Effects of Cropping History, Cultivar, and Sampling Date on the Internal Fungi of Soybean Roots

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ABSTRACT

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The incidence of fungi and bacteria occurring in the roots of six soybean (*Glycine max*) cultivars growing in fields cropped the previous 3 yr to either corn (*Zea mays*) or soybeans was determined at 2-wk intervals throughout the 1980 growing season. Incidence of fungi increased up to 96 days after planting, then decreased until harvest. Roots were colonized early by *Chaetomium* spp., two groups of *Fusarium* spp., *Gliocladium roseum*, *Penicillium* spp., and *Trichoderma* spp. Recovery of *Chaetomium* and *Penicillium* spp. declined rapidly, and only trace amounts were recovered after the sample at 82 days. *Fusarium* and *Trichoderma* spp. were recovered less often than *G. roseum* throughout the growing season. *Macrophomina phaseolina* and *Phomopsis* spp. are considered pathogenic, and their recovery differed among cultivars. Recovery of nonpathogenic early-season colonizing fungi did not differ among cultivars. Cropping history affected the recovery of *M. phaseolina, Phomopsis* spp., and *Trichoderma* spp. but not *Fusarium* spp. or *G. roseum*. Incidence of recovery from plants following soybeans was greater than from corn for *Phomopsis* spp. and nearly twice as great for *M. phaseolina*. Recovery of *Trichoderma* spp. was greater following corn than following soybeans.

Additional key words: pod and stem blight

At least 29 genera of fungi and five genera of bacteria occur internally on soybean (*Glycine max* (L.) Merr.) tissues (4). Many of the fungi reported are common pathogens of soybeans, but at least 16 genera are not considered pathogenic. Other crops such as beans (*Phaseolus* sp.), corn (*Zea mays* L.), and peas (*Pisum sativum* L.) have similar species of internal mycoflora (2,7,10). The succession of organisms that occurs on these crops is dependent on host plant age and environmental conditions. This succession consists of three communities in beans and peas and five in corn. An organism may be present in the original group of colonizers but not in subsequent groups, or it may be present from the time it invades the plant until the plant dies. Primary inoculum of most fungal pathogens of soybeans are such over-wintering structures as microsclerotia, pycnidia, and hypha that may be soilborne or in plant debris (6). Such carryover inoculum is especially important for diseases that develop late in the previous season. No genetic resistance in soybeans is known against these late-season fungal pathogens except for *Phialophora gregatum* (Allington & Chamber.) W. Gams and *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *caulivora* Athow & Caldwell (8). Chemical control of these pathogens usually is not economical except in seed production fields. Crop rotation and fall plowing are recommended control practices for these diseases.

The objective of this study was to compare the succession of microorganisms in soybean roots in relation to cropping history, cultivar, and sampling date. Various maturity groups were examined because many latent fungal infections of soybeans are favored by early or rapid senescence.

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MATERIALS AND METHODS

The six cultivars studied during the 1980 growing season were Harcor and Wells (maturity group II), Elf and Wayne (maturity group III), and Union and Clark 63 (maturity group IV). Elf, Harcor, and Union are less susceptible to Phomopsis seed, stem, and pod blight than either Clark 63, Wayne, or Wells. All cultivars are indeterminate except Elf, which is determinate and semidwarf.

Four replicates of the six cultivars were planted in a randomized complete block design on 30 May in adjacent fields at the University of Illinois at Urbana-Champaign Plant Pathology Research Center. Plots consisted of four 5-m rows on 76-cm centers. One field had been planted with soybeans and the other with corn the previous three growing seasons. Soil type was a Drummer silty clay loam. Both fields were chisel-plowed in November 1979 and chisel-plowed and disced in April 1980. Trifluralin (Treflan) was applied. Plots were hand-cultivated for additional weed control.

Five plants were removed from each plot at 2-wk intervals beginning 26 days after planting. Plants matured between 25 September and 9 October. Two 1-cm pieces were cut from each taproot 2–7 cm below the soil line. Pieces were placed in double-layered cheesecloth bags and washed in tap water for 8 hr in an automatic pipette washer. Pieces were dipped in 90% ethanol, surface-disinfested for 4 min in a 0.5% NaOCl solution, and rinsed in sterile distilled water for 1 min. One piece from each plant was transferred aseptically onto a 9-cm culture plate containing 20 ml of potato-dextrose agar (PDA) and the other onto a culture plate containing 20 ml of PDA amended with 200 μg/ml of potassium penicillin G (Eli Lilly, Greenfield, IN) and 200 μg/ml of streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) to inhibit bacterial growth. Plates were incubated in the dark for 12 days at 25 C. The number and type of fungi growing from each piece were recorded. Fungi were identified by cultural characteristics and fruiting structures to genera level. *Fusarium* spp. were divided into two groups based on coloration and colony morphology. The *Fusarium*-I group was floccose and had red, white, or yellow mycelia, or a mixture of the three colors, and turned the medium no color or pink to red. The *Fusarium*-II group produced colonies that were slightly oppressed and turned the media blue to purple.

Recovery incidence of the most commonly occurring fungi was analyzed as split-split plots with cultivars as main plots, isolation media as subplots, and sampling dates as sub-subplots (3). Cropping histories were not replicated and were analyzed separately. Correlation coefficients were computed for all possible combinations of the six most common fungal genera.

RESULTS

The total number of fungi recovered was greater following soybeans than following corn at each sampling date (Fig. 1). The number of fungi recovered following soybeans increased between 26 and 68 days after planting and fluctuated thereafter, and following corn, it increased between 26 and 96 days after planting. The number of fungi recovered from roots decreased from 96 days after planting to harvest at 110 days following both corn and soybeans. *Fusarium* spp., *Gliocladium roseum, Macrophomina phaseolina, Phomopsis* spp., and *Trichoderma* spp. were isolated most frequently. Species of *Alternaria, Aspergillus, Epicoccum, Eurotium, Mucor, Myrothecium, Rhizoctonia, Rhizopus,* and *Stilbum* also were recovered. *Chaetomium* spp. were present in 9% of the roots 26 days after planting, in 2% at 40 days, and in less than 1% thereafter (Fig. 2). *Penicillium* spp. were recovered from 1, 7, 6, and 3% of roots 26, 40, 54, and 68 days after planting, respectively, but recovery was never greater than 1% thereafter (Fig. 2).

Recovery of *Fusarium*-I group varied among sampling dates in both rotations, and recovery was greater on PDA than on antibiotic PDA at some sampling dates in the field previously cropped to soybeans (Tables 1 and 2, Fig. 3). Recovery of *Fusarium*-II group was not affected by any parameter in the field previously planted with corn (Table 1). Recovery of *Fusarium*-II group from plants in the field previously cropped to soybeans differed between sampling dates and was greater on PDA than on antibiotic PDA (Table 2, Fig. 3), which could not be explained.

Incidence of *G. roseum* ranged between 19 and 71% in individual plots and increased between 26 and 40 days after planting, then fluctuated until harvest (Fig. 3). Medium and sampling date affected its occurrence but cropping history and cultivar did not (Tables 1 and 2). Mean recovery of *G. roseum* from both rotations was 27 and 37% from PDA and antibiotic PDA, respectively. Recovery of *G. roseum* from plants grown in the field previously planted with soybeans differed between sampling dates (Fig. 3). These differences were affected by cultivar and isolation medium, with recovery from some cultivars greater on antibiotic PDA.

Recovery of *M. phaseolina* was affected by sampling date and cultivar with a cultivar × sampling date interaction (Tables 1 and 2). Recovery increased at each sampling date from each crop history (Fig. 4). Incidence following soybeans was almost twice that following corn (Fig. 4). Recovery was greater 82, 96, and 110 days after planting from cultivars Harcor and Wells (both maturity group II) than from Union (group IV). Recovery from Union, in turn, was greater than from Clark 63 (group IV) or Elf and Wayne (both group III) (Fig. 5).

Recovery of *Phomopsis* spp. from plants grown in the field previously planted with corn was not affected by any factor (Table 1). Recovery following soybeans was affected by sampling date, with recovery greater on antibiotic PDA than on PDA on some sampling dates and an interaction between sampling date, isolation medium, and cultivar (Table 2). The first recovery following corn was 82 days after planting, and recovery was never greater than 6.7%. The first recovery from roots of plants following soybeans was 40 days after

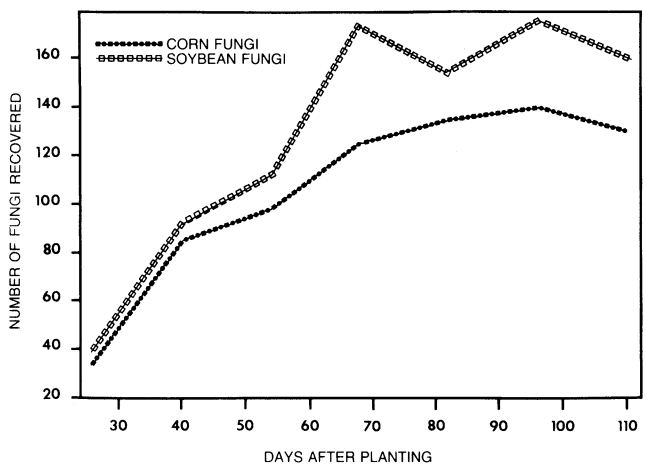

Fig. 1. Recovery of fungi from taproots of soybeans grown in two fields, one previously planted with corn and the other with soybeans.

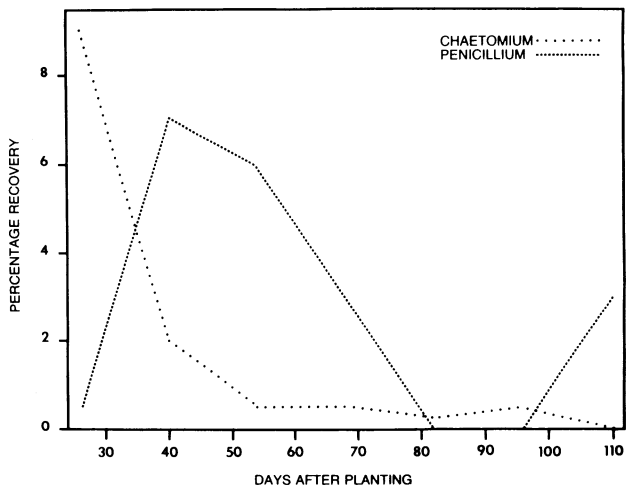

Fig. 2. Mean recovery of *Chaetomium* and *Penicillium* spp. from roots of soybeans sampled on seven dates from two fields, one previously planted with corn and the other with soybeans.

Table 1. Main effects and interactions of cultivar (cult.), isolation medium (med.), and sample date (S.D.) on recovery of two *Fusarium* groups, *Gliocladium roseum, Macrophomina phaseolina, Phomopsis* spp., and *Trichoderma* spp. from the taproots of soybeans grown in a field cropped the previous 3 yr to corn

Source variation	df	Mean squares					
		Fusarium I	*Fusarium* II	*G. roseum*	*M. phaseolina*	*Phomopsis* spp.	*Trichoderma* spp.
Block	3	246	776	184	439	8	200
Cult.	5	168	410	2,470*[z]	927**	41	1,132*
Error a	15	310	151	751	237	72	393
Med.	1	1	3,344	9,643**	525	119	1,905*
Cult. × Med.	5	82	133	141	115	14	469
Error b	18	145	174	565	135	44	539
S.D.	6	817**	2,246	6,178**	4,969**	153	7,039*
Cult. × S.D.	30	162	579	1,481	545**	64	448
Med. × S.D.	6	190	883	1,413	66	76	512
Cult. × Med. × S.D.	30	209	392	455	114	38	197
Error c	216	210	296	587	173	48	383
C.V.	···	188	136	81	120	527	123

[z] * = Significant at $P = 0.05$ and ** = significant at $P = 0.001$.

planting. Recovery was highest 96 days after planting and decreased between 96 and 110 days. At 96 and 110 days after planting, recovery of *Phomopsis* spp. was highest from Clark 63 (Fig. 6).

Incidence of *Trichoderma* spp. was affected by sampling date and isolation medium in both fields (Tables 1 and 2). Interactions of cultivar with isolation medium and sampling date were detected for recovery of *Trichoderma* spp. following soybeans (Table 2). Mean recovery of *Trichoderma* spp. following corn was 16% compared with 11% following soybeans over all sampling dates and cultivars (Fig. 7).

Recovery of *G. roseum* was positively correlated with recovery of *Fusarium*-I

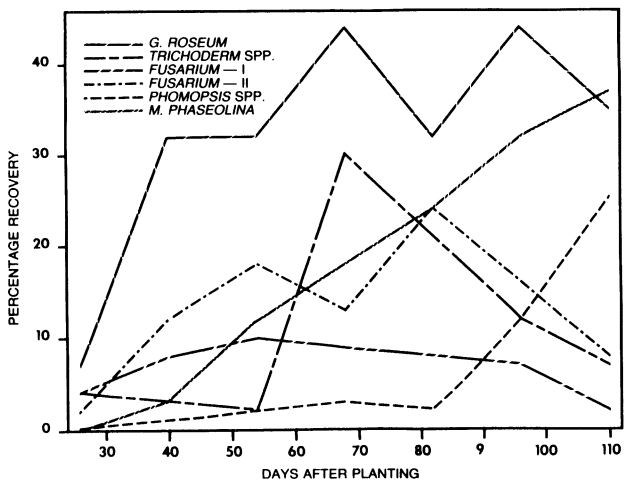

Fig. 3. Percentage of root pieces from which fungi were recovered at seven sampling dates from roots of soybeans grown in a field planted with either corn or soybeans the previous 3 yr.

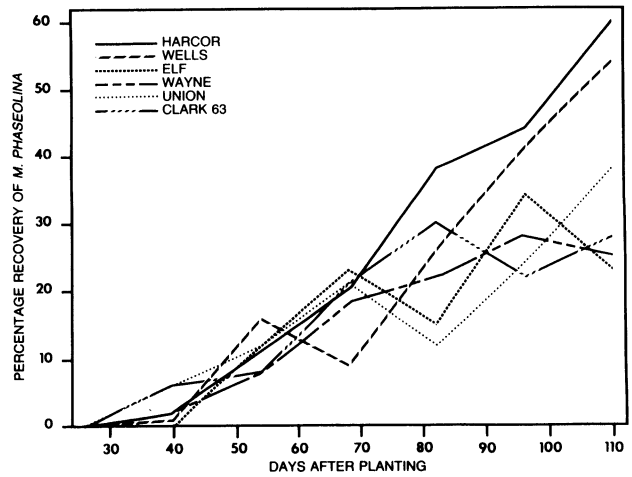

Fig. 5. Percentage of root pieces from which *Macrophomina phaseolina* was recovered at seven sampling dates from the soybean cultivars Clark 63, Elf, Harcor, Union, Wayne, and Wells grown in a field planted with either corn or soybeans the previous 3 yr.

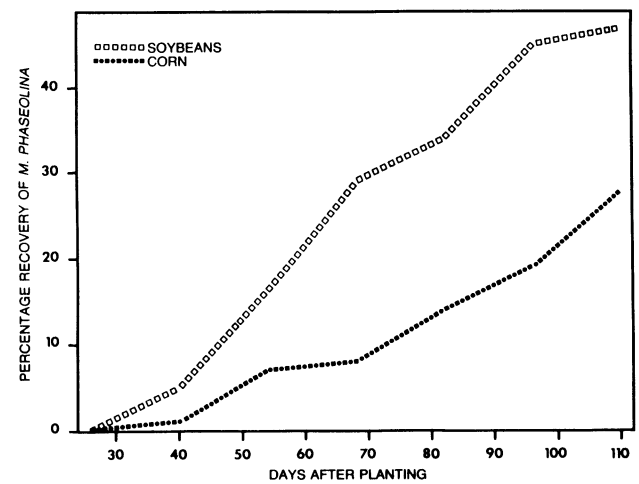

Fig. 4. Percentage of root pieces from which *Macrophomina phaseolina* was recovered at seven sampling dates from roots of soybeans grown in a field planted with either corn or soybeans the previous 3 yr.

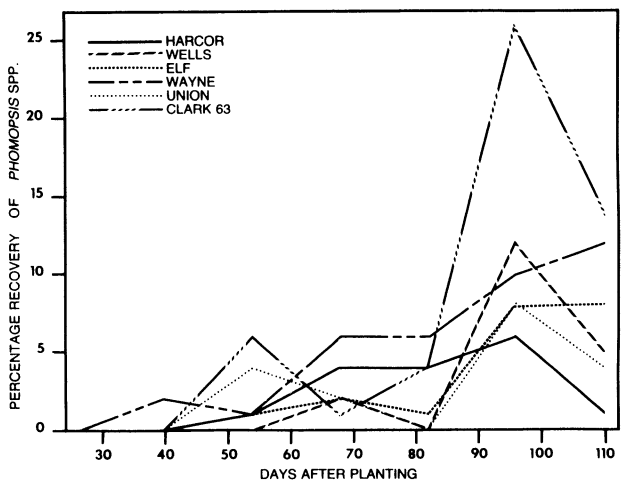

Fig. 6. Percentage of root pieces from which *Phomopsis* spp. were recovered at seven sampling dates from the soybean cultivars Clark 63, Elf, Harcor, Union, Wayne, and Wells grown in a field planted with either corn or soybeans the previous 3 yr.

Table 2. Main effects and interactions of cultivar (cult.), isolation medium (med.), and sample date (S.D.) on recovery of two *Fusarium* groups, *Gliocladium roseum, Macrophomina phaseolina, Phomopsis* spp., and *Trichoderma* spp. from the taproots of soybeans grown in a field cropped the previous 3 yr to soybeans

Source variation	df	Mean squares					
		Fusarium I	*Fusarium* II	*G. roseum*	*M. phaseolina*	*Phomopsis* spp.	*Trichoderma* spp.
Block	3	406	985	1,683	1,202	64	1,188**[z]
Cult.	5	206	297	569	2,212**	869**	341
Error a	15	293	392	572	540	41	212
Med.	1	5	6,344**	7,430**	268	1,296**	2,201**
Cult. × Med.	5	192	316	1,030*	206	102	217
Error b	18	380	414	374	177	129	224
S.D.	6	515**	3,534**	12,028**	16,690**	2,758**	5,500*
Cult. × S.D.	30	245	259	1,466**	896**	320	466
Med. × S.D.	6	631**	759	1,995**	79	149**	457
Cult. × Med. × S.D.	30	135	488	1,075**	259	162**	538**
Error c	216	178	370	519**	282	153	322
C.V.	···	215	140	66	67	190	156

[z] * = Significant at $P = 0.05$ and ** = significant at $P = 0.01$.

spp. (Table 3). Recovery of *M. phaseolina* was positively correlated with recovery of *Phomopsis* spp.; however, recovery of *Fusarium*-I group was not highly correlated with recovery of either *M. phaseolina* or *Phomopsis* spp.

DISCUSSION

Surface-disinfestation of plant parts ensured that this was a study of internal mycoflora instead of external mycoflora as in previous studies with beans and peas (2,7). We report the incidence but not the extent of fungal colonization.

Fungi were recovered from fewer than 10% of the plants 26 days after planting. Recovery of *Chaetomium* spp. was highest at the first sampling date, then decreased; *Penicillium* spp. was highest at the second and third sampling dates, then decreased. These two fungi apparently are initial colonizers of soybean roots but do not persist. Recovery of *G. roseum* increased rapidly as did that of *Trichoderma* spp., with the latter increasing about 28 days later. Recovery of the *Fusarium* spp. was intermediate between *G. roseum* and *Trichoderma* spp. All three genera were present from 28 days after planting until harvest. *G. roseum* was recovered more often than either *Fusarium* group or *Trichoderma* spp. at all sampling dates.

The linear increase of *M. phaseolina* incidence in roots in both fields on every sampling date was expected since the pathogen is favored by host senescence. *Phomopsis* spp. were not recovered from roots until 40 days after planting, which is later than first-recorded recovery from stems; however, recovery from roots followed the same pattern as in stems, being recovered at a later date from plants following corn than following soybeans (5).

M. phaseolina and *Phomopsis* spp. are pathogenic to soybean and were the only fungi whose recovery was affected by cultivar. This suggests that some level of resistance exists or that variation in maturity group, and therefore senescence rate, allows more colonization by these pathogens. Lower recovery of *M. phaseolina* and *Phomopsis* spp. from the field previously cropped to corn illustrates the role of crop rotation in reducing inoculum efficiency. *M. phaseolina* is pathogenic on corn, but the different recoveries between rotations confirms that some variability in virulence may exist on a subspecies level.

Fusarium spp., *G. roseum*, and *Trichoderma* spp. are more permanent constituents of the soybean-root mycoflora than are *Chaetomium* spp. or *Penicillium* spp. Lower initial recovery of *Trichoderma* spp. early in the growing season and the decrease in recovery late in the season indicates *Trichoderma* spp. may occur primarily during midseason. A similar pattern of occurrence of *Trichoderma* spp. was observed on corn (1,9). The greater recovery of *Trichoderma* spp. following corn than soybeans is consistent with previous reports on the recovery of *Trichoderma* spp. from corn (9). The positive correlation in the occurrence of *G. roseum, M. phaseolina,* and *Phomopsis* spp. suggests that increases in occurrence are due to plant senescence rather than to an interaction of the fungi leading to increased infection. The negative correlation between *Fusarium*-II group and *G. roseum, M. phaseolina*, and *Phomopsis* spp. probably is a result of *Fusarium*-II group being an early season inhabitant of soybean roots.

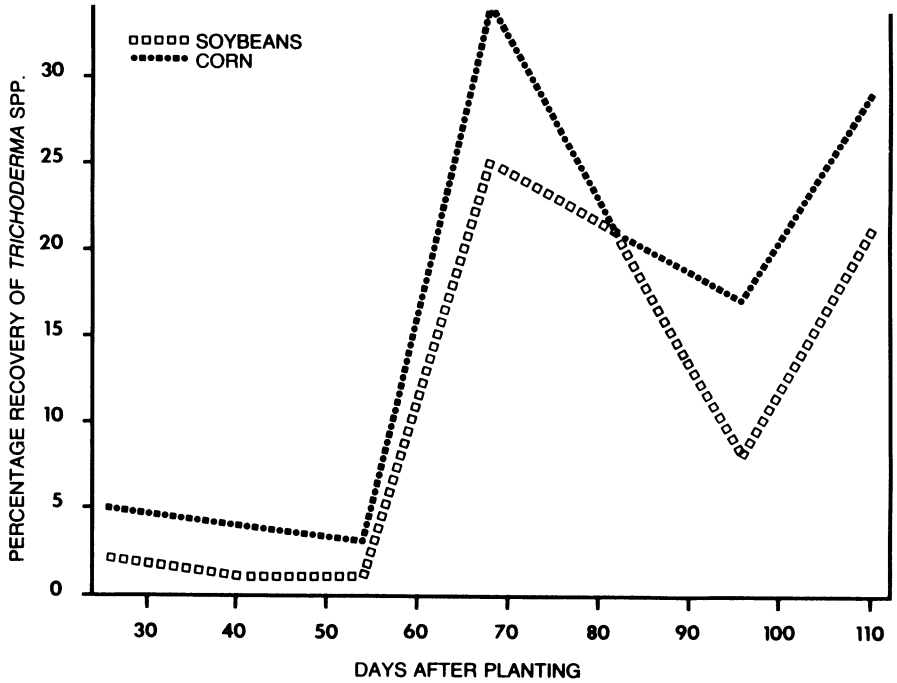

Fig. 7. Percentage of root pieces from which *Trichoderma* spp. were recovered at seven sampling dates from roots of soybeans grown in a field planted with either corn or soybeans the previous 3 yr.

Table 3. Correlation coefficients for recovery of the most frequently occurring fungi in soybean roots

	Correlation coefficients (r)[a]				
	Fusarium-II group	*Gliocladium roseum*	*Macrophomina phaseolina*	*Phomopsis* spp.	*Trichoderma* spp.
Fusarium-I group	0.26	0.63	0.10	0.04	0.13
Fusarium-II group	...	−0.03	−0.33	−0.27	−0.01
G. roseum	...	...	0.50	0.31	0.08
M. phaseolina	...	...	...	0.51	0.25
Phomopsis spp.	...	...	...	...	−0.03

[a] $N = 432$; all correlation coefficients were significant ($P = 0.01$) because of high degrees of freedom in the error term.

LITERATURE CITED

1. McFadden, A. G., and Sutton, J. C. 1975. Relationships of populations of *Trichoderma* spp. in soil to disease in maize. Can. J. Plant Sci. 55:579-586.
2. Parkinson, D., Taylor, G. S., and Pearson, R. 1963. Studies on fungi in the root region. I. The development of fungi on young roots. Plant Soil 19:332-349.
3. SAS Institute. 1979. SAS Users Guide. SAS Institute, Raleigh, NC. 494 pp.
4. Schiller, C. T., and Sinclair, J. B. 1984. Microorganisms associated with soybean vascular exudates and plant parts. Trop. Plant Pathol. 2:1-4.
5. Shortt, B. J. 1982. Epidemiology of Phomopsis seed decay of soybeans. Ph.D. thesis. University of Illinois at Urbana-Champaign. 92 pp.
6. Sinclair, J. B., ed. 1982. Compendium of Soybean Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN. 104 pp.
7. Stenton, H. 1958. Colonization of roots of *Pisum sativum* by fungi. Trans. Br. Mycol. Soc. 41:74-80.
8. Tisselli, O., Sinclair, J. B., and Hymowitz, T. 1980. Sources of Resistance to Selected Fungal, Bacterial, Viral and Nematode Diseases of Soybean. Univ. Ill. Urbana-Champaign, Coll. Agric. INTSOY Ser. 18, Urbana. 1,142 pp.
9. Windham, M. T., and King, S. B. 1983. Mycoflora of roots of maize plants at seedling and silking stages in Mississippi. Plant Dis. 67:1366-1368.
10. Young, T. R., and Kucharek, T. A. 1977. Succession of fungal communities in roots and stalks of hybrid field corn grown in Florida. Plant Dis. Rep. 61:76-80.