Interactions of Microsphaera diffusa with Soybeans and Other Legumes

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A portion of this research was supported by the Illinois Agricultural Experiment Station. The senior author is on leave to pursue graduate work with financial support from the Agricultural Experiment Station, University of Puerto Rico, Mayaguez Campus.

The authors acknowledge the assistance of Helen Sandberg, Center for Electron Microscopy, University of Illinois in the scanning electron microscopy.

This publication is a portion of the MS thesis of the senior author.

Accepted for publication 28 July 1977.

ABSTRACT

MIGNUCCI, J. S. and D. W. CHAMBERLAIN. 1978. Interactions of Microsphaera diffusa with soybeans and other legumes. Phytopathology 68: 169-173.

Conidia of *Microsphaera diffusa* produce as many as five germ tubes upon germination. Only the first germ tube forms an appressorium. The conidium is the center of the mildew colony and a conidiophore can arise directly from it. Development of the fungus from germination of conidia to production of conidia was followed at 3- and then 12-hr intervals. Twenty-one members of the 35 Leguminosae tested were hosts of the fungus. Different degrees of infection on susceptible plants were evident not only between species but also between cultivars of the same species. These hosts showed one or more of the following symptoms: curling of leaves, wilting, defoliation, chlorosis, necrosis, water-soaked lesions, earlier senescence, shriveled seeds, and incomplete pod filling. The production of conidiophores and conidia were inhibited in Flambeau but not in Acme soybeans.

Additional key words: histology, host range, symptomatology, asexual cycle, resistance.

The powdery mildew organism, *Microsphaera diffusa* Cke. & Pk. (3, 7, 10) infects soybeans, *Glycine max* (L.) Merr.; it grows profusely on cotyledons, stems, pods, and both sides of the leaf blade. In Delaware, Iowa, North Carolina, South Carolina, Texas, and Puerto Rico powdery mildew of soybeans has been reported as *Erysiphe polygoni* DC. Lehman, in 1947, reported that the cause of powdery mildew on soybeans in North Carolina was *Microsphaera* sp. and not *Erysiphe polygoni* as he had reported in 1931 (6, 7). Recently, soybean powdery mildew in Georgia also was identified by Demski and Phillips (3) who reported widespread occurrence of the parasite. Powdery mildew of soybean was reported in Michigan and South Carolina in 1974 (Epstein, *personal communication*) and in Wisconsin (1) in 1974. Since 1970, Chamberlain and others (*personal communication*) have observed powdery mildew on soybeans in Illinois. Infections in Illinois have been limited to scattered areas of isolated fields late in the growing season, but in 1976 powdery mildew was widespread early in the growing season. Infected soybean samples from four different locations in Illinois in 1976 showed cleistothecia of *Microsphaera diffusa*. Paxton and Rogers (10) first conclusively identified the cause of soybean powdery mildew as *Microsphaera diffusa* in 1974 and this was confirmed by others in 1976 (3, 8). *Microsphaera diffusa* has been reported on several genera of the Leguminosae (9, 12), in four species of the Caprifoliaceae (12), and on one species of the Solanaceae (4).

A brief description of the asexual life cycle on intact soybean plants, the host range interactions on other members of the Leguminosae, the plant reactions, and pathogen development on susceptible and resistant soybean cultivars are described in this paper.

MATERIALS AND METHODS

Production of disease.—The fungus originated from a single conidial isolate taken from naturally infected Harosoy soybeans in the greenhouse. Plants were inoculated at the primary leaf stage by shaking leaves of infected Harosoy soybean plants over them. The fungus was maintained on Harosoy plants in a growth chamber at 26 ± 2 C day temperature, 21 ± 2 C night temperature, and 14 hr of light.

Plants used for tests of host range, observation of symptom development, and comparative studies of the host-parasite relationships on susceptible and resistant cultivars were maintained in a growth chamber at 26 ± 2 C day temperature, 20 ± 2 C night temperature, and a day length of 14 hr. Control plants were kept in a growth chamber under similar conditions. Unless otherwise stated Harosoy was used throughout these investigations.

Leaf mounts.—In each leaf collection, four primary leaves were excised from the test plants 2, 3, 4, 5, 6, 8, 12, 36, 48, 96, 108, 120, 132, and 144 hr after inoculation. The excised leaves were placed in a clearing solution of glacial

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acetic acid and 95% ethanol (1:1, v/v). The solution was changed three times until the leaves turned white. Seventy-two hr after collection, the leaves were stained according to the procedure of Shobe and Lersten (11) for gymnosperm leaves. The entire upper leaf surface then was examined under a microscope for infected areas.

Leaf cross sections.—Primary leaves were harvested at 6, 12, and 132 hr after inoculation, cut immediately into 5 mm^2 pieces and immersed in formalin-ethanol-acetic acid. Twenty-four hr later, they were dehydrated and infiltrated with paraffin (5). Sections were cut 15 μm thick with a rotary microtome and stained with safranin-fast green (5). The number of cell layers penetrated by the fungus was determined and the shape and size of the haustoria were observed.

Scanning electron microscopy (SEM).—Soybean leaf tissue was cut into 3-5 mm^2 pieces, dehydrated in ethanol, critical-point dried, mounted on scanning electron microscope stubs, coated with gold palladium in a Denton DU-503 FP vacuum evaporator, and examined in a JSM U-3 scanning electron microscope.

Host range and symptomatology.—Thirty-five members of the Leguminosae were tested for susceptibility to the soybean powdery mildew fungus. In each case 10 plants were rated for infection using a 0-5 scale, where 0 is no infection; 1 equals a trace of mycelial growth; 2, 3, 4, and 5 represent fungal growth covering 20, 50, 75, and 100% of the leaf surface, respectively. Plants with no disease signs or symptoms were reinoculated. Leaves were always examined with the aid of a dissecting microscope.

Development of the fungus.—The susceptible soybean cultivar Acme, and the resistant cultivar Flambeau, were inoculated at the first trifoliolate leaf-stage. Two groups of samples were collected from each cultivar 24, 48, 120, and 380 hr after inoculation. The first sample consisted of tape impressions on the inoculated leaves. Three tape impressions were taken, each from a different primary leaf on a different plant. Sampled leaves were marked to avoid sampling the same leaves a second time. The second group of samples consisted of three leaflets excised from the inoculated plants and processed using the *Leaf Mount* technique (Described above under "Leaf mounts").

Data collected on both inoculated cultivars were: (i) number of germinated and nongerminated conidia at 24 and 48 hr after inoculation; (ii) number of conidia with appressoria at 24 and 48 hr after inoculation; (iii) length of the first germ tube formed 24 hr after inoculation; (iv) number of conidia with one, two, three, four, and five germ tubes 24, 48, and 120 hr after inoculation; (v) number of conidiophores produced at 120 hr after inoculation; and (vi) number of conidia produced 380 hr after inoculation.

RESULTS

Studies of the asexual life cycle.—The conidia of *M. diffusa* ranged from 27.7 μm to 54.1 μm long and 17.1 μm to 21.1 μm wide (avg. 40.3 μm × 18.2 μm).

Germination of some conidia was seen on Harosoy soybean leaves 3 hr after inoculation. The germ tube arose terminally on the conidium. Most conidia germinated within 6 hr after inoculation at which time the first sign of germ tube differentiation, appressorium formation, occurred (Fig. 1-B). However, before formation of the appressorium, a septum formed in the germ tube close to the cell wall of the conidium. The distance between the septum and the appressorium was variable.

Only the first germ tube formed an appressorium. At 8 hr the infection peg had penetrated the epidermal cells. A second germ tube was formed within 24 hr. It was formed either terminally and adjacent to the first germ tube or at the opposite end of the conidium. By 36 hr some conidia produced a third germ tube that arose terminally. A fourth germ tube (Fig. 1-A) sometimes appeared on some conidia by 48 hr. By 96 hr some conidia had produced the fifth germ tube that arose laterally on the center of the conidium. Swollen knobs (Fig. 1-A, arrow) appeared on hyphae above the sites of cell penetration and haustoria formation. Haustoria occurred only in the epidermal cells.

Colonies formed as the hyphae extended and branched. The germinated conidium was the center of the colony, and a conidiophore was formed in the center of some germinated conidia. Conidiophores (Fig. 1-C, D) were initiated 108 hr after inoculation. The first conidiophores formed around the center of the colony; younger conidiophores arose toward the periphery. At this stage, many conidiophores had three cells, the tip cell being the most elongated. At 144 hr, the cell at the tip of the conidiophore was a well defined conidium and there were from three to five cells per conidiophore.

Host range and symptomatology.—Of the 35 members in 11 genera of the Leguminosae tested, 14 were immune to the fungus (Table 1). Of the five wild soybeans evaluated, only *Glycine canescens* was immune. *Glycine canescens* has a more pubescent leaf blade than the other wild soybeans. Some conidia were suspended from the leaf hairs. None of the clovers of *Lotus* species became infected. Of four species of *Lespedeza* tested, *L. cuneata* was the only resistant species. Different degrees of susceptibility were observed not only at the species level but also within cultivars of the same species as shown by the reactions of *Phaseolus* and *Vigna* cultivars (Table 1).

Some hosts reacted with drying and curling of leaves or with wilting and/or defoliation (8). Most spp. tested fell into four reaction types: immune, chlorotic, necrotic, and water-soaked (depressed, sunken, translucent) lesions. The species tested and their reaction categories are:

(i) chlorotic:—*Cajanus cajan*; *Glycine clandestina*; *G. falcata*; *G. tabacina*; *G. tomentella*; *Phaseolus vulgaris* 'Bush Bountiful', 'Kentucky Wonder-Wax-Pale', 'Pale Kentucky Wonder'; *V. unguiculata* 'Field-Brown-Sugar-Crowder', 'New Era', and 'Prima'.

(ii) necrotic:—*Phaseolus aureus*; *P. vulgaris* 'Bush Bountiful', 'Early Golden Cluster', 'Pale Kentucky Wonder'; *V. unguiculata* 'Black Cowpea', 'Brown Crowder', 'Field-Brown-Sugar-Crowder', 'New Era', and 'Prima'.

(iii) immune:—*Glycine canescens*; *Lespedeza cuneata*; *Lotus pedunculatus*, *L. tenuis*, *L. uliginosus*; *Melilotus alba*, *M. dentata*; *M. officinalis*, *Phaseolus vulgaris* 'Bush Refugee Stringless'; *Trifolium hybridum*, *T. incarnatum*, *T. repens*, *V. unguiculata* 'Lalita'. No fungal growth was noted on any of the immune species.

(iv) water-soaked lesions:—*Phaseolus vulgaris* 'Kentucky Wonder-Wax-Pale', and *V. unguiculata* 'New Era' and 'Prima'.

The most severe susceptible reaction was shown by *Pisum sativum* 'Burpee'. The infected tissues not only dried but senescence seemed to occur earlier on infected than on the control plants. The pods were heavily infected with shriveled seeds and incomplete filling of pods.

Development of the fungus.—Acme was covered by profuse mycelium of the fungus, but on Flambeau, the fungus was not readily visible except under the dissecting microscope (×20) where a few colonies were observed on the occasional leaf. Fewer conidiophores (Table 2) and conidia were produced on Flambeau than on Acme. Three successive tape impressions per leaf of Acme 380 hr after inoculation, and replicated three times each, showed such an overload of conidia that a count was impossible. On the first tape impression taken on Flambeau, the counts for each replicate (total of 10 microscope fields) were three, one, and 60 conidia, respectively. Conidia were not found on 60 other microscope fields examined.

DISCUSSION

The asexual stage of this fungus plays an important role in the disease cycle. In Illinois and in Puerto Rico the disease has occurred repeatedly in the absence of cleistothecia (9). The infrequent occurrence of the sexual stage is common in other powdery mildew fungi (13). Some investigators (2, 13) have identified powdery mildew on morphological characters of the asexual stage, such as shape and germination pattern of the conidia, shape of the conidiophores, presence or absence of

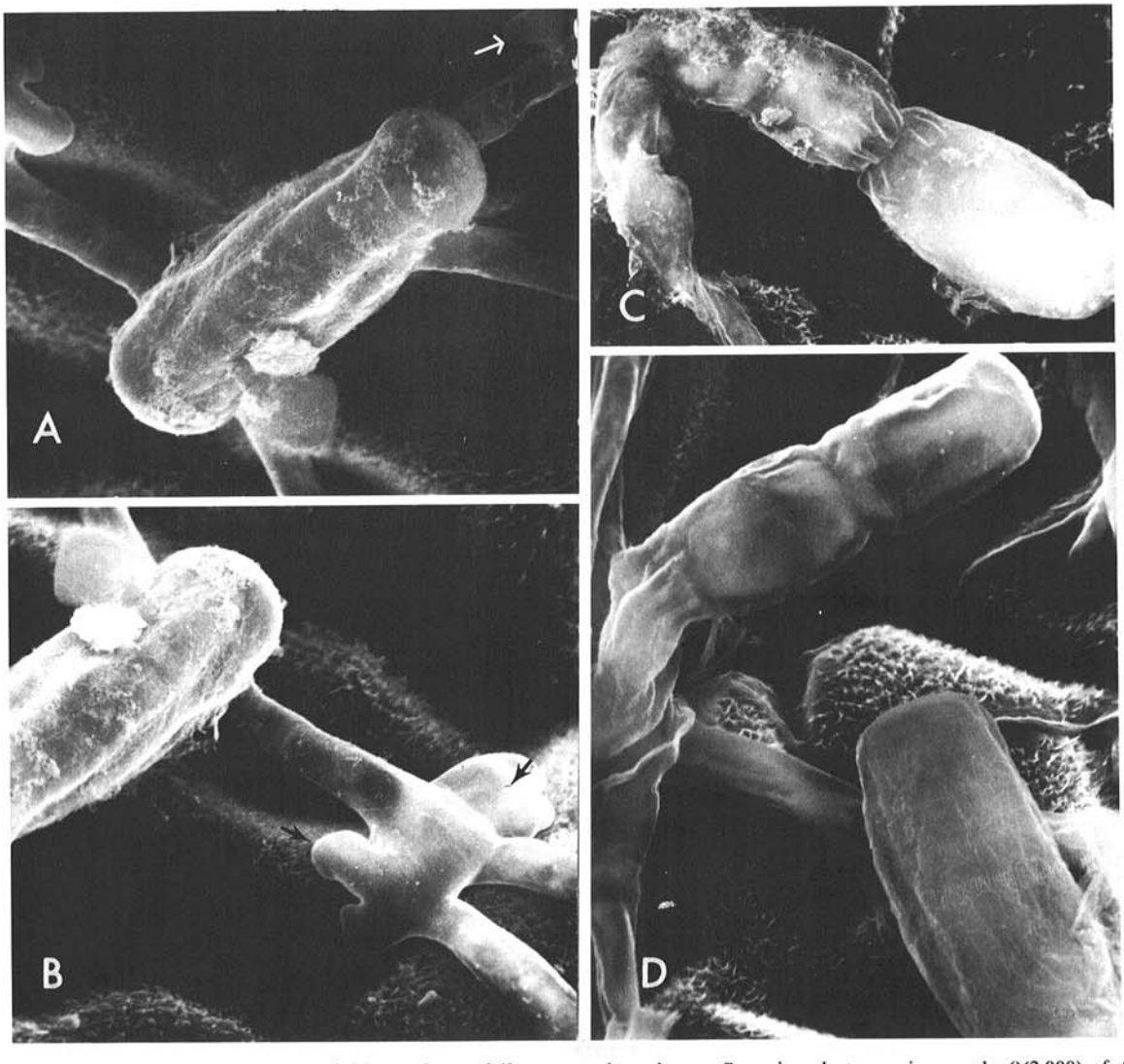

Fig. 1-(A-D). Asexual structures of *Microsphaera diffusa* on soybean leaves. Scanning electron micrographs (×2,000) of **A)** conidium with four germ tubes, **B)** appressorium (arrow), **C)** conidium constricting out from the conidiophore, and **D)** well-developed conidium.

TABLE 1. Reactions of some members of the Leguminosae to *Microsphaera diffusa*

Host	Infection rating[a]	Host	Infection rating[a]
Arachis hypogaea	1	*Phaseolus aureus*	3
Cajanus cajan	3	*P. vulgaris*	
Cyamopsis tetragonaloba	5	'Bush Refugee Stringless'	0
Glycine canescens	0	'Bush Bountiful'	4
G. clandestina	3	'Early Golden Cluster'	2
G. falcata	5	'Ky. Wonder-Wax-Pale'	5
G. tabacina	2	'Pale Ky. Wonder'	4
G. tomentella	2	*Pisum sativum*, 'Burpee'	5
Lespedeza cuneata	0	*Trifolium hybridum*	0
L. daurica schimidae	3	*T. incarnatum*	0
L. stipulacea	4	*T. repens*	0
L. striata	5	*Vigna unguiculata*	
Lotus pedunculatus	0	'Black cowpea'	5
L. tenuis	0	'Field-Brown-Sugar-Crowder'	3
L. uliginosus	0	'Lalita'	0
Melilotus alba	0	'New Era'	0
M. dentata	0	'Prima'	4
M. officinalis	0		

[a]Ratings on disease reactions were based on 0-5 scale: 0 = no infection; 1 = trace of mycelial growth; 2, 3, 4, and 5 = 20, 50, 75, and 100% of the leaf area covered by the fungus, respectively.

TABLE 2. Mean number of conidiophores produced on powdery mildew colonies on cultivars Acme and Flambeau, at 120 hr after inoculation

Technique	Conidiophores per soybean cultivar:[a]	
	Acme (no.)	Flambeau (no.)
Tape impressions	295	0
	506	2
	335	0
Leaf mount	180	96
	378	90
	299	0

[a]Mean number produced on six colonies examined.

fibrosin bodies, and others. This would be particularly helpful when cleistothecia are not present and when more than one species of powdery mildew fungi have been reported (13). The description of the asexual stage of *M. diffusa*, the SEM photomicrographs, and the methods described in this paper represent an attempt to provide additional information that may aid in the identification of this pathogen in the absence of cleistothecia.

A definite answer was not found on whether the resistant reaction of Flambeau and the susceptible reaction of Acme could be established on the basis of conidium germination, appressorium formation, length of the first germ tube, or number of conidia with one, two, three, four, and five germ tubes.

The soybean powdery mildew pathogen, when tested on several members of the Leguminosae, showed less specificity than expected. Seven of 11 genera tested had species that were susceptible to *M. diffusa*. Different degrees of infection on susceptible plants were evident, not only between members of the same genus but also between cultivars of the same species. In only three of the 11 genera (*Lotus, Melilotus,* and *Trifolium*) did all species show complete immunity to *M. diffusa*. In addition, one species of wild soybean, one species of *Lespedeza,* one cultivar of *Phaseolus vulgaris,* and two cultivars of *Vigna* were immune to the fungus. Hosts reacted with a wide array of symptoms. Since *M. diffusa* infects a number of leguminous plants, the possibility arises that one or more of these or other plant species may harbor this pathogen, serving as sources of inoculum during the growing season, and/or could be involved in over-wintering the fungus.

A luxuriant growth of the fungus occurred on all parts of *Pisum sativum* and resulted in drying, curling, and necrosis of leaves. This fungus also infected *Phaseolus vulgaris* and caused necrosis, defoliation, discoloration, water-soaked lesions, and wilting of leaves. *Erysiphe polygoni* has been reported as the organism causing powdery mildew on peas and beans. Whenever powdery mildew is observed on either of these hosts, the identity of the fungus must be verified by the cleistothecia produced.

The powdery mildew fungus on soybeans has been identified as *M. diffusa* in Illinois in 1974 (10) and confirmed in 1976 (8). Cleistothecia of *Erysiphe* have not been observed in any of the cases called to our attention since 1973 nor in field samples received in the University of Illinois Plant Disease Clinic. In all cases, the cleistothecia found were of *M. diffusa*. When the isolate used during these studies was inoculated on field-grown soybeans in June 1976, the cleistothecia formed in late August 1976 were of *M. diffusa*.

Reports on the occurrence of powdery mildew on field-grown soybeans, and the susceptibility of many commercial cultivars make this disease one that should be closely monitored.

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