open-field conditions the contamination with *Chlorella* could not be prevented and some of the experimental values obtained may thus be affected in an uncontrollable manner.

Composition of algal biomass is given in Tables 7 and 8. The Tables give no indication of any significant trend in the analysed quantities during the cultivation period. The mean content of N (7.8%) and P (1.05%) in the biomass (Table 7) is in keeping with the data of SOEDER (1972): 8.5% N and 1% P for autotrophic algae.

Bacteriological examination of samples of *Chlamydomonas* biomass showed the presence of saprophytic microflora. Mycological examination demonstrated the moulds *Mucor* and *Aspergillus* (60—2000 germs per 1 g biomass).

It was proved experimentally that the alga *Chlamydomonas geitleri* may be successfully used for removing N and P from waste water during the spring months, with a concomitant production of algal biomass. In view of the relatively long cultivation, the efficient removal of N and P would require the lowering of the concentration of the elements in the outlets of the treatment plants.

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Prepared at the Biotechnological Laboratory, Institute of Microbiology, Czechoslovak Academy of Sciences, Třeboň; Faculty of Agriculture of the Keszthely University of Agriculture, Mosonmagyaróvár.

K. VÉBER, B. PROKEŠ, K. LÍVANSKÝ, G. MÁRTON

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CONTRIBUTION TO THE BIOLOGY OF DIAPORTHE PHASEOLORUM (CKE. ET ELL.) SACC. VAR. SOJAE WEHM. (SYN.: D. SOJAE LEH.); IMP.: PHOMOPSIS SOJAE LEH., A PATHOGEN CAUSING A NEW SOYBEAN DISEASE IN HUNGARY

The import of seed for use in variety trials, breeding and commercial production represents a potential danger of introducing pathogens so far unknown in Hungary. The pathogen *Phyllosticta sojaecola* Massalem was described in Hungary by VIOLA (1969), while information on the occurrence of *Corynespora cassiicola* (Berk. et Curt.) Wei was given by ÉRSEK (1978a, b). An account of the appearance of *Ascochyta sojaecola* Abramov in Hungary was given by TÓTH—KÖVICS (1978).

Pod and stem blight in soybean is caused by the pathogen *Diaporthe phaseolorum* (Cke. et Ell.) Sacc. var. *sojae* Wehm. (Syn.: *D. sojae* Leh.); imp.: *Phomopsis sojae* Leh. (LEHMAN 1923, LUTTRELL 1947, ATHOW—CALDWELL 1954, HILDEBRAND 1956, WALLEN—SEAMAN 1962, PETERSON—STRELECKI 1965, NOBEL—RICHARDSON 1968, DUNLEAVY 1969, KMETZ *et al.* 1974, etc.).

The damage done by the pathogen in Hungary was observed by SZILI (1975) on the stems and pods of soybeans in Szolnok and Tolna counties. He found the degree of infection to be 6—12%, and the damage done to the variety G SZ.3 to be the most serious in both counties. The occurrence of the fungus in Győr-Sopron county was reported by SZALAY (1976). The appearance of the disease was observed in summer 1976 in Debrecen (East Hungary) in the soybean varieties Merit and Steele.*

The pathogen was first observed in the United States in 1920 (LEHMAN 1923). Its occurrence has since been reported from Brazil, Canada, Colombia, Guyana, India, Japan, China, Malawi, Taiwan and the Soviet Union (PATINO 1967, SIDDIQUI 1971, SINCLAIR—SHURTLEFF 1975).

Before 1960 the disease was considered to be of minor importance (SASAKI 1929, LUTTRELL 1947, ATHOW—CALDWELL 1954, HILDEBRAND 1956). It has since been found that the germinative ability of seeds severely infected by the fungus significantly decreases (WALLEN—SEAMAN 1963, ELLIS *et al.* 1974, 1976). Mouldy, inferior seeds infected by *Phomopsis sojae*, a pathogen causing considerable damage, are common in Brazil, Canada and the United States (WALLEN—SEAMAN 1963, CRITTENDEN—SVEC 1974, CHAMBERLAIN—GRAY 1974, BOLKAN *et al.* 1976). Besides *Diaporthe phaseolorum* var. *sojae*, major fungi infecting the seed of soybean are: *Diaporthe phaseolorum* var. *caulivora*, *Cercospora kikuchii*, *Alternaria* spp., *Aspergillus melleus*, *Aspergillus niger*, *Fusarium scirpi* var. *acuminatum*, *Fusarium gibbosum* and *Ascochyta sojaecola* (KILPATRICK 1957, MA 1967, WILCOX—ABNEY 1971, ATHOW 1973, KIS *et al.* 1977, TÓTH—KÖVICS 1978). In susceptible soybean varieties the *Phomopsis* infection of the seeds often exceeds 50%, especially when the harvest is delayed and wet, hot weather prevails (WILCOX *et al.* 1974, ELLIS *et al.* 1976, SINCLAIR 1977). As described by plant producers, physiologists and pathologists, delayed harvesting results in the "weathering" of soybean seeds (BAILEY 1964, GREEN *et al.* 1966, DE LOUCHE 1975, RACHIE—PLARRE 1975). According to the investigations of SZALAY (1976) the rate of infection by *Diaporthe phaseolorum* var. *sojae* in seed lots produced from imported seed was 1—3%.

The symptoms of the disease

Infection may occur on the stem, petiole, pod or seed, or less frequently on the leaf. Seriously infected plants may die. The fungus produces large numbers of pycnidia, mainly on the lower part of the stem, where branching occurs or on the pods at about the time of ripening.

On the stem the pycnidia mostly appear in a line ("tiger spots", Fig. 1) or in well defined lesions (Fig. 2), generally near the nodes.

On the maturing pods the pycnidia show a scattered arrangement (Fig. 3). In spite of strong infection of the stem SZALAY (1976) found no pods on which spores had developed.

On seriously infected seeds the spots become deeply cracked and dry out, while the seed are often partially or completely coated with the white mycelium of the fungus. Besides *Diaporthe phaseolorum* var. *sojae* the fungi *Cercospora kikuchii* (ROY—ABNEY 1976, MA 1967) and *Diaporthe phaseolorum* var. *caulivora* (KMETZ *et al.* 1978) also form mouldy coatings.

On the cotyledons of seedlings developing from infected seeds lesions ranging from nearly colourless to a light reddish-brown colour are formed and may even attain the full size of the cotyledon. Below the hypocotyl a narrow reddish-brown spot some 1.5 cm in length appears. The seedling may become deformed or totally destroyed.

Symptoms are less frequently found on the leaves; the infection usually takes place below the apical part of the leaflet and advances towards the petiole. The whole leaflet may eventually be destroyed (SINCLAIR—SHURTLEFF 1975).

* Data have recently been published by ÉRSEK (1978c, 1979) on the mycological characteristics of *Diaporthe phaseolorum* var. *sojae (Phomopsis sojae)*, a new pathogen in Hungary.

Fig. 1. Phomopsis sojae pycnidia arranged in a line on soybean stem ("tiger spots")

The morphology of the fungus

The imperfect form of the pathogen (*Phomopsis sojae* Leh.) is more frequent, while the perfect form [*Diaporthe phaseolorum* (Cke. et Ell.) Sacc. var. *sojae* Wehm.] seldom appears, probably because the fungus is heterothallic, i.e. a single specimen is not capable of sexual reproduction (WELCH—GILMAN 1948).

The pycnidia are found embedded in the black stroma under the epidermis. Their size and shape greatly depend on the plant part they are formed on. They are somewhat wider on the stem, nearly spherical on the pods and spherical on the leaves. The size of the pycnidia is $82-225\times82-375$ μm on the pods and stem, and $120-180\times135-240$ μm on the leaves. Most of the pycnidia have a single cavity, though some have two or more cavities. Each cavity opens on to the surface through an ostiolum (Fig. 4).

The conidia are unicellular, hyaline, usually binucleate, linear-elliptical and generally biguttulate (Fig. 5). According to SINCLAIR—SHURTLEFF (1975) they are $4.9-9.8\times1.7-3.2$ μm in size. While measuring the conidia formed in the pycnidia of the stem the average size was found to be 7.4×3.2 μm, ranging from 6.0 to 9.1 μm in length and from 2.6 to 4.3 μm in width. The conidia reach the surface in the thick fluid filtering through the ostiolum.

The perithecia, which are seldom formed, are spherical, smaller than the pycnidia and slightly flattened at the base; they are $48-282\times185-346$ μm in size (SINCLAIR—SHURTLEFF 1975). On the perithecium a long tapering ostiolum is found; the perithecia are arranged singly in the black stroma, which is $60-142$ μm wide and emerges to a height of 1.5 mm.

In the cavities of the perithecium large numbers of elongated-clavate, eight-spored asci with slightly thickened tips are found (Fig. 6A). They are $35-51\times3.3-10$ μm in size.

The ascospores are hyaline, one-septate, elliptical, with rounded ends, somewhat coarctated at the partition wall, with two nuclei in each cell, and $9-13\times2-6$ μm in size (Fig. 6B). The small germ tubes of the spores usually protrude from or near the end of the cell (Fig. 6C).

Disease cycle and epidemiology

The sources of primary inoculum are the infected plant residues, the soil and the seed. On areas where the disease has not yet occurred imported seeds infected by *Diaporthe phaseolorum* var. *sojae* represent the most important source of inoculum. SZALAY (1976) observed damage in plant stands developed from imported seeds of the soybean varieties Clay, Merit and Altona.

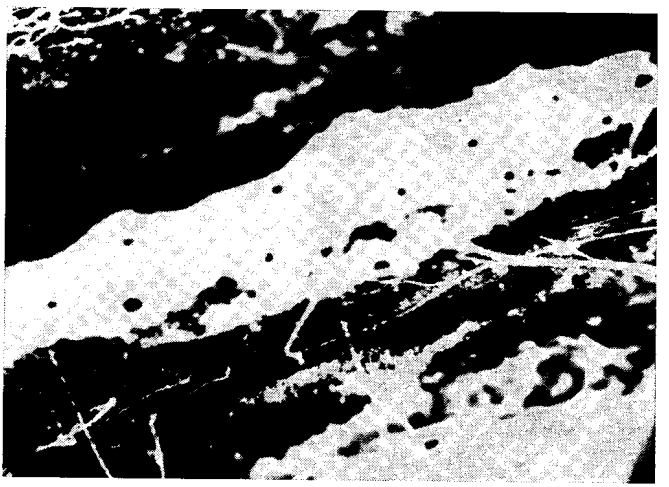

Fig. 2. Pycnidia may be formed in well defined lesions on the stem

Fig. 3. Infected soybean pod with scattered pycnidia

In seeds stored under cool, dry conditions the fungus survives for two years. The mycelium penetrates the ovule and interlaces the cotyledon, radicle and plumule. The disease can spread from plants grown from infected seeds.

An over-dense stand of plants promotes the infection of the seeds.

In the first phase of plant development the active penetration of the fungus through the thin-walled portion of the cortex is restricted. Later, however, it may even penetrate the thick-walled tracheae, thus becoming systemic.

Diaporthe phaseolorum var. *sojae* is able to infect many plant species, including *Phaseolus vulgaris*, *Phaseolus limensis*, *Vigna unguiculata*, *Allium sativum*, *Lespedeza* ssp., *Lupinus* ssp., *Arachis hypogaea*, *Hibiscus esculentus*, *Allium cepa*, *Capsicum frutescens* and *Lycopersicon esculentum*.

Fig. 4. Phomopsis sojae pycnidium with ostiolum

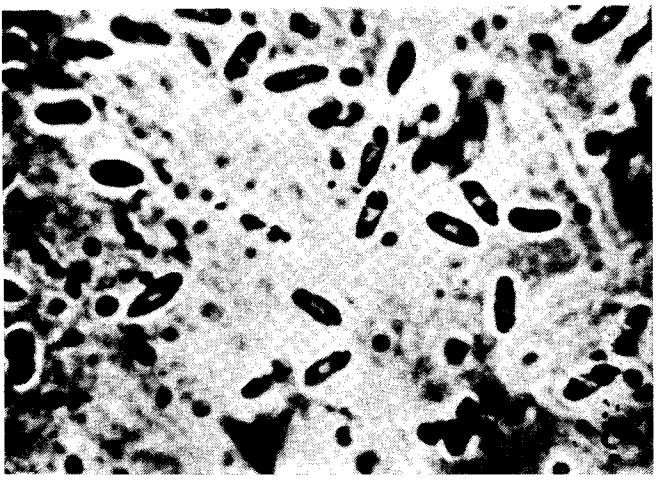

Fig. 5. Unicellular, binucleate conidia from pycnidia formed on soybean stem

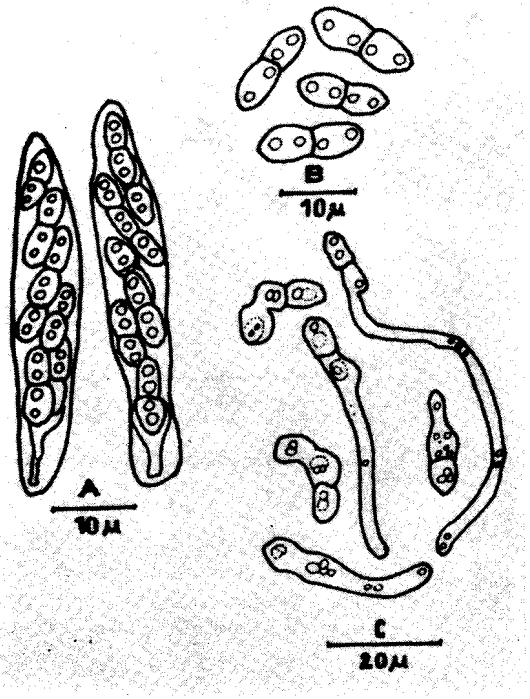

Fig. 6. A: eight-spore asci of *Diaporthe phaseolorum* var. *sojae*; *B:* bicellular ascospores; *C:* germination of ascospores (after Lehman)

Possibility of growing the fungus in culture

While studying the ecological requirements of the pathogen three culture media were tested. The fungus displayed optimum growth and fructification on potato dextrose agar (pH 4.5). Modified Leonian agar culture media (with 10 ml "Nektár" medicinal beer or ground, hydrolysed, germinated barley substituted for malt extract — Kövics 1978) are also suitable, though in this case growth is slower and the pycnidia appear later. Light is required if pycnidia are to form (Sinclair—Shurtleff 1975).

The heat optimum of the pathogen was found to be 25°C. At this temperature the organization of the pycnidia begins on the 12th day under neon tube illumination, and on the 15th day in a dark thermostat. Information on the intensity of development at a series of temperatures is given in Fig. 7. At temperatures of 15—20°C the fungus is still able to develop and fructify, while at 5—10°C development is greatly retarded. At a temperature of 30°C the curve of development is stepwise, with alternating phases of growth and stagnation, then when the culture medium dries up the development stops completely. At 35°C the fungus no longer grows.

Possibilities of controlling the disease

Since the fungus may cause considerable losses owing to its high pathogenicity, the following factors should be taken into consideration in control projects:

1. Good quality, healthy seed should be used.
2. An optimum potassium supply results in reduced seed decay.

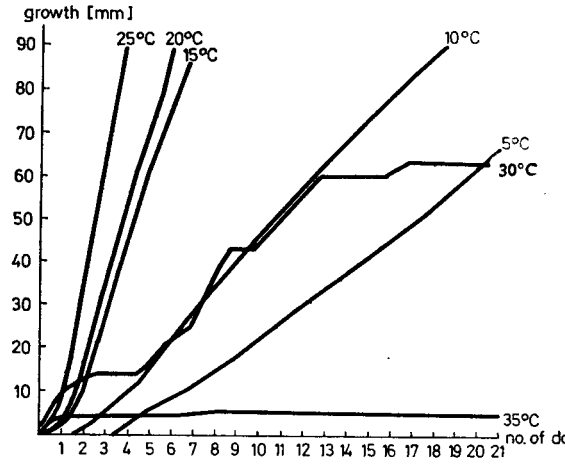

Fig. 7. Heat optimum diagram for *Diaporthe phaseolorum* var. *sojae*

3. Crop rotation; after harvest plant residues should be ploughed deep into the soil.
4. Spraying with fungicides should be applied at mid-flowering, then at the late pod stage to prevent pod and seed infection.
5. Varieties resistant to the pathogen should be grown (SINCLAIR—SHURTLEFF 1975).

In resistant varieties there is a lower extent of seed infection (WILCOX *et al.* 1974, SINCLAIR 1977).

Damage caused by delayed harvesting can be reduced by applying benomyl at mid-flowering and during seed formation (WALLA 1974, DE LOUCHE 1975, FOOR—SINCLAIR 1976, ILYAS *et al.* 1976).

In the case of seeds with or without symptoms, dressed with Thiram (TMTD), the development of *Phomopsis sojae* decreased, but the fungicide had a greater effect on fungi developing from symptom-free seeds than on those developing from seeds showing symptoms (HEPPERLY—SINCLAIR 1978).

When examining the toxicity of fungicides to N-fixing bacteria in laboratory experiments and field trials, KECSKÉS (1973) found that fungicides containing mercury and copper had the highest toxicity, while those containing thiram and captan only slightly inhibited these useful bacteria. CHAMBERLAIN—GRAY (1974) observed a 2—27% increase in germinative ability when infected seeds were dressed with captan and thiram. Fungicides containing benomyl, benomyl + captan, captan, bavistin and chloroneb were also found to be effective (GRAY—SINCLAIR 1970, ZINOVEV—BATALOVA 1976).

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Prepared at the Phytopathological Laboratory of the Research Centre for Agrobotany of the National Institute for Variety Testing, Tápiószele.

GY. KÖVICS

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