Fusarium Wilt of Susceptible and Resistant Tomato Isolines: Spore Transport

M. E. Mace, J. A. Veech, and F. Hammerschlag

Plant Pathologist, Plant Physiologist, and Plant Pathologist, respectively, Disease Resistance Investigations, Crops Research Division, ARS, USDA, Beltsville, Md. 20705.

Accepted for publication 15 December 1970.

ABSTRACT

The effects of the length of the inoculation period, inoculum concentration, and host plant age on spore uptake and distribution were determined for *Fusarium* wilt-susceptible and -resistant isolines of tomato. Stained spores were introduced directly into the shoot vascular system through a transverse cut in the hypocotyl 2 cm below the cotyledons. Within 15 min, the bulk of the spores was distributed over a 2.3-cm distance. The rate of transport then leveled off, and after a 90-min inoculation period, the bulk of the spores was distributed over a 3.2-cm distance. A similar, but more sensitive, experiment using unstained, viable spores and a bioassay procedure to measure transport distances indicated that some spores were transported up to 5 cm. Factors such as host plant age and inoculum concentration were investigated to determine their effect on spore transport. We conclude that morphological features of the stem xylem in the two cultivars affect spore distribution similarly, and that resistance is not manifested in the ability of the resistant cultivar to inhibit physically the systemic distribution of pathogen spores. Phytopathology 61:627-630.

Additional key words: vessel end walls, primary xylem.

The literature on *Fusarium* wilt of tomato is voluminous, and conflicting conclusions abound. Previously, some investigators (7, 8) considered resistance to *Fusarium* wilt as a root-localized factor; other workers (9, 10, 11) concluded that the resistance factor was also present in the stem. The use of cuttings as a way to introduce spores directly into the vascular system of the host stem has been widely used, and stem-inoculated resistant plants consistently retain resistance; therefore, the resistance factor is active in the stem although it may not normally be initiated there. Scheffer (9) recognized the possibility that the resistance factor could be initiated in organs other than the stem and systemically translocated.

Some effort has been made to determine the effect of host plant age on susceptibility and resistance (5, 10). In general, it appears that as plants become older they become more resistant. Scheffer & Walker (10) showed that in resistant plants there is a differential susceptibility between primary and secondary xylem.

Susceptibility and resistance to *Fusarium* wilt have been investigated from the standpoint of inoculum concentration. Haymaker (6) found that an inoculum concentration of less than 7×10^4 spores/ml would not cause infection when roots were inoculated. On the other hand, Scheffer & Walker (10) found no differences in disease development when they placed stem cuttings in suspensions containing 5×10^3 to 5×10^5 spores/ml. They also reported that there was no difference in disease development when as few as 4×10^3 spores or as many as 2.5×10^6 spores entered the host.

The nature of resistance of tomato to *Fusarium* wilt is one of the long-term projects under investigation in this laboratory. To determine the direction of future research, it is imperative that certain points be reinvestigated because of the conflicting conclusions in the literature and the absence of comparative studies of isolines of susceptible and resistant hosts. This paper reports the results of studies on the influence of the duration of inoculation, inoculum concentration, and plant age on the transport of *Fusarium* spores in the vascular tissue of isolines of susceptible and resistant plants.

MATERIALS AND METHODS.—*Host material.*—A major consideration in the selection of a host-pathogen complex was the availability of genetically similar susceptible and resistant plants. We selected 2 cultivars of tomato, *Lycopersicon esculentum* Mill., Improved Pearson (IP), and Pearson VF_{11} (VF), whose pedigrees were known and related. Seeds of both cultivars were donated by Peto Seed Co., Inc., Saticoy, Calif., USA. The two cultivars have comparable genetic backgrounds and may be considered isolines. The cultivars were developed by O. S. Cannon (2). IP is an inbred susceptible line, VF an isoline of IP carrying single gene resistance for *Fusarium* wilt incited by race 1 of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. The single gene resistance factor was introduced into the line by crossing IP with a selection from 59-1 × Southland (Southland carried the resistance factor). A selection from the resistant progeny bearing IP characteristics was backcrossed to IP. Subsequently selected resistant progeny were backcrossed seven times before the line was labeled Pearson VF_{11}. The seeds were sown in a sterile mixture of soil and fine vermiculite (3:1), and the plants were grown in the greenhouse for various intervals at 25-27 C. Plant age was measured from the time of seeding. Soluble 20:20:20 fertilizer was applied as part of the watering regime.

Pathogen material.—A culture of the pathogen, *Fusarium oxysporum* f. sp. *lycopersici,* race 1, was obtained from the American Type Culture Collection, Rockville, Md., (ATCC No. 16417). The genetic stability of the pathogen from one experiment to the next was insured by using lyophilized stock cultures. The ATCC isolate was grown in a shake culture on a pH 5.0 medium-B (4) amended with 0.1% yeast extract. After a growth period of 1 week, the bud cells (hereafter called spores) similar in size and shape to microconidia were aseptically separated from the culture medium, washed

several times in sterile distilled water, and suspended in 10% skim milk. One-ml aliquots of the suspension were aseptically transferred to 10 × 150-mm glass lyophilization tubes. The tubes of spore suspension were frozen in liquid nitrogen, lyophilized for 48-72 hr, and sealed under vacuum. The lyophilized content of one tube was used to seed 100 ml of shake culture medium. The seeded shake cultures were incubated for 5-7 days at 27 C. Spores were collected for inoculum by repeated centrifugation and washing with sterile distilled water. The final washing was filtered through glass wool and the filtrate was centrifuged and the supernatent discarded. The spores were washed several times with sterile water and brought to the desired concentration with the aid of a hemocytometer.

Transport distance determined with stained spores.—Approximately 30-day-old plants were removed from the soil and washed in tap water, and the main axis of the plants was transversely severed 2 cm below the cotyledons. The cuttings were inoculated by placing the cut ends in a suspension of ca. 5×10^5 spores/ml. The spores were freshly stained with aniline blue according to the procedure of Beckman et al. (1). The cuttings were held in the spore suspension for 15, 30, 60, or 90 min. The spores were kept suspended by intermittent stirring. Upon removal from the stained spore suspensions, the leaves were removed; the stem was washed with water to remove surface spores; and the main axis was transversely sectioned at 1-cm intervals. The sections were examined microscopically for the presence of stained spores. The experiment was repeated 5 times with 10 plants of each cultivar. The transport distance was determined from the section containing stained spores farthest removed from the point of inoculation.

Spore transport distance determined by bioassay.—Ten 30-day-old plants of each cultivar were surface-sterilized after washing with tap water by immersing them in 0.35% calcium hypochlorite for 3 min. The plants were then rinsed with sterile distilled water, and the main axis was transversely severed 2 cm below the cotyledons. The cuttings were placed in an axenic spore suspension of the same concentration used for the stained spore experiment, or in sterile water for the controls. The inoculation period was 15 min. Upon removal from the spore suspensions, the leaves were removed, and the main axis was again surface-sterilized and rinsed. Transverse sections were cut at measured intervals along the axis. The sections were transferred onto acidified (pH 4.0) potato-dextrose agar containing 20 ppm streptomycin. The plates were incubated for 1 week at 27 C, and examined for the presence of the pathogen. The experiment was repeated 3 times.

Effect of plant age on spore transport.—The rate of spore transport was determined for 21-, 27-, and 33-day-old plants of both cultivars. Except for an inoculum concentration of 5×10^4 spores/ml and the age of the plants, the procedures were like those described under the bioassay section.

Effect of inoculum concentration on spore transport.—The effect of inoculum concentration on spore transport was determined for 25-day-old plants inoculated with 5×10^6, 5×10^4, or 5×10^2 spores/ml. The inoculation period was 30 min. Other procedures were like those described under the bioassay section.

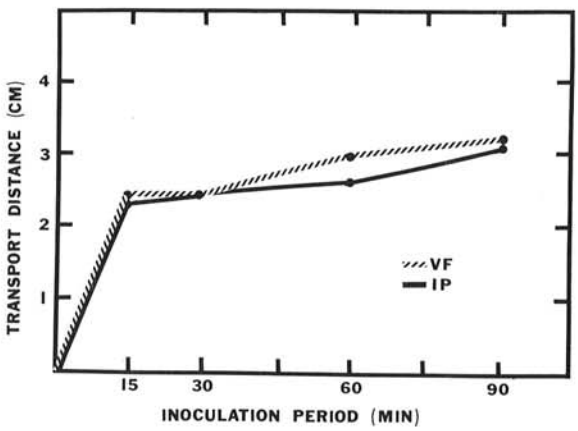

Fig. 1. The rate of transport of analine blue-stained *Fusarium oxysporum lycopersici* bud cells in the xylem of susceptible (IP) and resistant (VF) tomato isolines. Thirty-day-old plants were inoculated by transversely severing the main axis 2 cm below the cotyledons. The cuttings were allowed to take up bud cells (50,000/ml) by transpirational pull for the indicated times.

RESULTS.—*Stained spore transport.*—The average transport distance obtained at the four inoculation periods is presented in Fig. 1. The rate of spore transport was similar for the two cultivars at the 4 time periods sampled. Furthermore, the rate of spore transport was greatly reduced after the first 15 min of inoculation. Nearly 70% of the 3.3-cm transport that occurred during a 90-min inoculation period could be accounted for during the first 15 min.

The possibility existed that physiological shock from cutting the plants accounted for the leveling off of the rate of spore transport after about 15 min inoculation. Spore transport distance was determined for cuttings held in a sterile distilled water pretreatment for 45 min prior to the addition of stained spores. The transport distance observed in plants inoculated immediately after cutting was compared with the transport distance observed in the water pretreated plants. The experiment, repeated 3 times with 10 plants of each cultivar for each treatment, indicated that only a slight decrease in the rate of spore transport occurred in the plants pretreated in water before inoculation. Thus, cutting the stems does not seem to induce a delayed shock that could reduce the transpirational pull and thereby reduce the rate of spore transport.

The stained spore assay appears to afford a better index of the sites where spores are retarded rather than their true transport distance. Figure 2 is a longitudinal section of a tomato stem showing the accumulation of stained spores at a vessel end wall. The spores appeared to gradually leak past this barrier, and were rapidly transported to the next end wall. As additional spores accumulated, a blockage occurred and fewer spores passed through the barrier. This mechanism appears to account for the progressive decrease in spore transport.

Transport distance determined by bioassay.—The use of viable, nonstained spores to determine the transport

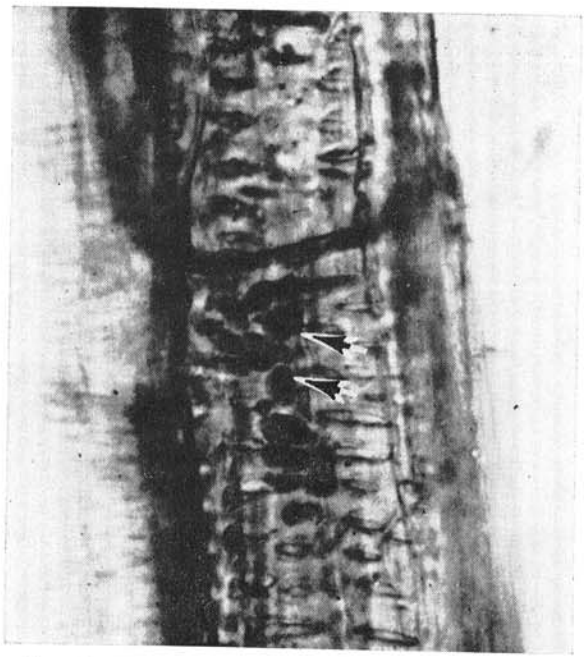

Fig. 2. A fresh longitudinal section of tomato stem showing the accumulation of spores at a xylem vessel end wall. The stained spores slowly pass through holes in the vessel end wall and are transported to the next vessel end wall.

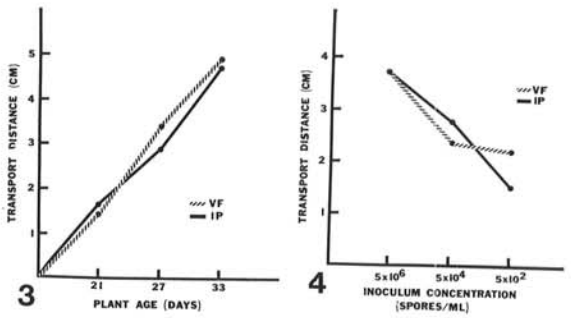

Fig. 3-4. **3)** The effect of plant age on spore transport distance. Susceptible (IP) and resistant (VF) tomato isolines of various ages were inoculated with 50,000 *Fusarium oxysporum lycopersici* bud cells/ml by cutting the main axis 2 cm below the cotyledon and immersing the cutting in the inoculum for 15 min. The cuttings were then sectioned at measured intervals along the axis and the sections transferred to agar (PDA) media for bioassay. **4)** The effect of inoculum concentration on spore transport distance. Twenty-five-day-old plants of susceptible (IP) and resistant (VF) tomato isolines were transversely severed 2 cm below the cotyledons. The cuttings were placed in various concentrations of inoculum and allowed to take up spores by transpirational pull for 30 min. The cuttings were then sectioned at measured intervals and the sections transferred to potato-dextrose agar medium for bioassay.

distance indicated that spore distribution was more systemic than that revealed by the stained spore tests. The average transport distance as determined by bioassay of 30-day-old plants inoculated for 15 min was 4.9 cm. The apparent increase in the rate of transport is not surprising when the greater sensitivity of the bioassay technique is considered. The bioassay data agreed with the stained spore data in that the transport distances were the same for the two cultivars.

Effect of plant age on spore transport.—The bioassay technique was used to determine the effect of plant age on spore transport. Figure 3 shows the average transport distance in 21-, 27-, and 33-day-old plants of both cultivars inoculated for 15 min with 5×10^4 spores/ml. Plant age had a direct effect on the spore transport distance: 33-day-old plants showed about 4.8 cm transport/15 min, whereas 21-day-old plants only showed 1.5 cm transport/15 min. This data suggested that vessel diam might be correlated with the rate of spore uptake. This was investigated by measuring the diam of 100 metaxylem vessels (five vessels in each of 20 plants) at 1 cm above the cotyledon in each cultivar at 21, 27, and 33 days of age. The average vessel diam of both VF and IP were 0.03, 0.05, and 0.06 mm in 21-, 27-, and 33-day-old plants, respectively.

Effect of inoculum concentration on spore transport.—The concentration of spores in the inoculum affects spore transport. Figure 4 shows the average spore transport distances of both cultivars inoculated for 30 min with 5×10^6, 5×10^4, or 5×10^2 spores/ml. The transport distances were determined by the bioassay technique. In previous experiments, the rate of spore transport was nearly equal for the two cultivars inoculated under similar conditions. In this experiment, however, the spore transport distance in plants treated with 5×10^2 spores/ml was 2.2 and 1.5 cm in resistant and susceptible plants, respectively. The situation was reversed for plants treated with 5×10^4 spores/ml. The spore transport distance in the resistant cultivar was 2.4 cm compared to 2.8 cm for the susceptible cultivar. At its maximum, however, the average variation was less than 1 cm.

DISCUSSION.—The concentration and distribution of the spores within the plant are important factors in pathogenesis. In this regard, both the bioassay and stained spore techniques have useful application. The bioassay procedure is useful in determining the distance spores are transported from the point of inoculation. This method, however, offers little indication as to whether spore distribution is uniform or discontinuous within the vascular system. On the other hand, the stained spore technique rapidly indicates the sites where spores lodge in the vascular tissue, but it does not provide accurate measurement of the maximum transport distance of the spores.

Spores were probably transported through a vessel at a uniform rate until they reached an end wall. Passage through the pores in the end walls was slower; hence a buildup of spores occurred at these sites. Spores that pass through the pores were transported to the next end wall where lodging again occurred. This process was probably continued throughout much of the stem. The progressive increase in the amount of spore lodging could cause a progressive decrease in the rate of transpiration, and thereby reduce the rate at which spores become systemically distributed. The proliferation of *Fusarium* hyphae and partial occlusion of

vessels below the end walls of vessels noted by Chambers & Cordon (3) in the susceptible Bonny Best tomato also indicate that the movement of the pathogen is retarded by the end walls. Regardless of the amount of lodging, some spores do pass through the pores in the end walls and continue up the stem in the transpiration stream. At some point along the way, the spores are too few in number to permit detection with the stained spore method, but the bioassay technique demonstrated that spores are transported several cm higher than the level indicated by stained spores.

It seems, therefore, that even during very brief inoculation periods nearly systemic distribution of spores could occur if the inoculated plants were allowed to continue to transpire. The limiting factors to systemic distribution of spores are probably the diam of the vessels and the pores in vessel end walls. It is highly improbable that tyloses or gum plugs could be induced in time to inhibit the systemic distribution of spores when cuttings are inoculated.

As might be expected, plant age has a direct relationship to the distance spores are transported. As plants become older, vessel diam increase. Enlarged openings in the xylem end walls of older plants could account for the more rapid spore transport. Beckman et al. (1) described such pores in the vessel end walls of banana. The increase with age in the diam of metaxylem vessels in both VF and IP would also facilitate spore transport. Any increase in vessel length with plant age probably would increase the spore transport distance, but no data was obtained on any such changes.

The similarity of experimental data from the two cultivars was noted consistently. At three different ages, the susceptible and resistant plants showed very similar transport distances for four different inoculation periods. Both cultivars supported similar transport distances at various inoculum concentrations. We conclude, therefore, that morphological features of the stem xylem in the two cultivars similarly affect spore distribution, and are not factors in determining resistance or susceptibility.

LITERATURE CITED

1. Beckman, C. H., M. E. Mace, S. Halmos, & M. W. McGahan. 1961. Physical barriers associated with resistance in Fusarium wilt of bananas. Phytopathology 51:507-515.
2. Cannon, O. S., & G. C. Hanna. 1959. New disease resistant tomatoes. Calif. Agr. 13:7.
3. Chambers, H. L., & M. E. Cordon. 1963. Semeiography of Fusarium wilt of tomato. Phytopathology 53: 1006-1010.
4. Esposito, R., & A. M. Fletcher. 1961. The relationship of pteridine biosynthesis to the action of copper 8-hydroxy-quinolate on fungal spores. Arch. Biochem. Biophys. 93:369-376.
5. Foster, R. E., & J. C. Walker. 1947. Predisposition of tomato to Fusarium wilt. J. Agr. Res. 74:165-185.
6. Haymaker, H. H. 1928. Pathogenicity of two strains of the tomato wilt fungus Fusarium lycopersici Sacc. J. Agr. Res. 36:675-695.
7. Heinze, P. H., & C. F. Andrus. 1945. Apparent Localization of Fusarium wilt resistance in the Pan American tomato. Amer. J. Bot. 32:62-66.
8. May, C. 1930. The effects of grafting on resistance and susceptibility of tomatoes to Fusarium wilt. Phytopathology 20:519-521.
9. Scheffer, R. P. 1957. Analysis of Fusarium resistance in tomato by grafting experiments. Phytopathology 47:328-331.
10. Scheffer, R. P., & J. C. Walker. 1954. Distribution and nature of Fusarium resistance in the tomato plant. Phytopathology 44:94-101.
11. Snyder, W. C., K. F. Baker, & H. N. Hansen. 1946. Interpretation of resistance to Fusarium wilt in tomato. Science 103:707-708.