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CHOOSING A SUITABLE TECHNIQUE FOR SCREENING SUNFLOWER
FOR RESISTANCE TO CHARCOAL ROT IN CLIMATIC CONDITIONS OF YUGOSLAVIA

By

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Summary

Different sunflower inoculation techniques were tested in field and greenhouse in 1976 and 1977 in order to determine the resistance to Macrophomina phaseoli Ashby. The following four techniques were tested in field:

- Toothpick method (Young, 1943)
- Suspension injecting (Czapek's liquid medium)
- Introduction of inoculi into soil during planting
- U.S.B.I. technique (Sackston and Chan, 1967)

C.H. Hsi's technique for sorghum was tested in greenhouse.

Introduction of fungi into plant tissue (toothpick, injecting) yielded unsatisfactory results because these techniques equalized the values for resistant and susceptible lines. Introduction of inoculi into the soil rendered unreliable results because of a poor competitiveness of the fungus and differences in pathogenicity among the isolates. U.S.B.I. technique differentiated resistant and susceptible lines only in conditions of high temperatures and less than 350 mm of rainfall. Satisfactory results were obtained by C.H. Hsi's technique for sorghum tested in greenhouse.

As neither technique offered completely satisfactory results it was decided that in our further work on the determination of sources for resistance to Macrophomina phaseoli Ashby we use comparative results of the toothpick method, U.S.B.I. technique, and C.H. Hsi's technique for sorghum.

Macrophomina phaseoli Ashby.(= Sclerotium bataticola Taub.), the agent of charcoal rot in sunflowers, is an important disease agent in Yugoslavia. In the region with high temperatures and low soil moisture (Macedonia), M. phaseoli is the principal cause of premature drying up of sunflowers. If the climatic conditions favor the development of the pathogen, the disease may become an epiphytotic -- the number of infected plants may range between 50 and 90% which decreases the yields by 20-50% (Acimovic, 1962).

Tests of sunflower cultivars grown in natural conditions indicated differences in the resistance among the tested cultivars (R.G. Orellana, 1970). We conducted a five-year study on the field resistance of the sunflower to M. phaseoli. The tested material varied considerably regarding this character-- 214 lines of different genetic origin remained uninfected. Field observations suggested that charcoal rot could be controlled by breeding for resistance.

As the disease does not occur regularly in any particular location, the planting of the material in infected plots is not a reliable test for resistance.

Materials and Methods

Considerable variability in the population of M. phaseoli regarding its pathogenicity, morphological and other characters, the prevalence of Sclerotinia sclerotiorum in some regions, low competitiveness of the pathogen, and a small possibility to control the limiting factors in field moved us to comparatively test the techniques of inoculation.

In 1976 and 1977, we tested a number of inbreds and hybrids for resistance to M. phaseoli using different techniques of inoculation in various climatic conditions.

The experiments were conducted in two climatically different localities (Table 1) -- at the Institute of Field and Vegetable Crops in Novi Sad and at the Agro-Industrial Combine Ovce Polje in Macedonia. Besides, an experiment in a greenhouse was also conducted in Novi Sad.

The experiments were conducted according to the system of randomized blocks, in four replications. Twelve plants were inoculated in each replication. For the toothpick method, we inoculated 24 plants in each replication.

Two susceptible lines, S-200 and S-1214, and VNIIMK 8931 were used as the control in the experiments.

Four techniques of inoculation were tested in field and one in greenhouse. The following techniques were tested in field:

- Toothpick method (Young, 1943)
- Suspension injecting (Czapek's liquid medium)
- Introduction of inoculi into the soil during planting
- U.S.B.I. technique (Sackston and Chan, 1967)

C.H. Hsi's technique for sorghum was tested in greenhouse.

The plants to be tested had been raised in a greenhouse during winter, at 25-27 C during the 16-hour light period and at 19-21 C during the 8-hour dark period.

"Unwounded stem inoculation" was performed by placing 3 gr of cornmeal sand inoculum around the basal part of the stem. The culture of M. phaseoli used for inoculation was seven days old.

The results were statistically calculated.

Results

The techniques based on the mechanical introduction of fungi into the stem and head tissue (toothpick method, suspension injecting) yielded unsatisfactory

results. Being insufficiently selective, these two techniques could not successfully differentiate the values for resistant and susceptible lines (Table 2).

TABLE 2. Resistance of S-lines to Macrophomina phaseoli Ashby - Toothpick Method. Locality: Novi Sad

Line Number	Number of plants		Total	% of Infected Plants	Resistance	Field Observation
	R	S				
S-15	8	71	79	90	S	R
S-77	12	57	69	83	S	R
S-150	12	65	77	84	S	MR
S-200	4	60	64	94	S	S
S-331	9	64	73	88	S	R
S-450	13	59	72	82	S	R
S-1214	1	70	71	99	S	S
cms-9	10	63	73	86	S	S
cms-13	9	59	68	87	S	MR
cms-43	9	66	75	88	S	MR
VNIIMK 8931	12	67	79	85	S	S

S = susceptible, R = Resistant

Post-infectional complications (S. sclerotiorum, Fusarium sp.) in the conditions with more than 550 mm of rainfall cause death of many test plants allowing these techniques to be used only in controlled conditions for determination of the sources of specific resistance.

Introduction of inoculi into the soil during planting in field renders unreliable results because of a poor competitiveness of the fungus and differences in pathogenicity among the isolates. The results of this technique were similar to those obtained by the previous two techniques.

The U.S.B.I. technique efficiently differentiates resistant and susceptible lines but only in conditions of high temperatures and less than 350 mm of rainfall (Table 3).

The results obtained in the experiments with inoculation approach in the values the results of field observations.

Satisfactory results were obtained when testing the breeding material by C.H. Hsi's technique for sorghum in the greenhouse. However, we cannot restrict ourselves to only this technique because of a limited space in greenhouses and the lack of vitality in the plants grown there.

After the analysis of the obtained results, we concluded that neither one of the techniques included into the experiment did not offer satisfactory results. Thus we decided to perform another comparative test of the following three techniques to be used in our search for the sources of resistance to Macrophomina phaseoli Ashby.:

- Toothpick method (Young, 1943)
- U.S.B.I. technique (Sackston and Chan, 1967)
- C.H. Hsi's technique for sorghum (1961)

Literature Cited

- ACIMOVIC, M., 1962. *Sclerotium bataticola* as a casual agent of sunflower wilt in Vojvodina. *Sastita bilja* 69-70:125-138.
- ACIMOVIC, M., 1964. The occurrence of *Macrophomina phaseoli* on some agricultural crops and morphological and ecological properties of the parasite. *Savr. polj.* 12:55-56.
- ALABOUVETTE, C., and P. BREMEERSCH, 1976. *Macrophomina phaseoli*, parasite du tournesol: etat actuel de nos connaissances at orientation des recherches. *Informations Techniques* 52:13-17.
- DIAZ, R.J., 1977. Personal correspondence. Catedratico de Patologia Vegetal, Cordoba, Spain.
- HSI, C.H., 1961. An effective technique for screening sorghum for resistance to charcoal rot. *Phytopathology* 51:340-341.
- MATHER, S.B., and W.E. SACKSTON, 1963. Effect of temperature and age of host on infection of sunflower by *Sclerotium bataticola*. *Phytopathology* 58:137:140.
- ORELLANA, R.G., 1970. The response of sunflower genotypes to natural infection by *Macrophomina phaseoli*. *Plant Disease Reporter* 54:891-893.
- SACKSTON, W.E., and Y. CHAN, 1969. Mechanisms of pathogenesis in *Sclerotium bataticola* on sunflowers. Production and translocation of a necrosis-inducing toxin. *Canadian Journal of Botany* 7:1147-1151.
- SACKSTON, W.E., and Y. CHAN, 1973. Penetration and invasion of sunflowers by *Sclerotium bataticola*. *Canadian Journal of Botany* 5:999-1002.

TABLE 1. Meteorological data for the Growing Season of 1977.

Month	Mean monthly t°C			Rel. air hum.			Precipitation			No. of rainy days		
	Novi Sad	Ovce	Polje	Novi Sad	Ovce	Polje	Novi Sad	Ovce	Polje	Novi Sad	Ovce	Polje
March	9.3	8.0		71	65.4		54.6	17.0		11	5	
April	10.2	11.1		69	58.0		58.8	16.2		10	5	
May	17.0	16.8		65	60.2		52.3	57.5		12	12	
June	19.9	20.7		65	54.7		84.3	33.2		10	5	
July	20.5	23.5		70	50.2		49.2	14.0		14	4	
August	20.1	21.7		74	55.7		107.0	28.6		10	5	
September	14.4	15.8		72	65.1		34.6	57.7		7	10	

TABLE 3. Resistance of S-Lines to *Macrophomina phaseoly* Ashby - USBI Method

Locality: Ovce Polje										Locality: Novi Sad				
Field Obser- vation	Resis- tance	Infected Plants	Total	No. Plants		Line Number	No. Plants		Total	Infected Plants	Resis- tance	Field Obser- vation		
				%			S	R					S	R
R	R	8	49	4	45	S-31	44	2	46	4	R	R		
R	R	12	43	4	38	S-59	43	7	50	14	R	R		
S	S	96	47	45	2	S-200	48	10	58	17	MR	S		
R	S	29	31	9	22	S-451	38	3	41	7	R	R		
S	S	96	50	48	2	S-1214	43	7	50	14	R	S		
R	R	6	49	3	46	B-7	47	5	52	10	R	MR		
R	R	9	47	4	43	B-16	33	1	34	3	R	R		
MR	S	35	54	19	35	NS-H-26	47	5	52	10	R	MR		
MR	MR	24	59	14	45	NS-H-27	43	4	47	9	R	MR		
S	S	55	58	32	26	NV11MK 8931	40	6	46	13	R	S		

S = susceptible R = Resistant