Fusarium Wilt Resistance in Sweet Potatoes

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ABSTRACT

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Experimental sweet potato clones were indexed for disease reaction to the wilt fungus *Fusarium oxysporum* f. sp. *batatas*. The clones exhibited a wide range of reactions with disease indices well correlated between tests (r = .94). Initial uptake of bud-cells of the fungus was similar in resistant and susceptible clones and not correlated with the disease index (r = 0.2). Distribution of bud-cells in the stems of resistant and susceptible cuttings was discontinuous, but they often reached a level of 15 cm 15 minutes after inoculation. When resistant stems were serially sectioned at 1-cm intervals after 6 days of incubation, *Fusarium* was recovered from about 50% of the sections and was still present after 48 days of incubation; but susceptible clones were completely invaded and often dead after 6-9 days. Development of tyloses in inoculated plants exceeded that in healthy plants. Tyloses were more abundant in inoculated resistant plants than in susceptible plants, but they rarely blocked all vessels completely. Although tyloses may function in resistance, they did not appear to be the primary resistance mechanism.

Forma speciales of soil borne *Fusarium oxysporum* (Schlecht) cause wilting, vascular necrosis, and death in many plant species. Development of resistant cultivars, the most successful means of control, has in turn led to studies to determine the mechanism of this resistance. The most extensive studies have been on bananas and tomatoes with sweet potatoes receiving less attention.

Resistance to Fusarium in many plant species has been associated with the early formation of physical barriers such as gels, gums, and tyloses. It is postulated that these barriers may localize the pathogen and prevent systemic distribution. Plants that fail to form these barriers in time to contain the pathogen become infected and the pathogen grows unimpeded through the vascular system. Evidence supporting this mode of resistance has been presented for banana (2), for tomato (1), and for sweet potato (15). However, physical barriers are formed infrequently or not at all in Fusarium wilt-resistant carnations (11), radish and cabbage (12), and watermelon (3). In addition, recent studies have shown that tyloses formed in resistant tomatoes are not induced in time to prevent systemic distribution of pathogen spores entering the vascular systems when stem cuttings are inoculated (9).

The production of chemical inhibitors by the host is considered a resistant mechanism to Fusarium wilts. Higher levels of fungitoxic acetic acid have been demonstrated in watermelons resistant to *F. oxysporum* f. *niveum* (3) and evidence has been given that expressed sap of resistant tomatoes is inhibitory to growth of *F. oxysporum* f. *lycopersici* (6). Mace et al. (9), after observing the systemic distribution of initial inoculum of the Fusarium wilt fungus in tomato cuttings, suggested that an inhibitory chemical may exist in resistant plants. This theory is further strengthened by Stromberg and Corden (14) who found that susceptible and resistant tomato plants contain fungitoxic materials in xylem extracts at concentrations that cause a rapid decrease in population of viable fungal cells. Evidently the fungitoxic material is detoxified with time in susceptible plants and the pathogen multiplies. In resistant plants the fungitoxic material persists and suppresses pathogen growth.

These chemical inhibitors, except for acetic acid in watermelon, have not been identified. One possibility is an increased level of polyphenols in resistant plants. These compounds occur in many plants, including sweet potatoes, and in some cases are toxic to fungi and bacteria. Polyphenols have been associated with resistance to many diseases including Fusarium wilts. Matta et al. (10) found that soluble phenols increase rapidly in resistant tomato plants inoculated with *F. oxysporum* f. *lycopersici* and increase slower in inoculated susceptible plants. Thus higher concentrations of phenols in resistant plants might inhibit fungus growth.

The objectives of these experiments were to compare Fusarium wilt-resistant and -susceptible sweet potatoes *(Ipomoea batatas* L.) as to (i) uptake and distribution of *F. oxysporum* f. *batatas* inoculum by fresh vine cuttings, (ii) the pathogen multiplication in the stems, and (iii) the relationship of tyloses to resistance.

MATERIALS AND METHODS

Three virulent isolates of *F. oxysporum* f. *batatas* were used in all inoculations. They were obtained from W. J. Martin, Louisiana State University, and maintained on potato-dextrose agar (PDA) slants at 8 C. Bud-cell inoculum was grown by seeding 250 ml of modified Richard's solution in a flask (16) with 10 ml of a spore-mycelial suspension washed from the surface of a PDA

slant; the flasks were left for 72 hours on a rotary shaker at 25 C. Bud-cells were collected on filter paper in a Büchner funnel, resuspended in distilled water and adjusted to 0.08 absorbance with a Bausch and Lomb Spectronic 20 colorimeter at 600 nm. Equal volumes of bud-cell suspensions from the three isolates were mixed for inoculations.

Roots from sweet potato clones were obtained from Alfred Jones and Philip Dukes, USDA, ARS, Southern Region Vegetable Laboratory, Charleston, S. C. The clones as categorized by Jones and Dukes represented a range from highly resistant to extremely susceptible to Fusarium wilt. Presprouted roots of these clones, the susceptible cultivar Porto Rico, and resistant P.I. 15635 (Tinian) were bedded in greenhouse flats in a mixture of loamy soil, sand, and peat moss (2:1:1, v/v). Vine cuttings or sprouts from these roots were planted in 15-cm diameter pots of the same soil mixture and the subsequent plant growth provided plant material for all experiments. Terminal vine cuttings 15-20 cm long with leaves attached were severed just below a node and inoculated by placing the severed ends in a beaker of the bud-cell suspension (approximately 800,000/ml) for 15 minutes.

The disease reactions of the experimental sweet potatoes were categorized using a scheme based, in part, on those previously described for evaluating resistance in breeding programs (4, 7), involving the rapidity of plant death and the extent of vascular browning in inoculated plants. Inoculated plants were examined at weekly intervals during a 3-week incubation period. Plants dead after 7, 14, and 21 days were given scores of 3, 2, or 1, respectively. After 21 days live plants were examined for vascular discoloration. Those with no discoloration at the basal internode were scored 0, and if discoloration was confined to this internode, they were scored 0.1. Plants with discoloration extending 25%, 50%, or 75% of the stem length were scored 0.2, 0.3, or 0.4, respectively. In tests with N plants per replication where n = number of plants with a given score the index was computed by the following formula:

$$\frac{3n + 2n + 1n + .1n + .2n + .3n + .4n}{N}$$

Inoculum uptake and multiplication in stems.—Terminal vine cuttings 15 cm long were inoculated to determine initial inoculum uptake and pathogen multiplication in the stems. As the cuttings were removed from the inoculum, leaves were removed by cutting the petiole base flush with the stem and the stem surface was disinfested in a 5% sodium hypochlorite solution for 3 minutes. Serial sections at 1-cm intervals were aseptically cut, placed on solidified acid-potato-dextrose agar (APDA) in petri dishes, and the dishes held at 25 C until *Fusarium* grew from the stem sections (usually 6-10 days). The number of sections with *Fusarium* was recorded. Pathogen multiplication in the stems was determined by similarly sectioning inoculated stems 0, 3, 6, 9, and 12 days after they were planted. All tests were performed at least twice.

Histological studies.—Stem sections of inoculated resistant and susceptible cuttings were collected after 3, 6, and 9 days in the soil mixture at 18-24 C. The first, third, and fifth centimeters were excised and fixed in formalin-aceto-alcohol solution (FAA) (8). Stem pieces were dehydrated through a standard tertiary butyl alcohol (TBA) series (8), embedded in paraffin, and cut in 20 to 25-μm longitudinal sections. Sections were mounted on glass slides and stained with a Triarch quadruple stain. They were examined microscopically for mycelial penetration and tyloses formation in vessels.

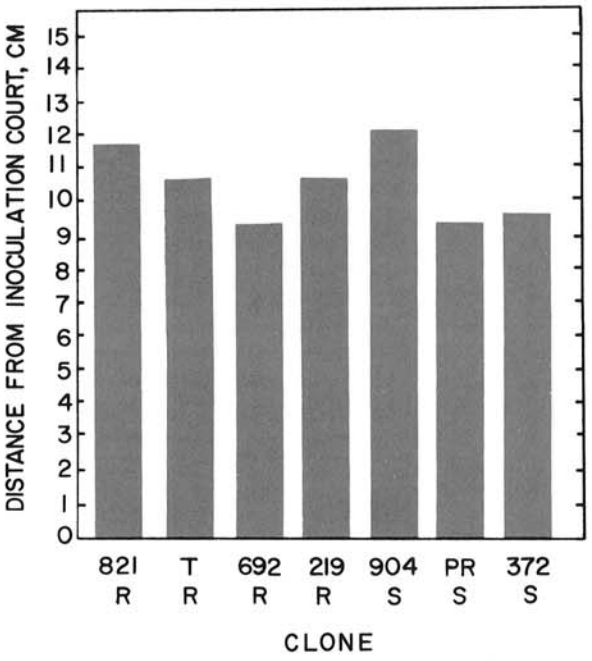

Fig. 1. Average distance from bases of cut stems at which *Fusarium oxysporum* f. sp. *batatas* was recovered from resistant and susceptible sweet potatoes immediately after inoculation.

TABLE 1. Disease indices of seven sweet potato clones inoculated with *Fusarium oxysporum* f. sp. *batatas* in two greenhouse tests

Clone	Disease index[a]	
	Test 1	Test 2
821	0.30	0.54
692	0.39	1.17
334	0.53	0.86
723	0.62	1.37
219	0.68	1.31
904	2.20	2.75
372	2.25	2.25
Standard error[b]	0.11	0.26
r	0.94	

[a]Mean of four replications per test; five plants per replication. Disease index ranges from the most susceptible reading of 3.00 (all plants dead in one week) to the most resistant reading of 0.00 (no plants dead after 3 weeks, and no vascular discoloration in surviving plants).

[b]Standard error is based on total population of clones tested in wilt reaction experiments from which these seven clones were selected.

RESULTS

Disease reaction of sweet potatoes.—The disease reactions of the seven numbered sweet potato clones used in these studies were evaluated in two experiments with five cuttings in each of four replications (Table 1). In both tests there was a gradual gradation of resistance from highly resistant (clone 821) to extremely susceptible (clone 372) as previously evaluated by Jones and Dukes. Clones with intermediate levels of resistance varied most in the two tests. However, the correlation coefficient (r) of 0.94 between the tests was significant ($P=0.01$) indicating that the sweet potatoes reacted similarly in the two tests.

Wilt-resistant Tinian and susceptible Porto Rico (5, 13) were included in a number of tests.

Inoculum uptake and multiplication in stems.—Ten terminal 15-cm stem cuttings of clones 904, 821, 692, 372, 219, and cultivars Tinian, and Porto Rico were immersed in the inoculum for 15 minutes to determine initial inoculum uptake and its distribution in the stems. There was no difference in the distance initial inoculum moved up the stems of resistant and susceptible sweet potato cuttings immediately after inoculation as indicated by fusarial growth from serial sections (Fig. 1). The average maximum distance for the 10 cuttings varied from 12.3 cm for susceptible clone 904 to 9.3 cm for resistant clone 692 (SE 7.40). The fungus was usually recovered from the four or five basal 1-cm sections of the cuttings, but fungus recovery from sections above about 5 cm indicated that the bud-cells were not present in each cm section. Although initial inoculum distribution up the stem was discontinuous, bud-cells often reached the fifteenth centimeter of resistant and susceptible cuttings within a few minutes after inoculation.

In this test 20-30 minutes elapsed between removal of the cuttings from the inoculum and placing the 1-cm sections on APDA. This time lag may have increased the distance inoculum moved in the transpiration stream. To avoid this and to determine whether the initial concentration of bud-cells in the upper portions of the cutting was sufficient to cause disease, 10 terminal 20-cm cuttings of resistant clone 723 and susceptible clone 372 were inoculated. As cuttings were removed from the inoculum, 0-10 cm of their bases were aseptically removed. The terminal remainders of the cuttings were planted and incubated three weeks. None of the resistant plants died. However, all 10 susceptible plants with 0 or 2.5 cm of the base removed died. The numbers of susceptible plants that died following removal of 5, 7.5, and 10 cm were 6, 4, and 4, respectively. Susceptible uninoculated control plants with 0 to 10 cm of the base removed remained healthy. These data confirm the above results that initial inoculum moved up the vascular system of some susceptible plants in excess of 10 cm in 15 minutes and the amount of inoculum that exceeded this distance was sufficient to cause disease.

To demonstrate that inoculum moves up the vascular systems of resistant cuttings also, 10 cuttings of resistant clone 821 and Tinian and susceptible Porto Rico and clone 372 were immersed in inoculum for 15 minutes and 5 cm of their bases removed before planting. In addition to recording dead plants, isolations were made by plating stem cross-sections taken at 2.5-cm intervals from plants living after 3 weeks. Again, more susceptible plants died and the numbers were similar to those that died in the above test with 5 cm of stem removed. The numbers of dead plants after 3 weeks were for clone 821, 1; Tinian, 0; Porto Rico, 3; and clone 372, 5. *Fusarium* was isolated

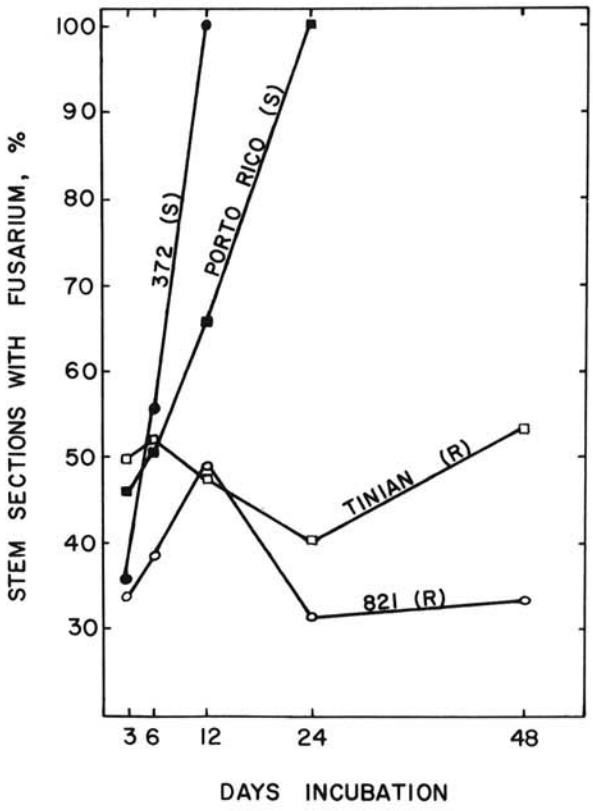

Fig. 2. Colonization of resistant and susceptible sweet potato stems by *Fusarium oxysporum* f. sp. *batatas*, as determined by plating 1-cm stem sections at different time intervals after inoculation.

TABLE 2. Indices of hyphal invasion and tylose development in vessels from the third and fifth centimeter from the base of inoculated resistant (R) and susceptible (S) sweet potato stems after 3, 6, and 9 days of incubation[a]

		Incubation period					
		3 days		6 days		9 days	
Clone		3 cm	5 cm	3 cm	5 cm	3 cm	5 cm
821	Hyphal	1	0	0	0	0	0
(R)	Tyloses	1	0	3	1	3	3
Tinian	Hyphal	0	0	0	0	0	0
(R)	Tyloses	1	0	3	1	4	1
Porto	Hyphal	1	0	4	1	3	2
Rico (S)	Tyloses	1	1	1	1	1	1
372	Hyphal	0	0	3[b]	2	4[b]	4
(S)	Tyloses	0	0	1	0	0	1

[a]Hyphae and tylose development indices are based on the following proportions of vessels affected: 0, none; 1, 1-25%; 2, 26-50%; 3, 51-75%; and 4, 76-100%.

[b]Sporulation present in vessels.

from all surviving resistant and susceptible plants thus showing that the initial inoculum moved up the vascular systems of resistant as well as susceptible plants in excess of 5 cm in 15 minutes.

Pathogen multiplication in resistant and susceptible stems was determined by plating serial sections from three stems of inoculated clone 821, Tinian, Porto Rico, and 372 after 3, 6, and 12 days of incubation and recording the number of sections with *Fusarium*. The test was performed four times with samples taken at these incubation intervals and once with the samples taken after 24 and 48 days. The percentage of one-cm sections that yielded *Fusarium* in culture during the first 12 days of incubation was similar in the four tests for all clones and these data were averaged for the respective clones (Fig. 2).

After 3 days of incubation, the frequency with which *Fusarium* was isolated from resistant and susceptible sections differed and the difference increased with time. The percentage of sections from resistant clone 821 and Tinian that yielded *Fusarium* remained somewhat constant through 48 days, but the percentage of sections from susceptible Porto Rico and clone 372 that yielded *Fusarium* increased with incubation time. After 9-12 days of incubation, susceptible stems were almost completely invaded and the plants were dead or dying. These data, although indirect, indicate that pathogen multiplication or growth is impeded in resistant plants but not in susceptible plants.

Histological studies.—Pathogen invasion of xylem vessels and the relative development of tyloses in vessels were studied in inoculated and noninoculated cuttings of clone 821, Tinian, Porto Rico, and clone 372. The first, third, and fifth centimeter from the stem base were taken 3, 6, and 9 days after inoculation and longitudinal sections were examined.

Hyphal invasion of the basal 5 cm of the vascular system was more rapid and extensive in the susceptible Porto Rico and clone 372 than in resistant clone 821 and Tinian (Table 2). Six days after inoculation the third centimeter of susceptible Porto Rico and clone 372 were heavily invaded. Sporulation was found in some sections, and hyphae were numerous in vessels in the fifth centimeter of stems. In contrast, hyphae were only observed in one third-centimeter section of resistant clone 821 after 3 days of incubation. No hyphae were observed in later samples of 821 or Tinian. Sporulation was seldom observed in the 1-cm sections of resistant clones.

Tylose development in inoculated plants exceeded that observed in healthy plants and was more abundant in inoculated resistant clones than in susceptible clones (Table 2), which confirmed a previous report (15). Although tyloses were more numerous in vessels of resistant clones they rarely blocked all vessels completely. There were always bordered pits available for the pathogen to grow into adjacent unobstructed xylem vessels. Some tyloses had developed in the basal centimeter of all healthy clones.

DISCUSSION

Previous studies on the nature of Fusarium wilt resistance in sweet potato attributed resistance primarily to the formation of physical barriers that might prevent systemic infection of the stem and restrict the pathogen to the site of initial invasion. In this study, bud-cells of the fungus were rapidly transported up the severed vascular systems of both resistant and susceptible vine cuttings to sites quite distant from the point of entry, agreeing with other studies on sweet potato (5) and tomato (9) which concluded that inoculum of the Fusarium wilt fungi enter vascular systems of susceptible and resistant plants with equal facility. Furthermore, inoculum sufficient to cause disease in susceptible plants was transported in excess of 10 cm from the entry site in 15 minutes. These data show (i) that tyloses formed after infection is initiated cannot serve to impede the initial uptake of inoculum by severed stems of Fusarium wilt-resistant or -susceptible clones, (ii) that uptake of initial inoculum was similar in resistant and susceptible clones, and (iii) that inoculum which reached the upper portions of the plants caused disease in susceptible plants, and its multiplication was inhibited in resistant plants. Tyloses formed after the inoculum entered the xylem vessels may retard further distribution of the pathogen by forcing it to grow through bordered pits into unobstructed vessels, or prevent the passage of secondary microconidia up the vessels. However, results of these studies agree with those of Mace et al. (9) who reported that in tomato tyloses alone cannot account entirely for resistance.

Since tyloses did not prevent inoculum uptake or lateral growth of the pathogen to unobstructed vessels in resistant sweet potato stems the chemical basis of resistance must be considered. The pathogen grew poorly, if at all, in vessels of resistant plants as evidenced by sparce hyphae in the vessels and no increase in percentage of 1-cm stem sections from which the pathogen was isolated after 48 days of incubation. Fungistatic conditions must prevail in vessels of resistant plants particularly in the early stages of infection. The results of this study support the theory of an inhibitory chemical which plays an active role in resistance in sweet porato to *F. oxysporum* f. *batatas*.

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