Resistance to Verticillium Wilt in Collections of Wild Helianthus in North America

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ABSTRACT

Resistance to Verticillium wilt caused by *Verticillium dahliae* occurred in all 46 collections of wild *Helianthus* tested. Six collections of *H. annuus* were from Manitoba and Saskatchewan in Canada, and 34 collections of *H. annus* plus 6 collections of *H. petiolaris* were from 12 states in the United States. Resistance was confirmed in F_1, F_2, and F_3 populations of crosses of resistant wild plants with a susceptible inbred. *H. petiolaris* was generally more resistant than *H. annuus*. Collections of *H. annuus* from Manitoba, Saskatchewan, and North Dakota were generally less resistant than those from more southern latitudes. A center of resistance is postulated to exist in the central and southern Great Plains, which coincides with the hypothetical center of origin of *H. annuus* and *H. petiolaris*. Frequency distributions of F_1, F_2, and F_3 populations suggested dominance of resistance, and recessiveness or lack or dominance of resistance.

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Sackston et al. (5) described leaf mottle or wilt in cultivated sunflower (*Helianthus annuus* L.) caused by the microsclerotial strain of *Verticillium albo-atrum* R. & B. (= *V. dahliae* Kleb.). The disease is widespread in the Red River Valley in Manitoba where sunflowers have been grown commercially since 1943. In susceptible cultivars like 'Commander' and 'Mennonite', the disease incidence is high, the symptoms severe, and yield losses are common and serious. Resistant cultivars exist and the inheritance of resistance from different cultivars of domesticated sunflower was studied by Putt (3). Besides occurring in domesticated sunflowers, resistance to wilt as well as to other diseases may be expected in wild material. Sunflowers are native to North America and seeds (achenes) of wild *Helianthus* were conveniently collected from many points in Canada and the United States. The collections were screened for resistance to Verticillium wilt under field conditions in Manitoba and the results are reported here.

MATERIALS AND METHODS.—Seeds of wild sunflower were collected at six locations in Manitoba and Saskatchewan in Canada, and at 40 locations in 12 states in the United States (Table 1). Forty collections belonged to *H. annuus*, while six belonged to *H. petiolaris* Nutt. The plants were identified according to Heiser et al. (2). Twenty-two collections were tested in 1967, and 24 were tested in 1969. The seeds were scarified to promote germination, treated with captan, and planted in a field near Morden, Manitoba, known to be heavily infested with *V. dahliae* pathogenic to sunflower. The inbred line CM 162, a single-stemmed, monocephalic cultivar which is highly susceptible to wilt, served as the control.

Although highly unlikely, seemingly resistant plants might have escaped infection and not been truly resistant. Consequently, to determine the existence of resistance more precisely in the collections tested in 1967, F_1, F_2, and F_3 populations of crosses between CM 162 and seemingly resistant wild plants were studied. Putt (3) observed 15 F_1 populations of single crosses between nine resistant and nine susceptible parents; each of 14 hybrids was clearly more resistant than the susceptible parent. However, one hybrid was as susceptible as the susceptible parent. In this work, it was therefore assumed that resistance had originated in the wild parent because the hybrid was clearly more resistant than CM 162. In 1967, healthy plants of CM 162 in another field were crossed with individual wild plants showing resistance to wilt. Usually three plants from each collection were

crossed. From the 52 crosses yielding seed, 28 seeds from 14 collections were randomly selected and planted in Chile during the 1967-1968 season to produce F_2 seed. In 1968, F_1 populations of the 52 crosses, and the 28 F_2 progenies produced in Chile were sown in the disease nursery. Sixteen F_1 plants and 16 F_2 plants showing resistance to wilt were selfed, and the resulting F_2 and F_3 progenies observed for wilt in 1969. Throughout the study, each plant was scored individually on a scale of 0-10 (0 = healthy, 10 = dead) after full bloom, which is a stage when symptoms of the disease are mature. Plants were divided into three classes: resistant, 0-4; moderately susceptible, 5-7; and highly susceptible, 8-10. Mean disease scores (MDS) were computed arithmetically.

RESULTS.—*Resistance occurring in collections and F_1 populations.*—Resistant, moderately susceptible, and highly susceptible plants occurred in 37 collections. Five collections of *H. annuus* (1117, 1338, 1350, 1354, 1355) and four of *H. petiolaris* (1337, 1349, 1351, 1353) consisted of resistant and moderately susceptible plants only. Forty-four collections all contained plants of score 0, but in collections 1053 and 1129 from Saskatchewan, the most resistant plants were scored 2 or 3. *H. petiolaris* was generally more resistant than *H. annuus*. In 1967, collection 903 from Oklahoma had the lowest MDS of 1.2, and 88% of the 25 plants were resistant. In 1969, of five other collections of *H. petiolaris* of MDS 0.5-1.8, 91% of 80 plants were resistant. In contrast, of the *H. annuus* collections tested in 1967 and 1969, 56% and 78%, respectively, of the plants were resistant.

Resistance was confirmed in F_1 populations. Fifty-two crosses produced branched, multi-headed wild type plants, indicating that the crosses had actually been achieved. Attempted crosses with *H. petiolaris* collection 903, and with *H. annuus* collections 1052, 1057, and 1058 were unsuccessful. Of 10 crosses from nine collections, all plants were as susceptible as the susceptible parent. Seven crosses of MDS 7.5-8.9 derived from six collections, did not include any resistant plants but nevertheless were less susceptible than CM 162. Thirty-five crosses of MDS 2.7-9.6 derived from 18 collections did include at least some resistant plants. There was no association between the disease score of the wild parent and the MDS of its F_1 offspring. Wild parents with disease scores over the range of 0 to 8 produced F_1 populations with high MDS of 8 to 9.

A correlation between degree of wilt resistance and geographical location was apparent in the case of the *H. annuus* collections tested in 1967 (Table 2). Individual plant scores were ranked and *t*-values from a modified Mann-Whitney test (4) indicated highly significant differences for both collections and their F_1 populations. Collections and F_1 populations from Colorado, Kansas, Nebraska, Ohio, Oklahoma, South Dakota, Texas, and Wyoming contained collectively higher proportions of resistant plants and lower proportions of highly susceptible plants than those from the North Dakota, Manitoba, and Saskatchewan locations north of the 45th parallel (Table 2). The most resistant of the southern collections were collection 1062 (MDS=2.0) from Colorado and collection 1054 (MDS=2.3) from South Dakota, each containing 80% resistant plants. Collection 1057 (MDS=3.8) from North Dakota with 56% resistant plants was the most resistant in the northern group. The Mann-Whitney test was not applied to the 1969 test of collections, because only one collection was from the northern latitude.

Resistance in F_2 and F_3 progenies.—Resistance occurred in 23 F_2 progenies out of seven collections and in 16 F_3 progenies from three collections (Table 3). The data show that the frequency distributions of resistance in some populations were skewed to the resistant side and others to the susceptible side. Populations of lower MDS generally descended from southern collections. Statistical analysis was not attempted, however, because only two collections came from northern latitude. The F_2 progenies tested in 1968 came from 28 random F_1 seeds. Eight seeds came from four northern collections and yielded one F_2 with resistant plants. Twenty seeds came from 10 southern collections, five of which yielded eight F_2's with resistant plants. Nineteen of the 28 progenies were completely susceptible. Three F_3 progenies out of collection 1059 from Kansas did not contain any highly susceptible plants of score 8-10. They consisted of 28, 34, and 48 plants of MDS 1.1, 1.1, and 1.4, and they included three, one, and four plants of moderate susceptibility, respectively.

DISCUSSION.—Resistance to *V. dahliae* occurring

TABLE 1. Collections of two wild *Helianthus* spp. tested for resistance to Verticillium wilt in 1967 or 1969

Species	Source	Accession numbers and year of test
H. annuus	Saskatchewan	1051, 1052, 1053, 1129 (1967)
	Manitoba	904, 906 (1967)
	N. Dakota	1056, 1057 (1967); 1334 (1969)
	Wyoming	1064 (1967); 1338 (1969)
	S. Dakota	1054, 1055 (1967); 1358 (1969)
	Nebraska	1058 (1967); 1339, 1342, 1348 (1969)
	Ohio	1181 (1967)
	Colorado	1062, 1063, 1179 (1967); 1335, 1341 (1969)
	Kansas	1059, 1060 (1967)
	California	1356, 1357 (1969)
	Oklahoma	901, 1117, (1967)
	Arizona	1340, 1344, 1345, 1350, 1354 (1969)
	New Mexico	1333, 1336, 1343, 1355 (1969)
	Texas	855 (1967)
H. petiolaris	Nebraska	1353 (1969)
	Kansas	1337, 1351 (1969)
	Oklahoma	903 (1967)
	New Mexico	1347, 1349 (1969)

TABLE 2. Resistance to Verticillium wilt in collections of wild *Helianthus annuus* and of F_1 populations of susceptible inbred × resistant wild sunflower, effect of sources and statistical significance

Latitude & sources of resistance	Material and year of test	No. of populations	Total no. plants	Mean disease score[a] Range	Mean disease score[a] Average	Percentage plants[a] Resistant	Percentage plants[a] Mod. susc.	Percentage plants[a] Highly susc.	Modified Mann-Whitney test: Mean rank of disease scores	Modified Mann-Whitney test: Disease score equivalent to mean rank
Northern										
904,906,1051,1052, 1053,1056,1057,1129	Collections - 1967	8	205	3.8-7.1	5.4	47	21	32	338.8[b]	4.5
As above, except 1052 & 1057	F_1 - 1968	11	225	4.5-9.6	6.9	20	14	65	485.8[c]	8.0
Southern										
855,901,1054,1055, 1058,1059,1060, 1062,1063,1064, 1117,1179,1181	Collections - 1967	13	370	2.0-4.8	3.1	61	25	14	259.9[b]	2.7
As above,except 1058	F_1 - 1968	24	603	2.7-9.0	5.8	37	14	49	387.9[c]	7.0
Northern										
1334	Collections - 1969	1	38		3.7	60	16	24	-	-
Southern										
1333,1335,1336, 1338-1345,1348, 1350,1354-1358	Collections - 1969	18	476	1.0-4.1	2.4	80	12	8	-	-

[a] Individual plants scored on a 0-10 scale (0 = healthy, 10 = dead); resistant (0-4), moderately susceptible (5-7), highly susceptible (8-10).
[b] Degrees of freedom is ∞; t = 5.46, and theoretical t (at P = 99.9%) = 3.29.
[c] Degrees of freedom is ∞; t = 5.24, and theoretical t (at P = 99.9%) = 3.29.

TABLE 3. Reactions to Verticillium wilt of F_2 and F_3 progenies of crosses between resistant wild *Helianthus annuus* and susceptible CM 162

Generation & year of test	Latitude & sources of resistance	Number of populations	Mean disease score[a] Range	Mean disease score[a] Average	Frequency of plants[a] Resistant	Frequency of plants[a] Moderately susceptible	Frequency of plants[a] Highly susceptible
	Northern						
F_2 - 1968	1053	1		7.8	11	5	41
	Southern						
	1054,1059,1062, 1063,1181	8	1.3-9.7	7.0	66	22	196
	Northern						
F_2 - 1969	1129	2	6.4-7.4	6.9	17	9	31
	Southern						
	1054,1062,1063,1181	12	1.5-7.5	4.6	94	35	72
	Northern						
F_3 - 1969	1053	4	1.5-4.2	3.1	40	10	11
	Southern						
	1179	2	7.0-7.7	7.4	18	16	51
	1059	10	1.1-4.8	2.3	331	29	40

[a] Plants were scored on a 0-10 scale (0 = healthy, 10 = dead); resistant (0-4), moderately susceptible (5-7), highly susceptible (8-10).

naturally in Manitoba was found in wild *H. annuus* and *H. petiolaris* from 46 widely separated locations in 14 provinces and states in Canada and the United States. Resistance was confirmed in F_1 crosses between susceptible CM 162 and resistant wild plants for each of the 18 *H. annuus* collections tested, and in 39 F_2 and F_3 progenies derived from eight collections (Tables 2, 3). Resistance to wilt is widespread and is easily recovered from wild sunflowers, at least in North America.

A correlation between geographical source and degree of resistance is indicated. Both F_1 populations and mother collections from Manitoba, North Dakota, and Saskatchewan had a higher MDS and

were generally less resistant than those from South Dakota and more southern areas (Table 2). The correlation is further supported by the 1968 data of F_2 progenies from randomly chosen F_1 seeds, indicating a greater frequency of resistance in material from southern sources (Table 3).

Even though based upon only 21 collections of *H. annuus* tested in only one location, the data suggest a center of resistance in the Great Plains area of Colorado, South Dakota, Nebraska, Kansas, and Oklahoma. The collections of *H. petiolaris* came from the same general area and from New Mexico and had a similar or even higher degree of resistance than the most resistant collections of *H. annuus*. Heiser et al. (2) recognized a western or southwestern center of origin of *H. petiolaris* and believed that *H. annuus* should be assigned to the same center. The proposed center of resistance to Verticillium wilt appears to coincide with the center of origin postulated by Heiser et al. (2). The lower frequency of resistance in collections away from the center may be due to remoteness from it. The postulation of a center for resistance presupposes selection pressure on sunflowers by *V. dahliae* indigenous to central or southwestern North America. These presuppositions are met by observations (i) that *V. albo-atrum* pathogenic to cotton is reported to occur in virgin soil in Arizona and Arkansas (1), areas near or part of the Great Plains; (ii) that strains of the fungus pathogenic to both cotton and sunflower which might also occur in the United States occur in South Africa (7); and (iii) that all 111 isolates from cotton and other plants in cotton-growing counties of New Mexico were of the microsclerotial type (6), which is the type that attacks sunflowers.

Resistance to wilt was found in both *H. annuus* and *H. petiolaris*. Both are annual diploids with 17 chromosomes, and they hybridize freely in many areas (2). Resistance might have arisen independently in both species or it is possible that one acquired it from the other. *H. petiolaris* was generally more resistant than *H. annuus*, which may indicate that resistance originated in the former species.

Frequency distributions of the various populations showed that genetic control of resistance varied greatly. Five out of 35 F_1 populations, 11/23 F_2 and 14/16 F_3 progenies had frequency distributions which were clearly skewed to the resistant side, indicating dominance of resistance. Sixteen F_1 populations and the other F_2 and F_3 progenies had frequency distributions clearly skewed to the susceptible side, indicating recessiveness or lack of dominance of resistance. Recessiveness of resistance is also suggested to occur in the 17 F_1 populations which lacked resistant plants. The emergence of three F_3 progenies which seemed pure breeding and which originated from the same cross suggests that some forms of resistance are simply inherited. Skewness of frequency distributions was more common in F_2 and F_3 progenies than in F_1 populations, presumably due to increased homozygosity and narrowing of the range of genotype caused by inbreeding. The collections were apparently highly heterogeneous and heterozygous for resistance to wilt, because populations of dissimilar frequency distribution were frequently obtained from the same collection or even from the same wild parent, and also because many F_1 populations contained plants of widely divergent disease score.

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