Survival of *Rhizoctonia* spp. and Root Diseases in a Rotation of Corn, Snap Bean, and Peanut in Microplots

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ABSTRACT

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The ability of several basidiomycetous fungi occurring naturally in Georgia coastal plain soils to survive and control *Rhizoctonia solani* anastomosis group 2 (AG-2) and AG-4 in field microplots was determined. Undisturbed profiles of soil in field microplots were fumigated with metam-sodium and infested with *Rhizoctonia solani* AG-2-1, AG-2-2, or AG-4, or with binucleate *Rhizoctonia* spp. CAG-2, CAG-3, CAG-4, or CAG-5. A corn-peanut rotation was then grown for 3 yr. In a second experiment, microplots were infested with AG-4 and AG-2 alone or in combination with the potential antagonists CAG-2, CAG-4, CAG-5, *Laetisaria arvalis,* and an unidentified orange basidiomycetous fungus. Two-year rotations of corn-snap bean (double crop)-peanut or peanut-corn-snap bean were grown for 3 yr. CAG-4 reduced stunting and leaf chlorosis induced by AG-2-2 in corn in the first year but not in the third year after infestation. In the third year, the orange basidiomycetous fungus and CAG-5 increased the yield of corn with low levels of crown and brace root rot. Root and hypocotyl rot was not decreased, and yield of peanut and snap bean were not increased by the antagonists. Population densities of all antagonists in infested soil remained at levels greater than in noninfested soil for 3–10 mo, but populations were variable 19–32 mo after infestation. Antagonists were isolated from 0–10% and AG-2-2 from 0–7% of the loose, sound, or decayed peanut pods each year. CAG-4, CAG-5, and the unidentified orange basidiomycetous fungus all show potential for surviving in soil and reducing deleterious effects of crown and brace root rot in corn in multiple-cropping systems in rotation with peanut.

Peanut, corn, and snap bean were grown on 316,000, 267,000, and 6,600 ha, respectively, in Georgia in 1990 (11,19). Corn is grown frequently in rotation with snap bean or peanut. Crown and brace root rot (CBRR) induced in corn (*Zea mays* L.) by *Rhizoctonia solani* Kühn anastomosis group (AG) 2 type 2 (AG-2-2) (29,30,34) and root rot of snap bean (*Phaseolus vulgaris* L.) and pod rot of peanut (*Arachis hypogaea* L.) induced by *R. solani* AG-4 (3,5,33,35) are widespread in the Georgia coastal plain. Both pathogens colonize peanut shells and seed, and the colonized pods and pod debris are sources of inoculum for the following crop (3). Both AG-2-2 and AG-4 are isolated more frequently from shells of detached pods remaining in soil than from shells of attached pods removed at harvest (2). The fungi may survive for more than 10 mo in peanut shells in fields with crops planted after peanut (D. K. Bell, *unpublished data*).

Binucleate *Rhizoctonia* spp., *Laetisaria arvalis* Burdsall, and other basidiomycetous fungi are indigenous to the soils of the Georgia coastal plain. They were found to be effective in controlling *R. solani* AG-4 in other areas (1,8,9,14–16,20). *R. solani* and binucleate *Rhizoctonia* spp. commonly colonize peanut shells, which can serve as symptomless carriers of the fungi in the Georgia coastal plain (4). Peanut shells decay slowly and have been observed in soil 1–3 yr after peanuts were harvested and other crops were grown. Colonized shells may serve as primary reservoirs for both pathogens and antagonists.

This research was initiated to investigate the ability of three binucleate *Rhizoctonia* spp., *L. arvalis,* and an unidentified orange basidiomycete to survive in soil and control diseases induced by *R. solani* AG-2-2 and AG-4. Preliminary reports have been published (4,29,32).

MATERIALS AND METHODS

Survival of *R. solani* and *Rhizoctonia* spp. Microplots were established on Fuquay sand (loamy, siliceous, thermic, Arenic Plinthic Kandiudults) at the Coastal Plain Experiment Station in 1985 in an agricultural field used previously for research plots or fallow. A metal ring 91 cm in diameter and 2 mm thick was pushed 15 cm into the ground to prepare a trench 2–8 mm wide. Fiberglass rings (15% acrylic; Plastics and Fiberglass Products Co., Raleigh, NC) were prepared by fastening 30-cm strips 5 mm thick into circles 91 cm in diameter with glue and rivets. The circles were inserted 15 cm deep into the soil leaving a 15-cm-tall rim aboveground to prevent soil movement from rainfall. Microplots were separated by 1.8 m, and areas between microplots were planted to centipede grass (*Eremochloa ophiuroides* (Menro) Hack.) and maintained as permanent sod during the experiments. Soil in the microplots was fumigated with metam-sodium to eliminate indigenous basidiomycetous fungi in February 1982 by applying 61.4 ml of 32.7% a.i. in 25 L of water to each microplot, the equivalent of 935.4 L/ha in 3.8 cm of water.

All *R. solani* and *Rhizoctonia* spp. used to infest the soil were identified to anastomosis group (7,22). CAG-2, CAG-3, CAG-4, and CAG-5 have recently been synonymized with AG-A, AG-E, AG-F, and AG-R, respectively (26). All cultures used in this research were isolated from roots of corn; from peanut seed, seedlings, or pods; or from roots of other crops in fields in Tift County, Georgia. Cultures of AG-2-2 were pathogenic on corn, and cultures of AG-4 were pathogenic on bean, cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata,* soybean (*Glycine max* (L.) Merr.), and cucumber (*Cucumis sativus* L.). Cultures of *R. solani* and binucleate *Rhizoctonia* spp. indigenous to the Georgia coastal plain and antagonistic to AG-2-2 or AG-4 (31) were grown on 3% cornmeal-sand (w/w) (CMS), and microplots were infested separately with cultures on 12 and 13 May 1982. A total of 200 cm^3 of CMS containing a mixture of one to four isolates of each anastomosis group was spread over the surface of a microplot and incorporated 2–5 cm deep with a rake. The soil was then turned 10–15 cm deep with a shovel and leveled to give a mixture of approximately one part CMS to 500 parts soil.

In this experiment, a randomized complete block design with 12 treatments and four replicates was used (Table 1). Eight treatments were *R. solani* AG-4, AG-2-1, and AG-2-2; binucleate *Rhizoctonia* spp. (*Ceratobasidium* spp.) CAG-2, CAG-3, CAG-4,

and CAG-5; and a noninfested control planted to peanut 20 May 1982; the other four treatments were AG-2-1, AG-2-2, CAG-4, and a noninfested control planted to corn 10 May 1982. AG-2-1 and AG-2-2 were included with both crops so that data on CBRR could be taken in each corn crop. CAG-4 was used with each crop because of its biocontrol potential shown in greenhouse experiments (31).

Peanut and corn were grown according to the recommendations of the Georgia Cooperative Extension Service (13,25). Ten corn or 20 peanut seeds were planted in each microplot. In 1983, microplots planted in peanut or corn in 1982 were rotated to corn or peanut, respectively; in 1984, microplots were rotated back to the original crop. Soil was collected from each microplot (10 cores, 2.5 cm in diameter, 15 cm deep) and assayed for *R. solani, Rhizoctonia* spp., and other basidiomycetous fungi on tannic acid-benomyl medium (10,28) with a multiple pellet soil sampler (12) on 13 January 1983, 5 March 1984, and 16 January 1985. Colonies (5–10) selected at random from each treatment 48 hr after incubation at 26 C were transferred to potato-dextrose agar and identified to anastomosis group. Colony-forming units (cfu) per 100 g of oven-dried soil were calculated. Detection levels were 1.4–1.7 cfu/100 g per treatment.

Each year, every other corn plant (4–5 plants) was dug, and roots were rated for CBRR (28,30). Fungi were isolated from root lesions (with tannic acid-benomyl medium) at 3–6 wk and again at 14–18 wk after planting. The total number of crown and brace roots, the number with lesions at different depths below ground, and the number with terminal decay were recorded. Five plants were left in each microplot and grown to maturity for yield. The height of the corn was recorded weekly or biweekly from approximately 6 wk after planting until 1 wk after tasseling. The average height in each microplot was determined by measuring each plant. Ears from each microplot were dried and shelled, and grain weight was adjusted to 15.5% moisture. All root and stalk residues were incorporated into the soil (10–15 cm) with a shovel after harvest, except in July 1984 when roots were inadvertently discarded. Peanut was planted in May; 4 wk after planting, plants were thinned to eight per microplot. Plants were harvested in September each year. Pods and vines were dried to 10% moisture and weighed. Basidiomycetous fungi were isolated from seeds (25–50), from visibly sound pods attached to plants at digging, from loose sound pods, and from loose decayed pods remaining in the soil after harvest. Two-seeded pods (approximately 85% of a commercial crop) were selected at random; so few decayed pods were available that all were used in each microplot. Shells and vines from each microplot were separately returned and incorporated into the soil each fall and winter after pods were shelled to remove seed.

Biocontrol with antagonists. Additional microplots were established and fumigated (12 March 1985) as described in the *Rhizoctonia* survival experiment. A randomized complete block design with four replicates of 13 treatments (Tables 2 and 3) was used. The isolate of AG-2-2 used was the same isolate used in previous research (30,34), and the two isolates of AG-4 used were isolated from lesions on peanut hypocotyls. The unidentified orange basidiomycetous fungus was floccose, had small (<0.5 mm) sclerotia, and resembled *Waitea circinata* Warcup & Talbot (21,26). Two to four isolates of each antagonist were used. Each microplot was infested on 12 April 1985 with 200 cm^3 of CMS cultures of the pathogens, with the pathogens plus 200 cm^3 of the antagonists, or was left noninfested. Cultures plus 150 g of dolomitic limestone ($MgCO_3 \cdot CaCO_3$) per microplot (the equivalent of 2,280 kg/ha to raise the pH from 6.0 to 6.2) were incorporated 15 cm deep with a shovel.

A 3-yr rotation was used. A corn-snap bean double crop (year 1), a peanut monocrop (year 2), and a corn-snap bean double crop (year 3) were grown in noninfested microplots or in those infested with AG-2-2. The same rotation beginning with peanut (year 1) was grown in noninfested microplots or in those infested

TABLE 1. Crown and brace root rot (CBRR) and yield in corn and plant stand and root and hypocotyl disease (RHD) in peanut in rotation in soil infested with different anastomosis groups (AG) of *Rhizoctonia* spp. in microplots

Rhizoctonia treatment	Year 1 Peanut Plants[w]	Year 1 Peanut RHD[x]	Year 2 Corn CBRR[y] (%)	Year 2 Corn Yield (g/m^2)	Year 3 Peanut Plants	Year 3 Peanut RHD
AG-4	11 b[z]	3.40 a	9 b	1,075	17	2.16 a
AG-2-2	19 a	1.47 c	60 a	835	16	1.66 bc
AG-2-1	19 a	1.56 c	6 b	1,063	17	1.90 ab
CAG-2	19 a	1.32 c	0 b	1,264	17	1.58 bc
CAG-3	18 a	2.07 b	1 b	1,188	17	1.61 bc
CAG-4	18 a	1.35 c	13 b	1,042	16	1.61 bc
CAG-5	18 a	1.34 c	0 b	957	14	1.67 bc
None	18 a	1.12 c	0 b	1,085	16	1.56 c

	Year 1 Corn CBRR	Year 1 Corn Yield (g/m^2)	Year 2 Peanut Plants	Year 2 Peanut RHD	Year 3 Corn CBRR	Year 3 Corn Yield (g/m^2)
AG-2-2	41 a	356 b	16 a	1.11	66 a	508
AG-2-1	10 b	440 ab	16 a	1.10	57 a	420
CAG-4	0 c	684 a	12 b	1.00	0 b	747
None	0 c	414 b	11 b	1.19	0 b	816

[w] At thinning, 4 wk after planting.
[x] Root and hypocotyl disease index: 1 = <2% discoloration and decay; 2 = 2–10%; 3 = 11–50%; 4 = >50%; and 5 = dead plants.
[y] Based on total number of crown and brace roots on 4–5 plants with lesions or terminal decay, 5–6 wk after planting.
[z] Numbers in columns followed by the same letter are not significantly different, according to Fisher's least significant difference; $P = 0.05$. No letters after a number indicates no significant difference.

TABLE 2. Root disease severity, root weight, plant height, and yield in corn in microplots infested with *Rhizoctonia solani* AG-2-2 and antagonists[v]

Pathogen	Antagonist	Year 1 Root apices killed[w] (%)	Year 1 Root wt[x] (g)	Year 1 Plant height[y] (cm)	Year 1 Yield (g/m^2)	Year 3 Root apices killed (%)	Year 3 Plant height (cm)	Year 3 Yield (g/m^2)
AG-2-2	None	63 a[z]	3.8 c	119 c	747 b	22	158	935 d
	CAG-2	58 a	9.0 b	129 bc	905 b	29	190	1,106 a–d
	Laetisaria arvalis	48 b	6.0 bc	129 bc	932 b	4	158	1,016 b–d
	Orange basidiomycetous fungus	58 a	7.5 bc	138 bc	974 b	8	192	1,120 a–c
	CAG-4	42 ab	19.8 a	154 ab	1,009 b	31	179	949 cd
	CAG-5	52 a	8.2 bc	125 c	691 b	8	186	1,136 ab
Control	None	4 c	19.0 a	178 a	1,492 a	16	190	1,211 a

[v] After initial infestation the first year and following 2 yr of a corn-snap bean double crop and a peanut monocrop.
[w] Root apices killed by *R. solani* AG-2-2 4–7 wk after planting.
[x] Oven-dried weight, 4–5 plants per plot, 32 days after planting. Data not taken in the third year.
[y] Final height 1 wk after tasseling.
[z] Numbers in columns followed by the same letter are not significantly different according to Fisher's least significant difference; $P = 0.05$. No letter after a number indicates no significant difference.

with AG-4. Corn was planted in March and harvested in July or August, snap bean was planted in August and harvested for green pods from September to November, and peanut was planted in May and harvested in September. Corn (4–5 plants) was rated for CBRR 3–7 wk after planting, and postemergence damping-off was recorded in snap bean. Peanut plants were removed at thinning 3–4 wk after planting and rated for root and hypocotyl discoloration and decay (3).

Soil cores were collected on 5 May and 11 July 1985, 31 January and 14 November 1986, and 21 August and 14 December 1987. Soil was assayed by the methods described above. After each harvest, all residues were incorporated into the soil before the next crop was planted. Corn residues were fertilized with ammonium nitrate (11 g per microplot, equivalent to 56 kg of N per hectare) 1–2 wk before snap bean was planted.

In all experiments, data were analyzed by PROC GLM (24). Fisher's least significant difference mean separation test was used. Data were transformed as necessary (square root transformations for small numbers [<100], $\log_{10}$ for large numbers [>100], or arcsine transformations for percentages [27]) for statistical analyses, but all data are reported as untransformed values. Data from each crop were analyzed separately, but when soil samples were taken simultaneously from all microplots within an experiment, data were analyzed together.

RESULTS

Survival of *R. solani* and *Rhizoctonia* spp. *Corn. R. solani* AG-2-2 caused severe CBRR in all years, whereas AG-2-1 caused slight CBRR the first two years and severe disease in the third year. CAG-4 caused no disease or slight root disease, and an increase in the yield of grain was seen only in the first year (Table 1). CAG-2, CAG-3, and CAG-5 caused negligible or no root discoloration and no differences in grain yield.

In the first year, AG-2-2 and AG-2-1 were isolated from 40 or 27% of rotted roots from 5- to 6-wk-old corn plants in plots infested with AG-2-2 or AG-2-1, respectively. AG-2-2 was not isolated and AG-2-1 was isolated infrequently (15%) from root lesions at harvest in the microplots infested with AG-2-2 and AG-2-1, respectively. In contrast, AG-2-2 and AG-2-1 were isolated rarely from lesions on corn roots during the second and third years after peanut and corn-peanut, respectively.

Plant height after tasseling was not different among plots in the first and third years. In the second year, CAG-3 increased the growth rate and final height significantly ($P = 0.05$) compared with the control (5.11 cm per day and 227 cm vs. 4.43 cm per day and 201 cm, respectively).

Peanut. In the first year, each fungus was isolated from 5-wk-old seedlings in each plot where soil was infested with the respective fungus. Root and hypocotyl disease severity was increased only by AG-4 and CAG-3, and the number of plants was decreased only by AG-4 compared with the control. In the second year, AG-2-2 and AG-2-1 improved emergence compared with the control and CAG-4; but in the third year, there were no differences in emergence. Root and hypocotyl disease at thinning was slight after the first year, and the antagonists reduced severity in the third year (Table 1). The yield of visibly sound, attached pods was not different among plots infested with different fungi for any year, but the weight of attached pods plus vines at harvest was reduced ($P = 0.05$) by AG-2-2 compared with the control (496 vs. 642 g per microplot) in the first year.

In the first year, each fungus was isolated from 1–6% of 100 seeds from visibly sound, attached pods grown in infested soil, except for CAG-4, which was not isolated. Similar trends were noted in the last two years, but only CAG-4 (3.8%) in the second year and CAG-5 (1.8%) in the third year were isolated more often from infested plots than from other plots ($P = 0.05$). The antagonists were usually isolated from more (3–18%) seeds from visibly sound pods loose in the soil than from visibly sound, attached pods. Basidiomycetous fungi, other than each fungus used to infest soil in a specific microplot, were isolated rarely.

Population densities of AG-4, AG-2-1, and CAG-5 were 5.4, 3.2, and 7.5 cfu/100 g of soil in their respective plots and were significantly higher than the undetectable levels in plots infested with other fungi 8 mo after infestation. AG-2-2, CAG-4, and CAG-5 were not recovered from any plots, and CAG-2 (1.6 cfu/100 g) was found only in the controls. *R. zeae* and *L. arvalis* were detectable at low levels (≤6.4 cfu/100 g) in some plots. At the beginning of the third cropping year, after 22 mo, population densities of the following were significant in their respective treatments (cfu/100 g): AG-2-1 (5.6) following peanut, undetectable in other treatments, and CAG-3 (15.8) and CAG-5 (3.4), undetectable following other treatments. Population densities of *R. zeae* and *L. arvalis* averaged 29.4 and 2.6 cfu/100 g, respectively, in all plots and were not different among treatments.

Population densities on 16 January 1985, after three cropping seasons and 35 mo after the soil was infested, are shown in Table 4. AG-2-1 and CAG-5 were found only in soil where they had been incorporated originally, but the other fungi were distributed sporadically throughout the plots. *R. zeae* was present in moderate to high populations in all treatments, but *L. arvalis* occurred sporadically at low levels.

Biocontrol with antagonists. *Corn.* The yield of grain was suppressed in all plots infested with AG-2-2, with or without antagonists (Table 2). The AG-2-2 plus CAG-4 treatment significantly improved growth and suppressed symptoms of leaf tip injury compared with AG-2-2 alone. The oven-dried weight of roots 31 days after planting was increased by the AG-2-2 plus CAG-2 and AG-2-2 plus CAG-4 treatments compared with the AG-2-2 treatment, and the AG-2-2 plus CAG-4 treatment was not different from the control (Table 2). The oven-dried weight of foliage 31 days after planting was increased by the AG-2-2 plus

TABLE 3. Emergence, root and hypocotyl disease (RHD), yield of attached, visibly sound pods, and the percentage of shells colonized by *Rhizoctonia solani* AG-4 in peanut in microplots infested with AG-4 and antagonists[w]

		Year 1						Year 3					
					Pod colonization[y]						Pod colonization		
Pathogen	Antagonist	Emergence at 4 wk (%)	RHD[x]	Yield (g/m²)	SAP (%)	LSP (%)	LDP (%)	Emergence at 4 wk (%)	RHD	Yield (g/m²)	SAP (%)	LSP (%)	LDP (%)
AG-4	None	66 c[z]	2.77 a	1,000	52	40	60	91	1.58	936	5	17	26
	CAG-2	86 ab	2.29 ab	960	54	47	50	89	1.30	765	16	20	16
	Laetisaria arvalis	80 a–c	2.09 b	1,006	54	53	38	83	1.08	842	13	23	18
	Orange basidiomycetous fungus	76 bc	2.24 ab	957	44	39	34	89	1.22	823	13	21	27
	CAG-4	77 bc	2.10 b	1,016	32	29	37	89	1.20	787	0	11	15
Control	None	96 a	1.00 c	984	20	20	18	74	1.50	887	8	6	22

[w] After initial infestation the first year and following 2 yr of a peanut monocrop and a corn-snap bean double crop rotation.
[x] Root and hypocotyl disease index: 1 = <2% discoloration and decay; 2 = 2–10%; 3 = 11–50%; 4 = >50%; and 5 = dead plants.
[y] SAP = visibly sound attached pods at harvest; LSP = visibly sound pods loose in soil at harvest; LDP = decayed pods loose in soil at harvest.
[z] Numbers in columns followed by the same letter are not significantly different according to Fisher's least significant difference; $P = 0.05$. No letters after a number indicates no significant difference.

CAG-4 treatment compared with AG-2-2 alone, but foliage weight was less in all plots infested with AG-2-2 compared with the control (data not shown).

In the second year, there was very little CBRR in plots with AG-4 following peanut. Plant growth was satisfactory in all microplots. Few roots had any kind of lesions, and there were no differences among treatments in final height or yield of grain (data not shown).

CBRR was slight to moderate in all treatments in the third year. Yield of grain was greater for the AG-2-2 plus the orange basidiomycete and the AG-2-2 plus CAG-5 treatments than for AG-2-2 alone (Table 2). Yield in microplots infested with AG-2-2 alone was 23% less (275 g/m^2) than in noninfested microplots. AG-2-2 was isolated from root lesions on plants in four of 24 microplots infested with AG-2-2 and from plants in one of four control microplots.

Peanut. Peanut yields were not different among treatments, nor were there differences in the colonization by AG-4 of visibly sound, attached pods or loose, decayed pods at harvest (Tables 3 and 5). In contrast, loose, visibly sound pods were colonized more commonly by AG-4 in the second year in microplots infested with AG-2-2 plus CAG-5 than in any other AG-2-2 treatment except AG-2-2 plus CAG-2 (Table 5). Colonization of all pods by AG-4 was much more common in the first year than in later years, but AG-4 was still isolated from 6–27% of the loose, sound or decayed pods in soil in the third year (Table 3).

The binucleate *Rhizoctonia* spp. were isolated infrequently from sound, attached pods in most microplots. CAG-4 was always reisolated from 5–8% of the pods each year in AG-4 plus CAG-4 or AG-2-2 plus CAG-4 treatments, and CAG-5 was isolated from 10% of the pods in the AG-2-2 plus CAG-5 infested microplots and was isolated rarely from other plots (Table 6). The orange basidiomycete was isolated more frequently from sound, attached pods in the AG-4 plus orange basidiomycete treatment in the first year but was isolated rarely from loose or sound pods in the second year and was never isolated in the third year. *L. arvalis* was isolated from attached, sound pods (2.5%) and loose, sound pods (7%) in the AG-4 plus *L. arvalis* treatment but not in other treatments in the first year, and this fungus was never isolated from pods after that.

Snap bean. Root and hypocotyl rot was moderate to severe in the first two years and slight in the third year, averaging 15, 15, and 4% postemergence damping-off, respectively ($P = 0.05$). It was not different among treatments. There were no differences among treatments in final stand of plants nor in yield of green pods.

Populations of basidiomycetous fungi in soil. In the first year, population densities of AG-2-2 38 days after infestation were 7–20 cfu/100 g of soil adjacent to plant roots in all infested plots. These levels were significantly greater than in the control plots, where no AG-2-2 was detected. At 90 days after infestation, levels had dropped to 4 cfu/100 g or lower in infested soil, and there were no differences among treatments. Population densities averaged 2.5, 0.6, 0.0, and 3.8 cfu/100 g at 10, 19, 28, and 32 mo after planting, respectively, and there were no differences among treatments in soils originally infested with AG-2-2 or those soils that were noninfested.

In soils infested with AG-4, population densities 3 mo after

TABLE 4. Population densities of basidiomycetous fungi in soil in January 1985, 35 mo after infestation with *Rhizoctonia* spp. in a corn-peanut rotation

Soil treatment[y]	Colony-forming units per 100 g of oven-dried soil							
	AG-4	AG-2-2	AG-2-1	CAG-2	CAG-4	CAG-5	*Rhizoctonia zeae*	*Laetisaria arvalis*
AG-4	1.5	0	0 b[z]	1.5	9.3 ab	0 b	7.7	0
AG-2-2	9.3	0	0 b	6.2	0 b	0 b	9.2	4.6
AG-2-1	0	1.5	3.1 a	0	0 b	0 b	38.6	1.5
CAG-2	3.1	0	0 b	1.5	0 b	0 b	21.6	0
CAG-3	0	0	0 b	0	7.7 ab	0 b	10.8	0
CAG-4	0	0	0 b	0	0 b	0 b	43.2	0
CAG-5	0	0	0 b	1.5	0 b	4.6 a	27.8	1.5
None	7.7	1.5	0 b	27.8	6.2 ab	0 b	9.2	0
AG-2-2	0	0	0 b	12.3	0 b	0 b	10.8	0
AG-2-1	0	0	1.5 ab	0	0 b	0 b	6.2	1.5
CAG-4	0	0	0 b	0	15.4 a	0 b	15.4	0
None	0	0	0 b	10.8	9.2 ab	0 b	10.8	0

[y] The first eight treatments were planted to peanut-corn-peanut and the last four treatments to corn-peanut-corn for 3 yr after soil was infested.
[z] Less than the detection level of 1.5 cfu/100 g of oven-dried soil. Numbers followed by the same letter are not significantly different according to Fisher's least significant difference; $P = 0.05$.

TABLE 5. Emergence, root and hypocotyl disease (RHD), yield of attached, visibly sound pods, and the percentage of shells colonized by *Rhizoctonia solani* AG-4 in peanut in microplots infested with *R. solani* AG-2-2 and antagonists[w]

Pathogen	Antagonist	Emergence (%)	RHD[x]	Yield (g/m^2)	Pod colonization[y]		
					SAP (%)	LSP (%)	LDP (%)
AG-2-2	None	94	1.40 ab[z]	665	7	3 bc	NP
	CAG-2	87	1.52 a	712	4	15 a–c	0
	Laetisaria arvalis	93	1.40 ab	721	1	0 c	NP
	Orange basidiomycetous fungus	93	1.15 c	715	2	4 bc	30
	CAG-4	91	1.35 b	683	0	8 bc	0
	CAG-5	90	1.25 bc	682	2	34 a	0
Control	None	95	1.40 ab	890	4	19 ab	16

[w] After 1 yr of a corn-snap bean double crop.
[x] Root and hypocotyl disease index: 1 = <2% discoloration and decay; 2 = 2–10%; 3 = 11–50%; 4 = >50%; and 5 = dead plants.
[y] SAP = visibly sound attached pods at harvest; LSP = visibly sound pods loose in soil at harvest; LDP = decayed pods loose in soil at harvest; and NP = no pods.
[z] Numbers in columns followed by the same letter are not significantly different according to Fisher's least significant difference; $P = 0.05$. No letters after a number indicates no significant difference.

infestation were 8–24 cfu/100 g of soil, and population densities in all infested plots were significantly greater than the undetectable level in the control. Population densities were 9–12 cfu/100 g in all infested plots after 10 mo and were variable with no significant differences among plots after that period. Populations were consistently low in the AG-4 plus CAG-4 treatment at 19 and 32 mo.

Population densities of CAG-2 and CAG-4 were significantly higher in soils infested with CAG-2 and CAG-4, respectively, than in noninfested soils 5 wk and 3 mo after infestations, but population densities were variable and were not different among treatments after that. The population densities of *L. arvalis,* CAG-5, and the unidentified orange basidiomycetous fungus remained significantly higher in soils infested with AG-2-2 plus *L. arvalis,* AG-2-2 plus CAG-5, or AG-2-2 plus orange basidiomycete, respectively, than in noninfested soils for 10, 10, and 3 mo, respectively. Populations were variable and were not different thereafter. CAG-5 populations remained high in infested soil for 19 mo, but the fungus was not detected after that. In contrast, *L. arvalis* was present in low to moderate levels in soils infested with AG-2-2 plus *L. arvalis* 10–32 mo after infestation.

DISCUSSION

Biological control of CBRR of corn was not evident from data taken on root disease severity in these tests. However, growth and yield of corn were greater for some combinations of AG-2-2 and the other basidiomycetous fungi than for AG-2-2 alone, both preceding and following peanut. The difficulty in isolating AG-2-2 from root lesions and soil and the relationship of CBRR to root growth and yield have been discussed previously (30,34). In vitro, no mycoparasitism or antibiosis of AG-2-2 or AG-4 could be observed. Similar observations have been reported with other binucleate *Rhizoctonia* spp. (8). We suspect that these basidiomycetous fungi are primarily competitors of *R. solani.*

Several indigenous binucleate *Rhizoctonia* spp. in Ohio were effective biocontrol agents of the AG-2-2 pathogen that causes crown and root rot of sugar beet (14,15). However, on the basis of isozyme polymorphism and DNA restriction analyses, the AG-2-2 pathogen on sugar beet is in an intraspecific group different from that of the AG-2-2 that induces CBRR on corn (17). Liu and Sinclair have proposed that AG-2-2 RHS36 be included in type AG-2-2 III B, intraspecific group 2B (18). Genetic differences within AG-2-2 may account for part of the differences in levels of biocontrol on sugar beet compared with that on corn.

All of the potentially antagonistic fungi selected for our experiments suppressed root rot or increased plant survival significantly in experiments with corn or snap bean in a greenhouse (31). In fields, however, there are substantial seasonal and yearly variations in environmental and microecological conditions, even with irrigated experiments. CAG-2 gave variable control of *R. solani* AG-4 in snap bean in other research in Georgia (33), but none of the antagonists reduced root and hypocotyl rot of snap bean in this investigation. Binucleate *Rhizoctonia* spp. are common in rye-soybean rotations in Florida (23) and are found frequently in low to moderate densities (1–10 cfu/100 g) in soils of the Georgia coastal plain in various cropping systems (*unpublished data*). We had previously shown that these fungi would survive several months in fallow soil in the field in plots (5), but this research documents the survival and potential benefits of these binucleate *Rhizoctonia* spp. in soil in specific cropping systems over several years.

L. arvalis was an effective biological control agent of *R. solani* in Nebraska for several weeks after soil was amended (1) but was less effective in Ohio (14). In both states, populations of the fungus dropped to low to moderate levels in a few months, and there was no effective disease control with time. *L. arvalis* has shown only limited potential as a biocontrol agent in coastal plain soils in both greenhouse and field experiments. The fungus produces laetisaric acid that is fungicidal against *R. solani* in culture (6). Perhaps the laetisaric acid is partially inactivated or the fungus does not produce adequate amounts of laetisaric acid for biological control of *R. solani* in the microenvironment in soils of south Georgia.

The unidentified orange basidiomycetous fungus is found infrequently in soils in the Georgia coastal plain, and treatments with this fungus mixed with AG-2-2 increased the yield of corn in the third year of the corn-snap bean-peanut rotation. It is not known whether the yield increase was related to decreased pathogenesis of AG-2-2 or to a stimulation of growth in corn. In greenhouse experiments, the fungus showed limited potential in controlling *R. solani* AG-2-2 and AG-4 (31). This fungus merits additional research, although it may have less potential for surviving and competing with *R. solani* than do the other basidiomyceteous fungi used in our experiments.

The colonization of peanut shells and seeds by these indigenous basidiomycetes and the effect of these colonized, loose pods in soil have interesting ecological ramifications. While peanut seeds decompose rapidly, the shells deteriorate slowly in soil (3), and potential antagonists may become established and survive in peanut shells for 2 or 3 yr, providing long-term competition for *R. solani.* However, *R. solani* also colonizes shells and seeds, and colonized peanut debris serves as an excellent reservoir for inoculum of *R. solani* (3). Both *R. solani* and binucleate *Rhizoctonia* spp. are isolated commonly from pieces of shells from the same peanut pod (*unpublished data*). The microplots were surrounded by a fence that kept out rabbits and deer, but field mice occasionally entered and dug in the peanuts, and that may account

TABLE 6. Isolation (%) of *Rhizoctonia solani* and binucleate *Rhizoctonia* spp. from peanut shells from visibly sound attached pods at harvest

		Year 1				Year 2				Year 3		
Pathogen	Antagonist	AG-2-2	CAG-2	CAG-4	OB[y]	AG-2-2	CAG-2	CAG-4	CAG-5	AG-2-2	CAG-2	CAG-4
AG-2-2	None	...	...	...	...	3	7	6	0 b[z]	...	...	...
	CAG-2	...	...	...	...	7	3	1	0 b	...	...	...
	Laetisaria arvalis	...	...	...	...	0	5	9	0 b	...	...	...
	Orange basidiomycetous fungus	...	...	...	...	0	7	1	0 b	...	...	...
	CAG-4	...	...	...	...	6	0	5	0 b	...	...	...
	CAG-5	...	...	...	...	1	0	4	10 a	...	...	...
Control	None	...	...	...	...	5	0	7	0 b	...	...	...
AG-4	None	0	0 b	0 b	1 b	...	...	...	...	0	0	0
	CAG-2	0	1 b	0 b	0 b	...	...	...	...	0	0	0
	Laetisaria arvalis	0	0 b	0 b	0 b	...	...	...	...	0	3	4
	Orange basidiomycetous fungus	0	0 b	0 b	2 a	...	...	...	...	0	1	0
	CAG-4	0	0 b	7 a	0 b	...	...	...	...	0	8	0
Control	None	0	3 a	2 b	0 b	...	...	...	...	1	1	0

[y] Unidentified orange basidiomycetous fungus.

[z] Numbers in columns followed by the same letter are not significantly different according to Fisher's least significant difference; $P = 0.05$. No letters after a number indicates no significant difference.

for some of the variability in populations of the basidiomyceteous fungi in soil among microplots after the first years of the experiments.

Several of the indigenous soil-inhabiting basidiomycetous fungi, particularly CAG-4, CAG-5, and the unidentified orange basidiomycetous fungus, show potential for reducing the deleterious effects of CBRR in corn planted in corn or corn-snap bean rotations with peanut. The variable levels of CBRR observed in corn in farm fields in the Georgia coastal plain (29,30) may be related to the population densities of these fungi in different cropping systems. Cropping systems that favor the maintenance of low to moderate levels of these basidiomycetous fungi may help reduce the survival of *R. solani* and the crop losses to root and hypocotyl diseases caused by *R. solani.*

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