Growth and Distribution of Fusarium oxysporum f. sp. lycopersici in Near-Isogenic Lines of Tomato Resistant or Susceptible to Wilt

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Senior author was Visiting Research Fellow and NATO Science Fellow, sponsored by the Netherlands Organization for the Advancement of Pure Research (ZWO), 1 February 1971 to 26 January 1972.

Supported in part by USDA Grant ARS 12-14-100-9896(34).

The assistance of Mrs. M. Harrison is gratefully acknowledged.

Publication No. 1429 of the Rhode Island Agricultural Experiment Station.

Accepted for publication 8 May 1972.

ABSTRACT

The buildup and distribution of *Fusarium oxysporum* f. sp. *lycopersici* in near-isogenic lines of susceptible and resistant tomato were investigated. Spore-size vinyl tracer particles introduced into the vascular systems showed no differences in the distribution pattern between the two isolines. Buildup of the pathogen, when calculated on the basis of infested vessels only, was found to be comparable in both isolines. Secondary distribution, however, was limited in the resistant isoline in contrast to the susceptible one in which all vascular bundles gradually became infected. It was concluded that a factor(s) which prevents distribution of the parasite is responsible for the single dominant gene type of wilt resistance in tomato.

Phytopathology 62:1232-1237.

Injury and infection are normal occurrences in the life of any plant and all higher plants have, of necessity, evolved recovery and defense systems (6, 7). These systems seem to be called into play only when, and where, and to the degree needed. Thus, some infections may be contained and overcome within a single cell, as in the case of hypersensitive reactions and lignituber formation (22, 23), whereas others, as in some delimited lesions and leaf spots and in vascular infections, may be contained only after considerable tissue involvement (5, 20). Even when infections are not successfully contained and the host plant develops severe disease symptoms, many of these symptoms (i.e., necrosis and hyperplasia) may actually reflect sustained and extensive responses of the host that are part of the total defense effort.

The defense systems in all higher plants appear to have many features in common. Thus, changes in permeability, increased respiration, increased levels of phenols, phenol oxidases and peroxidases, shifts in metabolism, and accumulation of nutrients and hormones are not only characteristic of diseased plants, but of plants in which the stimulus of injury or infection has set in motion processes of recovery and defense (1, 3, 6, 8, 13, 15, 24). Comparable responses have also been found in animals (21).

There are, of course, many innate features of structure and chemical composition in plants [i.e., epidermis, endodermis (22)] that provide a substantial degree of protection from many could-be pathogens. These features do not provide total protection, however, and it is unlikely that plants could function if they were to maintain total protection. Either the plants would be so insulated from their environment that needed supplies would be cut off, or the energy drain for total defensive mobilization would prevent the carrying out of other necessary functions. It is of considerable advantage, therefore, that plants respond to injury and infection only to a minimum in time and space and degree. Such modulation presumably involves genetic control.

Two modulated systems that commonly operate to minimize infections are:(i) chemical agents (phytoalexins) that inhibit the growth of the parasite; and (ii) changes in host structures that retard or prevent distribution of the parasite. The operation of one system in no way precludes the operation of the other. Rather, it is likely that both systems may be activated by infection. Phytoalexins can be detected within a matter of hours (1), and appear to accumulate to effective concentrations in 1 to 3 days after infection. Many wound barrier responses require several days to become effective (2). Therefore, these defense systems are probably effective to different degrees at different times, depending upon the host-parasite-environment interaction and especially the duration and extent of the infection stimulus. It is essential, however, that the infection site eventually be sealed off from surrounding healthy tissues so that these resistance mechanisms can be turned off.

Results elsewhere have shown that wound barrier formation and chemicals that inactivate hydrolytic enzymes may serve to limit secondary distribution of the parasite in plants resistant to vascular and other parasites (4, 9, 18). Limited distribution of the parasite could also result from inhibition of fungal growth by phytoalexins. Therefore, it seemed useful to establish the nature of resistance in a host-parasite system, such as Fusarium wilt of tomato, concerning which a considerable background of genetic information was available and for which seed stocks of near-isogenic lines were available that differed in a single dominant gene for resistance.

In this paper, the terms "initial uptake" and "secondary distribution" are frequently used and need some definition. The term "initial uptake"

herein refers to the passage of suspended spores into the transpiration stream of the vessels of a severed root or stem and on to the initial trapping site; i.e., the perforation plate or end wall upon which the spores are eventually trapped. Initial uptake is generally achieved within a few minutes and can be visually demonstrated by the use of spore-size tracer particles that are similarly carried in the transpiration stream and similarly trapped. Red vinyl particles (5) were mixed with spore suspensions in order to establish these uppermost limits of initial uptake. The term "secondary distribution" is used to connote distribution beyond these initial trapping sites. Secondary distribution occurs when spores of the pathogen produce hyphae that penetrate the membrane of the initial trapping site, and these hyphae, beyond the trapping site, produce spores which are carried in the transpiration stream to the next trapping site where this sequence is repeated. It is by such secondary distribution that vascular pathogens have the capacity for systemic invasion of the vascular systems of host plants.

The objectives of these experiments were (i) to determine in numerical terms the rates of growth of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans., race 1, in wilt-resistant and susceptible tomato plants (*Lycopersicon esculentum* Mill.) that are near-isogenic in other respects; (ii) to determine the rates of distribution of the parasite in the resistant and susceptible hosts; and (iii) to compare the growth of the parasite in only those vascular elements known to be infected. The final point could be examined by removal of the distribution factor either experimentally or mathematically. The distribution factor was minimized experimentally by a sampling of vascular tissue which was, as far as possible, completely infested during the initial inoculation procedure, or by sampling tissue from which secondary distribution was minimal. The distribution factor was minimized mathematically by dividing the numerical data for population buildup in the plant by the number of vascular bundles infested at the time of sampling. It was hoped that the data would indicate whether the resistance was due primarily to a process that (i) inhibited growth of the parasite; or (ii) prevented distribution of the parasite. Any inhibition of growth would, of course, tend to reduce distribution, and any restriction in distribution would tend to reduce growth, but these would be secondary effects. The object of these experiments was to determine whether the primary cause for resistance was growth inhibition or physical confinement of the parasite.

MATERIALS AND METHODS.—Improved Pearson (IP) and Improved Pearson VF-11 (VF-11) are near-isogenic lines (isolines) of tomato that are, respectively, susceptible and resistant to Fusarium wilt (14). Tomato seed of these cultivars were sown into a 1:3 sand:soil mixture and transplanted at the two-leaf stage to 10-cm-diam-pots containing a similar soil mix. These plants were grown in a greenhouse at 23-27 C. *Fusarium oxysporum* f. sp. *lycopersici*, race 1 (ATCC No. 16417), was grown on potato steep agar. Microconidia for inoculum were harvested after about 1 week of incubation at 27 C, and the concentration of spores was adjusted to the desired level with the aid of a hemocytometer.

Plants grown to the five- to six-leaf stage were used for inoculation. The root systems were gently washed free of soil and, in some experiments, submerged in a spore suspension of 10^6 microconidia/ml of water for 20 min. In other experiments, the taproots were first severed with a razor blade in the region where they were 1.5 to 2.0 mm in diam, then submerged in a spore susepnsion of 150,000 microcondia/ml of water. In this region, the vascular systems of the roots of these plants were mature enough to permit considerable inoculum uptake, and the entire secondary root systems were developed to such an extent that the roots removed represented only a loss of 20-25% of the root systems. We performed stem inoculations by making small incisions just above the cotelydons through which single drops of a spore suspension (500,000 spores/ml of water) were taken up. The inoculated plants were placed into environment chambers (Sherer-Gillett Co., Marshall, Mich., Model PEL-25) to provide ambient conditions of 2,500 ft-c of light during a 15-hr photoperiod/day, and a temperature of 28 ± 1 C.

In an initial experiment, we used the red-colored, spore-size, vinyl tracer particles (5) instead of spores to determine the relative capacities of the vascular systems to transport solid particles. We determined the extent of passage into individual vessels by cutting longitudinal sections 100 to 150 μ thick (5) and examining them under a dissecting microscope, using incident and transmitted light to detect the presence of the bright-colored particles. Ten plants of each isoline were so treated.

In a second experiment, we determined the extent of vascular browning 28 days after inoculation in each of 10 IP and 10 VF-11 plants by stripping the cortex off the stems, sectioning them longitudinally with a razor blade, and measuring the distance from the severed root to the topmost discoloration. Infection by *Fusarium* was verified by plating tissue bits on a potato steep (200 g/liter) agar.

In a third series of experiments, *Fusarium* propagules (spores and mycelial fragments that result from homogenization) in vascular tissues were determined at various intervals after inoculation to establish population growth curves in the resistant and susceptible isolines. The plants were uprooted, and soil particles were first removed from the root systems of inoculated plants by a tap water rinse. Leaves and lateral roots were then removed, and the plants were dipped into 70% ethanol and flamed. Tap root segments 5 mm in length and epicotyl segments 10 mm in length were cut (Fig. 1-A), and the cortex was removed aseptically. The segments were sliced as thinly as possible with a sterile razor blade and homogenized in 10 ml sterile water for 1.5 min by means of a Virtis (Model 45) homogenizer. Samples of the homogenized plant material were pipetted into petri dishes; and Czapek-Dox agar medium, which had been held at 45 C, was added. This medium contained

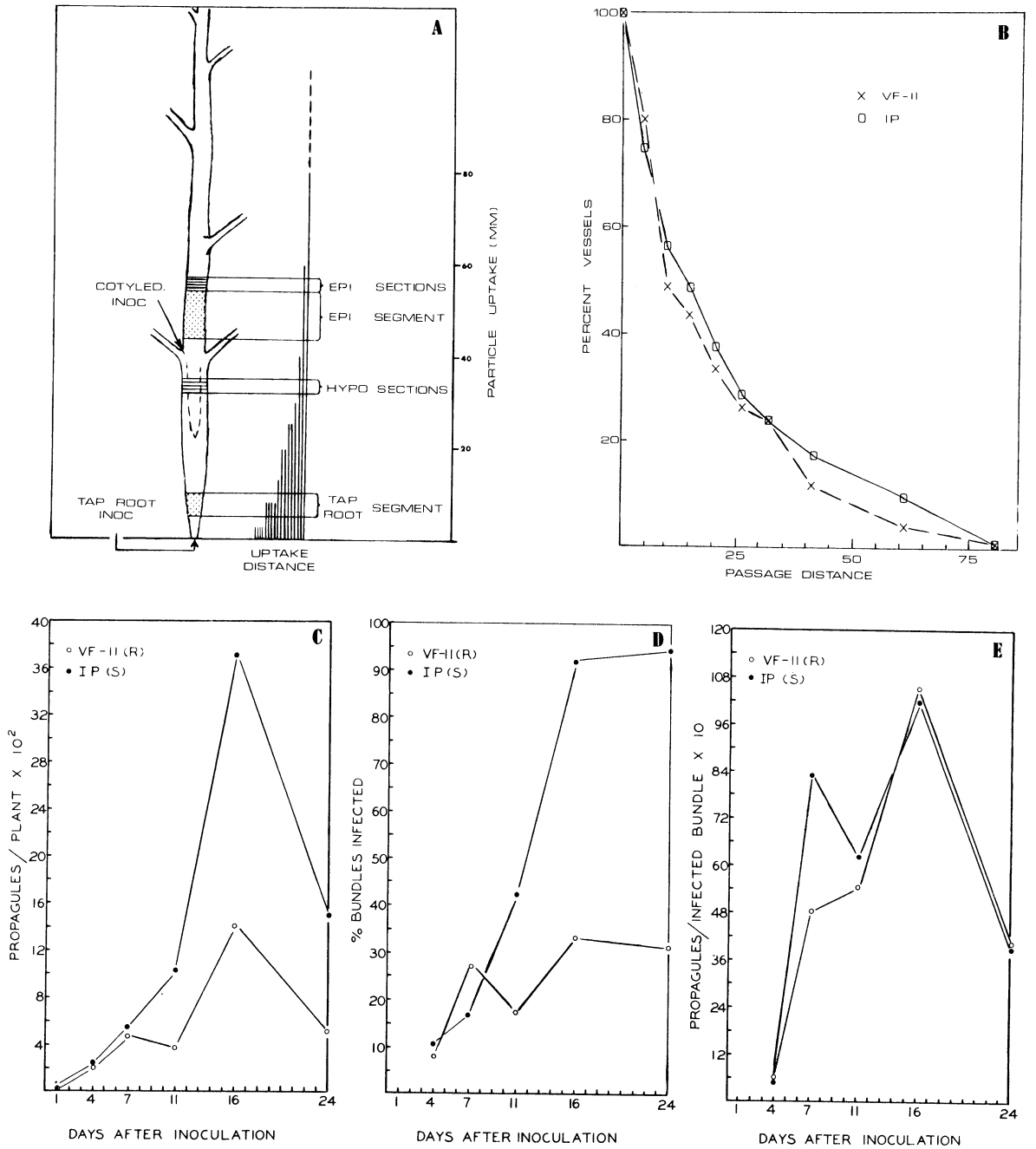

Fig. 1. A) Diagram of the main axis of a tomato plant showing points of inoculation and tissue harvested and the proportion of vessels that transports spore-size vinyl particles for various distances. Broken line represents infrequent uptake distances beyond 80 mm. Hypo = hypocotyl; epi = epicotyl. **B)** Transport distances in mm of spore-size, colloidal vinyl particles in vessels of susceptible (o) and resistant (x) tomato isolines. **C)** Average propagule counts per plant taproot segment at various intervals after root dip inoculation. **D)** Percentage of infected bundles in hypocotyl sections at various intervals after root dip inoculation. **E)** Average propagule counts per infected hypocotyl bundle at various intervals after root dip inoculation.

50 ppm sodium benzylpenicillin and 20 ppm streptomycil sulphate to minimize bacterial growth. Colony counts were made after 2 and 3 days of incubation at 23 C. This method was used in preference to other possible methods (19), since it seemed best for estimating the amount of viable fungus at any given time and place within the host plants. We determined the distribution of the fungus in the vascular tissue of

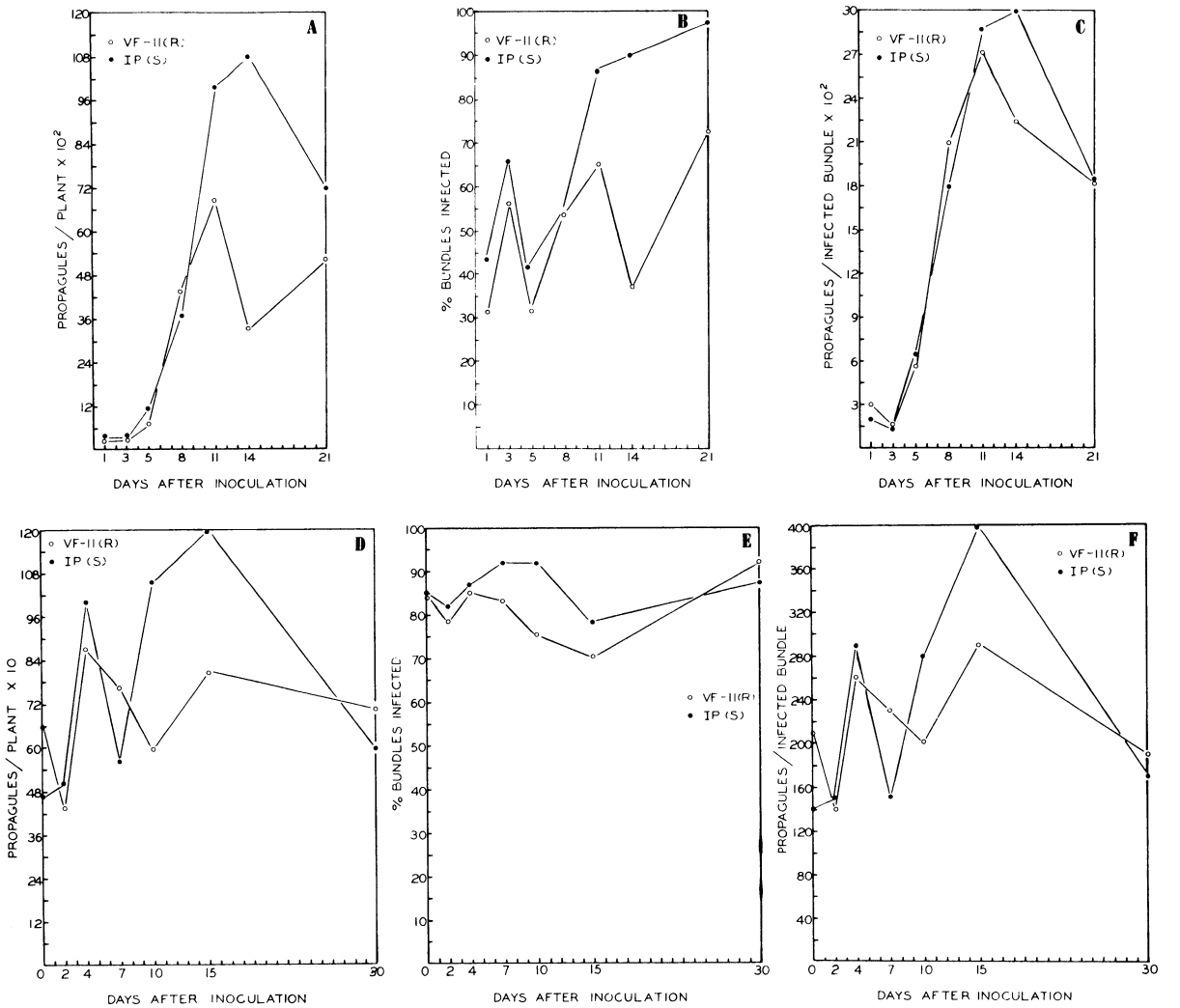

Fig. 2. A) Average propagule counts per plant taproot segment at various intervals after taproot inoculation of susceptible (•) and resistant (o) tomato isolines. B) Percentage of infected bundles in hypocotyl segments at various intervals after taproot inoculation. C) Average propagule counts per infected hypocotyl bundle at various intervals after taproot inoculation. D) Average propagule count per plant epicotyl segment at various intervals after inoculation of susceptible (•) and resistant (o) tomato isolines at the cotyledonary traces. E) Percentage of infected bundles in epicotyl segments at various intervals after cotyledonary inoculation. F) Average propagule counts per infected epicotyl bundle at various intervals after epicotyl inoculation.

these plants by making hand-cut cross sections of the hypocotyl or epicotyl region (Fig. 1-A) and placing them on sterile microscope slides. The slides were incubated in an inverted position in sterile moist petri plates for 24 hr at room temperature. The sections were then stained with 1% cotton blue in lactophenol, and the total number of vascular bundles, as well as those from which fungal hyphae had grown, were recorded. Generally, the four main bundles of the hypocotyl were counted in each of the nine susceptible and nine resistant plants sampled for each incubation period.

RESULTS.—An initial experiment was conducted to detect any differences in the physical capacity of IP and VF-11 plants to transport spore-size particulate matter. The tap roots of five- to seven-leaf IP and VF-11 plants were severed (Fig. 1-A) and placed in a red vinyl particle suspension to permit uptake. No differences were found in the capacity of vascular systems of these isolines to transport spore-size vinyl particles (Fig. 1-B). In both plant isolines, the average extent of transport of particles into vessels was ca. 19 mm, although particles were carried almost to the tops of the plants in one or two vessels. Differences in resistance of these two isolines, therefore, are probably not dependent upon the physical capacity of their vascular systems to transport spores.

An experiment was then conducted to determine the eventual distribution of *Fusarium* in resistant and susceptible hosts. IP and VF-11 plants were inocu-

lated by the severed tap root method. Red vinyl tracer particles were mixed with the spore suspension so that secondary distribution could be distinguished from initial uptake. After 28 days of incubation in environment chambers, foliar wilting and necrosis were 85% in the IP but neglible (small lowermost leaves) in the VF-11 plants. Vascular discoloration in IP plants extended an average of 580 mm from the point of inoculation. In VF-11 plants, the average extent of vascular discoloration was only 20 mm, a distance equivalent to the mean of initial passage distances (Fig. 1-B) of spores and vinyl particles. Thus, as evidenced by discoloration, there was virtually no advance of the fungus beyond the initial uptake of inoculum in the resistant isoline, but there was extensive secondary distribution beyond that of initial uptake in the susceptible isoline.

In the first of a final series of experiments, IP and VF-11 plants were first inoculated by the dipping of washed roots in a spore and vinyl tracer particle suspension. Changes in both the numbers of fungal propagules (Fig. 1-C) and of infected bundles (Fig. 1-D) were recorded at various times after inoculation. The data obtained were examined using the "Student" t-test to establish statistically significant differences between the two sample means at given harvest dates. Comparable increases in the numbers of propagules were found in IP and VF-11 plants during the first 11 days after inoculation ($P > .05$). During the period 11 to 16 days after inoculation, the difference in propagule counts was highly significant ($P < .01$), with propagule counts increasing dramatically in the IP plants compared to those in the VF-11 plants which increased only moderately (Fig. 1-C). There were also significant differences ($P < .05$) in the number of vascular bundles infected between the two isolines at 11 days after inoculation (Fig. 1-D), and highly significant differences ($P < .01$) thereafter. When propagule counts were calculated on the basis of infected bundles (Fig. 1-E), however, the number of propagules per infected bundle was found to be comparable in IP and VF-11 ($P > .05$).

The buildup of fungal propagules was then determined following inoculation directly into the lower portion of severed tap roots. Samples were taken 5 mm above the point of inoculation (Fig. 1-A). In these samples, 50 to 75% of the vessels were infested at the time of initial inoculum (spore and tracer particle) uptake. Differences resulting from secondary distribution were therefore reduced, although not totally eliminated. The data (Fig. 2-A, B, C) show no differences in the buildup or distribution of the fungus in resistant and susceptible hosts during the first 11 days after inoculation ($P > .05$). By the 14th day there were significant differences ($P < .05$) in both the number of vascular bundles infected and the number of fungal propagules isolated. Again, however, when propagule counts were calculated on the basis of infected bundles, no significant differences ($P > .05$) between growth of the parasite in resistant and susceptible plants were discernible (Fig. 2-C).

Finally, plants were inoculated in the stems just above both cotyledonary traces (Fig. 1-A). Generally three or four of the three major and three minor bundles of the epicotyl became infected by the procedure. Microscopic examination after such inoculations showed infection of only the early metaxylem elements of the bundles and little or no secondary distribution in either the IP or VF-11 isolines. Although the propagule counts were highly variable, there were no significant differences ($> .05$) in either the buildup or distribution of the parasite or in the number of propagules per infected bundle (Fig. 2-D, E, F).

Thus, the results of the final series of experiments showed that, although there were a few statistically significant differences in propagule buildup within the two varieties, these were always accompanied by statistically significant differences in distribution of the parasite. When the distribution factor was minimized by a calculation of the number of propagules per infected bundle, there were no statistically significant differences in the growth of the fungus in resistant and susceptible plants.

DISCUSSION.—The data presented indicate, first, that there were no physical differences in the vascular structures of the resistant and susceptible isolines and that the initial distribution of the pathogen from the point of inoculation was probably comparable in both isolines. Although initial uptake was found to extend into the upper stem region in a few instances in both isolines (one or at most two vessels/plant), the average uptake distance was 19 mm in both isolines. These results are in agreement with those of Mace et al. (14). Subsequent (secondary) distribution, however, was minimal in the resistant isoline and very extensive in the susceptible isoline.

The differences in eventual distribution of the parasite in these isolines could have resulted from differences in the ability of the fungus to grow or to penetrate barriers such as perforation plates or end walls, or they could result from differences in the ability of the resistant and susceptible hosts to seal off the infection sites quickly and permanently. The results of the third series of experiments rule out the first possibility. There were no differences in the ability of the parasite to grow in these hosts. The only discernible difference was that in the susceptible plants, the pathogen eventually infected many vascular elements in most bundles, whereas in the resistant plants the infection appeared to be restricted. In addition, although initial transport of the parasite to the upper parts of the plant occurred at the time of inoculation in a few vessels in both isolines, there seemed to be little further spread either laterally or longitudinally in the resistant plants. These results are in agreement with those of several other studies of resistance to vascular diseases (2, 4, 10, 12).

Since comparable growth of the fungus was found in these isolines, the role of phytoalexins in this single gene type resistance to *Fusarium* is doubtful. Fungitoxic compounds may well be formed, but presumably have little or no effect on this pathogen. Such a conclusion was drawn by Elgersma & Overeem

for Dutch elm disease in which both susceptible and resistant elm clones formed comparable amounts of fungitoxic compounds after infection with *Ceratocystis ulmi* (11). Although these compounds were toxic to several test fungi, they had little effect on *C. ulmi* (17).

On the basis of the data presented herein, we conclude that the single dominant gene type of resistance of tomato to Fusarium wilt is dependent upon rapid localization of infections. The limited spread of the parasite in the resistant plants probably results from physicochemical changes in the vascular tissue; i.e., the development of gels and tyloses, that provide mechanical barriers to secondary distribution of the parasite. The data do not rule out the possibility that substances are produced in the host that reduce the synthesis or action of hydrolytic enzymes and thereby prevent penetration of trapping sites by the parasite (9, 16, 18). These possibilities are currently under investigation.

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