

Methods of estimation of the pathogenicity of the fungus *Phoma exigua* var. *exigua*

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Abstract

During the period 1977-1981 33 isolates of *Phoma exigua* were recovered from diseased leaves, stems, root crowns and seeds of soybean cultivated in different localities of Poland. None of the obtained isolates caused disease symptoms on soybean and other plant species when they were inoculated by spraying with conidial suspension. Infection developed only on wounded plants after inoculation. *Phoma exigua* occurring on soybean in Poland is not a specialized pathogen of this plant, but a plurivorous weak or wound parasite which attacks all kinds of plants and so is *P. exigua* var. *exigua*. Among seven of the applied inoculation methods the best one seems to be the laboratory one, in Petri plates, with culture discs exposed on the seedling hypocotyl. Other methods varied in precision and were more time-consuming.

INTRODUCTION

On soybean plants (*Glycine max* ⟨L.⟩ Merrill) cultivated in Poland ⟨Marcinkowska et al., 1982⟩ and the USSR ⟨Bondarceva - Monteverde and Vasilevskii, 1940⟩, West Germany ⟨Frandsen, 1953⟩, Czechoslovakia ⟨Ondrej, 1976⟩ and Hungary ⟨Tóth and Kövics, 1978⟩ spots on leaves and pods as well as blight on seedlings and shoots of soybean were observed.

The cause of these diseases was described as *Ascochyta sojaecola* ⟨Abramov, 1931⟩, *A. sojae* ⟨Sawada, 1959⟩, *A. phaseolorum* ⟨Crossan, 1953; Melnik, 1977⟩ and lately as *Phoma exigua* ⟨Ondrej, 1976; Marcinkowska, 1983⟩.

Some investigators ⟨Abramov, 1931; Frandsen, 1953⟩ considered this fungus as a pathogen specific to soybean, others believed that this species may attack various plants ⟨Bondarceva - Monteverde and Vasilevskii, 1940; Crossan, 1953; Ondrej, 1976⟩. The question

arose whether the cause of leaf spot and blight on seedlings and soybean shoots in Poland is the polyphagous fungus of weak pathogenicity for soybean in Poland or whether it is a fungus infecting solely soybean. To find an answer to these questions the pathogenicity of a number of isolates obtained from the infected parts of soybean plants was tested by various inoculation methods in respect to soybean and other plant species.

MATERIAL AND METHODS

Fungus isolates for pathogenicity tests represented various morphological types of cultures. They were obtained in the period 1977-1981 from various infected parts of soybean plants cultivated throughout Poland (Marcinkowska, 1983). In the experiments 16 isolates from leaves, 11 from shoots, 2 from hypocotyls and one each from cotyledons, root neck and seed were studied. The fungus was kept on potato dextrose agar, PDA, at 5°C. Soybean plants of the variety 'Ajma' were infected. Usually all combinations included four replications. As replication served plants in one pot (5-6). During the experiments in the glasshouse temperature was maintained at 18-25°C and in the laboratory at 20-22°C as optimal temperature for the fungus development. The inoculated plants were covered for 48 h with plastic chamber to ensure 100 per cent relative air humidity. Later moisture was maintained within 65-85 per cent.

Germination capacity of the conidia was checked directly on sprayed leaves (Marcinkowska, 1977) and in hanging drops on concave slides parallelly with the experiment. The inoculum for plant and seed infection was prepared from 8- and 12-day culture on PDA. The concentration of conidia was 2 million spores in 1 ml of aqueous suspension. In the control combination the plants or seeds were treated with water, pure medium or used media were free from the fungus.

METHODS OF SOYBEAN PLANT INOCULATION

Method 1. Spraying of intact plants with conidial suspension

The plants were inoculated at the phase of 3rd pair of trifoliate leaves with conidial suspension of all the 33 fungus isolates. For each plant 1 ml of inoculum was used. The experiment was replicated four times.

Method 2. Rubbing soybean leaves with conidial suspension

Soybean leaves were powdered with carborundum at the phase of three trifoliate leaves and the inoculum was rubbed in with a cotton swab. The pathogenicity of 12 isolates obtained from soybean leaves was tested. Soybean

shoots were inoculated in the same way at the phase of the first pod. The shoots were inoculated with 11 cultures isolated from shoots.

The methods of spraying and rubbing the inoculum into the plants were used to investigate the influence of 100 per cent relative air humidity on infection of the plants and the further development of the disease. Cotyledones and leaves of seedlings in the phase of cotyledones and developing first leaves were inoculated with isolates from a leaf <L6>, hypocotyl <SK6>, and shoots <P9>. One half of plants inoculated by both methods was kept under foil chambers as usual for two days after inoculation, and a second one for six days to ensure 100 per cent relative air moisture.

Method 3. Drop-wise application of homogenate of culture on wounded shoot

The inoculum consisted of an aqueous suspension of conidia and mycelium from homogenized cultures with a part of the medium. A few drops of homogenate were placed on the lesion arising by pulling off three leaves of the central part of the plant at the phase of the first pod.

Method 4. Placement of culture discs on cotyledones

Discs of PDA cultures were placed on cotyledones of soybean seedlings. Then the mycelial disc was punctured with a sterile needle, wounding at the same time the cotyledone in one half of the inoculated plants. For a better development of the disease a 100 per cent relative air moisture was maintained for six days to the plants after inoculation.

METHODS OF INOCULATION OF SOYBEAN SEEDS

Seeds of the variety 'Ajma' healthy in appearance were disinfected after washing in water with a detergent, in ethyl alcohol <96%> and Perhydrol <15%> for 15 s. In each experiment 10 disinfected seeds were placed in a pot <replication>.

Method 5. Placement of seeds on infected substrate

Cultures of 13 fungus isolates from PDA were placed in pots filled to 3/4 of height with soil and peat. On each culture seeds were sown and covered with soil.

Method 6. Inoculation by contact in substrate with infected seeds

<D u n l e a v y, 1958>

The seeds were seeded in pots at a depth of 2 cm and lightly covered with soil <0.5 cm>. Over them were placed soybean seeds overgrown with mycelium of each of 12 isolates. The seeds serving as inoculum were covered with soil.

Method 7. Soaking of seeds in spore suspension

⟨Tòth and K ö v i c s, 1978⟩

Sterile seeds swollen after 48 h of soaking in dishes with moist filter paper were soaked for 30 min in a suspension of conidia from 12 isolates and then seeded in pots.

INOCULATION OF VARIOUS PLANT SPECIES

Various plant species were inoculated with isolates obtained from soybean by methods 1, 4 and 7. The experiments were to indicate the pathogenicity of the fungi isolated from soybean towards other plant species.

Spraying of intact plants with conidial suspension ⟨method 1⟩

Asters ⟨*Callistephus chinensis* Nees⟩, field bean ⟨*Vicia faba* subsp. *minor* Harz⟩, bean ⟨*Phaseolus vulgaris* L.⟩, pea ⟨*Pisum sativum* L.⟩, cabbage ⟨*Brassica oleracea* var. *capitata alba* L.⟩, maize ⟨*Zea mays* L.⟩, yellow lupin ⟨*Lupinus luteus* L.⟩, cucumber ⟨*Cucumis sativus* L.⟩, parsley ⟨*Petroselinum sativum* Hoffm.⟩, tomato ⟨*Lycopersicon esculentum* Mill.⟩, radish ⟨*Raphanus sativus* var. *sativus* Mansf.⟩, bird's-foot ⟨*Ornithopus sativus* L.⟩, vetch ⟨*Vicia sativa* L.⟩ and soybean in the phase of 3-5 leaves in dependence on the plant species were three time inoculated with 33 fungus isolates.

Placement of discs of cultures ⟨method 4⟩

In a glasshouse experiment discs of isolate cultures from leaves ⟨L6⟩ were placed on cotyledones or the two lowest situated leaves of field bean, bean, pea, lupin, cucumber, tomato, radish and soybean. Further procedure was the same as with soybean in method 4.

Laboratory experiments were performed in plates 12 cm in diameter. Into these plates lined with moist filter paper six healthy seedlings were placed with 2-3 cm sprouts. Discs of cultures were placed on injured hypocotyls of pea, lupin, cucumber, radish and soybean seedlings. For inoculation isolates representing various morphological types of cultures were used, that is L14 from leaves and SK6 from soybean root neck and PD 79/118 from chicory roots. All in five replications ⟨plates⟩.

Soaking of seeds in spore suspension ⟨method 7⟩.

The same plant species were inoculated as in the previous glasshouse experiment with a conidial suspension prepared from naturally infected shoots. The procedure was the same as with soybean in method 7.

RESULTS

RESULTS CONCERNING SOYBEAN PLANTS AND SEED INOCULATION

Method 1. After spraying of intact soybean plants with a spore suspension no disease symptoms were noted on any of the plants inoculated with the 33 isolates obtained from various soybean parts.

Method 2. On the leaves of soybean plants infected with 12 isolates from leaves and lesioned during inoculation by rubbing, brownish grey minute (2-3 mm) or larger (5-7 mm) diffuse spots appeared. The intensity of disease symptoms was estimated according to the number and size of the spots. It was highest with four isolates (L14, L18, L22, L24) and lowest for the isolate L10. The remaining seven isolates gave a moderate leaf infection, the leaf blades were destroyed in 20-30 per cent. On the two control plants spots were observed caused by mechanical damage to the tissue by rubbing. Prolongation of exposure time of the plants after inoculation to 100 per cent relative air moisture did not influence the disease symptoms on plants not subjected to lesions. Among the plants wounded with carborundum the number of infected plants was similar apart from incubation time under 100 per cent relative air humidity. For the isolate L6 the number of infected plants among the 24 inoculated was 22 and 23, for P9 — 19 and 20, for SK6 19 and 19, respectively. On the cotyledones and first leaves of plants incubated for 2 days there appeared, however, minute spots (ca. 2 mm), whereas on the cotyledones and first leaves of plants kept under the moist chamber for 6 days the spots were somewhat larger (ca. 3-4 mm in diameter) diffuse, with poorly delineated edges. All the infected plants had but few spots.

Methods 2 and 3. On the shoots of soybean plants damaged during inoculation by rubbing with a swab imbibed with inoculum brownish necrotic spots were visible on the shoots of most (83-100%) of the inoculated plants.

Table 1

Comparison of infection of soybean shoots inoculated
by various methods

Results for 11 fungus isolates	No. of plants with symptoms on shoots in relation to no. of plants inoculated by the method	
	of rubbing in conidial suspension	application of inoculum dropwise on lesion
Mean	21/24	15/24
Minimal	20/24	7/24
Maximal	24/24	23/24

Table 2

Influence of inoculation of cotyledones with discs of *Phoma exigua* var. *exigua* culture on infection of soybean seedlings

Fungal isolate	No. of dead seedlings as compared with no. of inoculated plants		No. of infected shoots of seedlings as compared with no. of inoculated plants	
	inoculation intact	cotyledones injured	inoculation intact	cotyledones injured
L2	14/28	17/28	1/28	1/28
L3	12/25	7/28	1/28	0
L6	19/24	13/28	5/24	0
P8	2/28	16/28	1/28	4/28
P9	4/24	4/24	7/24	6/24
K5	14/28	12/24	1/28	0
SK6	16/28	18/28	0	2/28

Table 3

Influence of various methods of inoculation of soybean seeds on seed germination and infection of seedlings

Fungal isolate	Methods of seed inoculation					
	Inoculation of substrate with:				Soaking of seeds in	
	culture of fungus		infected seeds		conidial suspension	
	no. of sprouting seedlings	no. of diseased seedlings	no. of sprouting seedlings	no. of diseased seedlings	no. of sprouting seedlings	no. of diseased seedlings
L2	13	0	20	0	22	2
L3	19	0	19	0	12	2
L6	17	1	16	0	20	3
L14	14	1	21	0	28	0
L21	17	2	20	0	24	4
L24	17	1	18	1	21	2
P8	17	0	22	0	24	2
P9	16	0	22	0	20	4
P23	21	0	23	0	23	4
K5	17	0	20	1	24	2
SK6	13	0	19	2	20	3
N43	16	1				
PD79/118	18	0	19	0	19	0
Control Sample	20	0	24	0	24	0

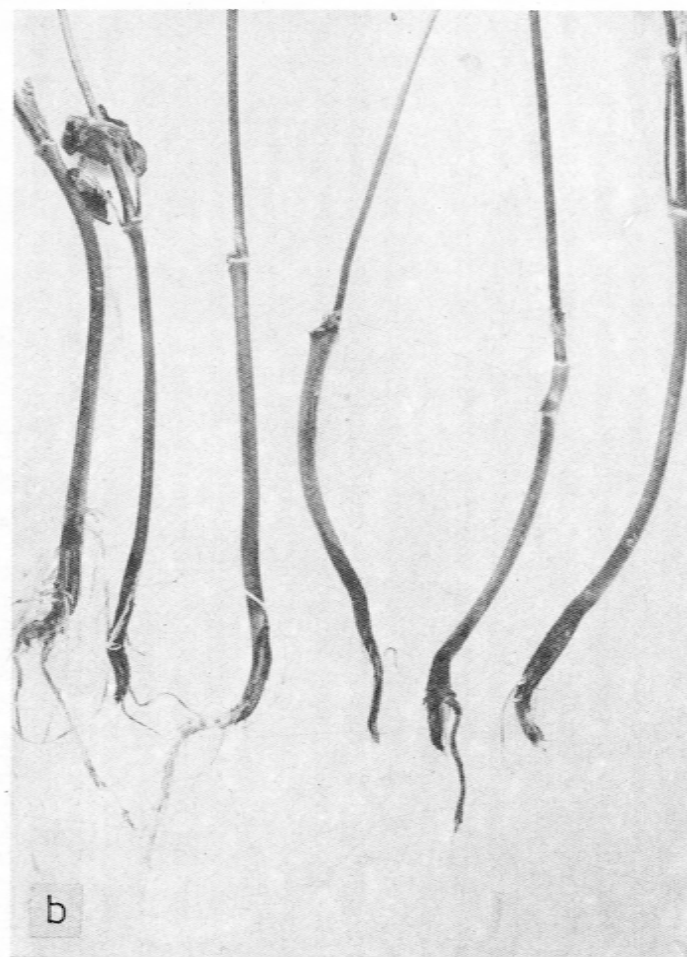
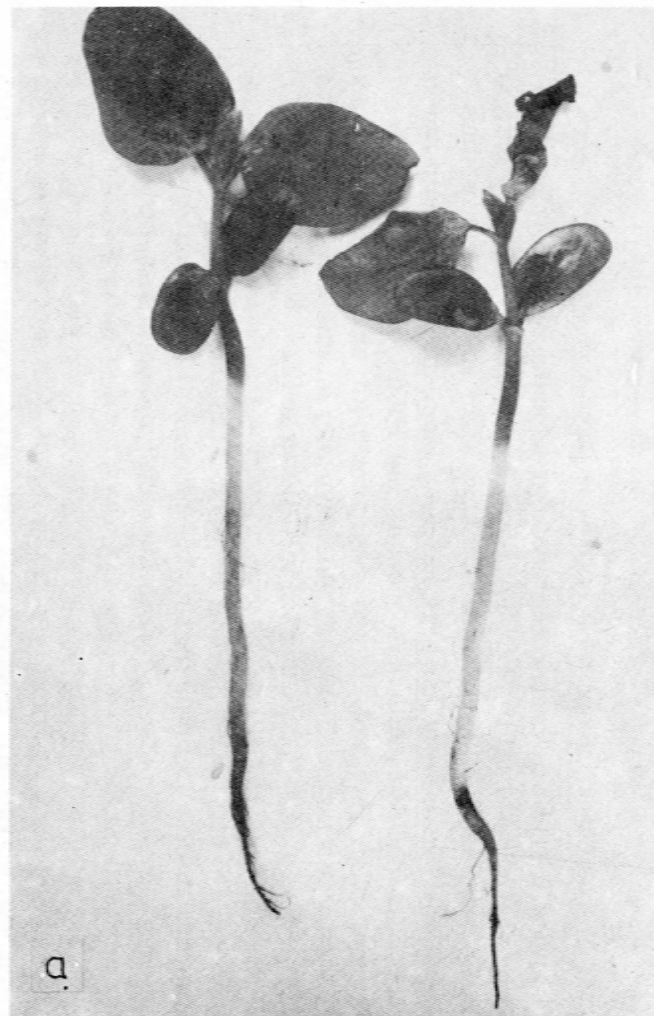


Fig. 1. Blight of root neck of soybean seedlings: a — symptoms on naturally infected plants, b — symptoms on inoculated plants

〈Table 1〉. As the result of shoot infection owing to application of inoculum dropwise at the site where the leaf was torn off there formed along the shoots on the surface brownish streaks. Disease symptoms appeared in 29-95 per cent of plants 〈Table 1〉. Sometimes only one or two streaks were observed.

Method 4. On some injured or unaffected soybean cotyledones disease symptoms appeared 〈Table 2〉. In part of the plants the fungus grew from the cotyledones on to the shoot. In the course of the experiment some seedlings died, others showed symptoms on the shoot, and others after shedding the infected leaves developed further as healthy plants.

Method 5. Most of the tested isolates decreased by 5-35 per cent soybean seed germination when the latter were placed on fungus cultures in the soil 〈Table 3〉. Only single seedlings, however, exhibited symptoms of surface blight of the root neck caused by five of the 13 tested isolates 〈Fig. 1〉.

Method 6. As the result of inoculation by contact of healthy seed in the substrate with seed overgrown with mycelium soybean germination decreased by 5-34 per cent in the case of all the 12 fungus isolates 〈Table 3〉. Blight of the root neck of the seedlings occurred only sporadically.

Method 7. Seeds soaked in a suspension of conidia of most isolates germinated worse than the control ones soaked in water 〈Table 3〉. Blight of the root neck developed only on the surface of seedlings. It was noted on several seedlings infected by 10 isolates of the 12 ones tested.

RESULTS CONCERNING INOCULATION OF VARIOUS PLANT SPECIES

Method 1. Intact aster, field bean, bean, pea, cabbage, maize, yellow lupin, cucumber, parsley, tomato, radish, bird's-foot, vetch and soybean plants sprayed with a suspension of 33 fungal isolates did not show any disease symptoms.

Method 4. Seedlings of field bean, bean, pea, lupin, cucumber, tomato, radish and soybean inoculated with discs of culture and left for six days under 100 per cent relative air moisture showed symptoms both on injured and intact cotyledones and leaves 〈Table 4〉. Nine days after inoculation some diseased seedlings died, others shed the infected cotyledones and developed further health, and in still others the fungus caused disease symptoms on the shoots, cotyledones and leaves. On many plants of field bean and bean and some soybean plants brown streaks were visible on the shoots, beginning with the node at which the dead leaf or cotyledone was situated. On radish and cucumber small necrotic spots appeared on the leaves at the site where the disc of fungus culture was placed. Similar spots were sporadically observed on pea plants.

In the laboratory experiments, where by means of discs of fungus culture the effectiveness of hypocotyl inoculation of seedlings of several plant species was tested, blight symptoms appeared on most of the inoculated plants 〈Table 5〉. The isolate PD79/118 from chicory *Cichorium intybus* L. produced usually

Table 4

Influence of leaf inoculation with discs of *Phoma exigua* var. *exigua* culture on seedling infection of some plant species under glasshouse conditions

Inoculated plant	No. of dead seedlings in relation to no. of inoculated plants		No. of infected leaves and shoots of seedlings in relation to no. inoculated plants	
	Leaf inoculation intact	injured	Leaf inoculation intact	injured
<i>Cucumis sativus</i>	11/28	6/30	3/28	4/30
<i>Glycinea max</i>	14/30	6/28	1/30	1/28
<i>Lupinus luteus</i>	28/30	24/28	0	3/28
<i>Lycopersicon esculentum</i>	1/30	4/28	0	2/28
<i>Phaseolus vulgaris</i>	2/32	6/28	28/32	19/28
<i>Pisum sativum</i>	0	1/30	0	4/30
<i>Raphanus sativus</i> var. <i>sativus</i>	0	0	11/30	20/28
<i>Vicia faba</i> subsp. <i>minor</i>	2/32	1/30	26/32	25/30

Table 5

Effectiveness of inoculation of several plant species with three isolates of *Phoma exigua* var. *exigua* in laboratory experiment

Inoculated plant	No. of diseased seedlings in relation of no. of seedlings inoculated with the isolates:		
	PD 79/118	L 14	SK 6
<i>Cucumis sativus</i>	30/30	30/30	28/30
<i>Glycinea max</i>	18/30	30/30	30/30
<i>Lupinus luteus</i>	16/30	29/30	28/30
<i>Pisum sativum</i>	18/30	26/30	24/30
<i>Raphanus sativus</i> var. <i>sativus</i>	18/30	30/30	30/30

surface blight on the root necks of pea, lupin, cucumber, radish and soybean. Isolate of SK6 from the root neck and L14 from soybean leaves in most cases caused blight of deeper lying tissues of the root neck in the tested plant species. Sometimes blight developed around the root neck. Usually the seedling was infected only on the segment equal in diameter to the disc of culture applied, but sometimes browning of tissues spread on a longer segment causing even dying back of the radicle (Fig. 2).

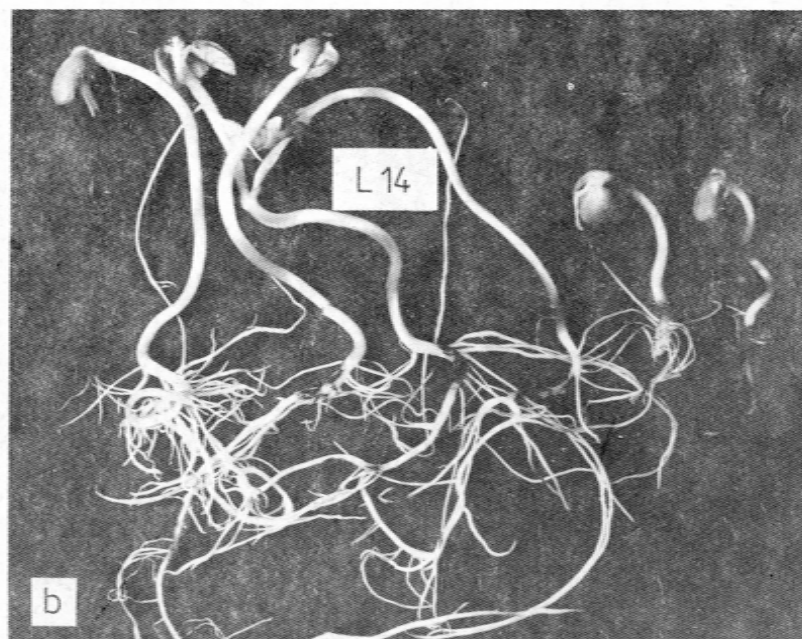
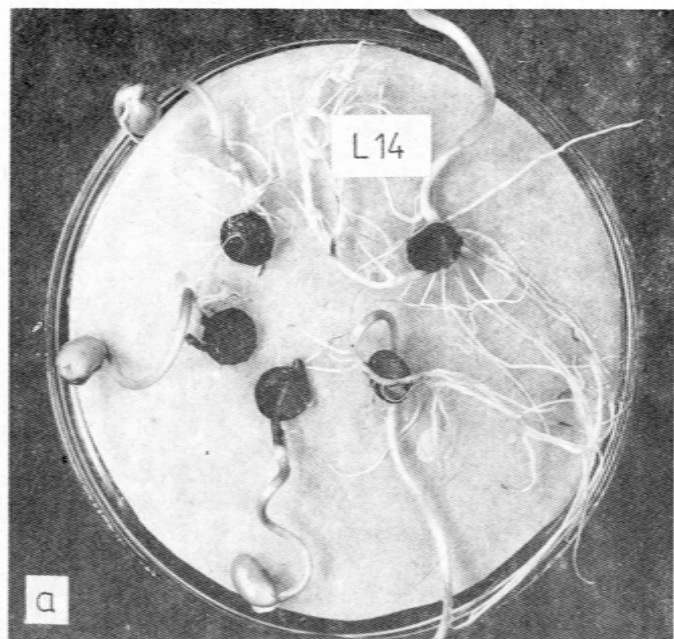


Fig. 2. Blight of root neck of soybean seedlings in laboratory experiment: a — necrosis of tissues at site of placement of discs of cultures and in their vicinity, b — symptoms on plants after removal of discs of fungal culture, plant on left healthy

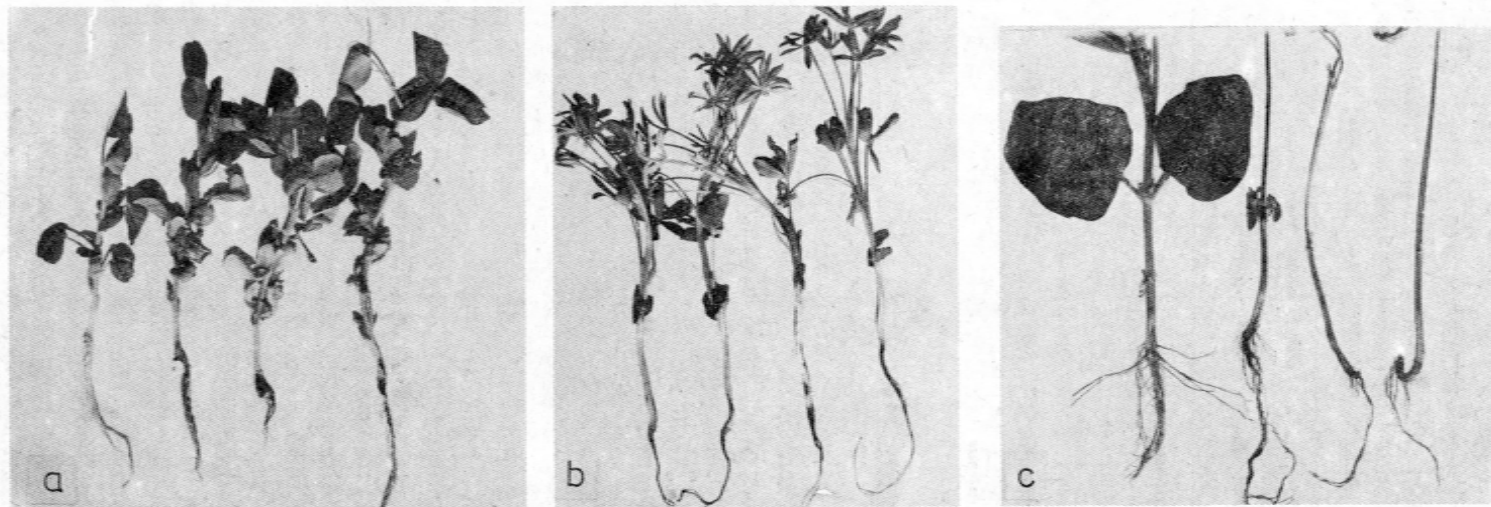


Fig. 3. Blight of root neck of seedlings of the following plants: a — pea, b — yellow lupin, c — soybean. Plant on left healthy

Table 6

Effectiveness of inoculation of several plant species by the method of soaking seeds in a conidial suspension from the fungus *Phoma exigua* var. *exigua*

Inoculated plant	No. of spouting seedlings after soaking of seeds in:		No. of diseased seedlings
	water	inoculum	
<i>Cucumis sativus</i>	37	19	1
<i>Glycinea max</i>	30	25	3
<i>Lupinus luteus</i>	45	39	4
<i>Lycopersicon esculentum</i>	35	30	0
<i>Phaseolus vulgaris</i>	36	24	4
<i>Pisum sativum</i>	46	38	5
<i>Raphanus sativus</i> var. <i>sativus</i>	39	41	2
<i>Vicia faba</i> subsp. <i>minor</i>	30	37	6

Method 7. Seeds of cucumber, lupin, tomato, bean, pea and soybean soaked in a conidial suspension germinated worse than those soaked in water (Table 6). The germination capacity of field bean and radish, however, was not depressed after soaking in the inoculum. Diseased seedlings grew from the seeds of most plants with the exception of tomato, soaked in conidial suspension (Fig. 3). The number of seedlings with symptoms of blight was small, barely 5-17 per cent.

DISCUSSION

Inoculation experiments demonstrated that the fungus tested in Poland is a weak pathogen since it infected only injured or weak soybean plants and those of other species, this indicating its polyphagous properties. These results additionally confirmed the correct conclusion, advanced on the basis of observation of the morphology and development of the cultures, that the tested fungus is the species *Phoma exigua* Desm. var. *exigua* (Marcinkowska, 1983). Bondarceva-Monteverde and Vasilevskii (1940) in the USSR while studying the fungus obtained from Abramov (1931) indicated on its low pathogenicity for the soybean and other plants of the *Leguminosae* family. It is, thus, not a pathogen specific for soybean, as believed by Abramov (1931) when he described this fungus as a new species of *Ascochyta sojaecola*. The lack of specialization of the isolates from the genus *Ascochyta* obtained from soybean and other plants is also reported by Crossan (1953) from the USA, and Ondrey (1976) from the Czechoslovak Socialist Republic for *Phoma exigua*. In West Germany

F r a n d s e n <1953> observed strong infection of soybean and bean by *Ascochyta sojaecola* in inoculation experiments. The infection of these two plant species may have indicated a lack of specialization, but the positive results of inoculation of healthy plants may be evidence of the high pathogenicity of the fungus. It is possible that F r a n d s e n <1953> isolated a fungus of high pathogenicity towards the tested plants since it is known <B o e r e m a et al., 1981> in the German Federal Republic that morphologically similar species of *Phoma exigua* var. *exigua* and *P. exigua* var. *diversispora* have been noted on bean plants. The latter fungus exhibited a strong pathogenicity towards beans. On the other hand, *P. exigua* var. *exigua* appeared usually at the end of the bean vegetation season <B o e r e m a et al., 1981>. In Poland the latter fungus has been observed on soybean with a greater intensity aslo on ripening plants as well as on weakened yellowing once at the stage of full vegetation and also around necroses produced by other fungi <Fig. 4> <M a r c i n k o w s k a et al., 1982>.

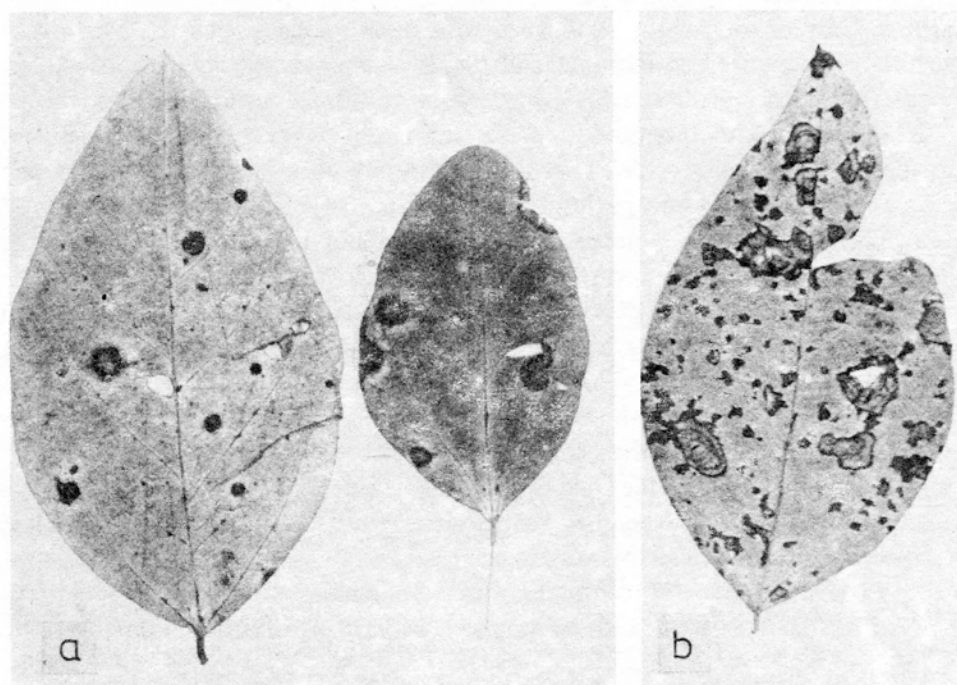


Fig. 4. Spots on soybean leaves from naturally infected plants: a — spots on leaves caused by fungus *Phoma exigua* var. *exigua*, b — on tissue infected by *Peronospora manshurica*, the fungus *Phoma exigua* var. *exigua* develops causing spread of necrotic spots on the leaves

In the case of each of the seed inoculation methods applied a depression of germination capacity of soybean seeds was observed. Most numerous seedlings were infected as the result of soaking in a suspension of conidia. This method was

considered as the best in agreement with the opinion of the authors (Tóth and Kovács, 1978), therefore, it was applied for inoculation of seeds of various plant species. Among the methods of soybean plant inoculation the most suitable is the laboratory method in which discs of the fungal culture were placed on injured seedlings, this giving the quickest and most precise results. Other methods are more time consuming and their results are difficult to compare because of difficulties in repeatability of the results. This may have also been caused by partial lack of precision in the methods used. In spite of application of methods varying in precision, none of the isolates exhibited a high pathogenicity for soybean and other plants, this being characteristic for the fungus *Phoma exigua* var. *exigua*, as reported by Boerema and Höweler (1967) in reference to other plants tested by them.

CONCLUSIONS

1. Among the methods of plant inoculation most suitable for estimation of the pathogenicity of the fungus *P. exigua* var. *exigua* is considered the laboratory method of inoculation with fungus culture discs, and among the methods of seed inoculation, soaking of seeds in a suspension of fungal conidia.

2. For successful inoculation of soybean and other plants the injury of inoculated organs was decisive. The tested fungus did not infect intact plants, this being evidence of its weak pathogenicity towards soybean and other plants.

3. The tested fungus isolated from soybean is not a specific pathogen of this plant since it infected injured plants not only of soybean but of other species.

4. The different time of exposure of the inoculated plants to conditions of 100 per cent relative air humidity had no influence on the incidence rate in intact and also injured plants.

5. Each of the three methods of seed inoculation depressed the germination capacity of the seeds and the health condition of the seedlings.

6. On the basis of the morphological features of the fungus and its pathogenicity towards soybean and other tested plants it was established that it is the species *Phoma exigua* Desm. var. *exigua*.

7. Various isolates of this fungus from soybean and the isolate from chicory behaved similarly as weak pathogens towards various infected plants.

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Metody oceny patogeniczności grzyba *Phoma exigua* var. *exigua*

Streszczenie

W latach 1977-1981 wyizolowano z liści, pędów, szyjek korzeniowych i nasion soi uprawnej w różnych okolicach Polski 33 izolaty grzyba *Phoma exigua*. Żaden z izolatów nie wywołał objawów chorobowych po inokulacji nieuszkodzonych roślin soi i roślin innych gatunków. Dopiero po inokulacji roślin uszkodzonych w różny sposób, obserwowano ich porażenie. Porażeniu uległy zarówno soja jak i inne rośliny, co świadczy o polifagicznym charakterze grzyba. Na podstawie tych czynników zidentyfikowano grzyb jako *Phoma exigua* var. *exigua*.

Po zastosowaniu siedmiu metod oceny patogeniczności uzyskano różną liczbę chorych roślin. Gdy inokulowano nasiona zazwyczaj mało siewek wykazywało objawy zgorzeli. Najefektywniejszą z trzech metod zakażania nasion okazała się metoda moczenia nasion w zawiesinie konidiów grzyba. Spośród metod inokulacji siewek oraz roślin starszych soi i innych gatunków za najlepszą uznano metodę wykładania krążków kultur na hypokotyl w warunkach laboratoryjnych. Wszystkie pozostałe metody, oprócz różnego stopnia precyzji, są bardziej pracochłonne, ponieważ wymagają uprawiania roślin w doniczkach i doświadczeń wykonanych w szklarni.