NEW METHODS OF TESTING RESISTANCE OF SUNFLOWER TO SCLEROTINIA SCLEROTIORUM /LIB./ DE BARY

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SUMMARY

Resistance tests with one single inoculum cannot give convincing resistance results. For this the plant material on different pathogenicity levels should be tested, so the deviations can be neutralized and the results are more precisely founded. The toothpick method, which was earlier seldom in use (KURNIK et al. 1978), may have a higher significance in the future. The same is valid for the use of mycelial suspension, being an easy and rapid way to increase inoculum, and make largescale inoculation.

INTRODUCTION

The diseases of sunflower caused by Sclerotinia sclerotiorum /seedling blight, stalk rot and head rot/ continously hazard the sunflower fields all over the world. Inspite of the efforts to create more resistant hybrids the results are not very encouraging. In the background we might suppose different problems. Here is the most important the pathogenicity and resistance expression relationship as it was outlined for wheat and corn fusariosis /MESTERHÁZY 1982, 1987/. Among isolates of Sclerotinia sclerotiorum there ara significant differences in morphology, formation of sclerotia /HUMPERSON-JONES and COOKE 1977/; also differences in pathogenicity were recorded, but no evidence for physiologic specialization was found (PRICE and COLHOUN 1975). Possibly this explains the very close electrophoretic relations between isolated (WONG end WILLETS 1975). We had, however, no information about the effect of the level of pathogenicity on the expression of resistance to the disease. Therefore the study of this problem was set into the center of this paper during tests in the greenhouse and field conditions, conducted in 1986 and 1987.

MATERIALS AND METHODS

Plant material, inbred lines and hybrids, originates from the Oil Crop Department of the CRI in Szeged. Isolates of the fungus were received from sclerotia collected from different parts of the country. The sclerotia were washed in top water, afterhat kept in 70 % alcohol for 5 minutes and the remaining alcohol was burned down. The sterilized pieces were placed on PDA agar in test tubes. The fungus has grown out in several daysand within one weak produced sclerotia again.
For the inoculation material different methods exist. Many authors work with ascospores produced on sclerotia under specific conditions. Others use ready collected sclerotia for soil infestation /PIRVU et al. 1985/. PRICE and COLHOUN /1975/ grown the fungus on wheat-oat seed mixture, and this served as inoculum. We, following the description of MESTERHÁZY /1977/, filled Erlenmeyer flasks with Czapek-Dox fluid medium (2/3rd) and supplemented with a glas tube to the bottom of the flask. After an autoclave treatment (2 bar, 1h) was inoculated with a needle and over a week sterile air was pressed through the liquid. The result is a highly concentrated mycelial suspension which was ready for use after blendoring. This suspension served as inoculum for the greenhouse test and with

this we infestated the toothpicks 3 days befor inoculation.
This was repeated several times according to the developmental phases of the genotypes in the field. This was necessary as the pathogenicity could be kept much more constant by this way as compared with the pathogenicity of this inoculum grown 2 weeks on toothpicks where the fungus lest pat ogenicity rather rapidly.
In the greenhouse test we used the method of MESTERHÁZY /1984/. Here ton pots of 7 cm diameter were filled 2/3rd with perlit. Before infestation the perlit was irrigated with distilled water. Thereafter the surface of the perlit was infestated with inoculum 1 ml/cm^2. On this pathogen film five seeds were placed. Four such replicates served for a treatment. For the greenhose test original inoculum and dilutions were 1:1, 1:2, 1:4 or 1:16, somewhat differing in various tests. For evaluation emergency, ratio of killed plants/also not germinated ones considered/ plant height, dry matter production, root length and root infection were taken into account. Later also mesocotyl infection was evaluated.
For the stalk inoculation the infestated toothpicks were inserted into the stalk at soil level into a hole made with an awl one week before flowering, (R3 stage by SCHNEITER and MILLER 1981).
For head inoculation the same toothpick method was used, it was inserted into the middle of the head et lemon developmental stage (R7).
For statistical analysis variance and correlation analyses were used.

RESULTS

In the first test two genotypes and 3 isolates (No.1,8,11) with different pathogenicity were used. Beside this the inocula were mixed in every possible way, so we had 7 inocula in the test. The results are shown in Table 1. Looking the date pairs of the two genotypes by different characteristics and isolates as well as isolate mixtures we find different reactions. Sometimes the A (HRO) genotypes is less attacked, sometimes the B(PR 97). This means that one single isolate is not enough to say something about differences in resistance. But the means for the varieties give a much more consequent picture, the genotype A is more susceptible for every parameter tested, added that this is not significant for root infection severity. The variance analyses show a significant isolate effect except the dry matter production where it is below the limit for P=5%: The effect of the dilutuion is significant, e.g. more diluted suspensions are less pathogenic. Its interaction with the genotype or isolate is mostly not significant. The two more replicates of this test brought the same consequences (Table 2). The mixing of the isolates gave generally more pathogenic suspensions, but there are also exceptions. It is sure, that the pathogenicity will not be equal with the arithmetical mean of the pathogenicities of the isolates mixed.
In the next experiment 13 genotypes were tested et normal and three diluted inocula of the isolates in four replicates (No 101,102,106). Here we presentonly the data of the data of liwing plants at the evaluation of the experiment.
Highly significant differences were found between genotypes, also the pathogenicites differed significantly and the dilution effect was remarkable, too. Considering the genotype by isolate and genotype by dilution interactions we can state that the genotype main effect is significantly differening from these. This means that the genotype differences are influenced by these effects, but fun-

damental changes do not occur. The dilution by isolate interaction is highly significant, but its value differing significantly from the main effects. The point is that the dilution differently influences the pathogenisity of the isolates. Also here we should state that the mean data for the more isolates give a more reliable picture than a single isolate would it do.
The similar picture is shown for stalk base infection, where 12 genotypes were inoculated with four isolates (No.1,5,7,20) in three replicates. The pathogenicity differences are large, but we should also recognize that the reactions of the genotypes to different isolates are not the same. This is shown also by the correlation coefficients, where values between r= 0,3171 an r= 0,6906 were calculated.
The same conclusions can be drawn from the head rot test, too. Again 13 genotypes (Isolaty 107,109,100) were used as in the previous test. It is interesting that the genotypes by isolate interaction has not been significant in this case.

DISCUSSION

From the four tests with seedlings and adult plants tested for resistance against different isolates or isolates mixtures we can conclude that 1/ the expression of the resistance will be influenced by the pathogenicity of the fungus isolates used. This is in full agreement with the results on wheat and corn inoculated with Fusarium spp. /MESTERHÁZY 1981, 1982, 1983 and 1987/. The reaction differences wary from isolate to isolate, therefore test based on a single cannot reflect real genetic differences for amount of resistance and differences in resistance.
The results of the test of mixed isolates /2/ showed that a mixture is not better or worse than a single isolate. This agrees well with the results received of wheat Fusaria /MESTERHÁZY 1977/. Conclusion: a misture of the isolates cannot replace the test on different pathogenicity levels. A very old and settled conviction become an illusion. We can draw the same consequenc e from the data RODINA /1987/ who found similar phenomena in wheat inoculated with isolates of Septoria nodorum. The mycelial suspencion is a good inoculating agent and easy to work with. It can be produced in large amount according to the needs of the tests. It depends only on the volume of the glass ballon you have.
The toothpick method combined with the infestation of the toothpicks by mycelial suspension helped to keep the pathogenicity of the inoculum stable during the inoculation period about two weeks.

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Table 2 Reaction of two sunflower inbreds to Sclerotinia sclerotiorum isolates and isolate mixtures measured by different characteristics in the greenhouse. Variance analyses, MQ values.

Source of variance	MQ-values for Living plants	Germination	Plant height	Root length	Root infection	Dry matter content
Isolate/A/	3,79^{+}	7,11^{+++}	1474,16^{+++}	17o4,6o^{+}	27,59^{+++}	1o78,54
Inbred/B/	6,11^{+}	17,72^{+++}	4327,14^{+++}	98o3,56^{+++}	17,61	2576,13^{++}
Dilution/C/	16,53^{+++}	76,6o^{+++}	9233,31^{+++}	54o8,97^{++}	39,55^{+++}	7233,65^{+++}
A x B	3,3o^{+}	5,66^{++}	885,13^{+}	16o6,39^{+}	1o,6o	131o,93^{+}
A x C	2,18	2,83^{++}	645,43	9o7,67	8,22	79o,o7
B x C	2,12	3,11	1221,84^{+}	14o8,64	4,32	817,63
A x B x C	1,96	1,9o	977,65^{++}	847,57	9,52^{+}	843,65
Error	1,49	1,31	368,57	664,67	5,3o	568,36

$^{+++}$P = o,1 %; $^{++}$P = 1 %; $^{+}$P = 5 %

Table 1 Reaction of two sunflower inbreds against Sclerotinia sclerotiorum isolates and isolate mixtures measured by different characteristics in the greenhouse

Isolate No.	No. of living plants at evaluation			Germination, %			Plant Height, % to the check			Root length, cm			Root infection, scale o-1o			Dry matter content, % to the check		
	Genotype																	
	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$	A	B	$\bar{x}$
1	o,68	1,37	1,o3	1,62	2,5o	2,o6	11,9o	26,oo	18,95	8,71	29,26	18,98	9,25	7,51	8,38	12,61	3o,25	21,43
8	1,37	o,7o	1,13	3,o6	2,31	2,69	26,41	22,o9	24,25	18,8o	17,15	17,98	8,o3	8,75	8,39	27,23	17,5o	22,36
11	1,31	2,12	1,72	2,81	2,93	2,88	24,8o	42,67	33,75	2o,49	5o,97	35,73	6,o5	6,26	6,16	22,44	38,44	3o,44
1 + 8/1:1/	o,62	o,94	o,78	1,81	2,56	2,19	14,66	22,3o	18,18	11,99	27,45	19,72	9,o1	7,83	8,42	14,78	18,79	16,78
1 + 11	o,12	1,31	o,72	o,62	2,44	1,53	2,4o	21,33	11,86	3,19	3o,59	16,89	9,81	7,69	8,75	2,86	25,7o	14,28
8 + 11	o,62	o,94	o,78	1,44	2,56	2,oo	11,76	25,98	23,38	1o,oo	16,8o	13,4o	9,o9	8,63	8,86	1o,79	16,2o	13,79
1 + 8 + 11 /1:1:1/	1,12	o,69	o,94	2,62	2,62	2,62	26,7o	19,91	16,42	19,48	13,o7	16,27	8,26	8,91	8,59	24,91	16,2o	2o,55
Means	o,85	1,18	1,o2	2,oo	2,56	2,28	16,97	25,76	21,37	13,24	26,47	19,86	8,5o	7,94	8,22	16,51	23,3o	19,91
LSD 5 %	o,84	o,84	o,59	o,79	o,79	o,57	13,3o	13,3o	9,41	17,87	17,87	12,76	1,6o	1,6o	1,13	16,52	16,52	11,8o
LSD 5 % for genotype means	o,32			o,3o			8,77			6,82			o,87			6,31		

Table 3 Reaction of sunflower inbreds and hybrids to three isolates of Sclerotinia sclerotiorum in the greenhouse. Data: surviving living plants, means.

Genotype	Isolate No 1o1	No.1o2	No 1o6	Mean
CHO x PR 97	2,62	1,44	2,94	2,33
PR 97	4,43	1,87	3,44	3,25
HRO	3,19	1,44	2,68	2,44
HRO x GA	2,75	1,44	3,25	2,48
P 2	3,87	2,25	3,31	3,15
P 1	4,81	2,12	3,93	3,63
CHO	3,5o	1,o6	2,25	2,27
PJ 2	3,81	3,56	3,87	3,75
PJ 6	3,12	4,62	3,12	4,29
CHO x P 1	3,69	1,87	3,31	2,96
TMA x 51/P 2	4,o6	2,25	3,69	3,33
TMA x 51/PJ 6	4,81	4,25	4,62	4,56
TMA x 51	4,43	3,75	4,75	4,31
Mean	3,78	2,46	3,48	3,24
LSD 5 %	o,2o	o,2o	o,2o	o,42

Variance analysis

Source of variance	SQ	DF	MS	F
Total	221o,o8	623	3,55	
Replicate	2,65	3	o,88	
Genotype/A/	358,29	12	29,86	$11,79^{+++}$
Isolate/B/	225,38	2	112,69	$44,49^{+++}$
Dilution/C/	475,72	3	158,57	$62,61^{+++}$
A x B	1o4,67	24	4,36	$1,72^{+}$
A x C	193,o7	36	5,36	$2,12^{++}$
B x C	165,1o	6	27,52	$1o,86^{+++}$
A x B x C	182,36	72	2,53	$2,34^{+++}$
Error	5o2,85	465	1,o8	

$^{+++}$P = o,1 %; $^{++}$P = 1 %; $^{+}$P = 5 %

Table 4 Reaction of hybrids and inbreds to Sclerotinia stalk base rot as a % related to the control.

Genotype	Isolates 1	5	7	2o	Mean	Head diameter % to the check
CHO x PR 97	34,52	92,22	42,45	52,o8	55,32	65,93
PR 97	26,71	78,13	28,13	2,o8	33,76	75,49
HRO	46,19	91,11	51,58	36,21	56,27	61,86
HRO x GA	51,48	91,11	41,67	47,78	58,o1	63,32
P 2	27,3o	93,27	34,39	22,oo	44,21	61,23
FE x P 2	58,33	95,24	67,2?	5o,75	67,29	54,45
FE x P 1	71,41	97,22	31,67	2,22	5o,62	65,9o
PJ 2	12,77	69,27	17,21	13,81	28,26	73,87
GK343 x PJ 2	6o,84	81,48	49,97	51,o6	6o,84	55,62
GK343 x PJ 6	62,18	1oo,oo	47,5o	33,41	6o,77	56,4o
GK343	47,22	83,33	2o,81	37,oo	47,o9	66,95
PJ 6	37,78	52,64	22,96	1,96	28,32	79,64
Mean	44,73	85,42	37,96	29,2o	49,33	65,o6

ISD 5 % for genotypes 12,64
LSD 5 % for isolates 7,3o
LSD 5o% for interact. 25,27

Variance analysis

Source of variance	SQ	DF	MS	F	F_{AxB}
Genotype/A/	22869,o6	11	2o79,o1	$8,51^{+++}$	$5,o1^{+++}$
Isolate/B/	66895,17	3	22298,39	$91,25^{+++}$	$53,72^{+++}$
A x B	13696,81	33	415,o6	$1,7^{+}$	
Error	23459,17	96	244,37		
Total	126920,22	143			

$^{+++}$P = o,1 %; $^{++}$P = 1 %; $^{+}$P = 5 %