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RELATIVE VIRULENCE AND SELECTIVE PATHOGENESIS AMONG ISOLATES OF VERTICILLIUM FROM SUNFLOWER AND OTHER HOSTS IN RELATION TO SUNFLOWER WILT

By

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The main objectives of this investigation were to obtain additional information on selective pathogenesis and on variation of virulence in sunflower isolates under environment-controlled conditions, and to determine if isolates from hosts other than sunflowers would infect sunflowers. Answers to these questions would be of considerable importance in sunflower improvement programs.

Materials and Methods.-*Sunflower plants.*-Varieties and lines tested for reaction to *Verticillium* wilt were the following: the Canadian varieties Mennonite and Commander, and the lines Peredovik (Blacklaw Selection), Lethbridge 159, CM-162, and CM-144; the American varieties Greystripe, Mingren, Arrowhead, Manchurian, Lyng Manchurian-26, Lyng Hybrid I, HO-1; and the Russian varieties Peredovik, VNIIMK 8931, VNIIMK 1646, Smena, Kubanec, Vostok, Armavirec, and Krasnodarets. The Canadian lines CM-162 and CM-144 are respectively highly susceptible and highly resistant to *Verticillium* wilt.

Verticillium isolates-Isolates of *V. albo-atrum* that were tested are listed in Table 1. Hyphal tips of these isolates were first transferred to nutrient agar and the resulting colonies increased on potato dextrose agar in petri plates for 2-4 weeks at 30 C. Inoculum was prepared by 2 methods: (a) by homogenizing the contents of 8 plates with 250 ml of distilled water in a Waring blender, and (b) by suspending in water conidia scraped from the colonies. Concentration of microsclerotia and of conidia was determined by means of an hematocytometer. Inoculum concentrations were regulated by centrifugation and resuspension of conidia or microsclerotia in given volumes of water. Homogenates contained about 0.1 million microsclerotia/ml and suspensions about 1.2 million conidia/ml for each isolate.

Methods of inoculation.- Inoculation tests were run in greenhouses at day and night temperatures respectively, of about 21 and 18 C as well as in environment-controlled rooms with a 16 hr photoperiod of 1,600 ft-c of light intensity and at day and night temperatures respectively of about 21 and 16 C.

Sunflower seedlings were inoculated when 10-15 days old by 2 methods: (a) soil from the roots was removed by rinsing, the root system was dipped in the fungus homogenate and the seedlings were transplanted to steam-sterilized soil in 6- or 8-inch clay pots with 3 or 4 seedlings per pot, and (b) seedlings were rinsed and transplanted, allowed to grow 3 weeks to a height of about 12 inches and to a stem diameter of 8-10 mm, and then injected with the conidial suspension by means of an hypodermic syringe in the stem, at the cotyledonary node and one inch below. Eighteen to 24 plants were inoculated by each method with each isolate. Plants dipped in sterile homogenated potato dextrose agar or injected with sterile water were used as controls.

The relative virulence or avirulence of a given *Verticillium* isolate was considered to be the ability of an isolate to induce wilt symtoms on test sunflower plants under given environmental conditions and was estimated on the basis of a calculated average disease index as shown in Table 1, for each isolate-host interaction.

Results.- Symtoms induced on sunflowers by stem injection with conidial syspensions or by dipping the roots in homogenates containing microsclerotia of *V. albo-atrum* from sunflowers, both in the greenhouse and in controlled-environment rooms, was characteristic of *Verticillium* wilt of sunflowers in the field, i.e., initial wilt of half of the leaf laminae, petiole epinasty, plant stunting, and wilt of the entire plant. Symptoms were, however, more severe and the plants died sooner when susceptible sunflowers were injected than when the roots were dipped.

Disease indices calculated for wilt reactions of the susceptible sunflower line CM-162 and the resistant line CM-144 inoculated with sunflower, potato, tomato, safflower, or cotton isolates of *V. albo-atrum* are shown in Table 1. Isolate 39 from Minnesota sunflowers was consistently more virulent on susceptible sunflowers than the Canadian isolates 1-2 or 4-3. Whereas all CM-162 plants died, CM-144 plants showed only slight symptoms and recovered or remained healthy.

Table 1 also shows that *Verticillium* isolates from sunflowers were consistently more virulent on CM-162 sunflowers than isolates from potato, tomato, safflower, or cotton. Potato isolate 119, which was of the 'dahliae' type, caused slight to moderate wilt symptoms on CM-162. Isolates from crops other than sunflowers were weakly virulent on the susceptible CM-162 and avirulent on the resistant CM-144 sunflowers. These results were confirmed by reactions of 20 sunflower varieties inoculated in the greenhouse with sunflower, potato, tomato, safflower, or cotton isolates of V. albo-atrum, as shown in Table 2. Varieties Mingren and Greystripe were almost as susceptible to the Minnesota sunflower isolate 39 and to the Canadian sunflower isolate 4-3 as was the susceptible line CM-162. Varieties, Arrowhead, Commander, and Smena were moderately susceptible. Peredovik and Mennonite were apparently mixed for resistance and susceptibility. The cotton isolate T-1 caused moderate wilt symptoms of Commander, Arrowhead, and Cm-162 but did not kill them. The other Verticillium isolates from potato, tomato, safflower, or cotton were weakly virulent on some sunflower varieties and avirulent on others.

Inoculations of 'Kennebec' potato, 'Campbell Soup' tomato, 'Gila' safflower, and 'Stardel' cotton, with sunflower isolates 39, 4-3, or 1-2 of V. albo-atrum showed that these isolates behaved like avirulent isolates even though these inoculated plants were known to be susceptible to Verticillium wilt.

Discussion.- Virulence in isolates of V. albo-atrum from sunflower was readily expressed on susceptible sunflower genotypes and unexpressed on resistant sunflower genotypes under conditions that approximate the environmental requirements of sunflowers in the field. Pathogenicity among Verticillium isolates from sunflower was nearly specific with respect to sunflower because these isolates were mostly avirulent with respect to potato, tomato, safflower, or cotton varieties known to be susceptible to Verticillium wilt. Virulence or avirulence among sunflower isolates of V. albo-atrum were, therefore, determined by the pathogen-host interaction. The present investigation also showed that highly susceptible sunflower varieties can be infected and/or diseased by some Verticillium strains parasitic on host species other than sunflower.

Evidence of heterokariosis in Verticillium albo-atrum, high frequency of mitotic recombination, anastomosis between hyphae, between conidia and hyphae, and between conidia in soil, and the possibility of mutation in Verticillium, suggest that new strains with different pathogenic capabilities can arise in nature.

Table 1. - Relative virulence and avirulence, expressed as average disease index[a/], of isolates of V. albo-atrum from sunflower, potato, tomato, safflower, or cotton on the susceptible CM-162 and the resistant CM-144 sunflower lines under greenhouse and controlled-environment conditions

Verticillium isolate	Host source	Sunflower line CM-162	CM-144
		Average disease	index
1-2	sunflower	3.6	0.2
4-3	"	3.9	0.3
39	"	4.4	0.5
119[b/]	potato	2.5	0.1
GR	"	2.0	-
V-8	"	0.9	-
V-78	"	1.6	-
22	"	0.8	-
V-81	tomato	0.9	-
T-1	cotton	1.6	0.1
C-5	"	0.7	-
SVA	safflower	1.2	-
15	"	0.8	-
11	"	0.7	-
6	"	0.5	-
4	"	1.0	-

a/ Disease index (number of infected plants X severity class ÷ number of inoculated plants) calculated for each isolate-host interaction in 2 greenhouse and 2 environment-controlled room experiments. Severity class: 5, plants killed; 4, severely stunted; 3, moderately stunted; 2, slightly stunted; 1, petiole epinasty and recovery; -, no symptoms.

b/ V. dahliae.

Table 2. - Relative virulence and avirulence expressed as average disease index[a/] of isolates of V. albo-atrum from sunflower, potato, tomato, safflower, and cotton on sunflower varieties under greenhouse conditions

Sunflower Variety	Verticillium isolates 39	4-3	119[b/]	GR	T-1	SVA	V-82	V-81
			Average disease index					
Peredovik	-	1.0	-	-	-	-	-	-
Peredovik (Blacklaw Sel.)	1.0	0.3	-	-	-	-	-	-
VNIIMK 8931	2.0	2.2	-	-	-	-	-	-
VNIIMK 1646	2.0	1.0	-	-	-	-	-	-
Kubanec	3.3	1.0	0.2	-	-	1.3	-	-
Vostok	1.0	-	-	-	0.5	-	-	-
Armavirec	1.0	1.0	-	-	0.2	-	-	-
Smena	2.0	5.0	0.3	-	-	-	-	-
Krasnodarets	3.3	1.5	-	-	0.3	-	-	-
Mingren	5.0	5.0	-	-	0.5	-	-	-
Commander	5.0	3.0	-	-	-	0.3	-	0.3
Arrowhead	1.3	5.0	-	-	3.0	-	-	-
Greystripe	5.0	5.0	-	-	2.6	2.0	0.2	-
Manchurian	1.3	3.0	-	-	-	-	-	-
Lyng-Manchurian 26	0.6	1.5	-	-	-	-	-	-
Mennonite	4.0	1.5	-	1.5	-	0.2	-	-
Lethbridge-159	1.3	-	-	-	-	-	-	-
HO-1	0.6	0.3	-	-	0.3	0.3	-	-
Lyng Hybr. I	3.0	1.0	-	-	-	1.3	0.3	0.3
CM-162	5.0	5.0	3.0	1.0	3.0	1/3	0.3	0.3

a/ Disease index (number of infected plants X severity class ÷ number inoculated plants) calculated for each isolate-host interaction in 3 greenhouse experiments. Severity class: 5, plants killed; -, no symptoms.

b/ V. dahliae.

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