Influences of Temperature and Plant Age on Differentiation of Races of *Fusarium oxysporum* f. sp. *tracheiphilum* on Cowpea

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ABSTRACT

Swanson, T. A., and Van Gundy, S. D. 1985. Influences of temperature and plant age on differentiation of races of *Fusarium oxysporum* f. sp. *tracheiphilum* on cowpea. Plant Disease 69:779-781.

In race-differential cultivars of cowpea (*Vigna unguiculata*) inoculated with isolates of races 1, 2, and 3 of *Fusarium oxysporum* f. sp. *tracheiphilum*, the extent of vascular discoloration in cross sections through the primary node was more reliable than foliar symptoms as a measure of plant reaction. Age of plants at inoculation (12 or 20 days) did not influence vascular discoloration. Plant death differentiated all three races when plants were grown at high temperatures (27 C), but at lower temperatures, vascular discoloration was the more reliable indicator of compatibility.

Fusarium wilt of cowpea, caused by *Fusarium oxysporum* f. sp. *tracheiphilum* (E. F. Smith) Snyd. & Hans., occurs throughout cowpea-growing regions of the southern United States (5). The disease was first described by Smith in 1899 (10). During the 1950s, three races of the pathogen were defined (1,4,6). No new races have been reported. Race 1 also attacks soybean and chrysanthemum (1,2).

We attempted to identify the races of isolates of *F. oxysporum* f. sp. *tracheiphilum* in the American Type Culture Collection and in collections previously made in California (12). In some experiments, it was hard to classify isolates to race from the incidence of external symptoms. We therefore examined the effects of temperature and plant age on the compatibility of selected isolates of the pathogen with the differential cultivars of cowpea (*Vigna unguiculata* (L.) Walp).

MATERIALS AND METHODS

Isolates of *F. oxysporum* f. sp. *tracheiphilum* coded 16608, 16609, and 16610, representing races 1, 2, and 3, respectively, were purchased from the American Type Culture Collection at Rockville, MD. These isolates originated in the southeastern United States. Isolates coded 016f, 1114-5, and 787 were obtained from S. Smith (University of California, Berkeley), D. Erwin (University of California, Riverside), and K. Kimbell (University of California, Davis), respectively. These isolates originated in California. All isolates were subcultured from single spores and stored at 5 C in soil (5). Inocula were prepared with conidia collected from cultures on potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI). The cultures were started with plugs from colonies growing from particles of infested soil sprinkled on acidified PDA. The plates were incubated at room temperature under continuous fluorescent light (72 μE m^{-2} sec^{-1}; 14,700 lux) for 4–7 days. The plates were filled with water, and the conidia were washed off the plates. The resulting supsensions were strained through cheesecloth to remove mycelial fragments. The inoculum concentration was adjusted to 0.5×10^6 conidia per milliliter.

Seeds of the cowpea differential cultivars Chinese Red, Groit, and Arlington were obtained from W. Hare, Mississippi State University. Chinese Red is susceptible to races 2 and 3, Groit is susceptible to race 1, and Arlington is susceptible to race 3. Seeds were germinated in vermiculite in a greenhouse after surface-sterilization by immersion in 0.5% NaOCl solution for 5 min. Plants were inoculated by the root-dip technique; roots were severed with scissors 5 cm below the root-shoot transition and immersed in inoculum for 5 min. After inoculation, the plants were grown in steam-sterilized sand, where they were given one application of Osmocote controlled-release fertilizer (Sierra Chemical Co., Milpitas, CA) and watered daily with deionized water.

Plant age. Plants 12 and 20 days old at inoculation were obtained by first planting seed for the 20-day treatment, and 8 days later, planting seed for the 12-day treatment. Both the 20- and 12-day-old plants were inoculated on the same day with isolate 16608, 16609, or 16610. Five plants of a treatment were planted together in sand in a 10-cm-square plastic pot. There were three replicates of each treatment, arranged in a completely randomized design on a bench in a growth chamber (air temperature 27 C, photoperiod 15 hr, light intensity 124 μE m^{-2} sec^{-1} [25,000 lux]). Twenty-one days later, the percentages of plants dead, of plants with vascular discoloration extending to the cotyledonary node, and of plants with vascular discoloration to past the primary node were recorded. Vascular discoloration was estimated visually from free-hand cross sections through the cotyledonary and primary nodes and scored on a scale pretransformed to arc sine (9), where 0 = 0, 1 = 10, 2 = 35, 3 = 65, 4 = 90, and 5 = 100% of vessels discolored. The experiment was analyzed as a $2 \times 3 \times 3$ factorial replicated three times in a completely randomized design with age, isolate, and cultivar as the factors.

Temperature. Two-week-old plants of the differential cultivars were inoculated with isolate 1114-5 (race 3) and grown in 10-cm pots as described earlier. The plants were then placed either in the growth chamber (conditions described earlier) or on a bench in the greenhouse (air temperature 18–30 C). Vascular discoloration ratings were made 21 days later as described earlier. Data from growth chamber and greenhouse were analyzed as separate experiments. The experiments were completely random designs with three replicates of each treatment (five plants per replicate).

In the second experiment, 2-wk-old plants were inoculated with isolate 16608, 16609, or 16610 as described earlier and placed in 10-cm pots in a growth chamber with a constant air temperature of 22 C. The light intensity at bench level was 124 μE m^{-2} sec^{-1} (25,000 lux). Vascular discoloration ratings were made 21 days later as described earlier. The fresh weight of the five plants in each pot was recorded. The experiment was repeated with the growth chamber temperature at 32 C.

Light. The influence of light on fungal growth was studied with cultures on PDA. Cultures of isolates 16608, 16609, and 16610 started with 2-mm plugs, were incubated at 22 C. One group of the plates was wrapped in aluminum foil; the other was continuously exposed to the fluorescent lights in the growth chamber as described earlier. Colony areas were calculated after 5 days. The experiment was replicated three times in a randomized

Accepted for publication 30 January 1985.

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Table 1. Reactions of plants of differential cultivars 12 and 20 days old at inoculation to isolates of races 1, 2, and 3 of *Fusarium oxysporum* f. sp. *tracheiphilum*

Isolate	Cultivar	Expected reaction[a]	Vascular discoloration (0–5)[b,c]				Percent dead plants[c]	
			Cotyledonary node		Primary node			
			12 Days	20 Days	12 Days	20 Days	12 Days	20 Days
16608	Chinese Red	−	0.4	1.3*	0.0	0.3	0	0
	Groit	+	5.0	5.0	5.0	5.0	100	100
	Arlington	−	0.3	0.7	0.1	0.0	0	0
16609	Chinese Red	+	3.3	4.0	2.9	3.5	40	27
	Groit	−	0.0	0.2	0.0	0.1	0	0
	Arlington	−	0.4	0.4	0.0	0.2	0	0
16610	Chinese Red	+	5.0	4.6	5.0	4.7	100	93
	Groit	−	0.1	1.3*	0.1	0.6	7	0
	Arlington	+	4.5	4.4	4.2	4.2	50	100

[a] − = Incompatible, + = compatible.
[b] Extent of vascular discoloration seen in cross sections through the cotyledonary and primary nodes, scored on a scale pretransformed to arc sine, where 0 = 0, 1 = 10, 2 = 35, 3 = 65, 4 = 90, and 5 = 100%. Means for 20-day-old plants followed by an asterisk differ significantly from means for 12-day-old plants by the LSD test ($P = 0.05$).
[c] Each value is the mean of three replicates (five plants per replicate).

Table 2. Influence of greenhouse and growth chamber environments on the reactions of differential cultivars of cowpea to isolate 16610 of *Fusarium oxysporum* f. sp. *tracheiphilum*

Differential cultivar	Vascular discoloration[x,y,z]		Percent dead plants[y,z]	
	Greenhouse	Growth chamber	Greenhouse	Growth chamber
Chinese Red	3.7 a	5.0 a	40	100
Groit	0.7 b	0.1 b	0	0
Arlington	2.9 a	5.0 a	13	100

[x] Extent of vascular discoloration seen in cross sections through the primary nodes, scored on a scale pretransformed to arc sine, where 0 = 0, 1 = 10, 2 = 35, 3 = 65, 4 = 90, and 5 = 100%. Means within a column followed by a common letter do not differ according to Duncan's multiple range test at $P = 0.05$.
[y] Each is the mean of three replicates (five plants per replicate).
[z] Temperature in the greenhouse ranged from 21 to 32 C. Temperature in the growth chamber was constant at 27 C.

block design. Data were analyzed as a 2 ×3 factorial with illumination and isolate as the factors.

In the second experiment, the cultures were started on PDA and placed in incubators set for 18, 21, 24, 27, 30, or 33 C. There were three replicates of each race-temperature combination. Colony areas were calculated after 4 days. Temperature response curves were fitted by polynomial regression (9).

RESULTS

Plant age at inoculation had no effect on vascular discoloration at the primary node but did affect discoloration ratings at the cotylendonary node (Table 1). Chinese Red challenged with isolate 16608, and Groit with isolate 16610, had significantly higher vascular discoloration scores when plants were 20 days old than when 12 days old at inoculation. Isolate 16610 killed more Arlington plants when plants were 20 days old than when 12 days old at inoculation.

Isolate 16610 could be identified as race 3 from the reactions of plants inoculated with the same inocula and at the same time but grown after inoculation in either the greenhouse or in the growth chamber (Table 2). The race type was clear from either incidence of death or vascular discoloration scores for the plants grown in the growth chamber, but only vascular discoloration permitted race identification for plants grown in the greenhouse.

In experiments with the growth chamber set for 32 and 22 C, the race 1 reaction was recognizable at both temperatures, whereas race 2 and 3 reactions were much clearer at 32 than at 22 C (Table 3).

Illumination significantly increased the colony sizes of isolates 16608 and 16610 but not of isolate 16609. Colony sizes of isolates 16608, 16609, and 16610 were 30, 24, and 29 cm^2, respectively, after 5 days in darkness versus 42, 22, and 38 cm^2 under fluorescent light. The interaction of illumination with isolate was significant ($P = 0.05$).

The optimum temperature for growth on PDA was similar for isolates 16608, 16609, and 16610, but colonies of isolate 16609 were significantly ($P = 0.01$) smaller than colonies of isolates 16608 and 16610 (Fig. 1).

DISCUSSION

Environmental factors influenced the expression of Fusarium wilt of cowpea caused by *F. oxysporum* f. sp. *tracheiphilum* and made it difficult to identify susceptible reactions of the differential cultivars in some experiments. Less difficulty was encountered if estimates of vascular discoloration rather than incidence of plant death were used to measure compatibility. For example, in the plant age experiment, isolate 16610 killed fewer Arlington plants that were 12 than 20 days old before inoculation, but the vascular discoloration ratings showed that plants in both age groups were extensively colonized by isolate 16610. Also, isolate 16609 was unable to kill many plants of either age and could only be recognized as race 2 with confidence from vascular discoloration ratings. Vascular discoloration ratings were also useful in identifying race reactions in the temperature experiments. At temperatures higher than 27 C, both plant death and vascular discoloration were reliable indicators of susceptibility, but at lower temperatures, which did not favor development of external symptoms of wilt, vascular discoloration was more reliable.

Vascular discoloration ratings have the additional advantage of being well suited for analysis of variance (ANOVA). Although incidence of plant death could also be subjected to ANOVA, each replicate would have to consist of a group of plants. A single plant can serve as one replicate for a discoloration measurement, and a comparatively small number of plants could be used to test the hypothesis that isolates of the pathogen are of different races (14). Evidence for differences in aggression among isolates of the same race type and interactions among races, cultivars, and environmental factors could also be evaluated from discoloration measurements (3,8,14).

The isolate of race 2 used in this study grew more slowly than the isolate of race 1 or of race 3 on PDA and caused less severe symptoms and less extensive vascular discoloration in susceptible plants. Armstrong and Armstrong (1) also found an isolate of race 2 that was less aggressive than race 1. Because isolates of *Fusarium* species that differ in aggressiveness occur in nature (11), other isolates of race 2 that cause severe disease in susceptible plants probably exist.

Table 3. Effects of growth chamber temperatures of 22 and 32 C on reactions of differential cultivars of cowpea to isolates representing races 1, 2, and 3 of *Fusarium oxysporum* f. sp. *tracheiphilum*

Isolate	Cultivar	Expected reaction[w]	Vascular discoloration[x,y]		Percent dead plants[y]		Plant fr wt (g)[y]	
			22 C	32 C	22 C	32 C	22 C	32 C
16608	Chinese Red	−	0.6	0.5	0	0	1.8	2.2
	Groit	+	4.9	4.7	100	93	0.4	0.3
	Arlington	−	0.5	2.8	0	53	1.8	1.1
16609	Chinese Red	+	1.2	5.0	7	87	1.9	0.1
	Groit	−	0.3	0.0	0	0	2.6	2.8
	Arlington	−	0.1	1.2	0	0	2.1	1.8
16610	Chinese Red	+	3.9	5.0	4.7	100	0.8	0.1
	Groit	−	1.5	2.2	20	33	2.2	1.8
	Arlington	+	2.8	4.7	40	93	2.2	0.3
LSD ($P = 0.05$)			0.9	1.1	...[z]	...	0.4	0.6

[w] − = Resistant, + = susceptible.
[x] Extent of vascular discoloration seen in cross sections through the primary nodes, scored on a scale pretransformed to arc sine, where 0 = 0, 1 = 10, 2 = 35, 3 = 65, 4 = 90, and 5 = 100%.
[y] Mean of three replicates (five plants per replicate).
[z] Data not subjected to analysis of variance.

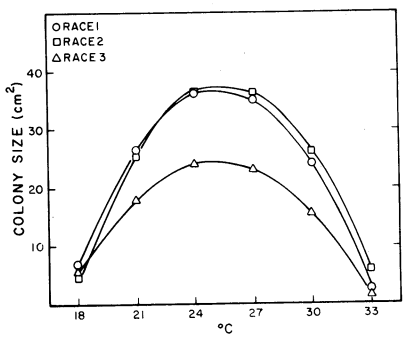

Fig. 1. Responses to temperature of races 1, 2, and 3 of *Fusarium oxysporum* f. sp. *tracheiphilum* on potato-dextrose agar. Race 1 $\hat{y} = 326.9 + 28.8(x) - 0.6(x)^2$ $r^2 = 0.94$, race 2 $\hat{y} = 210.4 + 18.7(x) - 0.4(x)^2$ $r^2 = 0.90$, and race 3 $\hat{y} = 340.8 + 29.6(x) - 0.6(x)^2$ $r^2 = 0.88$.

Symptoms of vascular wilt were more severe at higher temperatures (i.e., 27 C). This temperature effect showed that Fusarium wilt of cowpea is a warm-temperature disease in contrast to other *Fusarium* diseases such as cabbage yellows and pea wilt, for which temperatures from 22 to 24 C were best for identification of resistant and susceptible reactions (13). Fusarium wilt of cowpea is more like the tomato wilt and sweet potato stem rot diseases, also caused by *Fusarium* species, for which resistance is best determined at higher temperatures (13).

High temperature promoted severe symptoms of vascular wilt in Chinese Red cowpea inoculated with isolate 16609. Since Chinese Red is the only differential cultivar that succumbed to race 2 and since growth of race 2 on PDA was sharply inhibited as the temperature was raised from 30 to 33 C, the temperature effect was more likely on the host than on the pathogen. If the interaction of race 2 with Chinese Red is temperature-sensitive, this interaction could be used to study factors that control compatibility between the host and pathogen (7).

ACKNOWLEDGMENTS

We thank W. Hare, Mississippi State University, K. Kimbell, University of California, Davis, and S. Smith, University of California, Berkeley, for providing cowpea seed and fungal cultures and Kelly Moore and Marc Monson for technical help.

LITERATURE CITED

1. Armstrong, G. M., and Armstrong, J. K. 1950. Biological races of the *Fusarium* causing wilt of cowpeas and soybeans. Phytopathology 40:181-193.
2. Armstrong, G. M., and Armstrong, J. K. 1965. Wilt of chrysanthemum caused by Race 1 of the cowpea *Fusarium*. Plant Dis. Rep. 49:673-676.
3. Armstrong, G. M., and Armstrong, J. K. 1977. Reflections on the wilt *Fusaria*. Annu. Rev. Phytopathol. 13:96-99.
4. Armstrong, G. M., and Armstrong, J. K. 1980. Cowpea wilt *Fusarium oxysporum* f. sp. *tracheiphilum* Race 1 from Nigeria. Plant Dis. 64:954-955.
5. Booth, C. 1971. The Genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 237 pp.
6. Hare, W. W. 1953. A new race of *Fusarium* causing wilt of cowpea. (Abstr.) Phytopathology 43:291.
7. Holliday, M. J., Long, M., and Keen, N. T. 1981. Manipulation of the temperature-sensitive interaction between soybean leaves and *Pseudomonas syringae* pv. *glycinea*—implications on the nature of determinative events modulating hypersensitive resistance. Physiol. Plant Pathol. 19:209-216.
8. Kulkarni, R. N., and Chopra, V. L. 1982. Environment as the cause of differential interaction between host cultivars and pathogenic races. Phytopathology 72:1384-1386.
9. Little, T. M., and Hills, F. J. 1978. Agricultural Experimentation. Design and Analysis. John Wiley & Sons, New York. 350 pp.
10. Smith, E. F. 1899. Wilt disease of cotton, watermelon and cowpea (*Neocosmospora* nov. gen.) U.S. Dep. Agric. Div. Veg. Physiol. Pathol. Bull. 17:1-54.
11. Snyder, W. C., Nash, S. M., and Trujillo, E. E. 1959. Multiple clonal types of *Fusarium solani phaseoli* in field soil. Phytopathology 49:310-312.
12. Swanson, T. A. 1984. Root-knot nematode and Fusarium wilt diseases of cowpea and soybean. Ph.D. dissertation. University of California, Riverside. 125 pp.
13. Walker, J. C. 1965. Use of environmental factors in screening for disease resistance. Annu. Rev. Phytopathol. 3:197-208.
14. Zadoks, J. C., and Parlevliet, J. E. 1979. The integrated concept of disease resistance: A modeler's view of general and specific resistance. Pages 187-190 in: Proc. Symp. Int. Congr. Plant. Prot., 9th. Vol. 1. Plant Protection: Fundamental Aspects. T. Kommedahl, ed. 411 pp.