Results of Seed Tests. II. Occurrence of some Pathogenic Fungi in Plant Residues on Faba Bean Seeds

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Abstract

Seed decay of faba bean (*Vicia faba*) is caused by a complex of different fungi, some of them able to survive in plant residues. Material was scraped from seed surfaces and investigated in droplets of sterilized water to detect spores and hyphae. *Alternaria alternata* was the predominant fungus in residues represented by both mycelia and conidia. *Botrytis cinerea*, *B. fabae*, *Fusarium oxysporum*, *Phoma pinodella* and *Stemphylium botryosum* also were identified from the investigated plant material, and their pathogenicity to adult plants tested.

Introduction

Faba bean (*Vicia faba* L.) is one of the most important protein sources in many countries. Productivity of the crop is affected by a number of different factors, including seed-transmissible pathogens, and numerous fungi are known to occur on seeds of faba bean (Agarwal and Sinclair 1987; Neergaard 1977; Rádulescu and Negru 1971; Simay 1991). Some of these fungi are well-known saprophytic organisms, but a number of other pathogens also can survive on dead plant materials (Bánhegyi et al. 1985; Domsch et al. 1980; Ubrizsy 1965). Some fungal organs were observed in our seed health tests in plant residues attached to seed surfaces. The aim of the trials was to identify the fungi and investigate their pathogenicity.

Materials and Methods

The seeds investigated were harvested from the experimental plots and the samples were handled as normal seed. Seeds were stored in paper bags after cleaning at room temperature; laboratory investigations were conducted 2 to 4 weeks after the cleaning.

We removed the plant material with a pick to detect fungal organs that had occurred in the plant tissues and remained on the seed surface. The preparates were then investigated by microscope in droplets of sterilized water. If typical spores were available, the fungi were identified from the preparates; otherwise, the fungi were identified from cultures. The cultures were made by placing the scraped plant residues onto plates of potato dextrose agar (PDA) and 2% malt extract agar (MEA). The pure cultures were typed and their pathogenicity tested from some representative cultures found either on germinating seeds or on five-leaved faba beans grown in a greenhouse; 41 preparates were investigated for contaminants. The identification of fungi was made according to Bánhegyi et al. (1985), Booth (1971), Domsch et al. (1980), Ellis (1971), and Ellis and Waller (1974 a,b).

Pathogenicity tests were conducted for eight known pathogens, i.e., *Alternaria alternata* (Fr.) Keissler, *Botrytis cinerea* Persoon, *Botrytis fabae* Sard., *Fusarium oxysporum* Schlecht., *Peronospora viciae* (Berk.) Casp., Monatsber. K. Preuss, *Phoma pinodella* (L.K. Jones) Morgan-Jones et Burch, *Stemphylium botryosum* Wallr. and *Uromyces viciae fabae* (Persoon) Schroeter. Pathogenicity of culturable conidial fungi was tested by spraying the conidial mass washed from pure cultures onto faba bean plants grown in a greenhouse. The conidial suspension was adjusted to 10^5 conidia and the infected plants were covered with

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ينجم تعفن بذور الفول *(Vicia faba)* عن مجموعة من الفطريات المختلفة، التي يكون لبعضها القدرة على الحياة في الأجزاء النباتية المتبقية على بذور الفول. وقد كشطت مادة من سطوح البذور وفحصت في قطيرات من المياه المعقمة للكشف عن الأبواغ والخيوط الفطرية. وكان فطر التبقع الألترناري *Alternaria alternata* الفطر السائد في تلك البقايا ممثلاً بالغصينات الفطرية (mycelia) والأبواغ الكونيدية. كما أمكن تحديد *B fabae* و *Botrytis Cinerea* و *Stemphylium botryosum* و *Phona pinodella* و *Fusarium oxysporum* من المادة النباتية المدروسة، واختبرت قدرتها على إحداث المرض على النباتات البالغة.

polyethylene tents for 48 hours. Pathogenicity of *F. oxysporum* was investigated by soaking the seeds in the conidial suspension overnight. Plants were inoculated with *Peronospora* and *Uromyces* by taking the preparates containing the spores and placing them on the plants, then covering the plants with polyethylene tents for 48 hours.

Results and Discussion

Different fungal particles detected in the 41 preparates were grouped. The first group consisted of the three preparates containing oospores of *P. viciae*. The oospores were spherical and light brown with reticulations (Fig. 1a) developed singly or in clusters (Fig. 1b). Germination of oospores was not observed in the investigated preparates, but successful infections were made on two occasions using the preparates. Small yellowish spots were the first symptoms after the artificial infection; sporulation of fungus also was observed. Secondary infections were registered in the greenhouse. The fungus was identified by its characteristic sporangiophore branching and oospore structure (Mukerji 1975). Although the fungus is distributed on peas worldwide (Mukerji 1975), to the author's best knowledge only a few natural infections have been reported on *V. faba* (Blaeser-Diekmann 1982; Jamoussi 1968; Marras 1963; Sávulescu 1948). Rádulescu and Negru (1971) reported its seed transmission, and Jamoussi (1968) observed the occurrence of downy mildew on pods.

The second group of preparates contained the teleutospores of *U. viciae fabae*. This fungus is well known on faba bean (Gaunt 1983; Lelley 1964). Its occurrence on faba bean seeds was reported by Rádulescu and Negru (1971), but we do not have data on the economic importance of the seed transmissibility of *U. viciae fabae* in the case of *V. faba*. However, rust spores are a known primary source of infection on some other plants (Agarwal and Sinclair 1987; Emdal and Foldo 1979; Neergaard 1977). After the two preparates were placed on *V. faba* plants, development of aecia and, later, typical uredinia and telia were observed (Fig. 2a).

The third group of preparates also contained two samples in which chlamydospores were observed with some mycelia (Fig. 2b). *Phoma pinodella* was identified from pure cultures made from these preparates. The fungus sporulated well on both PDA and MEA media and the tests of pathogenicity resulted in symptoms similar to those observed earlier in this host-pathogen relationship (Simay 1988). *Phoma pinodella* is a well-known pathogen on different leguminous plants (Punithalingam and Gibson 1976) including faba bean (Bremer 1944; Hanounik and Maliha 1983; Simay 1988).

The other 34 preparates contained hyphae and mycelia. Some preparates were divided and plated onto agar media and stained with lacto-phenole-cottonblue to demonstrate the living parts (Fig. 3a,b). In pure isolates *A. alternata*, *B. cinerea*, *B. fabae*, *F. oxysporum* and *S. botryosum* were identified from 26, 3, 1, 1 and 3 preparates, respectively. The predominant *A. alternata* is distributed worldwide (Domsch et al. 1980), and is a known pathogen on numerous plants in addition to *V. faba* (Furgal-Wegrzycka 1984; Ibrahim and Michail 1968; Sumar et al. 1982). Its seed transmission is rather common on different plants (Neergaard 1977) and the seedborne nature of the pathogen was revealed in our earlier trials in the inner-seed tests (Simay 1987a). Isolates made from plant material harvested from the seed surface also were pathogenic on adult plants.

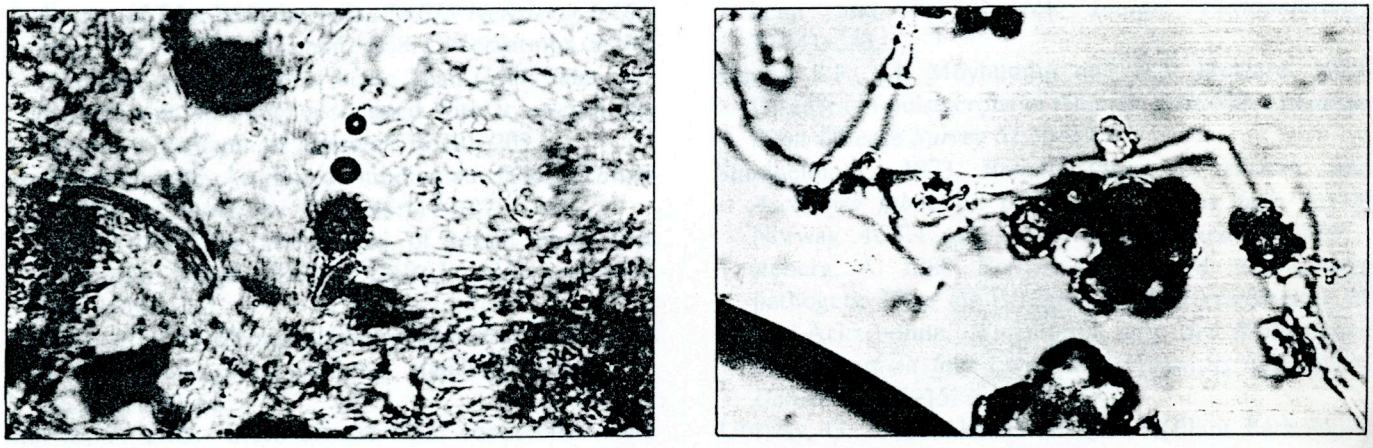

Fig. 1. (a) Oospore of *Peronospora viciae* in plant residue, (b) cluster of oospores of *Peronospora viciae*.

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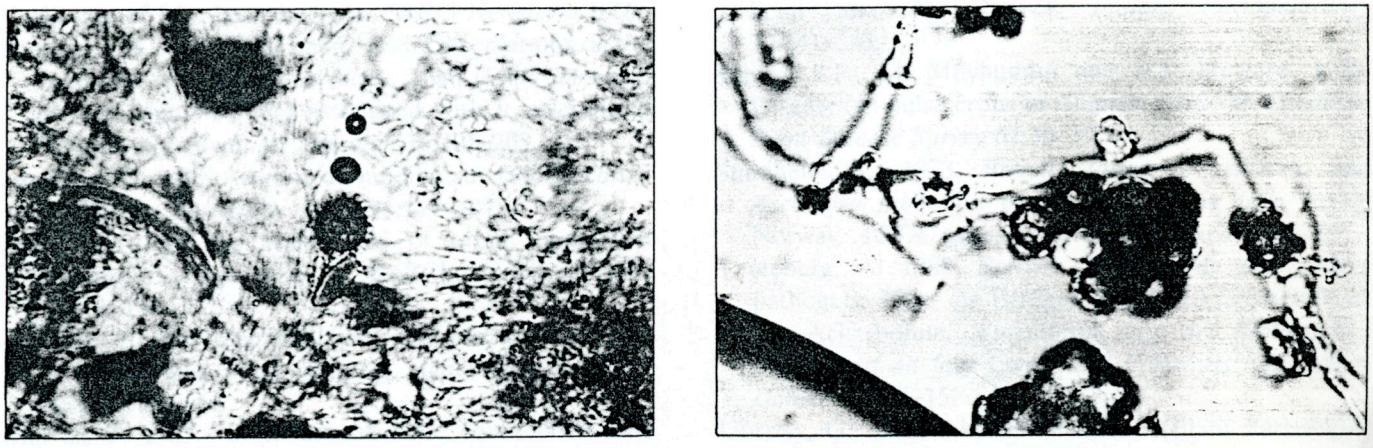

Fig. 1. (a) Oospore of *Peronospora viciae* in plant residue, (b) cluster of oospores of *Peronospora viciae*.

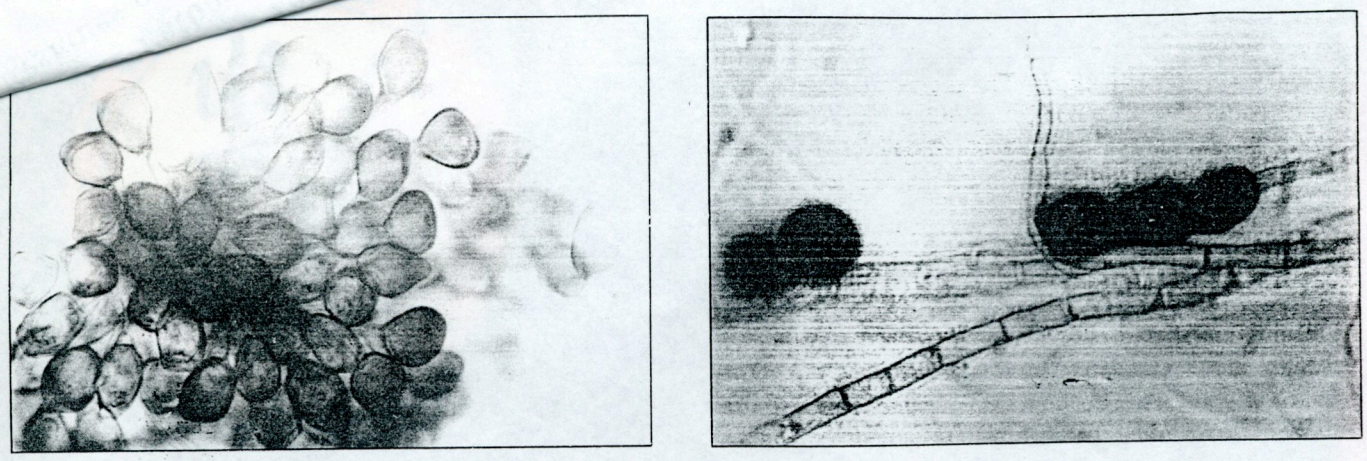

Fig. 2. (a) Teleutospores of *Uromyces viciae fabae*, (b) chlamydospores from seed surface.

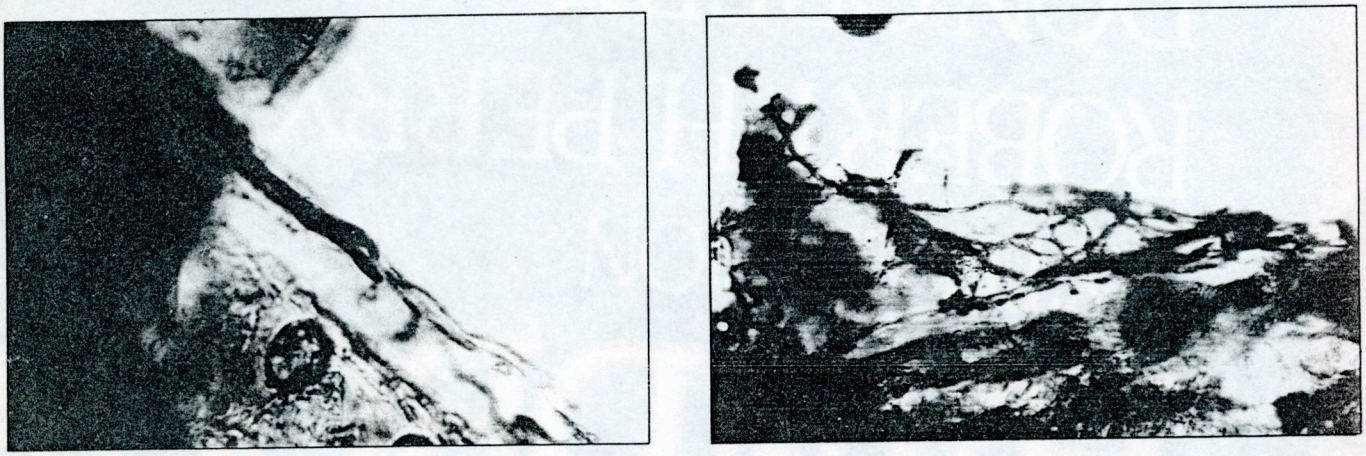

Fig. 3. (a) Hypha in plant material with cottonblue staining, (b) mycelium in plant material with cottonblue staining.

The two identified *Botrytis* spp. reported in Hungary (Simay 1987b) could cause serious damage to faba bean fields (Gaunt 1983). *Botrytis cinerea* is a polyphagous pathogen infecting different plants and fruits, while *B. fabae* can infect leguminous plants only (Ellis and Waller 1974 a,b). They both cause chocolate spot disease on leaves of *V. faba* (Gaunt 1983; Sundheim 1973). This disease also was observed on plants infected artificially by our isolates.

Fusarium spp. could cause root rot or wilt on faba bean (Salt 1983) and some may be seed transmitted (Simay 1991). *Fusarium oxysporum*, observed in dead plant material, caused wilt on different plants and on faba bean, while Singh and Singh (1986) claimed this fungus to be the most important of the seed-transmissible *Fusarium* spp.; it was observed on surface-sterilized seeds in our earlier tests (Simay 1986). The isolate made from plant residue caused no symptoms on leaves, but caused rot of primary roots. The germination of treated seeds was 13.5%, while it was 96% by treating the seeds with sterilized water, according to sowing tests using 50-50 seeds in two replicates.

Stemphylium botryosum is a widespread but rather minor pathogen of faba bean (Gaunt 1983), causing leaf spot on this host (Mansour 1980; Ruokola and Vestberg 1978; Simay 1988; Teuteberg 1980). Its seed transmission is common on different hosts (Neergaard 1977), but seed infection is not the most important source of infection as the fungus is known to occur on different substrates (Corlett et al. 1982; Domsch et al. 1980).

Other fungi, e.g., *Cladosporium* spp. and *Penicillium* sp., were observed from some preparates but determining their economic importance on this host requires further investigations.

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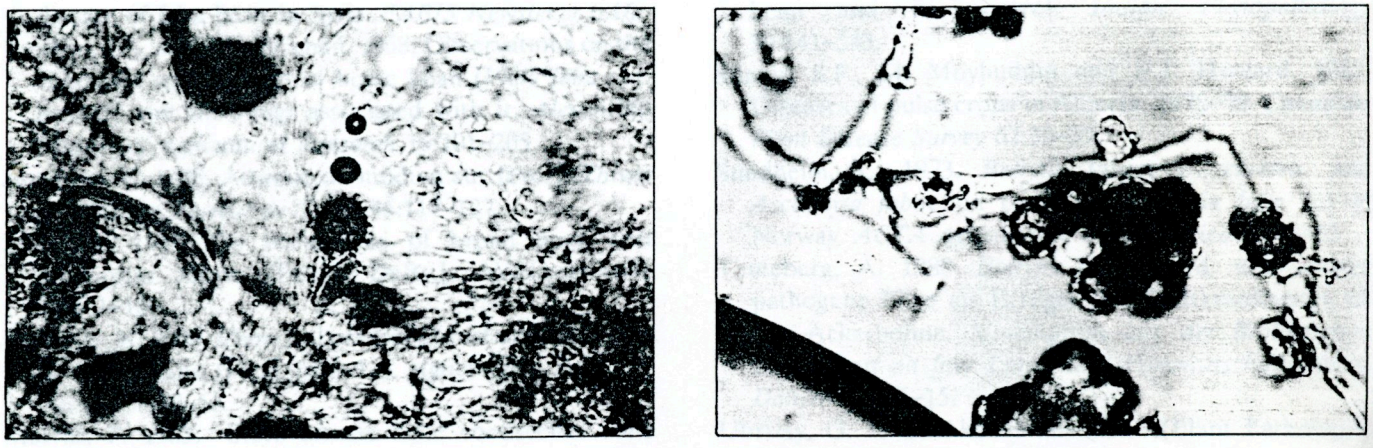

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polyethylene tents for 48 hours. Pathogenicity of *F. oxysporum* was investigated by soaking the seeds in the conidial suspension overnight. Plants were inoculated with *Peronospora* and *Uromyces* by taking the preparates containing the spores and placing them on the plants, then covering the plants with polyethylene tents for 48 hours.

Results and Discussion

Different fungal particles detected in the 41 preparates were grouped. The first group consisted of the three preparates containing oospores of *P. viciae*. The oospores were spherical and light brown with reticulations (Fig. 1a) developed singly or in clusters (Fig. 1b). Germination of oospores was not observed in the investigated preparates, but successful infections were made on two occasions using the preparates. Small yellowish spots were the first symptoms after the artificial infection; sporulation of fungus also was observed. Secondary infections were registered in the greenhouse. The fungus was identified by its characteristic sporangiophore branching and oospore structure (Mukerji 1975). Although the fungus is distributed on peas worldwide (Mukerji 1975), to the author's best knowledge only a few natural infections have been reported on *V. faba* (Blaeser-Diekmann 1982; Jamoussi 1968; Marras 1963; Sávulescu 1948). Rádulescu and Negru (1971) reported its seed transmission, and Jamoussi (1968) observed the occurrence of downy mildew on pods.

The second group of preparates contained the teleutospores of *U. viciae fabae*. This fungus is well known on faba bean (Gaunt 1983; Lelley 1964). Its occurrence on faba bean seeds was reported by Rádulescu and Negru (1971), but we do not have data on the economic importance of the seed transmissibility of *U. viciae fabae* in the case of *V. faba*. However, rust spores are a known primary source of infection on some other plants (Agarwal and Sinclair 1987; Emdal and Foldo 1979; Neergaard 1977). After the two preparates were placed on *V. faba* plants, development of aecia and, later, typical uredinia and telia were observed (Fig. 2a).

The third group of preparates also contained two samples in which chlamydospores were observed with some mycelia (Fig. 2b). *Phoma pinodella* was identified from pure cultures made from these preparates. The fungus sporulated well on both PDA and MEA media and the tests of pathogenicity resulted in symptoms similar to those observed earlier in this host-pathogen relationship (Simay 1988). *Phoma pinodella* is a well-known pathogen on different leguminous plants (Punithalingam and Gibson 1976) including faba bean (Bremer 1944; Hanounik and Maliha 1983; Simay 1988).

The other 34 preparates contained hyphae and mycelia. Some preparates were divided and plated onto agar media and stained with lacto-phenole-cottonblue to demonstrate the living parts (Fig. 3a,b). In pure isolates *A. alternata*, *B. cinerea*, *B. fabae*, *F. oxysporum* and *S. botryosum* were identified from 26, 3, 1, 1 and 3 preparates, respectively. The predominant *A. alternata* is distributed worldwide (Domsch et al. 1980), and is a known pathogen on numerous plants in addition to *V. faba* (Furgal-Wegrzycka 1984; Ibrahim and Michail 1968; Sumar et al. 1982). Its seed transmission is rather common on different plants (Neergaard 1977) and the seedborne nature of the pathogen was revealed in our earlier trials in the inner-seed tests (Simay 1987a). Isolates made from plant material harvested from the seed surface also were pathogenic on adult plants.

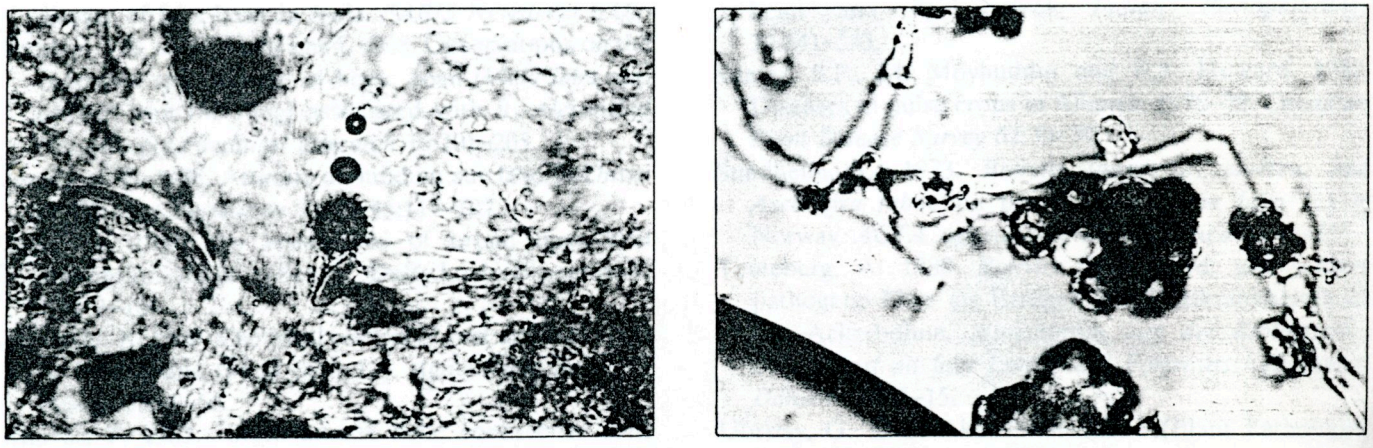

Fig. 1. (a) Oospore of *Peronospora viciae* in plant residue, (b) cluster of oospores of *Peronospora viciae*.

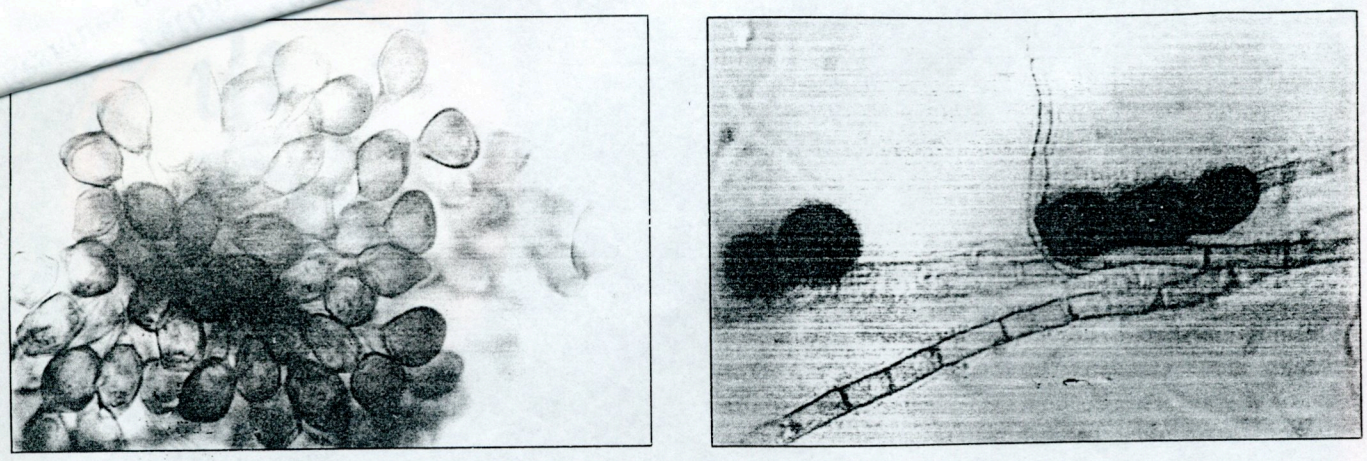

Fig. 2. (a) Teleutospores of *Uromyces viciae fabae*, (b) chlamydospores from seed surface.

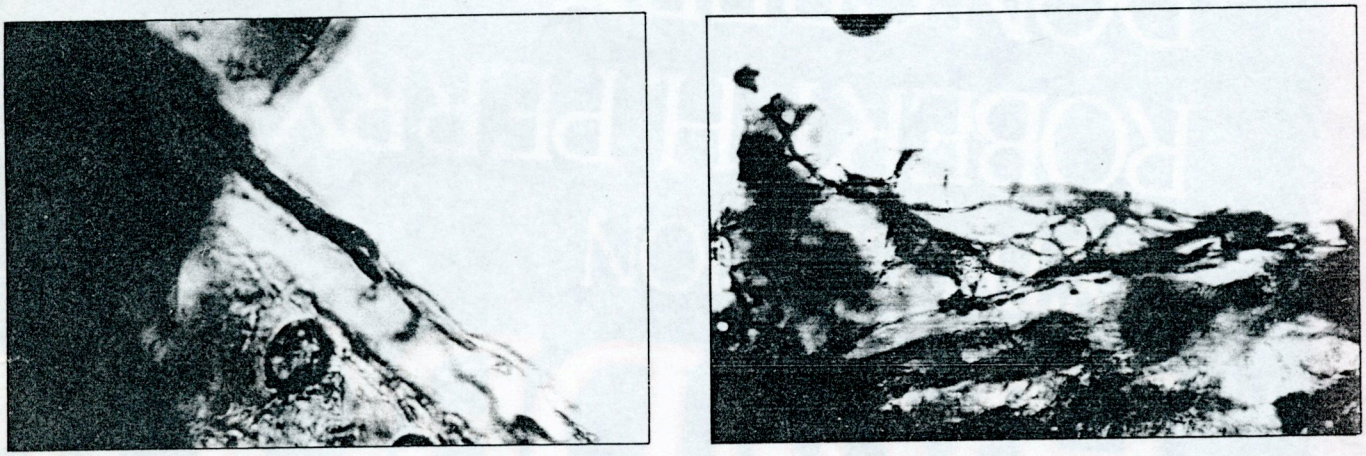

Fig. 3. (a) Hypha in plant material with cottonblue staining, (b) mycelium in plant material with cottonblue staining.

The two identified *Botrytis* spp. reported in Hungary (Simay 1987b) could cause serious damage to faba bean fields (Gaunt 1983). *Botrytis cinerea* is a polyphagous pathogen infecting different plants and fruits, while *B. fabae* can infect leguminous plants only (Ellis and Waller 1974 a,b). They both cause chocolate spot disease on leaves of *V. faba* (Gaunt 1983; Sundheim 1973). This disease also was observed on plants infected artificially by our isolates.

Fusarium spp. could cause root rot or wilt on faba bean (Salt 1983) and some may be seed transmitted (Simay 1991). *Fusarium oxysporum*, observed in dead plant material, caused wilt on different plants and on faba bean, while Singh and Singh (1986) claimed this fungus to be the most important of the seed-transmissible *Fusarium* spp.; it was observed on surface-sterilized seeds in our earlier tests (Simay 1986). The isolate made from plant residue caused no symptoms on leaves, but caused rot of primary roots. The germination of treated seeds was 13.5%, while it was 96% by treating the seeds with sterilized water, according to sowing tests using 50-50 seeds in two replicates.

Stemphylium botryosum is a widespread but rather minor pathogen of faba bean (Gaunt 1983), causing leaf spot on this host (Mansour 1980; Ruokola and Vestberg 1978; Simay 1988; Teuteberg 1980). Its seed transmission is common on different hosts (Neergaard 1977), but seed infection is not the most important source of infection as the fungus is known to occur on different substrates (Corlett et al. 1982; Domsch et al. 1980).

Other fungi, e.g., *Cladosporium* spp. and *Penicillium* sp., were observed from some preparates but determining their economic importance on this host requires further investigations.

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