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RELATIVE SUSCEPTIBILITY AND RESISTANCE OF SUNFLOWER (Helianthus annus L.) TO Fusarium sp., Phymatotrichum omnivorum and Macrophomina phaseolina

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A disease survey conducted in the area of Uvalde, Texas, in 1971, showed that irrigated plantings of sunflowers (Peredovik) were affected by a disease complex consisting of a wilt inciting Fusarium sp., Phymatotrichum omnivorum (Shear) Duggar, the Texas root rot fungus, and Macrophomina phaseolina (Tassi) Goid. (1) the charcoal rot fungus. A previous report indicated the Fusarium sp. as being the primary etiological agent (2). Early disease symptoms were not observed as the plantings were surveyed while maturing. A that time, plant mortality was high in large spots in fields where cotton had previously sustained significant losses. Surviving plants had brown to pink discoloration of the xylem of lower stems and roots and slight or moderate root rot. Occasionally, fungal strands of P. omnivorum and M. phaseolina were seen on the exterior of the roots and lower stems. A common feature of diseased plants was poor head development and shriveled seed. Heat rot caused by Rhizopus sp. or/and Botrytis sp. was infrequent. Although some diseased plants remained erect, most had collapsed.

In the southwestern United States, P. omnivorum reportededly (3) infects non-cultivated Helianthus species. M. phaseolina is destructive to the cultivated sunflowers particularly in warm soils throughout the south. Fusaria have not been thus far recognized as a serious sunflower pathogen. Elsewhere, F. solani in Argentina and Fusarium sp. in Peru have been isolated from sunflowers. T. Vrebalov, Agricultural Research Institute, Novi Sad, Yugoslavia, recently announced a destructive sunflower disease complex consisting of Macrophomina phaseolina, Fusarium sp. and other soil fungi at Novi Sad (4).

The purpose of this study was to determine the pathogenic behavior, with respect to sunflowers, of the isolates of Fusarium sp., P. omnivorum, and M. phaseolina as well as the possibility of identifying sources of resistance against this disease.

MATERIALS AND METHODS

Isolates

Fungal isolates used in pathogenicity studies conducted at Beltsville, Md., were Fusarium sp., P. omnivorum, and M. phaseolina obtained from random samples of diseased sunflowers collected at the farms of J. Miyakawa, O. Strube, and R. Nelson and at the varietal trial planting of S. Smith in the Uvalde, Texas, area.

These isolates were grown on potato-dextrose-agar in petri plates, and inoculum was prepared by homogenizing the cultures in a ratio of 3 plate cultures/100 ml water. Inoculum consisting of reciprocal mixtures of these isolates was prepared in equivalent ratios for each isolate. Inoculum was also prepared by suspending the conidia of Fusarium in water at an approximate density of 10^6/ml. This Fusarium isolate is on deposit at the American Type Culture Collections, Rockville, Md., as Accession 22782.

PATHOGENICITY TESTS

Sunflower plants used in these tests were seedlings up to 15 days in age grown from surface-disinfected seed and older plants up to 40 days in age grown from non-disinfected seed. The inoculation methods used were the following :

1 - For seedling reaction to either of the three isolates, the roots were dipped in an homogenate or the conidial suspension and the seedlings were replanted, four per pot.

2 - For older plant reaction, plants growing in pots were inoculated as follows :

a) for reaction to either of the three isolates, by partially uncovering the roots and pouring an homogenate or the conidial suspension upon them,

b) for reaction to the Fusarium isolate alone, by injecting the conidial suspension into the stem just below the growing point or above or below the cotyledonary node,

c) for reaction to M. phaseolina alone by placing an agar culture disc 1 cm in diameter cut out from a growing culture, on the wounded or non-wounded stem and supporting it with film and aluminum foil.

The inoculated plants were kept in the greenhouse or in an environment-controlled room with 14-hr photoperiods, day and night temperatures of respectively 18 and 24 C and fluorescent-incandescent illumination of 1 600 ft-c intensity.

EVALUATION OF DISEASE REACTION

Sunflower genotypes evaluated for disease reaction were 59 inbred lines from College Station, Texas. Test plants inoculated were seedlings and older plants prior to flowering by the methods described. A disease index was calculated for each genotype.

RESULTS

Disease symptoms incited by means of root-dip inoculation (Method 1) of sunflower plants with homogenates of Fusarium, P. omnivorum and M. phaseolina or with reciprocal mixtures of these isolates are described (2). Even though differences in symptom expression were not readily discernible in preliminary root-dip inoculations with conidial suspension of the Fusarium alone or in mixture with the other two pathogens, the percentage of seedling plants killed by Fusarium alone or by Fusarium in mixture were greater than the percentages of plants killed by either P. omnivorum or M. phaseolina alone (Table 1). M. phaseolina when used in root-dip inoculation (method 1) caused, in most cases, only cortical necrosis and chlorosis and occasionally seedling blight. The percentage of plants killed by the mixture of P. omnivorum and M. phaseolina were unsuspectedly low. In general, symptom-response of older plants to inoculation with fungal homogenates (method 2-a) was less severe than symptom-response of seedlings similarly inoculated. Stem injection (method 2-b) of older plants with the standardized conidial suspension of Fusarium brought about foliar wilt and leaf distortion. Although most plants recovered, some underwent severe wilt and died soon after flower initiation. Stem inoculation with M. phaseolina above the cotyledonary node (method 2-c) caused the collapse of both wounded and non-wounded stems. Stem inoculation below the node was often ineffective.

Disease reaction, expressed as disease index, of the 59 sunflower genotypes inoculated in the seedling stage with homogenates of the two fungal isolates are given in Table 2. From this material 23 genotypes were found to have considerable seedling resistance to the Fusarium, whereas the remaining genotypes varied in susceptibility from moderate to high. These genotypes were also resistant to Fusarium when

older, in the pre-flower stage. All genotypes had root and basal stem resistance to M. phaseolina, but with the exception of the CM 303 selections, namely HA 37, HA 89, HA 90, HA 94 and the V 3883-2-1 selections, namely HA 123 and HA 124 none had stem resistance above the cotyledonary node. All genotypes tested were highly or moderately susceptible in the seedling stage to P. omnivorum and quite variable in reaction when older in the pre-flower stage.

DISCUSSION

Results of controlled inoculation of sunflowers with Fusarium sp., Phymatotrichum omnivorum, and Macrophomina phaseolina isolated from sunflowers and with reciprocal mixtures of these isolates indicated that the Fusarium was the primary etiological agent of the disease at Uvalde, Texas in 1971. Foliar chlorosis and early killing of seedling sunflowers and of certain tomato cultivars inoculated with this Fusarium suggests that its pathogenicity may be related to the production of a toxin. The apparent pathogenic synergism between Fusarium and P. omnivorum as suggested by the data (Table 1) may not, however, be a common field problem. Optimum high soil temperature for the growth of P. omnivorum, which is apparently also required by M. phaseolina, does not usually prevail until the plants are older and less prone to attack. Soil temperature requirements for Fusaria are, however, usually wider in range. Pathogenic synergism between Fusarium and P. omnivorum may nevertheless occur with sunflowers grown under predisposing environmental conditions that may affect the host-pathogen interaction. In the case of sunflower infection by P. omnivorum alone, delayed seed germination and late planting may result in significant losses. Conversely, the low percentage of plants killed by the P. omnivorum - M. phaseolina mixture may have been related to fungal antagonism. Root-dip inoculation with M. phaseolina, which in most cases incited only cortical necrosis and foliar chlorosis, did not bring about consistent differences in disease reaction. It is therefore apparent that besides the pathological histology of the disease, the relation of fungal toxins and cellulolytic and pectolytic enzymes secreted by the fungus ought to be considered in the disease syndrome as a measure of resistance or susceptibility. The differentiation, within the same sunflower plant or within a single genotype, of root reaction and upper stem reaction to M. phaseolina is promising but is yet to be correlated to resistance or susceptibility under natural infection conditions. The identification of sunflower genotype possessing seedling resistance to Fusarium reported here may aid the sunflower program of breeding for disease resistance. Earlier tests (2) indicated also that Peredovik (66), P-21 VR2 X HA 60, P-21 ms X HA 60, and Romania RS-52 were less affected by the Fusarium.

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Table 1 - Average percentages of Peredovik sunflower seedlings killed by means of root dip inoculation with fungal homogenates of Fusarium sp., Phymatotrichum omnivorum, and Macrophomina phaseolina or with reciprocal equivalent mixtures of these fungi. Averages of four experiments in an environment-controlled room.

Inoculant	Non-wounded	Wounded (1)
M	0	7.6
P	37.6	66.6
F	54.0	75.0
P + M	12.0	16.6
F + M	56.0	8.3
F + P	87.5	100.0
Controls	0	0

(1) Lower stem punctured with a needle four times

Table 2 - Disease reactions, expressed as disease index(1) of sunflower selections inoculated by dipping the roots in homogenates of Fusarium sp. and Phymatotrichum omnivorum. Average of two greenhouse tests.

HA n°	1969 Source	F. sp.	P. o.	HA n°	1969 Source	F. sp.	P. o.
61/2604-T1							
HA 60(2)		4.5	4.0	HA 62	2 607	4.2	5.0
953-88-3 X A3497							
HA 61	2 413	5.0	4.0	63	2 609	3.2	4.0
"	2 415	3.8	4.5	"	2 610	5.0	5.0
"	2 421	4.2	2.5	"	2 612	3.7	5.0
"	2 422	1.7	4.0	C 64-112			
"	2 513	3.8	5.0	HA 67	2 619	0.5	3.0
"	2 514	1.7	4.5	70	2 623	2.6	4.5
62	2 521	3.1	5.0	CM 303			
"	2 522	2.5	5.0	HA 87	4 209	0.2	3.2
"	2 523	3.0	5.0	89	4 211	0.2	2.4

(1) Disease Index (Number of infected plants X severity class : number of inoculated plants) calculated for each genotype in two greenhouse experiments. Severity class : 5, plants killed ; 0, no symptoms. Disease reaction : (Disease Index of : 0-1.0, resistant ; 1.1-2.0, moderately resistant ; 2.1-3.0, moderately susceptible ; 3.1-4.0, susceptible ; 4.1-5.0, highly susceptible.

(2) Sources of ten HA 60 selections : 11, 2 208, 2 218, 2 219, 2 222, 2 223, 2 307, 2 311, 2 320 and 2 321.

Table 2 (continued)

HA n°	1969 Source	F. sp.	P.o.
CM 303 (continued)			
HA 90	4 212	0.2	3.5
91	4 213	0.2	2.0
92	4 214	0.2	3.2
93	4 215	1.8	4.0
94	4 216	0.1	2.5
95	4 218	0.1	2.0
97	4 220	0.1	2.2
98	4 221	0.3	2.5
99	4 222	0.3	0.5
100	4 223	0.5	3.5
101	4 307	0.1	2.0
104	4 310	0.5	2.5
V 1646-8-41-1-1			
HA 107	4 314	4.0	5.0
108	4 315	3.7	4.5
109	4 316	2.3	4.0
Smena 2 X RR			
HA 232	3 420-B	0.4	4.5
234	3 422-B	1.8	3.5
235	3 423-B	0.7	4.5
V 1646-8-41-1-1			
HA 110	4 318	1.1	4.3
111	4 319	1.8	4.0
112	4 321	1.9	5.0
113	4 322	1.7	5.0
114	4 407	0.6	4.5
J 8281-1-4-3			
HA 115	4 410	0.6	4.5
116	4 411	1.8	5.0
117	4 412	0.1	5.0
121	4 419	0.4	5.0
V 8883-2-1			
HA 122	4 420	1.8	3.5
123	4 421	0.9	2.5
124	4 510	1.5	4.5
125	4 511	1.8	3.5
126	4 512	0.5	3.5