Macrophomina phaseolina - One of The Main Pathogens of Sunflower Crop in Romania

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Abstract

This paper presents the plant host range of *Macrophomina phaseolina* fungus enlarged with new host cultivated species as alfalfa , rape, cotton, sesame, sorghum , potato, okra pods, green pepper. Also, some biological aspects concerning this fungus were studied *in vitro* conditions: growth on different culture media, the influence exerted by abiotic factors on fungal growth and sclerotia development. By using cross inoculations, the virulence of the isolations obtained from different host plants possibilities are discussed which imply the joint use of the general methods of integrated control: technological, biological and chemical ways.

Introduction

Sunflower is for Romania as well as for many other countries, the main source of edible oil and that is why it ranks among the most widely cultivated crops.
Getting yields below the biological potentiality of the cultivars is due to unfavourable conditions, to shortcomings in applying the technologies and mostly to pathogens attack. The fungus *Macrophomina phaseolina*, is considered as a pathogen of debility which can, under certain pedoclimatic circumstances as well as based on the cultivars sensibility, cause major losses (Alabouvette & Bremeersch, 1975, Bekesi 1981).
The rich enzymatic endowment gives the fungus the characteristic of a parasite for various species of both cultivated plants or from spontaneous flora and that makes it difficult to establish forecasting and attack control measures.
So far, in Romania, charcoal rot has been evidenced on sunflower, soybean, bean (Bontea, 1985,1986), sugarbeet (Puscasu, 1988), castor oil (Ciurea et al.) and faba bean (Puscasu et al. in press).

Materials and methods

In order to know the range of host plants, observations were done in many production fields where plants with the rot symptom were drawn from.
Isolation and fungi purification were done on PDA; influence of abiotic factors on fungus growth was observed on synthetic media and with natural extracts, at temperatures ranging between 15^0 and $30\ ^0C$ and pH values of 3-10.
The virulence of *Macrophomina phaseolina* as obtained from different hosts was registered under controlled conditions, by means of crossed inoculations.
Being quite a virulent and aggressive pathogen, therefore a real danger for many cropping plants, there were studied elements which might be the basis of a new integrated control system these being: density, planting time, biological control. As with sunflower have been registered the most severe damages, under the conditions in Romania, the experiments were carried out with this crop. The influence of genotype and of some agrophytotechical factors as well as the attack frequency have been registered for three experimental years under natural infection conditions, for which Turbo and Select hybrids were planted in two different periods, at densities ranging between 40 and 70,000 plants/ha. The attack frequency was also recorded.

From the rhizosphere of tomato plants which had been cultivated in lasting monocropping, isolations were made on King B medium. Fluorescent isolates of *Pseudomonas* were tested for protection activity and also for enhancing sunflower plants growth (Florom 350 hybrid).
Bacteria were cultivated on KO liquid medium shaking it horizontally; after 24 hours they were centrifugated for 20 minutes at 5000 rpm and the bacterial mass was resumed on phosphate buffer pH 6.8. The titre was adjusted to 10^7 cell/ml. Sunflower seeds treated by dipping (soaking) for 30 minutes were planted in soil artificially inoculated with *M. phaseolina*. Observations were carried on for the number of sound plants emerged after 7 and 14 days form planting as well as on the dry weight of plants (14 days following planting).

Results and discussion

Based on the observations performed in the field, on the isolations from harvest biological material and also of the pathogenity test, the fungus *M. phaseolina* was first evidenced in Romania on new cropping plants ant that is: on winter oil rape, sesame, saffron, pumpkin, alfalfa, cotton, potato, sorghum, okra, cucumbers and sweet pepper.
It was considered by studying the influence of certain abiotic factors on *M.phaseolina* that the fungus developed very well and it also formed out many sclerotia both by sublayers with natural potato and bean extracts and on synthetic ones (Leonian and Czapek) on which the sclerotia had the largest size (Table 1). The abundant formation of sclerotia on agar with onion decoction or with oats flour as well as on PDA was also observed by Acimović (1963) and Waseer et al. (1990).

Table 1

Cultural characteristics of *Macrophomina phaseolina* on different culture media
(3 days following inoculation)

Culture medium	Isolate	Colonies characteristics				
		form	colour	diameter (cm)	no. sclerotia (mm^2)	sclerotia size μ
Leonian	M_1	round	light grey	8.33	61.91	97.0x58.0
	M_2	round	dark grey	7.54	52.86	87.3x67.9
Czapek	M_1	stellated	dark brown	6.53	39.23	87.3x87.3
	M_2	stellated	dark brown	4.97	23.05	145.5x97.0
PDA	M_1	round	dark grey	9.00	75.15	87.3x87.3
	M_2	round	brown	9.00	72.17	97.1x97.1
Bean extract	M_1	round	light grey	9.00	72.17	77.6x77.6
	M_2	round	light grey	9.00	70.48	77.6x67.9
Soil extract	M_1	round	greyish white	8.67	53.57	77.6x77.6
	M_2	round	greyish white	7.35	49.55	97.1x83.3

No matter the host plant, *in vitro* the fungus developed very well and it formed sclerotia at temperatures ranging between 15^0-30^0C, with an optimum between 25^0 and 30^0C (Table 2). Acimovic (1963), working with soya isolates reached the conclusion that the optimum temperature is 30^0C and Waseer et al. (1990) at 35^0C.
M.phaseolina tolerated a large range of pH (Table 3) and showed a good development from slightly acid values to alcaline values, with an optimum at pH 6; thus the results confirm those obtained by Ratnoo and Bhatnagar (1991).
Following the artificial inoculations crossed with isolates from various cropping plants, it could be observed that the highest virulence was shown by the soya isolate, the attack frequency being 63% for castor bean and 89% for soya (Table 4).

Plants density was positively correlated with attack frequency of *M.phaseolina*, the highest values being registered at the hybrids under study, at densities of 60-70 thousands plants/ha (Table 5). That is due both to plants weakness following competition and also to maintaining a warm and humid microclimate favourable for fungus development.

Although between the two planting periods there was only one month difference, at the end of flowering stage when observations were done, no significant differences could be noted with respect to the percentage of sick plants (Table 6).

Table 2

Influence of temperature on *Macrophomina phaseolina* isolated from different cropping plants (PDA, pH 6,5)

Isolates source	Temp. °C	Colony growth (cm) 2 days	4 days	6 days	8 days	Sclerotia formation in: 2 days	4 days	6 days	8 days
Sunflower	15	2.9	4.1	5.4	8.1	-	+	+	++
	20	3.9	6.3	8.2	8.9	+	+	++	+++
	25	6.9	8.7	9.0	9.0	+	++	+++	+++
	30	7.4	9.0	9.0	9.0	+	+++	+++	+++
Castorbean	15	3.1	4.6	6.4	8.7	-	+	++	+++
	20	4.3	6.9	9.0	9.0	+	++	+++	+++
	25	7.9	9.0	9.0	9.0	++	+++	+++	+++
	30	7.6	9.0	9.0	9.0	++	+++	+++	+++
Soya	15	3.3	4.6	6.4	8.6	-	-	+	++
	20	4.7	7.1	8.7	9.0	+	+	++	+++
	25	8.2	8.9	9.0	9.0	++	+++	+++	+++
	30	8.9	9.0	9.0	9.0	++	+++	+++	+++
	DL 5%	1.34	1.15	1.09	0.95				
	DL 1%	2.22	1.42	1.50	1.58				
	DL 0.1%	4.31	3.74	2.10	2.95				

Table 3

Development of *Macrophomina phaseolina* at different pH values on Leonian medium

pH	Sunflower isolate (M1) Colony diameter (cm) 2 days	6 days	Biomass (mg)	Soya isolate (M2) Colony diameter (cm) 2 days	6 days	Biomass mg
3.0	-	-	80.660^{ooo}	-	-	80.74^{ooo}
4.0	2.93^{ooo}	6.400^{ooo}	87.28^{ooo}	2.44^{ooo}	6.28^{ooo}	89.64^{ooo}
4.5	2.97^{oo}	7.79^{ooo}	99.34	2.69^{oo}	7.54^{ooo}	102.22
5.5	3.44	9.00	103.22	3.35	9.00	105.08
6.0	3.68	9.00	102.08	3.21	5.81^{ooo}	102.40
6.5	3.17	7.20^{ooo}	100.86	2.61^{ooo}	6.78^{ooo}	99.00
7.0	2.95^{ooo}	6.44^{ooo}	97.76°	2.28^{ooo}	5.84^{ooo}	93.74^{ooo}
7.5	2.76^{ooo}	6.19^{ooo}	97.82°o	2.38^{ooo}	5.35^{ooo}	91.30^{ooo}
8.0	2.29^{ooo}	5.40^{ooo}	22.58^{ooo}	2.17^{ooo}	5.18^{ooo}	96.08°
9.0	1.98^{ooo}	5.19^{ooo}	86.86^{ooo}	2.22^{ooo}	5.37^{ooo}	85.58^{ooo}
10.0	1.41^{ooo}	4.35^{ooo}	79.54^{ooo}	2.44^{ooo}	4.76^{ooo}	78.82^{ooo}
5 (mt)	3.37	8.70	101.36	3.06	8.84	102.24
DL 5%	0.23	0.33	3.23	0.22	0.23	4.67
DL 1%	0.31	0.44	4.32	0.30	0.31	6.26
DL 0.1%	0.41	0.58	5.66	0.40	0.41	8.19

Table 4

Virulence of certain isolates of *Macrophomina phaseolina* in crosssed inoculations

Isolate	Attack frequency (%) on: Sunflower	Soya	Castorbean	Bean	Maize
Sunflower	72.5	76.5	29.5	39.0	42.5
Soya	89.0	75.0	63.0	65.5	59.5
Castorbean	63.5	64.5	71.5	64.0	45.0
Bean	80.0	83.5	26.5	76.5	42.0
Maize	59.5	46.0	52.5	65.5	75.0
DL 5%	8.35	5.24	9.68	7.26	3.65
DL 1%	13.34	7.98	14.05	10.67	5.74
DL 0.1%	15.86	9.74	18.96	14.68	7.89

Table 5

Influence of genotype and certain agrophytotechnical factors on attack frequency of *Macrophomina phaseolina* with sunflower

(field, natural infection)

Hybrid	Density plants/ha	Attack frequency (%) 1992	1993	1994	Yield (kg/ha) 1992	1993	1994
Turbo	40,000	46.5	69.9	68.8	1930	2310	2410
	50,000	59.6	75.4	75.5	2040	2214	2250
	60,000	63.5	77.4	79.5	1820	2109	2040
	70,000	64.3	89.5	92.7	1710	2110	2000
Select	40,000	49.1	46.4	46.9	2118	2150	2100
	50,000	54.4	52.5	48.2	2200	2010	2100
	60,000	55.3	55.6	59.1	2055	1980	1930
	70,000	52.4	59.5	55.7	1890	1860	1830
Turbo	optimum	53.7	63.8	61.3	1990	2200	2130
Select	period	54.0	52.9	59.3	2190	2100	2150
Turbo	when plants in 1st period	55.0	58.9	60.3	2000	1980	2090
Select	have 2-4 true pairs of leavs	54.8	55.7	59.6	2200	2050	1990
DL 5%		2.63	9.83	4.28	130.15	44.18	124.63
DL 1%		3.42	15.93	6.05	203.65	63.16	212.45
DL 0.1%		7.05	22.15	11.86	340.18	89.17	405.01

In case of strains RR 19 and RR 105, plantlets protection against fungus attack from soil could be observed 7 days after treatment, while with RR 3c, RR 7c, RR 20c and RR 73, although they had distinct and very significant differences compared to the control, their activity was manifest only 14 days after treatment application. Under such circumstances, one may suppose that a long lasting process of adjusting the microbian balance from rhizosphere in favour of saprophyte bacteria is implied, and it is not the matter of an antibiotic direct activity on the pathogen. The strains RR 7c, RR 73 and RR 105 favoured plants growth expressed by the increase of dry weight with distinct and very significant values. If the

activity of the inoculants lasts to 40-45 days after planting, as Hebbar et al. (1991) observed, the efficient strains could be used to prepare biological products.

Table6

Influence of seed treatment with strains of fluorescent pseudomonases of sunflower plantlets (greenhouse, 1995)

Variant	Plant percentage (%)				Dry weight of plant in 14 days (g)	
	emerged in 7 days		sound in 14 days			
	Average	Difference	Average	Difference	Average	Difference
Non inoculated soil	23.67	-5.33^{000}	20.67	-5.33	304.66	- 22.00
Inoculated soil with *M. phaseolina*	29.00	-	26.00	-	326,66	-
Chemically treated seed	27.33	-1.67	28.00	2.00	390.00	63.34**
RR 21	27.00	-2.00	26.33	0.33	354.00	27.34
RR 23	31.00	2.00	25.00	-1.00	350.33	23.67
RR 3c	30.33	1.33	30.00	4.00**	361.66	35.00
RP 7c	28.67	-0.33	28.67	2.67*	440.66	114.00**
RR 20c	27.00	-2.00	30.67	4.67***	391.66	65.00**
RR 19	31.67	2.67*	32.67	6.67**	355.33	28.67
RR 73	30.00	1.00	29.33	3.33**	437.66	110.00***
RR 105	32.00	3.00*	31.00	5.00***	369.66	43.00*
	DL 5%	2.59		2.35		36.34
	DL 1%	3.52		3.19		49.38
	DL 0.1%	4.78		4.33		66.94

Conclusions

- *M.phaseolina* was for the first time evidenced in Romania on new hosts: oil seed rape, sesame, saffron, pumpkin, alfalfa, cotton, potato, sorghum, faba bean, cucumbers, green pepper;
- *M.phaseolina* developed very well and it formed plenty of sclerotia on media with natural extracts, at temperatures ranging between 15^{0} and 30^{0}C with an optimum value at 30^{0}C and it tolerated pH values of 3-10, with an optimum around neutral value;
- the isolates drawn from sunflower, castorbean, soya, bean, maize showed different virulence, the most virulent being the one from soya;
- the great density of plants is a factor which favours attack frequency;
- planting period did not influence significantly the attack frequency;
- seed treatment with strains of fluorescent pseudomonases is useful and it can be also an element of integrated control when a long lasting efficacity up to 40-45 days is observed.

Bibliography

Aćimović, M., 1963 - *Sclerotium bataticola* as a parasite of Soybean in Yugoslavia. Savr. Polijopr., 11(4):271-280.

Alabouvette, C.; Bremeersch, P., 1975 - Les degâts provoqués par *Macrophomina phaseolina* dans les cultures de Tournesol. Def. Veg., **173**: 125-131.

Bekesi, P., 1981 - A napraforgo makrofominas szartorothadasa. Magyar Mezogazdasag, **36**:7.

Bontea, Vera, 1985, 1986 - Ciuperci parazite si saprofite din România. Vol.I,II. Edit.Acad.RSR, Bucureşti.

Ciurea, A.; Oprea, M.; Cojocaru, D., - Două boli păgubitoare pentru ricin şi sorg în România. Anal. ICPP, vol.XXIV, : 77-84.
Puscasu, A., 1988 - Putrezirea cărbunoasă - o nouă boală a sfeclei în România. Buletin Prot. Plant., 1(2): 13-15.
Hebar, P., Berge, O., Heulin, T., Singh, S.P., 1991 - Bacterial antagonists of sunflower (Helianthus anuus L.) fungal pathogens. Plant and Soil, 133: 131-140.
Puscasu, A., Ionita, A., Iordache, E.,- 1990 - Bobul - o gazdă nouă pentru *Macrophomina phaseolina* în România.(in press)
Ratnoo, R.S.; Bhatnagar, M.K., 1991 - Effect of temperature and pH on growth and sclerotia formation of *Macrophomina phaseolina*. Ind. J. Mycol. Plant Pathology, 21(3): 279-280.
Waseer, N.A.; Pathan, M.A.; Wondiar, M.; Solangi, G.R., 1990 - Studies on charcoal rot of soybean caused by *Macrophomina phaseolina* Tassi (Goid.). Pakistan J. Phytopathology, 1990 publ. 1992, 2(1-2): 22-30.