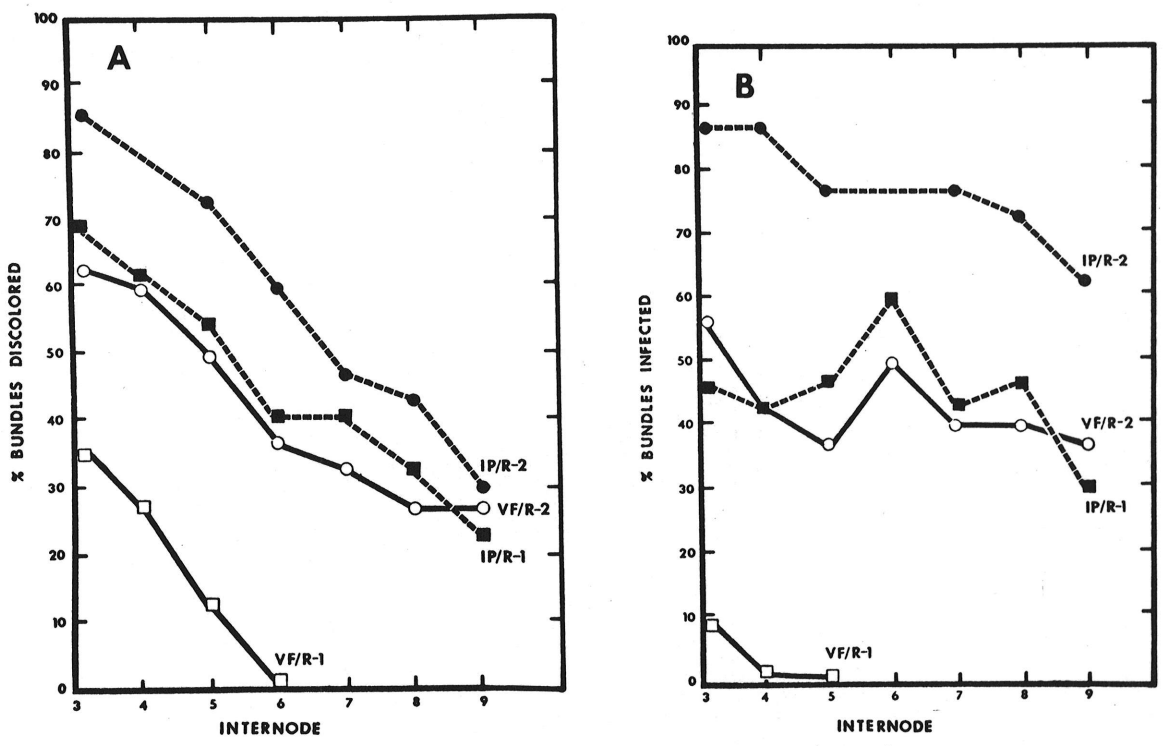


**Fig. 1-(A, B).** Visual evidence of the activity of *Fusarium oxysporum* f. sp. *lycopersici* at various internodes along the plant axis 30 days after inoculation of Improved Pearson (IP) and Pearson VF-11 (VF) with race 1 or race 2. Percentage of vascular bundles **A)** discolored or **B)** infected (exhibiting fungal growth). Cultivar IP is susceptible to both races; VF is resistant to race 1 but susceptible to race 2.

**Detection of Fusarium in vascular bundles.**—The extent of fungal invasion of the hypocotyl or epicotyl was determined by observing and recording the number of vascular bundles infected. Free-hand cross sections were removed from the hypocotyl or epicotyl region adjacent to the tissue used in determining fungal growth curves. The sections were incubated, stained, and examined according to the procedure described above. Fifteen IP and 15 VF plants were sampled for each incubation period.

## RESULTS

**Distribution of Fusarium and vascular discoloration in the upper plant axis.**—Discoloration of vascular bundles was used as an indicator of the spread of the pathogen.

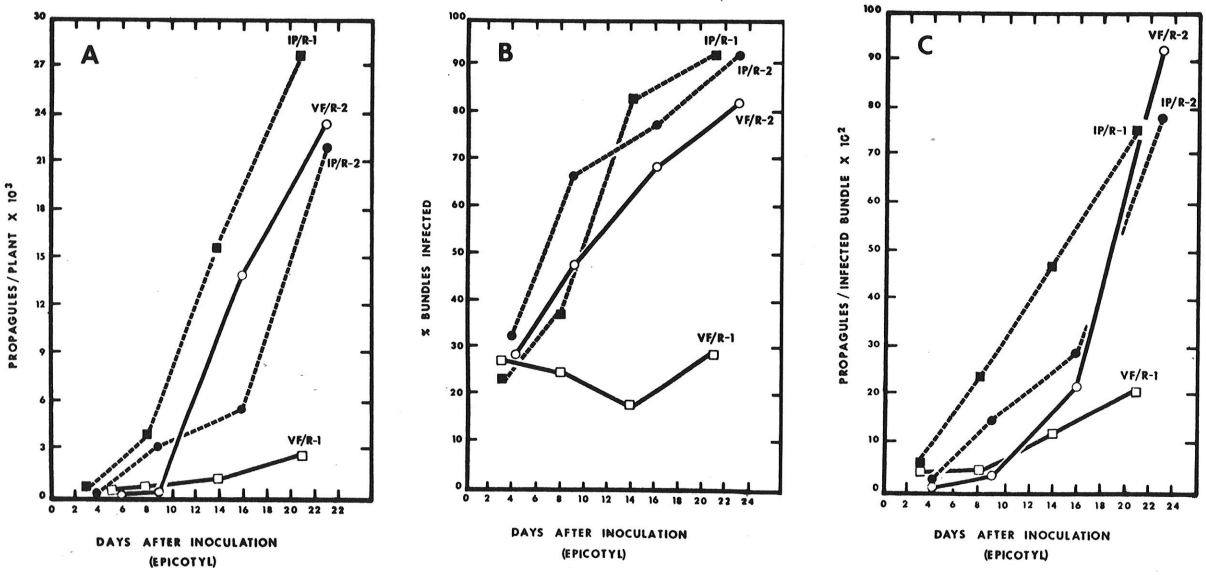


**Fig. 2-(A to C).** Measurements of the presence of *Fusarium oxysporum* f. sp. *lycopersici* race 2 within hypocotyl tissue at various time intervals following taproot inoculation of Improved Pearson (IP) or Pearson VF-11 (VF). **A)** Average propagule count per 1-cm segment, **B)** percentage of vascular bundles infected, and **C)** average propagule count per infected vascular bundle. Cultivars IP and VF are susceptible to race 2.

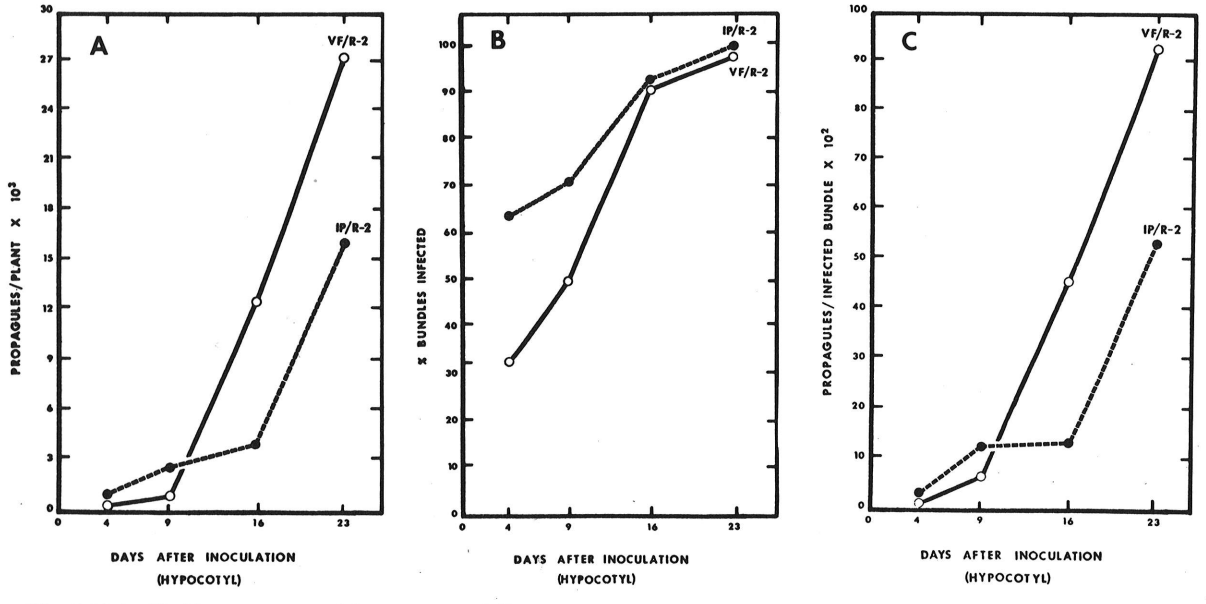


**Fig. 3-(A to C).** Measurements of the presence of *Fusarium oxysporum* f. sp. *lycopersici* within epicotyl tissue at various time intervals following taproot inoculation of Improved Pearson (IP) or Pearson VF-11 (VF) with race 1 or race 2. **A)** Average propagule count per 1-cm segment, **B)** percentage of vascular bundles infected, and **C)** average propagule count per infected vascular bundle. Cultivar IP is susceptible to both races; VF is resistant to race 1 but susceptible to race 2.

The bundles of IP inoculated with race 2 (susceptible) exhibited the highest degree of vascular browning with 87% of the bundles showing browning at the third internode and 30% showing browning at the ninth internode (Fig. 1-A). The bundles of VF inoculated with race 2 and IP inoculated with race 1 (both susceptible) had similar percentages of discolored bundles, ranging from 63-78% at the third internode to 25-28% at the ninth internode. Pearson VF-11 inoculated with race 1, the only resistant combination, had only 37% browning at the third internode and complete absence of vascular browning at the sixth internode.

The percentage of bundles infected (Fig. 1-B) was determined by counting the number of bundles with mycelial growth after cross sections were incubated for 24 hr. The curves for the percentage of bundles infected and percentage of bundles discolored at internodes 3-9 are similar (Fig. 1). Fungal growth was detected in 87% of the bundles within the third internode and 63% of the bundles within the ninth internode of IP inoculated with race 2. Again Pearson VF-11 inoculated with race 2 and IP inoculated with race 1 were similar to each other, with approximately 50% of the bundles infected at the third internode and 34% of the bundles infected at the ninth internode. Pearson VF-11 inoculated with race 1 showed little incidence of fungal hyphae (10% of the bundles with fungal growth) at the third internode and no incidence of fungal growth by the fifth internode.

**Determination of race 2 population and distribution patterns within hypocotyl tissue.**—The number of propagules per hypocotyl segment was similar within both isolines through the 9th day, at which time the growth of race 2 in VF accelerated rapidly until sampling was terminated on the 23rd day (Fig. 2-A). This rapid acceleration of growth did not begin within IP inoculated with race 2 until after the 16th day (Fig. 2-A). Although colonization was extensive in the hypocotyl segments of both isolines, there was significantly less population increase (Student's $t$-test, $P = 0.05$) of race 2 within IP, as compared to VF, by the 23rd day.

Initially, IP inoculated with race 2 had a greater percentage of bundles infected compared with VF inoculated with race 2. However, by the 16th day after inoculation the percentage of bundles infected in the two isolines was similar and this similarity continued until the experiment was terminated (Fig. 2-B). When propagule counts were calculated on the basis of infected bundles (Fig. 2-C), the build-up of propagules per infected bundle was greater in the VF/race 2 combination than the IP/race 1 combination.

**Determination of race 1 and race 2 population and distribution patterns within epicotyl tissue.**—The number of propagules per plant in all host/pathogen combinations was similar at the initial sampling period, 3-4 days after inoculation (Fig. 3-A). Between days 3-4 and days 8-9, there were moderate increases in fungal growth in epicotyl tissue of IP inoculated with race 1 or race 2, but fungal growth increased only slightly within VF inoculated with either race 1 or race 2. From days 8-9 until termination at days 21-23, there were dramatic increases in propagule build-up in the three susceptible host/pathogen combinations compared with only a slight increase in the resistant combination (VF/race 1). Within the epicotyl segments, as within the hypocotyl segments, propagule build-up in IP inoculated with race 2 was delayed in comparison to propagule build-up in the VF/race 2 combination.

Initial sampling, 3-4 days after inoculation, indicated that the distribution of fungal spores within epicotyl bundles following inoculation was similar in all combinations (Fig. 3-B). However, after 3-4 days there was again a dramatic increase in the colonization of IP by races 1 and 2 and of VF by race 2. In contrast, there was no increase in the number of infected bundles within the epicotyl of VF inoculated with race 1. Propagules per infected bundle in the epicotyl, calculated by dividing the total propagules per segment sampled by the number of infected bundles, was significantly lower in the resistant (VF/race 1) combination when compared with the three susceptible combinations (Fig. 3-C).

## DISCUSSION

The data indicate that susceptibility in tomato to *F. oxysporum* f. sp. *lycopersici* relates to the extent to which the pathogen colonizes the hypocotyl vascular tissue and subsequently spreads and colonizes the upper axis. Continued distribution of the pathogen following initial uptake of race 1 conidia is limited in VF (resistant combination) in contrast to race 2 which spreads readily throughout the vascular system of VF (susceptible combination).

The tomato isoline VF has a single-dominant-gene (I) for resistance to *F. oxysporum* f. sp. *lycopersici,* race 1; IP is genetically similar to VF but lacks this resistant gene. Elgersma et al. (2) concluded that the resistance gene is expressed in a host-response process that results in rapid localization of infection. Results of the present study support this localization hypothesis, and indicate further that the virulence of race 2 toward VF is related to its capacity to overcome the localization process within the hypocotyl. Colonization by race 2 was comparable in both isolines, and often even more extensive and intensive in the VF host, when the spread of infection was measured by: (i) percentage hypocotyl or epicotyl bundles infected, (ii) percentage propagules per hypocotyl or epicotyl segment, (iii) propagules per infected hypocotyl or epicotyl bundle, and (iv) percentage bundles infected and discolored in internodes three through nine (Fig. 1, 2, 3).

The comparable number of propagules per infected bundle within resistant and susceptible host/pathogen combinations is interpreted to mean that a fungal inhibitor is not involved in resistance or that if one is being produced, it occurs in equal concentrations in the various hosts. Previous work (2) has shown that viability of race 1 is comparable in the hypocotyl of both resistant and susceptible isolines following root dip or taproot inoculations and within epicotyl segments following stem inoculation. In the present study, the greater number of propagules per infected bundle in the three susceptible combinations compared to the one resistant combination (Fig. 3-C) at first glance appears to indicate that an inhibitory substance is operating in the epicotyl of the one resistant combination. However, these data were determined assuming that the number of infected vessels within each bundle was similar. Although this is a valid assumption when examining hypocotyl segments, this assumption may not hold true when epicotyl segments are

examined following taproot inoculations. Visual inspection of many epicotyl cross sections consistently revealed greater numbers of vessels containing viable fungal material in the susceptible reactions, although numerical data were not collected to quantify this observation. However, Mace and Veech (4) found that four times as many vessels were colonized in the epicotyl of the susceptible host when compared with the resistant host following stem inoculations. Thus, it is not unreasonable to assume that a similar ratio of colonization between susceptible and resistant reactions occurred in the present study. If so, and if the data presented in Fig. 3-C are compared on a per-vessel basis by dividing the twenty-day figures for the susceptible combinations by four, then growth of race 1 within VF vessels would be comparable to the three susceptible host/pathogen combinations; i.e., propagules per infected vessel per infected bundle for all combinations would only range from 1,900 to 2,300.

Beckman et al. (1) produced evidence that resistance is manifested within the hypocotyl by rapid physical blockage of vessels by tyloses and that susceptibility occurs when this process is delayed. In their study tylose initiation was similar in the resistant and susceptible host following race 1 inoculations, but complete vessel occlusion was delayed 7 or more days in the susceptible reaction. Hammerschlag and Mace (3) have recently reported that IP and VF root extracts exhibit antifungal activity. The extract from the resistant VF roots was nearly twice as active against race 1 as the extract from the susceptible IP roots, whether or not the plants were infected with race 1. The authors concluded that the fungitoxic substance, believed to be $\alpha$-tomatine, may contribute to resistance. Stromberg and Corden (5) reported the presence of a fungitoxic material in stem extracts of Jefferson and Bonney Best tomato cultivars, and suggested that this material may contribute to tomato wilt resistance. A fungal inhibitor may play a role in resistance, but probably not the role of a primary determinant of resistance because once the physical localization process within the taproot/hypocotyl region is disrupted, the pathogen can build up, spread into the upper stem, and cause symptoms even though there is antifungal activity (4, 5) along the plant axis. Thus, the extensive distribution and build-up of race 2 within VF epicotyls is strong evidence that antifungal compounds within the epicotyl do not effectively stop the pathogen once the localization process within the taproot/hypocotyl region has been overcome.

Perhaps a sequential two-component resistance process involving both physical barriers and antifungal compounds is operating to restrict pathogen invasion: first the pathogen propagules are trapped by vessel end walls followed by the rapid development of physical barriers (gels and tyloses) that prevent further upward fungal movement, and this allows for the accumulation of antifungal compounds that inhibit continued fungal development and lateral spread. Additional work is needed to determine the manner by which race 2 overcomes the localization mechanism in the two isolines and to evaluate the hypothesis that a sequential two-component resistance mechanism involving both physical barriers and antifungal compounds restricts movement of a vascular invader in resistant-type reactions.

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