Isolation of *Gaeumannomyces graminis* var. *graminis* from Soybeans in the Midwest

K. W. ROY, Former Research Assistant, Botany and Plant Pathology Department, T. S. ABNEY, Plant Pathologist, USDA, ARS, D. M. HUBER, Professor, and R. KEELER, Former Research Assistant, Botany and Plant Pathology Department, Purdue University, West Lafayette, IN 47907

ABSTRACT

Roy, K. W., Abney, T. S., Huber, D. M., and Keeler, R. 1982. Isolation of *Gaeumannomyces graminis* var. *graminis* from soybeans in the Midwest. Plant Disease 66:822-825.

Gaeumannomyces graminis (= *Ophiobolus graminis*), the cause of take-all disease of wheat (*Triticum aestivum*) and other cereals, was isolated from immature field-grown pods of six soybean (*Glycine max*) cultivars in Indiana. The soybean isolates produced typical take-all symptoms on wheat roots and culms and were reisolated from soybean roots inoculated in the greenhouse. The soybean isolates produced lobed hyphopodia, perithecia, and ascospores (71.6 × 2.6 μm) on potato-dextrose agar. The presence of lobed hyphopodia and ascospore size indicated that the isolates were *G. graminis* var. *graminis* rather than varieties *tritici* or *avenae*. Aminopeptidase profiles for the three varieties supported the identification of the soybean isolates as *G. graminis* var. *graminis*, as well as the separation of *G. graminis* into three varieties. *G. graminis* var. *graminis*, previously reported only on monocotyledonous plants, may be potentially significant in the epidemiology of the take-all disease in the Midwest.

A lobed hyphopodial fungus thought to be *Gaeumannomyces graminis* (Sacc.) Arx et Oliver (= *Ophiobolus graminis* Sacc.), the cause of take-all of cereals, was isolated during a survey of the mycoflora of soybean reproductive structures in Indiana. Walker (8) has proposed the following classification for fungi causing take-all of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and other grasses: a) *G. graminis* var. *graminis*—lobed hyphopodia, ascospores 75–100 μm; b) *G. graminis* var. *tritici* Walker—simple hyphopodia, ascospores 75–100 μm; and c) *G. graminis* var. *avenae* (Turner) Dennis—simple hyphopodia, ascospores 110–130 μm. Walker found *G. graminis* var. *graminis* and *O. oryzinus* Sacc., the cause of brown sheath rot of rice, to be synonymous (8).

Nilsson (4) was the first to isolate a lobed hyphopodial strain of *G. graminis* from wheat in Sweden and northern Europe. This lobed hyphopodial strain was virulent to wheat, oats, barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), and maize (*Zea mays* L.). Furthermore, Nilsson (4) reported that because environmental and cultural conditions might influence the production of lobed hyphopodia, additional data were required to determine whether the Swedish isolate was *G. graminis* var. *graminis* or a mutant of *G. graminis* var. *tritici* capable of producing lobed hyphopodia and having an unusually high pathogenicity to wheat, oats, rye, maize, and other grasses.

Walker (8) also reported a lobed hyphopodial strain from Australia. However, the Australian isolate referred to as *G. graminis* var. *graminis* was weakly pathogenic on wheat. The conidial state of the common take-all fungus (*G. graminis* var. *tritici*) has been reported to be *Phialophora radicicola* Cain (7); however, there are differing concepts of this species held by different authors (7,9,10).

Our objectives were to report the occurrence of *G. graminis* isolated from soybean pods and to determine whether these *G. graminis* isolates with lobed hyphopodia could be distinguished from two nonlobed strains commonly isolated from cereals.

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MATERIALS AND METHODS

Natural incidence of *G. graminis* on soybean reproductive structures. Amsoy 71, Wells, Wayne, Williams, Cutler, and P.I. 80837 soybeans (*Glycine max* (L.) Merr.) were planted in a randomized block design at the Purdue University agronomy farm near Lafayette, IN, to allow study of the mycoflora of soybean reproductive structures. The soybean cultivars were planted in four-row plots 76 cm apart and 1 m long, replicated three times. Because the six soybean cultivars were of different maturity groups (II, III, and IV), they were planted at different dates to synchronize the time of flower, pod, and seed development for all six cultivars. The first flower samples were collected 7 August. At that time, stage of growth for the six cultivars ranged from full bloom to young pods developing in lower leaf axils. Subsequently, pod and seed samples were collected seven times at approximately 2-wk intervals, with the last samples being collected after normal harvest.

At each sampling time, 10 plants were collected from each replicate of the cultivars and stored at 5 C overnight. The following day, main stems of each plant were divided into four equal sections, and a random sample of 20 flowers, pods, or seeds was selected from each section. A 5-mm^2 disk of tissue from the peduncle and stylar ends and middle of pods was removed using a 6-mm-diameter paper punch. The flowers and three pod disks and seeds from each pod were submerged in 95% ethanol for 10 sec and then in 1% sodium hypochlorite for 1 min. The flowers, pod disks, and seeds were agitated during treatment. Flowers and pods contaminated with soil were avoided during sampling. Flowers, pod disks, and seeds from each pod position were plated separately on potato-dextrose agar (PDA) and incubated for 10 days at 22 C. Percentage of *G. graminis* pod infection was based on the most frequent occurrence of the fungus among the three pod positions.

Pathogenicity of *G. graminis*. Pathogenicity tests were conducted in a growth chamber and in the greenhouse with a representative isolate (116) of the lobed *G. graminis* obtained from soybean pods.

Table 1. β-Naphthylamide substrates utilized in obtaining aminopeptidase profiles

β-Naphthylamide substrate	Abbreviation
Alanine	ALA
Argine	ARG
Benzol-argine	BANA
Aspartic acid	α-ASP
Glutamic acid	γ-GLU
Cystine	CYS
Glycine	GLY
Histidine	HIS
Hydroxyproline	HYPRO
Leucine	LEU
Isoleucine	ILEU
Lysine	LYS
Methionine	MET
Methoxyleucine	4-M-LEU
Phenylalanine	PHE
Proline	PRO
Pyrrolidone	PYR
Serine	SER
Threonine	THR
Tyrosine	TYR
Valine	VAL

A 10-day-old culture of the fungus growing on PDA was placed at the bottom of each of 10 clay pots (10 cm diameter) containing an unsterilized sand-soil mixture in which five 1-wk-old Amsoy 71 seedlings were growing in a growth chamber. An equal number of pots containing five seedlings were treated with PDA as controls. The five seedlings in each container were selected for uniformity from eight seeds planted. Treatments were replicated three times. Ten days after inoculation, portions of the secondary roots and taproot of each plant were washed with running water, surface sterilized as previously described, and plated on PDA.

Lemhi wheat seedlings, grown in a sand:soil:vermiculite mixture (1:1:1, v/v) in plastic pots (10 cm diameter) in the greenhouse, were inoculated with mycelium taken from 10-day-old cultures of the fungus growing on PDA by inserting mycelial fragments around the base of tillers. Plants were observed periodically for expression of symptoms and compared with wheat seedlings inoculated with *G. graminis* var. *tritici*. After 14 days, portions of the roots and basal culms were examined for signs and symptoms of disease, surface sterilized as previously described, and plated on PDA.

Characterization of isolates. In addition to comparing morphological characteristics, aminopeptidase profiles of isolates from soybean were compared with profiles of *G. graminis* var. *tritici* (isolated from wheat in Indiana) and var. *avenae* (isolated from barley in England). Isolates were grown for 2 wk on 0.45-nm millipore filters overlaying PDA plates. The filters were removed from the plates and placed on sterile paper, and the mycelium was harvested from the millipore filters with a scalpel. The mycelium was weighed, suspended in 0.05 M tris-HCl buffer (pH 8.0), ground, and the resulting homogenate adjusted to 0.1 g of mycelium per milliliter of buffer.

Twenty-one 1.9-ml aliquots of β-naphthylamide substrates (Table 1) in 0.05 M tris buffer (pH 8.0) were each inoculated with 0.1 ml of the mycelial homogenates and incubated for 4 and 24 hr at 22 C. Aminopeptidase activity was determined fluorometrically by measuring the hydrolyzed β-naphthylamine from

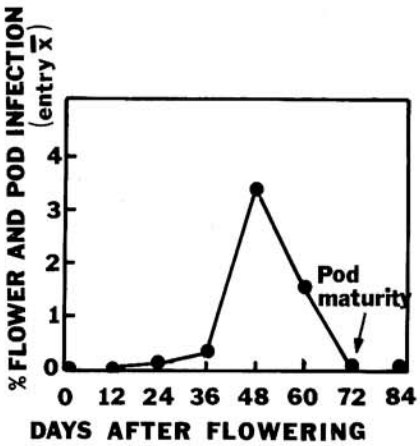

Fig. 1. Average incidence of *Gaeumannomyces graminis* var. *graminis* isolated from reproductive structures of six soybean varieties.

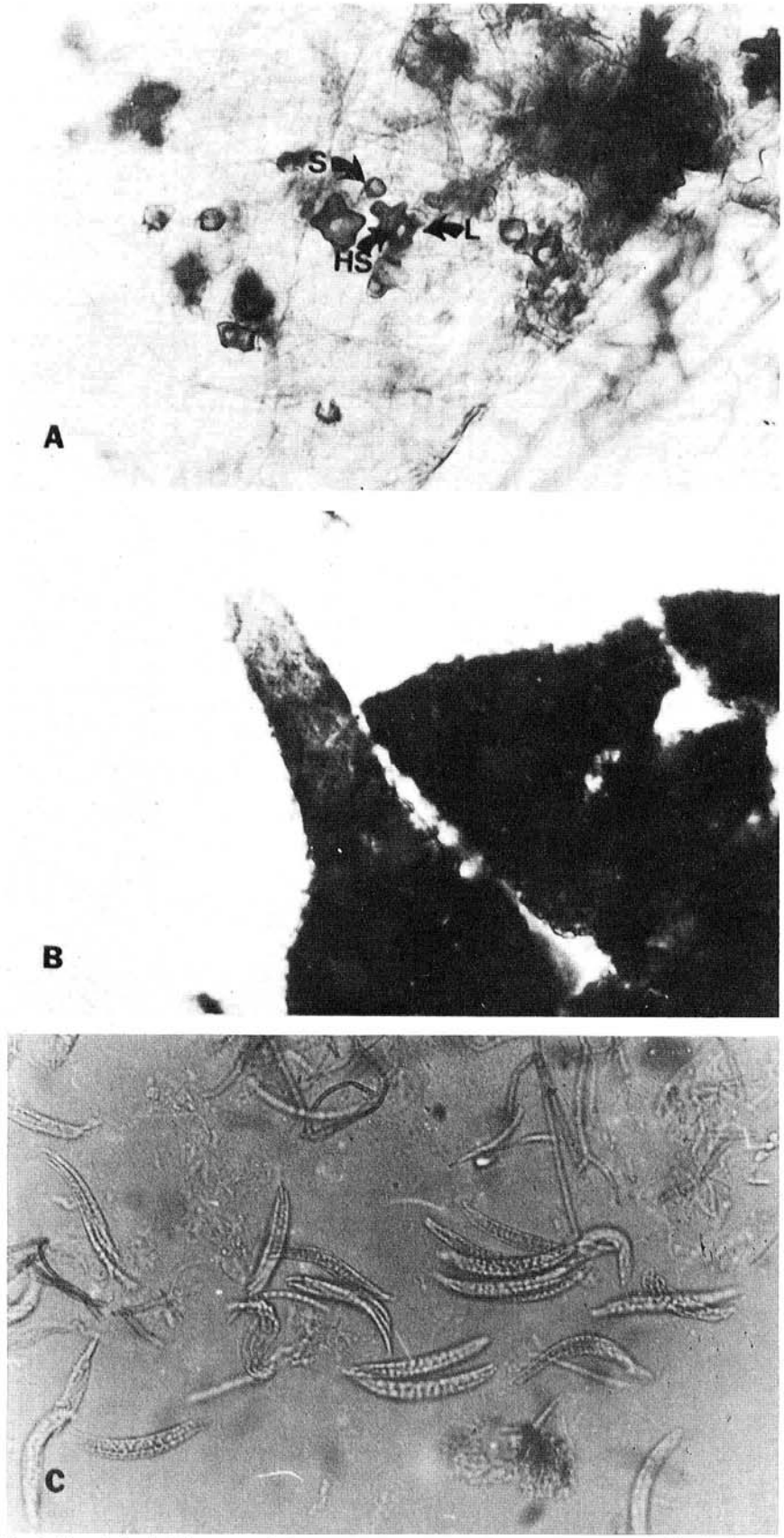

Fig. 2. Morphological features of *Gaeumannomyces graminis* var. *graminis*: **(A)** Simple (S) and lobed (L) hyphopodia. Note hyaline sphere in center of hyphopodium (HS). **(B)** Perithecium. **(C)** Asci and ascospores.

each substrate with a fluoromicrophotometer (American Instrument Co., Silver Springs, MD) fitted with a Corning 7-60 narrow-band-pass primary filter and a Wratten 2A secondary filter. Tris-HCl buffer alone or with β-naphthylamine was also inoculated to determine background fluorescence and maximum fluorescence with complete hydrolysis at both incubation periods.

The data were analyzed by Duncan's new multiple range test. Significance was determined at the 0.05 level.

RESULTS

Natural incidence of *G. graminis* on soybean reproductive structures. *G. graminis* was first isolated from pods 24 days after flowering. It reached its highest frequency 24 days later and then decreased (Fig. 1). It was isolated from pods of each soybean cultivar, with the highest frequency (8.3%) in Amsoy 71. The fungus was isolated in similar frequencies from pods on the four stem positions, but was not isolated from flowers or seeds. In this initial study, *G. graminis* was not isolated from mature pods; however, we have isolated it sporadically from mature pods of soybean cultivars in the course of other studies.

Table 2. Aminopeptidase activity of *Gaeumannomyces graminis* var. *avenae*, var. *tritici*, and var. *graminis* after incubation for 4 and 24 hr in β-naphthylamide substrates

β-Naphthylamide substrate	Hydrolysis (%)					
	Incubated 4 hr			Incubated 24 hr		
	avenae	*tritici*	*graminis*	*avenae*	*tritici*	*graminis*
ALA	49.3 a[z]	26.9 b	17.3 c	107.0 a	89.5 ab	76.4 b
ARG	41.6 a	38.8 a	25.5 b	45.9 a	46.1 a	38.9 b
BANA	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
ASP	6.4 a	6.1 a	4.2 a	12.7 a	6.8 b	6.8 b
GLU	23.6 b	35.3 a	5.4 c	95.0 b	110.5 a	16.3 c
CYS	6.6 a	7.2 a	5.4 b	11.1 a	10.4 a	7.0 b
GLY	10.5 a	5.5 b	5.6 b	31.5 a	19.9 b	13.0 c
HIS	3.2 a	3.3 a	1.4 a	16.4 a	7.4 b	4.9 c
HYPRO	12.8 a	12.1 a	5.1 b	44.3 a	39.0 b	16.6 c
LEU	76.7 b	95.4 a	38.4 c	90.8 a	91.8 a	97.5 a
ILEU	1.6 a	24.2 b	1.4 a	11.0 a	84.5 b	6.5 a
LYS	110.2 a	120.3 a	73.0 b	112.5 a	120.0 a	108.9 a
MET	36.9 a	32.2 a	11.5 b	109.6 a	101.1 a	58.7 b
4-M-LEU	105.9 a	102.4 a	22.6 b	264.5 a	275.9 a	71.2 b
PHE	36.7 a	19.3 b	9.6 c	83.6 a	65.0 b	39.1 c
PRO	47.6 a	38.6 a	11.1 b	93.7 a	73.2 b	67.6 b
PYR	6.4 a	0.0 b	0.0 a	1.7 a	0.0 b	0.0 b
SER	6.2 a	4.6 ab	1.8 b	27.7 a	19.8 b	12.3 c
THR	1.6 a	2.6 a	0.0 b	6.6 a	15.0 b	3.3 a
TYR	4.4 a	2.0 b	1.4 b	16.4 a	11.3 b	8.2 c
VAL	1.6 a	17.9 b	1.1 a	13.1 a	90.8 b	6.5 a

[z] Means followed by the same letters within a row are significantly different, $P = 0.05$.

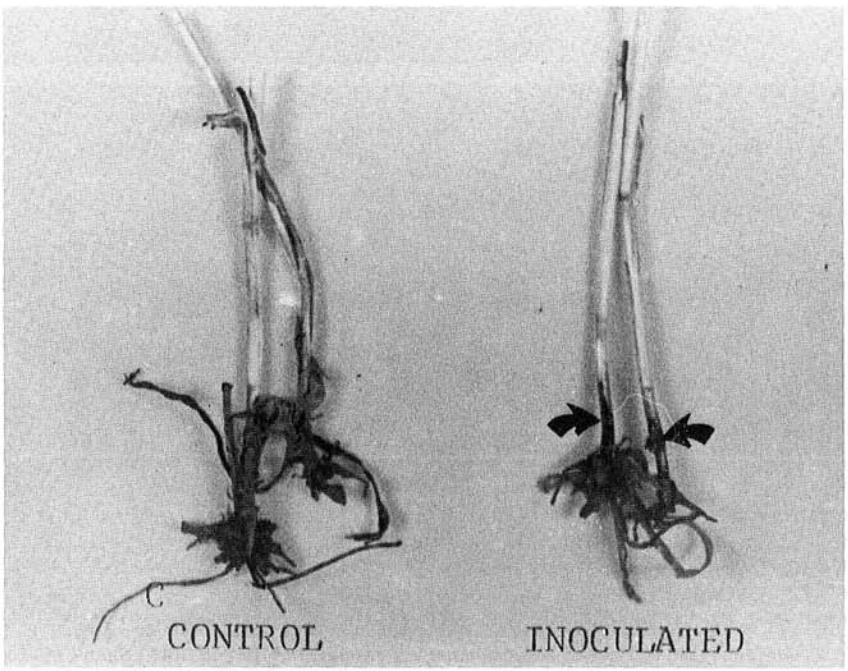

Fig. 3. Necrosis (arrows) of wheat culm following inoculation with *Gaeumannomyces graminis* var. *graminis*.

Morphological characterization of *G. graminis* from soybean. The fungus was recognized in cultures incubated at 22 C by its spreading mycelium, aggregations of hyphae on and below the agar surface, and production of hyphopodia, which imparted a "peppered" appearance to the underside of PDA cultures and along the sides of the petri dishes. The aerial mycelium was at first white but turned gray after several days. Submerged and surface mycelial aggregations were dark green to black. Hyphopodia usually had one or more lobes, were brown to olive brown, and each contained a hyaline sphere (Fig. 2A). Perithecia occurred only on pod disks and rarely formed during the normal 10-day bioassay period. Although perithecia were produced in PDA cultures incubated 6 wk, perithecial development occurred more rapidly on a thiamine biotin basal salt medium. Perithecia were light to dark brown; some were globular with a short, papillate ostiole, and others were pyriform, occasionally with long, cylindric beaks that curved near their tip (Fig. 2B). Ascospores (Fig. 2C) were hyaline, multiseptate, filiform, and attenuated toward one end; they were oriented in a parallel fascicle within the cylindric ascus and measured 71.6 (± 6.8) × 3.6 μm (± 0.5). Conidia were not observed in any of the soybean isolates of *G. graminis* during the initial 10-day bioassay period or after subsequent subcultures in excess of 6 wk.

The *G. graminis* soybean isolates were stable in cultural appearance on subculturing and differed from *G. graminis* var. *tritici* and var. *avenae* in cultural appearance because of their hyphopodial development, rapid growth rate, and hyphopodial morphology. Lobed hyphopodia were never produced by isolates of *G. graminis* var. *tritici* or var. *avenae* in this study.

Pathogenicity of *G. graminis* to wheat and soybean. The lobed hyphopodial *Gaeumannomyces* was recovered from 100% of the roots and culms of inoculated wheat plants. Infection was mild, but discolored roots, necrosis, and vascular occlusion adjacent to infected cortical tissue were typical of take-all (Fig. 3). Disease symptoms were not apparent on soybean leaves, stems, or roots, but the fungus was reisolated from roots and hypocotyls (Fig. 4) of 70% of the inoculated plants.

Aminopeptidase profiles. Aminopeptidase profiles representative of the three *G. graminis* isolates are given in Table 2. Profiles for the isolates were similar in some respects, but each differed from the other with specific substrates. There were more similarities between *G. graminis* var. *avenae* and var. *tritici* than between either of these varieties and the soybean isolate. Each isolate differed from the other in the extent of its hydrolysis of alanine, glutamic acid,

leucine, phenylalanine, and proline β-naphthylamides after 4 hr of incubation; these differences were also shown at 24 hr for glutamic acid and phenylalanine. The hydrolysis of glycine, histidine, hydroxyproline, serine, and tyrosine at 4 hr was similar among isolates, but each differed from the other in hydrolysis of these substrates at 24 hr. The hydrolysis of arginine, glutamic acid, histidine, hydroxyproline, methionine, 4-methoxyleucine, and phenylalanine by the lobed hyphopodial isolate was less than that by *G. graminis* var. *avenae* and var. *tritici*; the latter two hydrolyzed several of these substrates to a similar extent. Values in excess of 100% hydrolysis resulted from equating all intensities to the β-naphthylamine blank, which was lower than calculated because of sublimation during autoclaving.

DISCUSSION

Morphological characteristics of the lobed hyphopodial strain of *G. graminis* isolated from soybean were similar to the description of *G. graminis* var. *graminis* reported by Walker (8–10) and the description of a lobed strain of *G. graminis* reported by Nilsson (4). The soybean isolates did not produce conidia, but Nilsson's report indicates that conidial production by his lobed isolates was variable (4). Aminopeptidase activities of the three *G. graminis* types indicated that *G. graminis* var. *tritici* and var. *avenae* were more closely related than was the soybean isolate to them and also indicated that the three were basically different from each other. Thus, according to the morphological and physiologic data, the three types are separate varieties as proposed by Walker (8–10).

Our previous report (6) is the first record of the isolation of *G. graminis* var. *graminis* from Indiana and the Midwest. This paper presents a detailed account of the occurrence and identification of *G. graminis* var. *graminis* on soybean. To our knowledge, this is the first report of this fungus on a dicotyledonous plant. It was found frequently on soybean pods in this study (8.3%). By comparison, the highest frequency of *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm. and *Cercospora kikuchii* (Matsumoto & Tomoyasu) Gardner, two common pathogens of soybean pods, was 22% and 7.5%, respectively, in the same year (5). Most important, however, is the potential significance of the presence of *G. graminis* var. *graminis* on soybean.

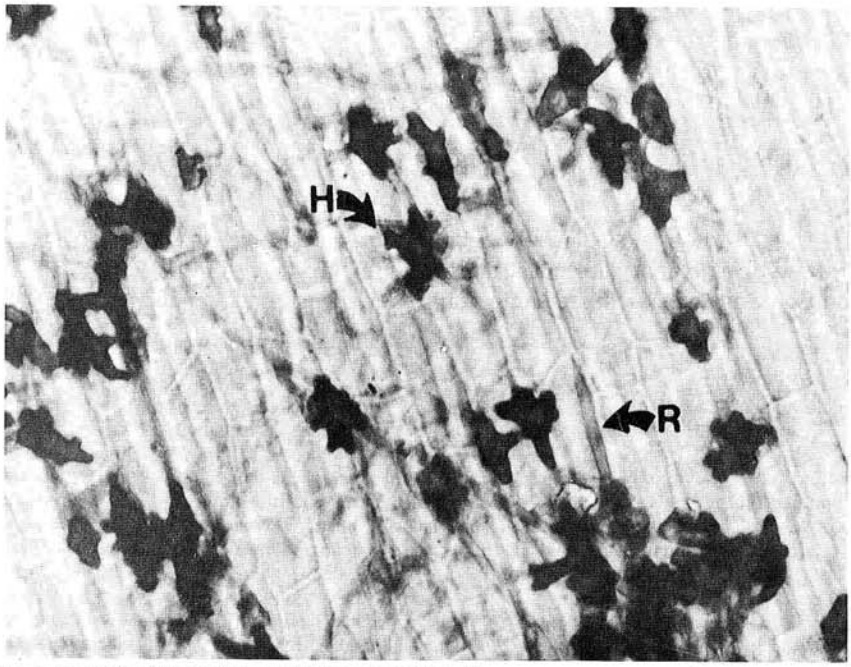

Fig. 4. Colonization of Amsoy 71 hypocotyl by *Gaeumannomyces graminis* var. *graminis*. Hyphopodia (H). Runner hyphae (R).

Our data indicate that the fungus can occur as a parasite on soybean. However, additional studies are needed to establish the importance of these observations. The occurrence of *G. graminis* var. *graminis* on soybeans may suggest a potential role for it in the epidemiology of the take-all disease. The report by Nilsson (4) indicates that the lobed strain of *G. graminis* is capable of causing heavy infection of wheat under natural conditions. Although we have not isolated the lobed hyphopodial strain of *G. graminis* from field-grown wheat, take-all is frequently severe on early seeded wheat grown after soybeans in the Midwest (1,2). In addition, the planting of wheat following other legumes has been reported to increase the incidence of take-all (1–3). This increase in take-all has been partially attributed to changes in soil nitrogen levels due to the legumes. Our study indicates there is reason to suspect that the inoculum of *G. graminis* may increase through its survival on soybean tissues.

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