Ecology of a Sterile White Pathogenic Basidiomycete in Corn, Peanut, Soybean, and Snap Bean Field Microplots

D. K. BELL and DONALD R. SUMNER, Department of Plant Pathology, University of Georgia, Coastal Plain Experiment Station, Tifton 31793

ABSTRACT

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A sterile, white basidiomycete (SWB) caused a slight necrosis of seedling and mature corn roots. Crop rotation of soybean and corn increased the number of black lesions characteristic of SWB infection on corn roots. The fungus was recovered after 21 mo from soil planted to corn, peanut, and soybean but not from snap bean or fallow soil 16 mo after infestation. Fumigation of microplots with DD-MENCS before this study began did not eliminate *Rhizoctonia solani* AG-4, and this fungus caused extensive root-hypocotyl necrosis of peanut, soybean, and snap bean seedlings. Fewer colonies of AG-4 were isolated from peanut seed in pods attached to the plant at harvest in soil infested with the SWB than in the control. Fewer colonies of AG-4 were recovered from soil in peanut-corn and snap bean-corn than in corn-peanut and soybean-corn cropping systems. Although the SWB can cause extensive necrosis of corn roots in localized areas where inoculum potential is high, damage over a broad area of the Georgia coastal plain is probably slight.

A sterile, white basidiomycete (SWB) resembling *Sclerotium rolfsii* Sacc. in culture was described as a pathogen of snap bean (*Phaseolus vulgaris* L.) seedlings in Florida (4). A similar fungus was pathogenic to seedlings and older plants of pigeon pea (*Cajanus cajan* L.) in Puerto Rico (5). In Georgia, the same type of fungus has been isolated from roots or hypocotyls of corn (*Zea mays* L.), snap bean, and squash (*Curcurbita pepo* var. *melopepo* (L.) Alef) and from peanut seed (*Arachis hypogaea* L.) (6,8,10). One or more isolates of the SWB were highly virulent to Funks G-522 sorghum (*Sorghum vulgare* L.), Pioneer 3369A corn, Eagle snap bean, Athens Abruzzi rye (*Secale cereale* L.), and Coker 136 soybean (*Glycine max* L.) in a greenhouse test. It was less virulent to Purple Hull Pinkeye southern pea (*Vigna unguiculata* (L.) Walp.), Dade polebean, Early Bunch, Starr, and Florunner peanut, and Gemini Hybrid 353 cucumber (*Cucumis sativus* L.). The fungus produced black lesions on host roots or hypocotyls. Patches of white mycelium and small rhizomorphs were frequently seen at the juncture of stem and roots, especially on corn, sorghum, and rye (8).

The SWB did not produce sclerotia in culture or on infected host tissue (4,5), and the teleomorph has not been positively identified (8). Hyphae submerged in agar were predominantly monilioid cells, whereas *S. rolfsii* produced straight, slender hyphae. The SWB is characterized by prominent clamp connections, dolipore septa, binucleate aerial hyphae, and wide-angle hyphal branching. Aminopeptidase profiles of the SWB and *S. rolfsii* (8) indicated little physiological resemblance between the two fungi. The SWB was more virulent on test hosts at a night-day temperature range of 15–24 C than *S. rolfsii* (8).

The SWB has been frequently associated with multiple or monocropping systems in Georgia (9). The objectives of this study were 1) to determine the pathology and virulence of the SWB on corn, snap bean, peanut, and soybean in field microplots, 2) to determine populations of the SWB in soil about 4 mo after cropping, and 3) to study survival of the SWB in fallow soil.

MATERIALS AND METHODS

The study was established in field microplots consisting of concrete cylinders (surface area 0.3 m^2, length 1.2 m, and volume 0.1 m^3) filled with Alapaha loamy sand (about 77, 20, and 3% sand, silt, and clay, respectively). About 0.4 m of each cylinder was above the ground. The microplots were established in 1963 and were cropped to legumes or grasses each year. In 1977, 1978, and 1979, the microplots were infested with *Rhizoctonia solani* Kühn, *Pythium myriotylum* Drechs., or *Fusarium solani* (Mart.) Appel & Wr. and planted to peanut. In January 1980, microplots were fumigated with 315 L/ha of DD-MENCS (Vorlex) injected at about 25 cm deep. A split-plot experiment with a randomized complete block design and three replicates was established. The various crops constituted the whole plots and soil infested or uninfested with the SWB constituted the subplots.

Preplant fertilization (5-10-15; N, K_2O, P_2O_5) was 1,120 kg/ha for corn, 560 kg/ha for peanut, and 672 kg/ha for snap bean and soybean. Fertilizer was incorporated 20–25 cm deep. Thereafter, corn was fertilized with 56, 93, 56, and 28 kg N/ha using NH_4NO_3 at 16, 32, 42, and 47 days, respectively, after planting. Soybean and snap bean each received 56 kg N/ha 35 days after planting.

Peanut plants were sprayed as needed for leaf spot and insect control. All crops were watered as needed to prevent drought stress when rain was inadequate. Rainfall and irrigation were measured at each occurrence and maximum-minimum soil temperatures in the upper 9 cm of soil were recorded weekly.

On 2 May 1980, one-half of the subplots were infested with a mixture of six isolates of the SWB grown on 3% (w/w) cornmeal-sand at 1:500 (v/v) inoculum:soil, and the inoculum was incorporated about 15 cm deep. Soil samples were collected from four plots 12 May 1980. A multiple-pellet soil sampler (3) was used to deposit 1.7 g of oven-dry (OD) soil on each of five petri plates of tannic acid-benomyl agar (TABA), a

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selective medium for *R. solani* and other soilborne basidiomycetes (7).

The crops were planted 5 May 1980. Test plants were Funks G-4507 corn, Florunner peanut, Bragg soybean, and Eagle snap bean. Stand counts were made 14 and 35 days after planting. Seedlings removed in thinning were given a root-hypocotyl disease index rating (RHDI). The scale was 1–5, where 1 = <2%, 2 = 2–10%, 3 = 11–50%, 4 = >50% root or hypocotyl discoloration or necrosis, and 5 = dead plant. Corn, peanut, and soybean were thinned to four, five, and five plants per subplot, respectively, and snap bean was not thinned. Weeds were removed periodically by hand.

The height of corn plants was measured 29 and 35 days after planting. Snap bean pods were harvested for fresh market 63, 74, and 87 days after planting. Corn, peanut, and soybean were harvested 93, 147, and 163 days after planting, respectively. Mature plants were given an RHDI rating with the same scale used for seedlings. Yields of shelled corn and soybean seed were based on 15.5 and 12% moisture, respectively. Peanut yields, based on 10% moisture, were determined for vines plus pods attached, hand-picked sound pods, and sound and rotten pods loose in the soil.

Fifty peanut seeds from pods attached to plants and from sound pods loose in the soil in each replicate at harvest were surface-disinfested for 3 min in 0.53% (w/v) NaOCl and plated on TABA to assay for the SWB and other basidiomycetes. Fifty seeds were not available from loose rotten pods for each replicate; all available seeds were plated.

After yields were measured, the entire plants of snap bean, corn (except seed), soybean, and peanut (except seed and pod shells) were returned to the respective microplots. Plant debris was chopped and incorporated into the upper 15–20 cm of soil and allowed to decay through natural weathering until March 1981.

On 23 and 26 February 1981, 10 soil cores 15 cm deep were collected from each microplot and composited. About 2.5 g of OD soil from each microplot was plated on each of 20 plates of TABA with the multiple-pellet soil sampler to enumerate the populations of the SWB and other basidiomycetes.

In 1981–1982, major emphasis was on monitoring survival of the SWB in the susceptible hosts corn and peanut and assaying soil for populations of the fungus. No additional inoculum was added to the soil. Funks G-4507 corn was planted in subplots following peanut, soybean, and snap bean; Florunner peanut was planted in subplots following corn. Cultural practices for corn and peanut were the same as in 1980.

Corn was planted on 12 March 1981 and stand counts were made 29 days after planting, when plants were thinned to three per plot. Peanut was planted on 9 May 1981 and stand counts were made after 10 days, when plants were thinned to five per plot. A RHDI rating was given to corn seedlings removed at thinning. Height measurements were made on corn 46, 75, and 111 days after planting. The RHDI was omitted for peanut seedlings removed at thinning and for mature plants.

Corn was harvested 132 days after planting. An RHDI rating was given to mature corn plants and the yield of shelled corn was determined. Peanut was harvested 150 days after planting. Yields were determined for vines plus pods attached, hand-picked sound pods, and sound and rotten pods loose in the soil.

All plant debris except corn seed and peanut seed and pod shells was returned to the respective microplots, incorporated into the upper 15–20 cm of soil, and left exposed to natural weathering. On 22 February 1982, soil in all microplots was sampled and assayed by plating 1.2 g OD soil on each of 15 plates of TABA using the same methods described for February 1981.

In 1980, 96 cm of rain fell on the microplots from May through October and was supplemented with 52 cm of irrigation water. The average maximum and minimum soil temperatures in the upper 9 cm were 31 and 20 C, respectively, and the range was 13–35 C.

In 1981, 67 cm of rain fell on the microplots from March through October and was supplemented with 100 cm of irrigation water. The average maximum and minimum soil temperatures in the upper 9 cm were 36 and 23 C, respectively, and the range was 8–43 C.

Survival of the SWB in fallow Tifton loamy sand (about 85, 10, and 5% sand, silt, and clay, respectively) was determined in a separate location. Clay pots (25 × 19 cm) of heat-treated (77 C, 30 min) soil were infested singly with five isolates of the SWB in cornmeal-sand inoculum at a 1:60 inoculum/soil concentration. Pots were buried within 5 cm of the rim in a field, watered with 400 ml/pot and left fallow and exposed to natural weathering. Rainfall was recorded at each occurrence and maximum-minimum soil temperatures in the upper 9 cm of soil were recorded weekly.

Soil of each isolate was sampled when infested and after 86, 211, 283, and 483 days. Three cores measuring 1.5 × 10 cm were collected for each isolate and composited. A multiple-pellet soil sampler was used to place 1.7 g of OD soil of each isolate on five petri plates of TABA at infestation and after 86 days. Ten plates per isolate were used after 211 days and 15 each after 283 and 483 days. Pots were kept fallow by hand weeding. Glyphosate was used periodically to keep the soil adjacent to the pots weedfree. The pots were covered to prevent any herbicide from entering them. No other pesticides were used.

On 10 September 1981, the soil in each pot was stirred and 139 kg/ha of 5-10-15 fertilizer plus 397 kg/ha of $CaCO_3$ were added. Five Funks G-4507 corn and five Eagle snap bean seeds were planted in each pot. Seeded pots each received 400 ml of water, and were watered thereafter as needed to prevent drought stress. Plants were harvested after 2 wk and given an RHDI rating using the scale listed previously. Isolations were made from roots to determine if the SWB was present. After plants were removed, pots containing the SWB were left for natural weathering.

Fallow soil was sampled for the last time on 29 March 1982, or 483 days after infestation. On 30 March 1982, the soil in each pot was stirred and 1,075 kg/ha of 5-10-15 fertilizer was added. Five Funks G-4507 corn and 10 Eagle snap bean seeds were planted in each pot. Seeded pots received 400 ml of water each and were watered thereafter as needed to prevent drought stress. Plants were removed after 2 wk and given an RHDI rating. Isolations were made from roots to determine if the SWB was present. Average maximum and minimum soil temperatures were 25 and 10 C, respectively, and the range was −4 to 40 C. Rain in the pots totaled 146 cm. Data were analyzed with a least-squares analysis of variance.

RESULTS

Populations of the SWB averaged three propagules per 100 g of OD soil (P/100 g) on 12 May 1980, 10 days after the soil was infested. Soil fumigation with DD-MENCS did not eliminate all background *R. solani*, binucleate *Rhizoctonia*-like fungi, and *Fusarium* spp. from the soil. Incorporation of preplant fertilizer 20–25 cm deep brought partially decomposed peanut pods near and onto the soil surface. This material apparently was the source of *R. solani*, binucleate *Rhizoctonia*-like fungi, and *Fusarium* spp. Isolations from hypocotyls of snap bean seedlings in 1980 yielded cultures of *R. solani* AG-4. *F. oxysporum* Schlecht, *F. solani*, and AG-4 were isolated from soybean hypocotyls. Peanut hypocotyls yielded cultures of AG-4, *F. solani*, and one culture of the SWB. *F. oxysporum, F. solani*, and two cultures of the SWB were isolated from corn seedlings. The SWB was not reisolated from snap bean and soybean seedlings.

For corn in 1980, there was a significantly ($P = 0.05$) greater RHDI rating of mature plants in microplots infested with the SWB than in the control (Table 1). Stand counts, seedling RHDI, height measurements, and sound seed weights were not significantly different.

For peanut in 1980, there were more colonies of the SWB isolated from seed from loose and attached pods in the SWB treatment than in the control (Table 2). There were more colonies of AG-4

isolated from seed from loose pods in the control than from the SWB treatment (Table 2). Stand counts, seedling and mature RHDI ratings, and weights of vines plus pods, hand-picked sound pods, and loose sound and loose rotten pods were not different. Isolations from 41 seeds from loose rotten pods yielded 11 isolates of AG-4 from SWB and control treatments and no colonies of the SWB.

There were no differences in stand counts, seedling and mature plant RHDI ratings, and mature plant weights in soybean, or in stand counts, seedling RHDI, and weights of pods picked for the fresh market in snap bean.

The SWB was recovered from soil following corn and soybean but not following peanut and snap bean in February 1981 (Table 3). It was recovered from soil in all crops in 1982 (except corn following snap bean) but there were no significant differences among cropping systems either year. The fungus was recovered from the SWB subplots in 1981 and 1982 and was never recovered from uninfested subplots. Populations of *R. solani* AG-4 were higher with all crops except corn in February 1981 than in 1982. Populations of *R. solani* dropped drastically in 1982 where corn followed peanut and snap bean but maintained moderate to high levels where corn followed soybean and peanut followed corn. We consider population ranges of <10, 10–20, and >20 P/100 g of OD soil to be low, moderate, and high levels, respectively, in soils of the Georgia coastal plain. Populations of AG-4 were high in both the SWB and control subplots in 1981 but had dropped to moderate levels in 1982 (Table 3).

The binucleate *Rhizoctonia*-like fungi and binucleate *Ceratobasidium* anastomosis group (CAG) 2 in Table 3 are not comparable. In 1981, the binucleate fungi were not identifed to CAG, and in 1982, the only binucleate *Rhizoctonia*-like fungus recovered from soil was CAG-2.

Low populations of *R. zeae* Voorhees were recovered across cropping systems and soil treatments (except in corn after soybean) in 1982 (Table 3) but not in 1981. The anamorph of the fungus labeled *Laetisaria arvalis* Burdsall in Table 3 was indistinguishable in culture from a known anamorph of *L. arvalis* (2). This fungus was recovered from soil in all cropping systems in 1982. Significantly higher populations of this fungus were isolated from SWB-infested than from control soil (Table 3).

In 1981, there were no differences in stand counts of corn following peanut, soybean, or snap bean. Corn plant height 111 days after planting was greater in SWB than in control subplots (227 vs. 215 cm) following peanut. There were no significant differences in height measurements 46 and 75 days after planting. The RHDI rating of mature plants in 1981 was not different among cropping systems. The seedling RHDI rating of corn following soybean was significantly greater in the SWB subplots than the control in 1981 (Table 4). Also, there were more black lesions characteristic of SWB infection on mature corn roots of plants in SWB subplots than in the control in 1981 following soybean. Differences were not significant following snap bean or peanut (Table 4). Also, the SWB was isolated from seedling corn roots only following soybean. There were no differences in grain yield among treatments in 1981.

In the 1981 peanut subplots following corn, there were no significant differences in weights of vines plus pods, hand-picked sound pods, loose sound pods, and loose rotten pods. There were no SWB, *R. solani*, or binucleate *Rhizoctonia*-like fungi isolated from peanut seed from pods attached to plants at harvest. Few seeds were available for plating from loose sound and loose rotten pods recovered from the soil at harvest. Nineteen seeds from loose sound pods were plated. One SWB culture and one of *R. solani* AG-4 were recovered from seed of peanut planted in soil infested with the SWB and following corn. One binucleate

Table 1. Root-hypocotyl disease severity and yields of four crops in soil infested or uninfested with a sterile, white basidiomycete in 1980

Crop	Soil treatment	Root-hypocotyl disease index[x] Seedlings	Mature Plants[y]	Yield (g/0.3 m²)
Corn	SWB[z]	1.5 a	1.8 a	378 a
	Control	1.2 a	1.1 b	322 a
Peanut	SWB	3.1 a	1.3 a	233 a
	Control	2.5 a	1.0 a	311 a
Soybean	SWB	2.5 a	···	352 a
	Control	2.3 a	···	280 a
Snap bean	SWB	4.0 a	···	161 a
	Control	4.0 a	···	249 a

[x] Scale: 1 = <2%, 2 = 2–10%, 3 = 11–50%, 4 = >50% root or hypocotyl discoloration or necrosis, and 5 = dead plant.
[y] Means within columns of soil treatment on each crop bordered by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Differences in root and hypocotyl disease severity among crops were not analyzed.
[z] Sterile, white basidiomycete.

Table 2. Fungi isolated from 50 surface-disinfested seeds from peanut pods loose in the soil or attached to the plant at harvest in 1980

Soil treatment	Loose pods[x] SWB[y]	*R. solani* AG-4[z]	Attached pods SWB	*R. solani* AG-4
SWB	13.0 a	3.0 b	6.5 a	0 a
Control	0 b	16.3 a	0 b	0.7 a

[x] Means within columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Sterile, white basidiomycete.
[z] *Rhizoctonia solani* anastomosis group 4.

Table 3. Populations in propagules per 100 g of fungi isolated from soil in 1981 and 1982 following different cropping systems in 1980 and 1981

Cropping system 1980[t]	1981[t]	23 February 1981 RS SWB[u]	RS AG-4[v]	RS RBN[w]	22 February 1982 RS SWB	RS AG-4	CAG-2[x]	*RZ*[y]	*LA*[z]
Corn	Peanut	0.7	13.0	42.0	7	46 a	9	1 b	25
Peanut	Corn	0	93.0	5.3	29	1 b	20	4 a	13
Soybean	Corn	0.7	108.0	6.0	7	17 b	12	0 b	5
Snap bean	Corn	0	62.0	0	0	0 b	25	14 a	17
Soil treatment[t]									
SWB		0.7	70.0	20.0	21 a	13	21	5	28 a
Control		0	68.2	6.7	0 b	19	32	5	3 b

[t] Means within columns in a cropping system or soil treatment followed by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test. No letters indicates no significant difference.
[u] Sterile, white basidiomycete.
[v] *Rhizoctonia solani* anastomosis group 4.
[w] Binucleate *Rhizoctonia*-like fungi.
[x] Binucleate *Ceratobasidium* anastomosis group 2.
[y] *Rhizoctonia zeae.*
[z] The anamorph of this fungus is indistinguishable in culture from that of *Laetisaria arvalis.*

Table 4. Influence of crop sequence on seedling root-hypocotyl disease index and lesions on mature corn in 1981

Crop sequence	Soil treatment	Seedling RHDI[x]	Black lesions[y]
Soybean-corn	SWB[z]	1.4 a	2.7 a
	Control	1.1 b	0.0 b
Snap bean-corn	SWB	1.2 a	0.7 a
	Control	1.2 a	0.0 a
Peanut-corn	SWB	1.0 a	1.0 a
	Control	1.3 a	1.0 a

[x] Root-hypocotyl disease index. Scale: 1 = <2%, 2 = 2–10%, 3 = 11–50%, 4 = >50% root-hypocotyl discoloration or necrosis, and 5 = dead plant.

[y] Number of corn plants with black lesions on mature roots characteristic of those produced by the sterile, white basidiomycete. Numbers within columns of each crop-sequence or treatment bordered by the same letters are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Sterile, white basidiomycete.

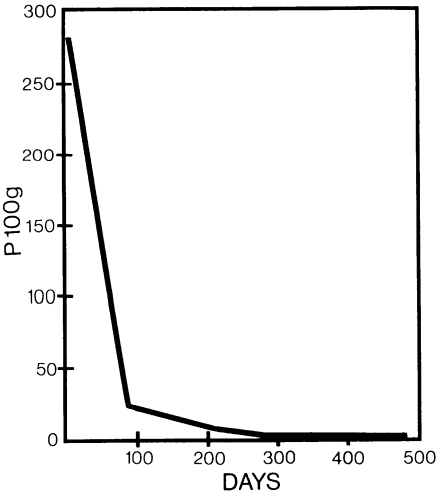

Fig. 1. Survival of a sterile, white basidiomycete in artificially infested Tifton loamy sand at infestation and after 86, 211, 283, and 483 days of natural weathering in pots buried in a field. Corn and snap beans were planted in the soil after 298 days, maintained for 2 wk, and removed.

Rhizoctonia-like culture was recovered from seed in the control subplots following corn. Ninety-one seeds from rotten pods were plated. From these, only two AG-4 cultures grew from plants in the control subplots.

In buried pots, all five isolates of the SWB were recovered from soil plated on TABA at infestation and after 86 days, with an average of 264 and 24 P/100 g, respectively (Fig. 1). Three isolates were recovered 211 days after infestation and one after 283 days, with an average of 11 and 2 P/100 g, respectively (Fig. 1). The SWB was not recovered from soil after 483 days.

The RHDI rating of corn and snap bean was 1 and 1.3, respectively, 298 days after soil was infested with the SWB. The SWB was isolated from one corn plant and no snap bean plants. The RHDI of corn and snap bean was 1 and 1.7, respectively, 505 days after the soil was infested, and the SWB was not reisolated from either corn or snap bean.

DISCUSSION

Results indicate that the SWB will survive in field soil through two growing seasons (21 mo) and >9 mo in fallow soil; however, the inoculum density declined to moderate or low levels in a few months. The inoculum potential of the fungus was low on snap bean, peanut, and corn, and the pathogen had no measurable effect on crop yields in field microplots. The fungus was isolated from soil in a field of Bonifay sand in 1983 following 5 yr of continuous double-cropped corn. The SWB caused moderate root disease severity in the second crop of corn in 1978 but only slight to moderate injury in 1979 and 1980 (D. R. Sumner, *unpublished*). Carbofuran significantly increased root disease severity by the SWB on 5-wk-old Pioneer 3369A corn compared with atrazine and butylate in a greenhouse test (10). The SWB has been isolated from corn roots in nine counties and occasionally from peanut seed and snap bean, sorghum, soybean, and southern pea roots in the Georgia coastal plain.

Other researchers have reported that the SWB was pathogenic on snap bean (4) and probably the same fungus was pathogenic on pigeon pea (5), but to our knowledge, no one else has monitored the survival and inoculum potential of the SWB in soil. Our results show that the fungus is indigenous to the soils of the Georgia coastal plain and apparently survives saprophytically in root and crown tissues of susceptible hosts and in visibly healthy and decayed peanut seed. This pathogen possibly causes slight to moderate root disease severity in crops in numerous fields, but crop losses are probably small. It is possible, however, that severe crop losses could occur in some fields where the microhabitat is especially suited to survival of the pathogen and susceptible crops were planted previously.

The fact that the SWB is seedborne in mature, visibly healthy peanut seed in low to moderate levels indicates that the fungus may be transmitted and spread in such seed. This risk, however, is probably not great because commercial peanut seed in the United States is normally treated with broad-spectrum fungicides. The isolation frequency of the SWB from commercial fungicide-treated peanut seed has been <0.5% (D. K. Bell, *unpublished*).

Presence of moderate to high background populations of *R. solani* AG-4 and binucleate *Rhizoctonia*-like fungi in the microplots following fumigation with DD-MENCS was unexpected. There was, however, an 82% reduction in isolation of AG-4 from peanut seed from loose sound pods in 1980 in plots infested with the SWB, which may have been caused by competition or antagonism by the SWB.

Populations of *R. solani* AG-4 recovered from soil in 1982 where corn followed peanut dropped sharply, possibly because corn is not a good host for *R. solani* AG-4 (7). Populations of AG-4 in soil in 1982 where peanut followed corn rose sharply, probably because loose peanut pods containing seed are a good substrate and inoculum reservoir for *R. solani* (1). The sharp drops in populations of AG-4 recovered from soil in 1982 where corn followed soybean and snap bean were possibly because mature soybean and snap bean are not good hosts for *R. solani* and the loose peanut pods were removed from the soil in 1980.

R. zeae was isolated from soil in all cropping systems in 1982 except corn following soybean. Although the fungus is a weak pathogen of corn (7), it did not produce any recognizable symptoms in this study.

A basidiomycete tentatively identified as *L. arvalis* was recovered from soil in all cropping systems in 1982, and populations of the fungus were significantly higher in the SWB treatment than in the control. *L. arvalis* has been used experimentally as a biocontrol agent of *R. solani* and *Pythium* spp. (2). The reasons for the higher populations of *L. arvalis* in soil infested with the SWB are not known. The fungus may have been parasitizing the SWB or it may be that the microhabitat was more favorable for growth of *L. arvalis* in subplots infested with the SWB.

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