THE EFFECT OF SOIL TEMPERATURE ON DEVELOPMENT OF VERTICILLIUM WILT OF SUNFLOWERS

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Screening of sunflowers (Helianthus annuus L.) for resistance to wilt caused by Verticillium albo-atrum Reinke et Berth (2) may not be a simple task when variability in wilt reaction may be influenced by factors other than the host's genotype and/or the pathogen's level of virulence. Putt (1) suggested that the breeding behavior of sunflower resistance could be best measured under controlled conditions and with pure cultures of the pathogen and that controlled testing would be required to determine genes, besides gene V_1, which may account for wilt resistance.

Because of the seriousness of this disease, attempts were made at Beltsville, Maryland, to ascertain if, by controlling soil temperature during pathogenesis, reliable symptom reaction of resistant and susceptible lines could be obtained so that a uniform testing method could be developed. Such a method would be applicable in general to breeding material and in particular to material that on the basis of field testing would merit strict evaluation.

MATERIALS AND METHODS

Sunflower assay hosts. - Sunflowers tested for reaction to Verticillium wilt were California gray stripe and the lines CM 162 and CM 144. The latter two lines were furnished by E. D. Putt as, respectively, the most susceptible and highly resistant lines.

Verticillium isolates. - The following were tested: isolates 4, 6, 11, 15 and SVA from safflower, supplied by D. E. Zimmer, and isolates 1-2 and 4-3 from sunflower supplied by J. A. Hoes. These isolates were grown in potato dextrose agar medium supplemented with 0.5 g/l of dextrose in petri plates for 4 weeks. Inoculum was prepared by grinding the contents of eight plates in 250 ml of water.

Methods of inoculation. - Individual sunflower seedlings were grown in soil in 3-inch pots in a growth room with a 9 hour-day at 65°F in daytime and 55 F at night. Under these conditions seedlings grew to a height of 4-5 inches in three weeks. Two methods of inoculation were used: seedlings with the ball of soil intact were placed in partially filled 8-inch pots, four per pot, and inoculated by pouring the fungus mixture on the ball of soil and covering; or soil was removed from the root system by rinsing in water, inoculated by dipping the roots in the fungus mixture, and transplanting to pots or 1-gallon glazed crocks.

Four types of replicated experiments were conducted:

(A) California gray stripe and CM 162 seedlings, inoculated by both methods with each of the seven isolates were grown in a controlled environment room with 1000 ft-c light for a 16 hr/day at 80F; (B) California gray stripe and CM 162, inoculated with isolates 1-2 and 4-3 by the root dip method, were grown in the greenhouse in crocks in controlled temperature tanks at 15, 20, 25, and 30 C; (C) CM 162 and CM 144 seedlings, root-dipped in isolate 4-3 inoculum, were grown in controlled temperature tanks at 13, 22, 27, and 32 C; and (D) CM 162 and CM 144 seedlings, root dipped in inoculum 4-3, were grown in 8-in clay pots in the greenhouse at 80 F. All experiments were completed in approximately 5 weeks after inoculation.

RESULTS

No isolate incited moderate or severe wilt of California gray stripe sunflower when inoculated by pouring the inoculum on the ball of soil. By the root-dip method, however, wilt was moderate to severe in this variety inoculated with isolates 4-3 or 1-2. The safflower isolates, except isolate 15 which was slightly pathogenic, failed to incite wilt symptoms on this variety.

Wilt reaction of California gray stripe was not as severe as that of CM 162 when root-dip inoculated with isolate 4-3 at 15C in experiment B (Table 1). In experiment C with isolate 4-3, (Table 2), wilt reaction of CM 162 was extremely severe at 22, less severe at either 13 or 27, and very slight or absent at 32 C. The resistant line CM 144 was slightly affected by wilt at 22 and very slightly or unaffected at 13, 27, or 32C.

Wilt reaction of CM 162 and CM 144 plants in clay pots in experiment D, root-dip inoculated with isolate 4-3 and incubated under warm greenhouse conditions, was very severe on the former and mild on the latter line.

Table 1 The effect of soil temperature on Verticillium wilt reaction of sunflowers.

Sunflower line	Verticillium isolate	Soil temperature in C			
		15	20	25	30
		Wilt severity 1/			
Calif. gray stripe	1-2	2	2	4	1
	4-3	1-2	4	4	2
CM 162	1-2	2-3	2-3	4	1
	4-3	4	5	4-5	2

1/ 0, wilt absent; 5, plants severely wilted or killed

Table 2 Reaction of a susceptible and a resistant sunflower line to Verticillium isolate 4-3 at constant soil temperatures.

Sunflower line	Soil temperature in C			
	13	22	27	32
	Wilt severity[1]/			
CM 162	2-3	5	4	1
CM 144	0-1	1	0-1	0-1

1/ 0, wilt absent; 5, plants severely wilted or killed

DISCUSSION

Thes results show that maximum wilt development in susceptible sunflower by Verticillium occurred at or near 22 C. Although the highly susceptible line CM 162 was most affected at this temperature, wilt was severe also at 20 and 27. On the resistant line CM 144 wilt reaction was mild at 20 and 22 and negligible at the other soil temperatures. These experiments showed also that California gray stripe was slightly less susceptible than CM 162 when tested at I5 C with isolate 4-3.

To detect field tolerant types, plants with less resistance than that of CM 144, it may be necessary to use sub-optimum soil temperatures and/or isolates of a specific level of virulence. The present results indicate that low sub-optimum temperatures may be more useful in detecting tolerance than high sub-optimum temperatures.

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LITERATURE CITED

1. Putt, E. D. 1964. Breeding behavior of resistance to leaf mottle in sunflowers. Crop Science 4 : 177-179.

2. Sackston, W. E., McDonald, W. C., and J. Martens. 1957. Leaf mottle or Verticillium wilt of sunflower. Plant Disease Reporter 41 : 337-343.

DISCUSSION

Sackston: What was your spore load? Did you try sub-optimum spore loads to get essentially the same range of discrimination?

Orellana: The inoculum I used was standardized as far as growing the fungus. We did not count the spores but all the inoculum used in all experiments was the same for all the tests. We don't know how many spores we had and we did not think that was necessary.

Sackston: We have found in various tests with resistant, susceptible, and intermediate degrees of reaction of various hosts to various kinds of Verticillium, not specifically sunflowers, that inoculum level was a significant factor. You can get at this kind of discrimination you asked for at optimum temperature conditions and lower inoculum dosages if you wish to do it that way and get your results faster.

Orellana: Probably.

Hoes: You say that you do not know the actual spore concentration but yet you say that it was equal. How then did you determine that?

Orellana: The pathogen was grown under similar conditions, the same media and the same number of plates were homogenized and in the same amount of water and the same amount of this spore suspension was poured on each plant. Actually, we did not think it necessary to go into calibration for this particular type of test.

Hoes: I may confirm what Dr. Sackston has said that spore concentration is quite effective. I tested in respect to the root immersion technique, concentrations ranging from about 65,000 spores per ml. to over 5 million and there was a very definite difference. At the 2.6 million there was actually less disease than at the lower concentration of 65,000.

Orellana: I believe that is true for most diseases. You can use the more concentrated spore loads and you obtain more disease and more opportunity for infection, but at the same time the type of spores you have in your spore suspension may vary. My spores may be younger than others and so on and so forth so that is a very difficult thing to standardize. Actually if you prepare enough inoculum at once you do not expect any variation due to that and we have obtained no differences by that method.

Hoes: At what stage of plant growth did you check your results?

Orellana: We made a rating when the plants were four weeks old.

Hoes: Were they flowering then?

Orellana: Yes, they were flowering.

Bondshu: Could we expect from a field standpoint or a grower's stand-

point that if we were to consider our soil conditions at planting time and plant at a higher soil temperature that we may get away a little bit from this condition, or from a wilt condition than if we were to plant at cooler temperatures?

Orellana: I suppose a correlation of this nature will have to come from more research.

Bondshu: I see. I was trying to put it down to a grower's level where this information would apply to us.

Sackston: Can I hazard at least a guess at an answer there. I think the temperature at which this pathogen is fairly effective is such that if you waited until the temperatures went above a suitable level you would be seeding, talking in terms of Fahrenheit, at temperatures above 75-80°. You see this is quite a virulent pathogen, especially the dark microsclerotial form at anything up to about 70 or 80 and it will attack lightly at above that and then will become relatively severe again if the temperature drops to a favorable level.

Kinman: Our soil temperatures do not even get that high.

Sackston: Well, he is asking from California but I think even in California the possibility of seeding when the temperature was too high would be very, very limited.

Orellana: I would have to add that this sort of testing would be applicable to material that merits such strict evaluation and that is exactly the objective of this test to detect all the levels of resistance or susceptibility that cannot be detected by other methods less refined.

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