Reaction of Sunflower and Safflower Germ Plasm to *Verticillium dahliae*

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ABSTRACT

Klisiewicz, J. M. 1981. Reaction of sunflower and safflower germ plasm to *Verticillium dahliae*. Plant Disease 65:237-239.

Growing sunflower and safflower plants in a medium infested with *Verticillium dahliae* was an effective inoculation method in growth chamber and greenhouse tests to differentiate resistant and susceptible germ plasm. A *V. dahliae* isolate from sunflower (ND) induced severe disease in open-pollinated sunflower cultivars and inbred lines except the highly resistant inbred line HA 89. Most cultivars and lines had resistance to an isolate from safflower (T-1) and an isolate from cotton (SS-4). HA 89 was susceptible to T-1. Sunflower oilseed hybrids were highly resistant to T-1 and SS-4, and five hybrids were highly resistant to the sunflower isolate. Severe disease developed in most of the safflower introductions inoculated with SS-4 and T-1. Safflower cultivars and breeding lines were susceptible to SS-4 and ranged from highly susceptible to highly resistant to T-1. Reactions differentiating pathotypes were observed in sunflower and safflower.

Sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) are important oilseed crops that are susceptible to Verticillium wilt (2,5). Expansion of sunflower and safflower production in the same or to different geographic areas in the United States may subject the crops to infection by different pathotypes of *Verticillium dahliae* Kleb. Their susceptibility to *Verticillium* isolates from other susceptible crops (1,2,10) creates a possible rotation problem and a potential threat to expanded production that could be alleviated by resistant cultivars.

Field screening is effective for evaluating a large quantity of materials for resistant germ plasm (7), but unless the tests are conducted in different growing areas, resistance to different pathotypes of *Verticillium* may be overlooked and escape identification. Field evaluation should be supplemented by greenhouse tests in which plants are tested for resistance to specific pathotypes by using an effective inoculation method.

In greenhouse pathogenicity tests, sunflower (1,2) and safflower (3,9) plants have been inoculated with *V. dahliae* by dipping roots in a spore suspension or culture medium homogenate or by injecting stems with conidial suspensions in sterile water. Although these techniques are effective, a more natural method of inoculating plants was sought that would also minimize injury to roots. This paper describes a method for infecting young plants by growing them in an infested medium and describes the reaction of sunflower and safflower germ plasm to pathotypes of *V. dahliae*.

MATERIALS AND METHODS

Pathotypes. Isolates of *V. dahliae* from infected sunflower (ND) in North Dakota, from safflower (T-1, severe on cotton), and from cotton (SS-4, mild on cotton, from W. C. Schnathorst) in the San Joaquin Valley of California were used in this study. Cultures were maintained on potato-dextrose agar in petri dishes or culture tubes.

Plants. Twenty-one sunflower cultivars, inbred lines, and selected hybrids were tested for resistance to ND, T-1, and SS-4. Thirty-one safflower introductions and breeding lines were tested for reaction to T-1 and SS-4.

Inoculum. A medium of vermiculite and V-8 juice was prepared for growth of *V. dahliae*. Dry vermiculite (250 ml) was placed in 400-ml beakers and saturated with 110 ml of V-8 juice solution (800 ml of water, 200 ml of V-8 juice, and 2 g of $CaCO_3$). Excess juice was decanted and the beakers were covered with aluminum foil and autoclaved for 30 min. The vermiculite was loosened by gently shaking the beaker and inoculated with 12 pieces (2 × 2 mm) of a *V. dahliae* culture on potato-dextrose agar. The inoculum pieces were mixed in the vermiculite by gentle agitation.

V. dahliae was allowed to grow throughout the medium for 14 days at 24 C; then foil covers were removed and seeds that were surface-sterilized in 1% sodium hypochlorite and rinsed in sterile water were planted in the vermiculite. As many as 12 sunflower or 18 safflower seeds were planted per beaker.

Ten milliliters of sterile distilled water was added, and the beakers were covered with loosely fitting plastic bags and placed under artificial lights (4,088 lux) in the laboratory at 24 C. When plants were 7 days old, the vermiculite was washed from the roots and the plants were transplanted into autoclaved loam soil. A standard transplanting age of plants was based on development of wilt symptoms in 100% of the plants of a susceptible cultivar that were transplanted when 2 to 7 days old. The plants were kept in a growth chamber at 21 ± 1 C illuminated (21,000 lux) for 14 hr daily for 7 days; then for 14 days, day and night temperatures were 28 ± 1 C and 21 ± 1 C, respectively. Plants were then transferred to the greenhouse at average ambient night and day temperatures of 21 and 28 C, respectively, and observed for symptom development.

Disease index. Disease was rated on a 0–5 scale, 0 indicating no disease and 5 indicating dead plants. A score of 1, 2, or 3 indicated chlorosis and necrosis of foliage on 25, 50, and 75% of the plant, respectively. Development of symptoms to the top of safflower plants and stunting of sunflowers were indicated by a score of 4. Plants with an index of 0 or 1 were considered highly resistant to *V. dahliae*.

RESULTS AND DISCUSSION

Efficacy of inoculum. Plants emerged 3–4 days after sowing in vermiculite infested with *V. dahliae*. Wilt symptoms did not develop between the time of plant emergence and transplanting. The incidence of wilt on plants that were transplanted 2–5 days after emergence ranged from 8 to 90%, but 100% of the plants transplanted 6–7 days after emergence developed wilt symptoms. The incidence of disease after transplanting plants of different ages suggests that at least 6 or 7 days are needed for infection of all the plants. Because microsclerotia were present on the roots of the plants, however, infection could also occur after transplanting.

The vermiculite medium supported good plant growth, although plant hypocotyls were abnormally elongated because of low light intensity. Contamination of the medium was not a problem but was occasionally evident on surface vermiculite particles and empty seed pericarps.

Symptoms and disease in sunflower. The first symptom in sunflower was chlorosis of cotyledons and first true leaves. Leaf necrosis and stunted growth of plants were evident during the second week at day and night temperatures of 28 ± 1 C and 21 ± 1 C, respectively. Stunted plants often did not flower because the head did not develop, and they had extensive vascular discoloration in the roots and stems. Lethal reactions

Table 1. Average disease on sunflowers infected by *Verticillium dahliae*

Entry	*V. dahliae* strains: Disease index[a] T-1	SS-4	ND	Disease index range T-1	SS-4	ND
Cultivar						
Mingren	2.3	1.9	4.0	0–5	1–2	4
Sundak	0.7	0.7	3.7	0–1	0–1	3–4
Sputnik	0.4	0.2	3.3	0–1	0–1	2–4
Peredovik	0.3	0.0	2.9	0–5	0	0–4
Inbred line						
HA 89	3.1	0.7	0.6	1–5	0–1	0–1
CM 90RR	2.7	0.6	4.0	0–5	0–1	4
HA 60	1.3	1.0	5.0	0–2	1	5
HA 232	1.1	1.0	4.2	1–4	1	4–5
RHA 266	0.4	1.3	3.7	0–4	1–2	3–5
RHA 265	0.3	0.0	3.6	0–4	0	3–4
HA 234	0.2	0.0	5.0	0–1	0	5
Oilseed hybrid						
Sunbred 212	0.0	0.5	3.4	0	0–1	3–4
Hybrid 204	0.3	0.1	1.0	0–1	0–1	0–2
Sun Gro 372	0.3	0.5	1.8	0–1	0–1	0–4
Sunbred 232	0.5	1.7	4.0	0–1	1–2	3–5
IS 8984	0.6	0.8	2.8	0–2	0–1	2–4
IS 891	0.6	0.8	1.5	0–1	0–1	1–2
Sun Hi 301 A	1.0	0.2	0.8	0–2	0–1	0–2
DO 410	1.1	0.5	4.7	1–2	0–1	4–5
Sun Gro 380	1.4	0.5	0.4	0–4	0–1	0–1
Sun Hi 304	1.8	1.0	2.5	1–5	1	1–4

[a] Disease severity index: 0 = no disease; 1, 2, and 3 = leaf chlorosis and/or necrosis on 25, 50, and 75% of a plant, respectively; 4 = stunted, and 5 = dead plants (LSD 0.01 = 2.3 for cultivars and inbred lines, 0.01 = 2.0 for oilseed hybrids).

Table 2. Distribution of safflower introductions, cultivars, and breeding lines by disease reaction to *Verticillium dahliae*

Entries	*V. dahliae* isolate	No. of entries/disease rating[a] 0	1	2	3	4	5
Plant introductions	T-1	0	0	6	6	3	4
	SS-4	0	1	4	10	3	1
Cultivars and breeding lines	T-1	1	2	2	2	0	2
	SS-4	0	0	0	9	0	0

[a] Rounded to nearest whole number.

Table 3. Reaction of selected safflower cultivars, breeding lines, and introductions to *Verticillium dahliae*

Entry	Disease index[a] for isolate T-1	SS-4
VFR-1	0.0	3.0
VFstp-1	0.3	3.0
14-5	1.1	2.6
PI 251398	1.5	2.1
PI 306596	3.0	3.3
PI 209285	3.6	1.4
Gila	5.0	3.0
PI 250823	5.0	5.0

[a] Disease index: 0 = no symptoms; 1, 2, 3 and 4 = chlorosis and necrosis of leaves on 25, 50, 75, and 100% of the plant, respectively; 5 = dead plants (LSD 0.01 = 1.0).

occurred only among plants that were stunted.

The ND isolate induced severe disease of open-pollinated cultivars and inbred lines except inbred line HA 89 (Table 1). The resistance of HA 89 to ND corresponds to its resistance in field trials in naturally infested soils in North Dakota (11). Stunting and killing of plants infected by ND was extensive but was only occasional in plants infected by T-1. Most of the cultivars and lines had resistance to T-1 and SS-4 (Table 1). The average disease index of 3.6 for 11 sunflower cultivars and inbred lines infected by ND was significantly (P = 0.01) greater than those of 1.2 and 0.7 for T-1 and SS-4, respectively. The differential reaction of HA 89 to T-1 and ND was the reverse of the reactions of other inbred lines and cultivars to the isolates.

The average disease indexes of 10 oilseed sunflower hybrids inoculated with ND, T-1, and SS-4 were 2.3, 0.8 and 0.7, respectively. Five hybrids were highly resistant to ND, and all hybrids were highly resistant to T-1 and SS-4 (disease index less than 2.0) (Table 1). ND induced stunting and killing in plants of some hybrids. The high resistance of hybrids to ND indicates that progress toward incorporating HA 89 resistance has been achieved in breeding programs.

Symptoms and disease in safflower. Chlorosis of cotyledons and first true leaves of safflower plants was mild at 21 ± 1 C but developed rapidly on leaves after 3–4 days at 28 ± 1 C and 21 ± 1 C day and night temperatures, respectively. Stunting of plants was mild, and lethal reactions occurred when plants were 4 to 8 wk old. Extensive disease developed in plants of some safflower introductions infected by T-1 and SS-4, but most introductions had disease indexes of approximately 2.0 and 3.0 (Table 2). Comparison of the reactions of 19 introductions showed that the disease index was higher in eight infected by T-1 and six infected by SS-4 and that five were equally susceptible to both isolates. Cultivars and breeding lines were similar in their reaction to SS-4 but varied in reaction to T-1 from highly susceptible to highly resistant (Table 2).

Safflower germ plasm VFR-1 and VFstp-1 possessed high resistance to T-1 (Table 3). They were previously selected for Verticillium wilt resistance (6,8), but their susceptibility to SS-4 in these tests suggests that this germ plasm was selected for resistance principally to T-1. Although some safflower introductions, cultivars, and breeding lines showed no significant difference in reactions to T-1 and SS-4, the reactions of others differentiated the two pathotypes (Table 3). In comparison, the differential reactions of PI 209285 and Gila to the two pathotypes was the reverse of the reactions of VFR-1 and VFstp 1. Severe and mild reactions differentiating T-1 and SS-4, respectively, have been observed in an unspecified safflower cultivar (4). Although germ plasm with resistance to T-1 is available, identification of other sources of resistance would offer a broader genetic base for breeding programs. Germ plasm with resistance to SS-4 is also needed. Safflower introductions with a disease index of 2.0 might be of value for improving resistance to SS-4. Because only a few plant introductions have been evaluated for resistance to both SS-4 and T-1, a search for germ plasm resistant to specific pathotypes should probably concentrate on introductions in the world safflower collection.

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