

THE EFFECT OF PHYLLOSHERE MICROORGANISMS ON THE HEALTHINESS OF ABOVEGROUND PARTS OF SOYBEAN (*Glycine max* (L.) Merrill)

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Summary. The studies were conducted in the years 1998–2000 on soybean, cv. Polan. The purpose of the studies was to conduct microbiological and mycological analyses of the leaves and pods of soybean. Bacteria and fungi were obtained much more often from the diseased soybean parts as compared to the healthy ones. 778 fungi isolates were obtained as a result of the mycological analysis of the leaves and pods of soybean. *Fusarium* spp. and *Phomopsis sojae* were most frequently isolated within pathogenic fungi. Their proportion was 17.5% and 7.8% of all the fungi, respectively. On the other hand, the isolated saprophytic fungi included for example the species from the genera of *Acremonium*, *Cladosporium*, *Gliocladium*, *Penicillium* and *Trichoderma* as well as *Aureobasidium pululans* and *Epicoccum purpurascens*. Besides, about a four times lower number of antagonistic microorganisms (*Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.) was isolated from the infected soybean parts in comparison to the number of microorganisms isolated from the healthy parts.

Key words: phyllosphere, soybean, antagonistic microorganisms, pathogenic fungi

INTRODUCTION

The phyllosphere of plants is a dynamic ecosystem inhabited by specific bacteria, yeasts and fungi. Their activity is related to various interactions between the biotic and abiotic factors of the environment [Behrendt et al. 1997, 1999]. The interactions of microorganisms living on the surface of the plants' aboveground parts are based on antibiosis, competition and parasitism [Chakraborty et al. 1994, Markellou et al. 1995, Sobiczewski et al. 1996, Stromberg et al. 2000, Larena et al. 2002, Sobiczewski 2002]. A positive role is played by phyllosphere antagonistic microorganisms, which protect the plants from pathogenic microorganisms and in this way improve their healthiness.

It follows from the information contained in the literature on the subject that the aboveground parts of soybean are infected by such fungi as *Fusarium* spp., *Alternaria* spp., *Colletotrichum dematium* (Pers. ex Fr) Grove var. *truncata* (Schw.), *Peronospora*

manshurica (Naum.) Syd., *Septoria glycines* Hemmi, *Phoma exigua* Desm. var. *exigua*, *Phomopsis sojae* Lehman and *Sclerotinia sclerotiorum* (Lib.) de Bary [Sinclair and Backman 1989, Boerema and de Gruyter 1998, Pidi et al. 1999, Pięta et al. 1999]. These fungi are especially dangerous when inhabiting the pods and seeds of soybean because they contribute to a considerable drop of the emergencies as well as worse healthiness of the seedlings and plants in the later developmental stages.

A considerable role in limiting the populations of these pathogenic fungi inhabiting the aboveground parts of plants is played by antagonistic microorganisms. Such properties are first of all exposed by the bacteria from the genera of *Bacillus* and *Pseudomonas* and by the fungi *Trichoderma* and *Gliocladium* [Ahmed et al. 2000, Fiddman et al. 2000, Hag and Khan 2000, Kredics et al. 2000, Mc Quilken et al. 2001].

The accessible literature of the subject does not provide any information concerning the relations within the populations of phyllosphere microorganisms of soybean, that is why studies were undertaken the aim of which was to search for the microorganisms that are antagonistic towards the pathogens of this plant and those that have a positive effect on its healthiness.

MATERIALS AND METHODS

The studies were conducted on soybean, cv. Poland in the years 1998–2000 at the Experimental Station of Czesławice near Nałęczów. They concerned the leaves of soybean from the second and third floors in the period of anthesis and the pods in the phase of maturation.

In each year of the studies both the plant material that did not show any disease symptoms and that with clear necrotic spots were subjected to microbiological analysis according to the method described by Sobiczewski et al. [1991]. Besides, the leaves and pods of soybean were subjected to mycological analysis, according to the method described by Pięta [1988] in order to establish the species composition of the fungi occurring on those parts. The bacteria and saprophytic fungi obtained from the microbiological analysis were used to determine their effect on the fungi pathogenic to soybean, such as *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *Phomopsis sojae* and *Sclerotinia sclerotiorum*, according to the methods described by Patkowska [2001].

The results concerning the numbers of bacteria and fungi occurring in the phyllosphere of soybean were statistically analysed, and the significance of differences was established on the basis of Tukey's confidence intervals [Oktaba 1987].

RESULTS AND DISCUSSION

The composition of the populations of microorganisms inhabiting the phyllosphere of soybean was very differentiated like it was in the case of studies devoted to the phyllosphere of winter wheat [Wachowska 2000].

The microbiological analysis of soybean leaves and pods showed that the total number of bacteria in 1 g of fresh weight ranged from $2.19 \cdot 10^6$ to $6.32 \cdot 10^6$ colonies (tab. 1).

Bacteria from the genera of *Bacillus* and *Pseudomonas* inhabited the soybean leaves with necrotic spots in greater numbers than the infected pods. The number of *Bacillus* spp. on the surface of the studied parts ranged from $0.52 \cdot 10^6$ to $3.81 \cdot 10^6$ colonies, while the number of *Pseudomonas* spp. ranged from $1.36 \cdot 10^6$ to $2.24 \cdot 10^6$. The total number of fungi obtained from the surface of the examined leaves and pods ranged from $18.70 \cdot 10^3$ to $54.28 \cdot 10^3$ colonies, and they were isolated twice as often from the infected parts than from the healthy ones (tab. 1).

Table 1. Number of bacteria and fungi in the phyllosphere of soybean (mean from the years 1998–2000)

Tabela 1. Liczebność bakterii i grzybów występujących w fyllosferze soi (średnia z lat 1998–2000)

Number of bacteria and fungi Liczebność bakterii i grzybów	Leaves – Liście		Pods – Strąki	
	with spots z plamami	without spots bez plam	with spots z plamami	without spots bez plam
Total number of bacteria (mln · g ⁻¹ fresh weight) Ogólna liczebność bakterii (mln · g ⁻¹ świeżej masy)	6,32 ^b	2,19 ^a	4,28 ^B	3,79 ^A
Total number of <i>Bacillus</i> spp. (mln · g ⁻¹ fresh weight) Ogólna liczebność <i>Bacillus</i> spp. (mln · g ⁻¹ świeżej masy)	3,81 ^b	0,52 ^a	2,13 ^B	1,56 ^A
Total number of <i>Pseudomonas</i> spp. (mln · g ⁻¹ fresh weight) Ogólna liczebność <i>Pseudomonas</i> spp. (mln · g ⁻¹ świeżej masy)	2,24 ^b	1,36 ^a	1,95 ^A	2,05 ^A
Total number of fungi (thous. · g ⁻¹ fresh weight) Ogólna liczebność grzybów (tys. · g ⁻¹ świeżej masy)	54,28 ^b	20,15 ^a	43,39 ^B	18,70 ^A

Mean in lines differ significantly ($P \leq 0.05$), if they are not marked with the same letter

Średnie wartości w wierszach różnią się istotnie ($P \leq 0.05$), jeśli nie są oznaczone tą samą literą

As stated by Philipp [1988], the variety within the population of microorganisms inhabiting the phyllosphere of plants is related to different physiological properties of the host plant as well as by the interactions between the microorganisms. Besides, the changing environmental conditions such as humidity, temperature or sun exposure affect the number of bacteria and fungi inhabiting the phyllosphere of plants in different periods of their vegetation [Dickinson 1986, Ercolani 1991]. Behrendt et al. [1997] observed the high numbers of bacteria, especially *Pseudomonas* spp., in the phyllosphere of grasses.

778 fungi isolates were obtained as a result of the mycological analysis of soybean leaves and pods (tab. 2). Slightly more fungi (275 isolates from the leaves and 193 isolates from the pods) were obtained from the infected parts than from the healthy ones. *Fusarium* spp. and *Phomopsis sojae* were most frequently isolated within the pathogenic fungi. Their proportion constituted 17.5% and 7.8% of all the fungi, respectively. The fungi from the genera of *Fusarium* were represented by *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. solani* and *F. sporotrichioides*. On the other hand, the isolated saprophytic fungi included the species from the genera *Acremonium*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Gliocladium*, *Penicillium* and *Trichoderma* (tab. 2).

Within the microorganisms antagonistic towards the examined pathogenic fungi and inhabiting the surface of soybean leaves and pods, the dominating were bacteria *Pseudomonas* spp. (108 isolates) and saprophytic fungi *Gliocladium* spp. (45 isolates), *Penicillium* spp. (142 isolates) and *Trichoderma* spp. (121 isolates). About four times as many antagonistic microorganisms were obtained from the healthy plant parts than from the infected ones (tab. 3).

Table 2. Fungi isolated from leaves and pods of soybean (total from the years 1998–2000)
 Tabela 2. Grzyby wyizolowane z liści i strąków soi (suma z lat 1998–2000)

Fungus species Gatunek grzyba	Number of isolates – Liczba izolatów						total razem
	leaves – liście			pods – strąki			
	1	2	total razem	1	2	total razem	
<i>Acremonium kiliense</i> Grutz	2	-	2	1	-	1	3
<i>Acremonium murorum</i> (Corda) W. Gams	4	-	4	2	-	2	6
<i>Acremonium strictum</i> W. Gams	1	-	1	1	-	1	2
<i>Alternaria alternata</i> (Fr.) Keissler	12	2	14	10	3	13	27
<i>Alternaria tenuissima</i> (Fries) Wiltshire	5	1	6	2	-	2	8
<i>Aspergillus flavus</i> Link	3	-	3	1	-	1	4
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	37	10	47	21	8	29	76
<i>Botrytis cinerea</i> Pers.	6	-	6	4	-	4	10
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	15	3	18	7	1	8	26
<i>Cladosporium herbarum</i> (Pers.) Link	12	1	13	6	-	6	19
<i>Cladosporium sphaerospermum</i> Penzig	7	-	7	2	-	2	9
<i>Epicoccum purpurascens</i> Ehr. Ex Schl.	11	3	14	8	2	10	24
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	20	5	25	13	3	16	41
<i>Fusarium equiseti</i> (Corda) Sacc.	18	6	24	12	4	16	40
<i>Fusarium oxysporum</i> Schl.	16	3	19	11	2	13	32
<i>Fusarium solani</i> (Mart.) Sacc.	7	2	9	5	-	5	14
<i>Fusarium sporotrichioides</i> Sherb.	5	1	6	3	-	3	9
<i>Gliocladium catenulatum</i> Gilman et Abbott	3	15	18	1	7	8	26
<i>Gliocladium fimbriatum</i> Gilman et Abbott	2	9	11	-	5	5	16
<i>Gliocladium roseum</i> Bainier	-	2	2	-	1	1	3
<i>Humicola fuscoatra</i> Traaen	-	-	-	1	-	1	1
<i>Humicola grisea</i> Domsch	-	-	-	1	-	1	1
<i>Mucor hiemalis</i> Wehmer	2	-	2	1	-	1	3
<i>Mucor racemosus</i> Fresenius	1	-	1	1	-	1	2
<i>Penicillium decumbens</i> Thom	4	8	12	2	5	7	19
<i>Penicillium janczewskii</i> Zaleski	6	11	17	3	8	11	28
<i>Penicillium janthinellum</i> Biourge	7	10	17	4	6	10	27
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling) Samson, Stolk et Hadlok	5	13	18	2	10	12	30
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i> Samson, Stolk et Hadlok	8	16	24	5	9	14	38
<i>Phoma eupyrena</i> Sacc.	4	-	4	10	-	10	14
<i>Phoma exigua</i> Desm. var. <i>exigua</i>	9	-	9	15	2	17	26
<i>Phomopsis sojae</i> Lehman	27	1	28	30	3	33	61
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	5	-	5	4	-	4	9
<i>Torula herbarum</i> (Pers.) Link ex Fr.	1	-	1	2	-	2	3
<i>Trichoderma aureoviride</i> Rifai	-	3	3	-	1	1	4
<i>Trichoderma hamatum</i> (Bon.) Bain	1	6	7	-	2	2	9
<i>Trichoderma harzianum</i> Rifai	2	5	7	-	2	2	9
<i>Trichoderma koningii</i> Oud.	4	19	23	-	8	8	31
<i>Trichoderma polysporum</i> (Link ex Pers.) Rifai	1	11	12	-	4	4	16
<i>Trichoderma pseudokoningii</i> Rifai	-	8	8	2	3	5	13
<i>Trichoderma viride</i> Pers. ex S.F. Gray	2	28	30	-	9	9	39
Total – Razem	275	202	477	193	108	301	778

1 – with spots – z plamami

2 – without spots – bez plam

Table 3. Antagonistic microorganisms isolated from leaves and pods of soybean (total from the years 1998–2000)

Tabela 3. Mikroorganizmy antagonistyczne wyizolowane z liści i strąków soi (suma z lat 1998–2000)

Bacteria and fungi Bakterie i grzyby	Number of isolates – Liczba izolatów				total of isolates suma izolatów
	leaves – liście		pods – strąki		
	1	2	1	2	
<i>Bacillus</i> spp.	19	32	13	28	92
<i>Pseudomonas</i> spp.	7	66	4	31	108
Total bacteria – Razem bakterie	26	98	17	59	200
<i>Gliocladium catenulatum</i> Gilman et Abbott	3	15	1	7	26
<i>Gliocladium fimbriatum</i> Gilman et Abbott	2	9	-	5	16
<i>Gliocladium roseum</i> Bainier	-	2	-	1	3
<i>Penicillium</i> spp.	30	58	16	38	142
<i>Trichoderma aureoviride</i> Rifai	-	3	-	1	4
<i>Trichoderma hamatum</i> (Bon.) Bain	1	6	-	2	9
<i>Trichoderma harzianum</i> Rifai	2	5	-	2	9
<i>Trichoderma koningii</i> Oud.	4	19	-	8	31
<i>Trichoderma polysporum</i> (Link ex Pers.) Rifai	1	11	-	4	16
<i>Trichoderma pseudokoningii</i> Rifai	-	8	2	3	13
<i>Trichoderma viride</i> Pers. ex S.F. Gray	2	28	-	9	39
Total fungi – Razem grzyby	45	164	19	80	308

1 – with spots – z plamami

2 – without spots – bez plam

Information from the literature on the subject points to the effect of *Trichoderma* spp., *Gliocladium* spp., *Bacillus* spp. and *Pseudomonas* spp. in the protection from pathogenic fungi [Ahmed et al. 2000, Fiddman et al. 2000, Mc Quilken et al. 2001]. These microorganisms can be used as components of biopreparations that limit the occurrence of the populations of pathogenic microorganisms [Seasan et al. 1998, Singh and Mukhopadhyay 2000, Sobiczewski 2002].

The results suggest that the presence of antagonistic bacteria and fungi on the surface of the studied soybean parts limits the growth of pathogens and has a positive effect on the healthiness of leaves and pods.

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WPLYW MIKROORGANIZMÓW FYLLOSFEROWYCH NA ZDROWOTNOŚĆ ORGANÓW NADZIEMNYCH SOI (*Glycine max* (L.) Merrill)

Streszczenie. Badania przeprowadzono w latach 1998–2000 na roślinach soi odmiany 'Polan'. Przedmiotem badań były liście i strąki soi, które poddano analizie mikrobiologicznej i mikologicznej. Z chorych organów soi znacznie częściej, aniżeli ze zdrowych, uzyskiwano bakterie i grzyby. W wyniku analizy mikologicznej liści i strąków soi uzyskano 778 izolatów grzybów. W obrębie grzybów chorobotwórczych najczęściej wyisobnionymi okazały się *Fusarium* spp. i *Phomopsis sojae*, bowiem ich udział stanowił odpowiednio 17,5% i 7,8% wszystkich grzybów. Natomiast spośród grzybów saprotroficznych izolowano m.in. gatunki z rodzajów *Acremonium*, *Cladosporium*, *Gliocladium*, *Penicillium* i *Trichoderma* oraz *Aureobasidium pullulans* i *Epicoccum purpurascens*. Ponadto z porażonych organów soi izolowano około czterokrotnie mniej mikroorganizmów antagonistycznych (*Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.), aniżeli ze zdrowych.

Słowa kluczowe: fyllosfera, soja, mikroorganizmy antagonistyczne, grzyby patogeniczne

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