Symptomless Carriers of the Tomato Fusarium Wilt Pathogen

J. Katan

Department of Plant Pathology and Microbiology, Faculty of Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel.

This research was partially supported by a grant from the Research and Development Authority of The Hebrew University.

The author gratefully acknowledges the encouragement and valuable interest of I. Wahl, and also thanks A. Z. Joffe for identification of *Fusarium* isolates.

Accepted for publication 17 May 1971.

ABSTRACT

Various weeds grown in soils naturally infested with *Fusarium oxysporum* f. *lycopersici* did not show wilt, although their tissues harbored *Fusarium*. Roots of weeds belonging to the genera *Oryzopsis, Digitaria, Amaranthus,* and *Malva* were colonized with *F. oxysporum* f. *lycopersici,* which constituted a part of the natural population existing in these soils. When planted under controlled conditions in soils naturally infested with races 1 or 2 of the pathogen, these plants were also colonized with the respective races. Roots of the four weeds were colonized to various degrees when artificially inoculated with any tested isolate of the pathogen originating either from the roots of the weeds or diseased tomato plants. Roots of other wild or cultivated plants were also colonized with the pathogen to various degrees when artificially inoculated, eggplants being distinguished by an extensive colonization and partial stunting. Quantitative estimation of the pathogen in the tissues, by maceration and subsequent dilution, showed that the population in inoculated weed tissues was 1-4% of the quantity in diseased tomato tissues. A method is suggested for determining the natural occurrence of symptomless carriers of a soil pathogen. Phytopathology 61:1213-1217.

Additional key words: quantitative estimation of *Fusarium* in plant tissues, survival.

Persistence of soil-borne pathogens in soil depends largely on their ability to survive in the absence of the host. Fusarium wilt of tomatoes, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans., is a severe disease in all areas of Israel. The pathogen persists in soils in which tomatoes have not been grown for more than 10 years.

It has been well established that Fusaria can persist in soils for considerable lengths of time in a "passive", dormant state by means of chlamydospores (12). An "active" mechanism for prolonged survival of this pathogen would be the infection of plants other than tomato. Various wild and cultivated plants are symptomless carriers of several soil pathogens, including some fusarial pathogens such as the tomato wilt fungus (1, 2, 7, 12, 16, 18). Colonization of a tissue enables the fungus to reproduce and to increase its population without being subjected to antagonism by soil saprophytes. Since *F. oxysporum* f. *lycopersici* causes disease in tomatoes only (2, 5, 7), we searched for plants which are invaded by the pathogen under natural conditions but do not show wilt (symptomless carriers), and studied the nature of *Fusarium* fungi isolated from these plants. Race 2 of this pathogen has recently been found in Israel (8), and it has been included in this study because it is a serious threat to cultivars endowed with gene *I* for resistance.

MATERIALS AND METHODS.—*Isolation.*—Roots and stems of the tested plants were washed under running water for 10 min, blotted, surface-disinfested, cut into segments 0.5-cm long, and plated out on potato-dextrose agar (PDA) in petri dishes. Different methods of surface disinfestation were used for the various types of plant material. Mature organs (stems and roots) of adult tomato, eggplant, or pepper plants as well as adult weeds such as *Malva* and *Amaranthus* spp. were dipped in 70% ethanol and flamed. After the peeling off of the outer layer, tiny pieces of deeper tissues were plated out. Young seedlings and small roots were immersed in 1% sodium hypochlorite for 2 min, then rinsed with sterile water. The dishes were incubated at 27 C for 5-7 days, and *Fusarium* isolates obtained were transferred separately to PDA for further tests.

Pathogenicity tests.—Fusarium isolates were grown on PDA at 27 C for 8 days, and assayed for pathogenicity. Seeds of susceptible and resistant tomato cultivars, Marmande (*ii*) and Rehovot-13 (*II*), respectively, were sown in methyl-bromide treated soil. Eight days after sowing, the seedlings were removed, and their roots were washed in tap water. After they were dipped in an inoculum suspension of one of the respective isolates, they were transplanted to methyl-bromide-treated, loamy sand soil, and were grown for 3 weeks in a greenhouse at a temperature of 25-31 C. Unless we have stated otherwise, the inoculum suspension was obtained by macerating the culture of one plate with 100 ml of water in a Waring Blendor. Isolates of the pathogen were considered to belong to race 1 when inoculation resulted in disease symptoms on seedlings of the susceptible cultivar Marmande only. When symptoms appeared on both Marmande and the resistant Rehovot-13, the isolate was considered as being race 2 (8). Seedlings inoculated with standard isolates of races 1 and 2 were grown under the same conditions, for comparison.

Unless otherwise stated, seedlings of plants other than tomato were inoculated by the same procedure, and the inoculated seedlings were planted in a naturally noninfested loamy sand soil.

The rate of colonization of plant tissues with *Fusarium* was determined either relatively—by plating out tissue segments on PDA and calculating the per-

centage of colonization by the fungus; or by a direct assessment of the number of propagules per g of fresh tissues (14). Here, the tissue was surface disinfested, cut into small pieces, and macerated with cold sterile water in a Waring Blendor. The macerated tissue was then diluted and 0.5 ml aliquots were poured onto plates of selective medium (10). After incubation the number of colonies obtained was counted and inoculum density in the tissue was calculated. Preliminary tests showed that blending for 4 min gave optimal counts.

RESULTS.—*Symptomless carriers of the tomato Fusarium wilt fungus in naturally infested soils.*—Weeds of the genera *Amaranthus, Digitaria, Erigeron, Malva, Molucella, Oryzopsis, Polygonum,* and *Solanum* were collected from a field which in the previous year had carried a tomato crop heavily attacked by *F. oxysporum* f. *lycopersici.* The weeds were examined for visible symptoms, and from each species, 50 stem and 50 root segments were plated out. Roots were colonized to a greater degree than stems by various species of *Fusarium,* mainly *F. solani* and *F. oxysporum.* The graminaceous weeds were especially densely colonized, and in some plants up to 40% of their root segments harbored *Fusarium.* Isolates of *F. oxysporum* pathogenic to tomato seedlings were obtained only from plants belonging to the following species: *A. graecizans* L.; *A. retroflexus* L.; *D. sanguinalis* (L.) Scop.; *M. silvestris* L., and *O. miliaceae* (L.) Asch. et Schw. Frequency of isolation was much higher from roots than from stems. All plants harboring pathogenic isolates showed no visible symptoms. Similar results were obtained from weeds collected in the same field, 6 weeks after the first sampling.

Tests were carried out in order to prove the reliability of the results. The pathogenicity of over 300 randomly selected *Fusarium* isolates previously derived from the roots of the above weeds was tested on tomato seedlings in two parallel experiments in separate greenhouses. Results in both greenhouses were identical. Noninoculated tomato seedlings showed no wilting, and did not harbor pathogenic isolates of *Fusarium.* In all cases, isolates found to be pathogenic belonged to race 1 of the pathogen. *Fusarium oxysporum* f. *lycopersici* isolates derived from wilted tomato plants grown in this soil or isolated from it by the dilution technique also belonged to the same race. Similar results were obtained using different inocula for pathogenicity tests; i.e., macerated PDA cultures, macerated washed mycelium mats grown on potato-dextrose broth, or washed conidia. The pathogenicity of 10 isolates, derived from weeds and previously defined as pathogenic, was also tested on 28-day-old tomato plants instead of 8-day-old ones normally used. Since results obtained were similar to the above, the pathogenicity of these isolates is not restricted to young seedlings. Pathogenic stability of eight isolates originating from weeds was tested by inoculating tomato seedlings and subsequent isolation and reinoculation. This procedure was repeated 4 times, and no change in pathogenicity was observed.

Since the pathogenic isolates from the weeds were identified as *F. oxysporum,* we concluded that they constituted a part of the population of *F. oxysporum* f. *lycopersici* existing in this naturally infested clay loam soil and had been responsible for the wilt disease of tomatoes in this field. The weeds harboring these pathogenic isolates were considered symptomless carriers.

These studies were repeated in three other naturally infested fields. Isolates of *F. oxysporum* f. *lycopersici* were obtained from roots of *A. lividus* L. and *M. parviflora* L. in the first field, from *A. lividus* and *D. sanguinalis* in the second, and from *A. retroflexus* in the third field. The soil types in these fields were clay, sandy loam, and clay loam, respectively. Again, all isolates of the pathogen derived from either the symptomless carrier weeds, or from the soil or from wilted tomatoes in each field, belonged to race 1.

Seeds of *O. miliaceae, D. sanguinalis, A. graecizans,* and *M. silvestris* were sown in soils naturally infested with race 1 of the pathogen taken from five locations, four of them being those previously described. The plants were lifted after 7- to 12-weeks growth in the greenhouse and portions of their roots and stems were plated out. Isolates belonging to race 1 were obtained from all these weeds. This method enabled us to confirm, under controlled conditions, our previous results regarding field-grown plants, and with a variety of infested soils taken from various locations. Similar experiments were carried out with soils taken from two fields in which plants of the resistant tomato cultivars were previously attacked by race 2. The isolates derived from the four weeds grown in these soils belonged to race 2.

Artificial inoculation of symptomless carrier plants with F. oxysporum f. lycopersici from different sources.—Twenty seedlings from each of five plant species were artificially inoculated by dipping their roots in inoculum suspensions of each of eight tested isolates of the pathogen from different sources (Table 1). During the first 5 weeks after inoculation, one-fifth of the plants were lifted at weekly intervals, and 8-20 portions of the roots of each individual plant were plated out on PDA. Results in Table 1 are the average of five tests, and show that the roots of the various weeds were colonized with the pathogen at different rates (11-42%), confirming the results obtained with weeds under field conditions. The colonization percentage was not correlated with the origin of the isolate or the species of the inoculated weed. No significant difference, at the 5% level, in the ability to colonize weeds or tomatoes was found between isolates derived from weeds or wilted tomatoes. Thus, no specific relationship could be shown between isolate and weed plant with various combinations under artificial inoculation. The pathogen race derived from inoculated plants was identical with the race of the isolate which served for inoculation. In no case did the per cent colonization of roots of the symptomless carriers approach that obtained on diseased tomatoes. Moreover, the use of another inoculation technique in a soil treated with methyl bromide did not signifi-

TABLE 1. Colonization by *Fusarium oxysporum* f. *lycopersici* of root portions of four symptomless carriers and of tomatoes, inoculated with isolates of the pathogen from different sources

Source of the isolate	% Colonization of hosts[a]					
	Oryzopsis miliaceae	*Digitaria sanguinalis*	*Amaranthus graecizans*	*Malva silvestris*	Avg	Tomatoes[b]
O. miliaceae[c]	21	31	25	19	24	100
D. sanguinalis[c]	28	26	20	18	23	100
A. retroflexus[c]	18	11	17	26	18	100
M. silvestris[c]	31	21	14	22	22	100
Tomatoes (A)[d]	41	23	21	24	27	100
Tomatoes (B)[d]	26	29	24	14	23	100
Tomatoes (C)[d]	42	26	33	24	31	100
Tomatoes race 2	31	15	26	20	23	100
Average	30	23	22	21	24	100
Tomatoes (A)[e]	30	41	18	33	30	100

[a] Average of five tests at weekly intervals. In each test, 40-80 portions of roots of each plant species were plated out on potato-dextrose agar, and after incubation, per cent colonization by the pathogen was determined.
[b] Wilted seedlings of the cultivar Marmande plated out 8, 10, and 15 days after inoculation. Per cent wilt with the different isolates was 93-96.
[c] Belonging to race 1 of the pathogen. Inoculation was carried out by dipping the roots in suspension of the inoculum.
[d] Tomatoes A, B, C denote three different isolates of the pathogen belonging to race 1. Inoculation as in [c].
[e] Plants inoculated by dipping the roots in the inoculum suspension of the pathogen and subsequent planting in a soil previously treated with methyl bromide and mixed with 100,000 conidia/g soil.

cantly change the results (Table 1). Colonization of tap roots was higher than the average, ranging from 60 to 90%.

Seedlings of the four weeds were inoculated with 10 different isolates of race 1 of *F. oxysporum* f. *lycopersici* obtained from wilted tomatoes. Isolation tests from the roots of inoculated plants essentially confirmed the above-mentioned results. Penetration of weed roots after artificial inoculation is therefore not restricted to a specific isolate of the pathogen.

Isolations were also made from stems of inoculated plants. Degree of colonization was lower than that of the roots, being 5-20% in the lower part of the stem, and 0-3% on upper parts. In wilted tomatoes, colonization reaches 100 and 94% in the lower and upper parts of the stem, respectively. Thus, this pathogen had a limited ability to advance in the stems of these weeds.

Inoculated weeds generally showed no wilt or stunting. In a few cases some inoculated *Malva* plants were stunted.

Artificial inoculation of additional wild and cultivated plants with F. oxysporum f. lycopersici.—A variety of wild and cultivated plants were artificially inoculated with an isolate of race 1 of the pathogen, to determine their potential as symptomless carriers. Five to eight root portions from each of 5-8 inoculated plants of each species were plated out at weekly intervals during the first 3 weeks after inoculation. The average colonization per cent of each plant was as follows: wild plants: *Aegilops* sp., 60; *Arrhenatherum* sp., 0; *Bromus scoparius* L., 12; *Hordeum marinum* Huds., 22; *Lolium rigidum* Gaud., 3; *Phalaris paradoxa* L., 10; *Datura stramonium* L., 15. Cultivated plants (name of the crop and the variety are given in parentheses): *Triticum durum* Desf. (wheat, Heiti), 17; *T. aestivum* L. (wheat, Florence × Aurore), 50; *Hordeum sativum* Jess (barley, Nigret), 50; *Avena sativa* L. (oat, Mulga), 28; *Citrullus vulgaris* Schrad. (watermelon, Malali), 15; *Daucus carota* L. (carrot, Nantes), 22; *Solanum melongena* L. (eggplant, Black Beauty), 80; *Gossypium hirsutum* L. (cotton, Acala 4-42), 25; *Capsicum annuum* L. (pepper, California Wonder), 50; *Zea mays* L. (corn, Dent), 25; *Lycopersicon esculentum* Mill. (tomato, Marmande), 100. Isolates of the pathogen derived from these plants belonged to race 1. Of all species inoculated, only tomato plants wilted. Inoculated plants of other species, with the exception of eggplants and peppers, did not show symptoms. Eggplants, and to a lesser extent peppers, were stunted. Five additional inoculation tests were carried out with these two species, and this phenomenon recurred 3 times with eggplant and once with pepper. In two experiments in which stunting of eggplants was noticed, their weight as compared to control plants was decreased by 41 and 35%, and their length by 25 and 18%, respectively. These differences were significant at the 5% level. Woolliams (18) reports similar stunting to various degrees in weeds inoculated with *Verticillium dahliae*. As eggplants and peppers are both Solanaceae and were relatively highly colonized, we examined the possibility that also they may act as symptomless carriers under more natural conditions. Eggplant and pepper seedlings were planted in pots filled with a naturally infested soil. After 4 and 10 weeks of growth in the greenhouse, portions of their roots and stems were plated out. We obtained isolates of *F. oxysporum* f. *lycopersici* from each individual plant tested. In naturally infested soil, these two species were colonized by this pathogen to a greater extent than the other four symptomless carriers.

Cotton and watermelon plants were colonized in our experiments at the rate of 25 and 15%, respectively, in contrast to 0 and 2% reported by Armstrong et al. (2). Three further inoculation experiments with these two species confirmed our earlier results. Sowing in soil artificially infested with the pathogen (instead of

dipping seedling roots in inoculum suspension), resulted in similar results.

Quantitative estimation of the population of F. oxysporum f. lycopersici in tissues of inoculated tomatoes and symptomless carriers.—Populations of *F. oxysporum* f. *lycopersici* in tissues of artificially inoculated symptomless carriers and tomatoes were quantitatively estimated by maceration and subsequent dilution. These experiments were repeated several times, and average results regarding the number of propagules per gram of fresh root tissue were as follows: *O. miliaceae,* 7900; *D. sanguinalis,* 3700; *M. silvestris,* 2100; *A. graecizans,* 2000; diseased tomatoes, 210,000; and stems of diseased tomatoes, 170,000. These results show that the level of colonization of the four weeds was much lower than that of the infected suscept. Fungal populations in weed tissues decreased as plants became older. Moreover, as was found in the isolation experiments, fungal populations estimated by the direct method were much smaller in weed stems than in their roots, normally less than 250 propagules/g. A variability between experiments was noticed similar to that found by Stover & Waite (15), who worked with infected banana roots.

DISCUSSION.—In our studies, *F. oxysporum* f. *lycopersici* invaded the roots and stems of numerous species, but was pathogenic only to tomatoes. This dual capacity of the fungus evokes questions concerning its relationships with different host plants, the evolution of parasitism and pathogenicity, and the methods of proving the existence of carrier plants.

The invasion of a plant by a pathogen, not accompanied by symptom development, is in accordance with the idea that parasitism and pathogenicity are not necessarily related. A similar phenomenon was also observed when plants of varieties resistant to fusarial diseases or different plant species were inoculated with noncompatible *Fusarium* pathogens (1, 5, 9). One or more of the mechanisms offered for explaining this selective pathogenicity (4, 5), e.g., the inability of fungi to produce toxins or enzymes in the noncompatible plants (5, 9), may also be involved in the nonappearance of disease in invaded symptomless carrier plants.

The relationships between the parasite and the symptomless carriers are commensal and therefore, balanced. Hence, the living substrate is available to the parasite for a long time. Moreover, the plant is not pressed to develop more resistant types by selection which might adversely affect the invader. Pathogenic relationships lack these two advantages, but result in others. The pathogen produces greater amounts of inoculum in diseased tissues; it reaches the upper parts of the stem, and therefore has better chances of dispersal in space. Invasion of plant roots by nonpathogenic microorganisms appears to be widespread in nature, and the idea that susceptibility in plants is the exception rather than the rule has often been suggested (3, 4, 17, 19). Taking into account all these considerations, three evolutionary possibilities may be suggested. One is that commensal association between *Fusarium* and plants preceded the pathogenic association between the fungus and its respective suscept, pathogenicity being a rather accidental, extreme deviation from the common balanced relationships between plants and most microorganisms in nature. A second, but contrary, possibility is that this fungus was first a pathogen of the tomato, and only later was its host range extended to its carriers as a result of the intermittent occurrence of the host when the crop is in rotation. Odum (11) pointed out that beneficial ecologic relationships may also have evolved from adverse ones. A third possibility is that parasitism and pathogenicity evolved simultaneously from primitive forms. More information on the gene(s) which determine parasitism and pathogenicity is required to determines the evolutionary pathway involved.

Koch's postulates are not adequate to prove the existence of a symptomless carrier of a certain pathogen, since they are based on the reproduction of symptoms. The use of artificial inoculation (sometimes using sterile soil), followed by isolation, cannot be used as the sole test for this purpose, since it does not prove that the organism can successfully invade the plant under natural conditions where inoculum potential is low. We suggest that a plant may be considered a carrier of a soil pathogen if (i) the pathogen can be isolated from the plant grown in naturally infested soil; (ii) this phenomenon can be reproduced under controlled conditions by planting the suspected carrier in naturally infested soil; (iii) the pathogen can be reisolated equally well from plants, after artificial inoculation with isolates derived from (i) or (ii) or from a diseased suscept; (iv) the suspected carrier does not show disease symptoms in (i, ii, or iii). To exclude the possibility that an isolate in (i) or (ii) is an exceptional one, its identity with the pathogen existing naturally in the soil must be proved. If the results obtained in (i) and (ii) can be repeated in different soils, and if in (iii) a successful colonization can be obtained with any isolate tested, this will indicate also that the ecological importance of this phenomenon is not restricted to a specific isolate, soil, or condition. The existence of symptomless carriers was proved in our studies according to these suggested criteria.

We have not excluded the possibility that this pathogen may also live in the rhizosphere of carriers (13), although this might be less effective for survival than penetration into the tissues.

The wide parasitic host range of this pathogen rather than its limited pathogenic range should be taken into consideration when planning crop rotation, and when grouping it in the ecological groups of soil fungi according to Garrett (6).

LITERATURE CITED

1. ARMSTRONG, G. M., & J. K. ARMSTRONG. 1948. Nonsusceptible hosts as carriers of wilt fusaria. Phytopathology 38:808-826.
2. ARMSTRONG, G. M., B. S. HAWKINS, & C. C. BEN-

NETT. 1942. Cross inoculations with isolates of Fusaria from cotton, tobacco and certain other plants subject to wilt. Phytopathology 32:685-698.
3. BAWDEN, F. C. 1957. The role of plant hosts in microbial ecology, p. 299-314. *In* R. E. O. Williams & C. C. Spicer [ed.] Microbial ecology, 7th Symp. Soc. Gen. Microbiol. Cambridge Univ. Press.
4. BECKMAN, C. H. 1964. Host responses to vascular infection. Annu. Rev. Phytopathol. 2:231-252.
5. DAVIS, D. 1963. Investigations on physiology of selective pathogenicity in Fusarium oxysporum in test tube culture. Phytopathology 53:133-139.
6. GARRETT, S. D. 1956. Biology of root-infecting fungi. Cambridge Univ. Press, London & N.Y. 293 p.
7. HENDRIX, F. F. JR., & L. W. NIELSEN. 1958. Invasion and infection of crops other than the forma suscept by Fusarium oxysporum f. batatas and other formae. Phytopathology 48:224-228.
8. KATAN, J., & I. WAHL. 1966. Occurrence in Israel of new dangerous isolates of the Fusarium tomato wilt pathogen. First Congr. Mediterranean Phytopathol. Union Proc. Bari-Naples, p. 425-430.
9. MUSSELL, H. W., & R. J. GREEN, JR. 1970. Host colonization and polygalacturonase production by two tracheomycotic fungi. Phytopathology 60:192-195.
10. NASH, SHIRLEY M., & W. C. SNYDER. 1962. Quantitative estimations of propagules of the bean root rot Fusarium in field soils by plate counts. Phytopathology 52:567-572.
11. ODUM, E. P. 1959. Fundamentals of ecology. [2nd ed.] W. B. Saunders Co. Philadelphia & London. 546 p.
12. PARK, D. 1965. Survival of microorganisms in soil, p. 82-98. *In* K. F. Baker & W. C. Snyder [ed.]. Ecology of soil-borne plant pathogens. Univ. Calif. Press, Berkeley.
13. SCHROTH, M. N., & F. F. HENDRIX, JR. 1962. Influence of nonsusceptible plants on the survival of Fusarium solani f. phaseoli in soil. Phytopathology 52:906-909.
14. STOVER, R. H., & B. H. WAITE. 1953. An improved method of isolating Fusarium spp. from plant tissue. Phytopathology 43:700-701.
15. STOVER, R. H., & B. H. WAITE. 1954. Colonization of banana roots by Fusarium oxysporum f. cubense and other soil fungi. Phytopathology 44:689-693.
16. WAITE, B. H., & V. C. DUNLAP. 1953. Preliminary host range studies with Fusarium oxysporum f. cubense. Plant Dis. Reptr. 37:79-80.
17. WILHELM, S. 1959. Parasitism and pathogenesis of root-disease fungi in soil, p. 356-366. *In* C. S. Holton et al. [ed.]. Plant pathology problems and progress. Univ. Wisc. Press.
18. WOOLLIAMS, G. E. 1966. Host range and symptomatology of Verticillium dahliae in economic, weed, and native plants in interior British Columbia. Can. J. Plant Sci. 46:661-669.
19. YARWOOD, C. E. 1967. Response to parasites. Annu. Rev. Plant Physiol. 18:419-438.