Efficacy of Pencycuron Against Isolates Representing Different Anastomosis Groups of *Rhizoctonia solani* and *Rhizoctonia*-like Binucleate Fungi

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ABSTRACT

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Pencycuron was evaluated for efficacy against isolates of *Rhizoctonia solani* and *Rhizoctonia*-like binucleate fungi in soil in the greenhouse at 10.0, 1.0, and 0.1 $\mu g/g$ of soil. Soil was infested separately with isolates of *R. solani* (AG-2 type 1, AG-2 type 2, and AG-4) and isolates of *Rhizoctonia*-like binucleate fungi (CAG-2, CAG-3, CAG-4, and CAG-5) from soil and plants in Georgia. Of the 14 isolates, only one isolate of *R. solani* AG-2 type 1 was resistant to pencycuron and caused root and hypocotyl rot in snap bean at all dosages tested. Root and hypocotyl rots of snap bean caused by isolates of *R. solani* AG-2 type 2, AG-4, a second isolate of AG-2 type 1, and isolates of CAG-5 were controlled by pencycuron at 10.0 and 1.0 $\mu g/g$ of soil. Colony-forming units of only one isolate each of AG-2 type 1, CAG-4, and CAG-5 were detected in soil 3 wk after treatment at 10 $\mu g/g$.

Numerous anastomosis groups (AGs) of *Rhizoctonia solani* Kühn (10,12) and *Rhizoctonia*-like binucleate fungi (RLBF), designated CAGs (3,11), have been identified, and isolates representing several of the AGs and CAGs are pathogenic to snap bean (*Phaseolus vulgaris* L.) and other vegetables (5,17). Few soil or seed treatments are available to control Rhizoctonia root and hypocotyl rots (2,4,6,8,9), and research on control of *R. solani* with soil fungicides has given variable results. Isolates from AGs and CAGs differ in pathogenicity and cultural morphology (3,5,10–12) and may respond differently to fungicides in vitro (9). In Georgia, most vegetables are not treated with soil fungicides and Rhizoctonia damping-off is common. Fungicides are needed to control *R. solani* and RLBF, which decrease plant stands and reduce yield and quality of vegetables.

Pencycuron is a nonsystemic protective fungicide that has been evaluated for control of *R. solani* in many crops. Pencycuron has controlled Rhizoctonia

crown rot in sugar beet (14) and has suppressed crown and brace root rot in corn caused by *R. solani* AG-2 type 2 and root rot in vegetables caused by *R. solani* AG-4 at the Coastal Plain Station (18; D. R. Sumner, *unpublished*). This research was initiated to determine the efficacy of pencycuron (BAY NTN 19701) in controlling root and hypocotyl diseases caused by the different AGs of *R. solani* and the CAGs of RLBF isolated from plants and soils of the Georgia coastal plain and to determine the influence of pencycuron on populations of different *R. solani* and RLBF isolates in soil.

MATERIALS AND METHODS

Two split-split-plot experiments with randomized complete block designs were done in a greenhouse. Isolates of *R. solani* and RLBF were whole plots (two replicates), two inoculum densities were subplots, and three dosages of pencycuron and a control were sub-subplots.

Seven isolates of *R. solani* and seven isolates of RLBF from soils and plants in the coastal plain were tested against pencycuron. In the first experiment, all isolates were from soil except an isolate of AG-4 from peanut seed (Table 1). In the second experiment, one isolate of AG-4 from soil and one from sorghum, one isolate of AG-2 type 1 from sweet sorghum, one isolate of AG-2 type 2 from corn, one isolate of CAG-3 from onion, and one isolate each of CAG-4 and CAG-

5 from soil were used. *R. solani* AG-2 is divided into type 1 and type 2 based on frequency of hyphal anastomosis (10); AG-2 type 2 is virulent on corn, snap bean, and sugar beet, whereas AG-2 type 1 is avirulent on corn but virulent on snap bean, cowpea, crucifers, and flax (10,16,17). Cultures were grown on corn-meal-sand (3 g of cornmeal, 100 g of sand, and 15 ml of deionized water) in Erlenmeyer flasks. In the first experiment, cultures were grown 10–19 days at 20–30 C. In the second test, cultures had been stored dry for 12–15 wk at 20–30 C in the laboratory. Inoculum densities used were 1:500 and 1:2,000 (v/v) cornmeal-sand to soil. A Tifton loamy sand soil, pH 5.9, treated 30 min at 70–75 C with aerated steam was used in both experiments.

Dosages of pencycuron were 10.0, 1.0, 0.1, and 0 μg a.i./g of soil. Soil was blended with pencycuron and 19, 38, and 57 mg of fertilizer (NPK, a commercial 5-10-15 blend commonly used in the Georgia coastal plain) per kilogram of soil (0.6 mg/kg, about 1 kg/ha) for 5 min in a concrete mixer. Each soil mixture was stored 1–5 days until it could be mixed separately with each inoculum density of each fungus. A soil sample from each mixture was collected and assayed on a tannic acid agar medium modified with benomyl (TABA) (17) to determine populations of fungi in soil at planting.

For each sub-subplot, 1,200 cm^3 of soil was placed into a black plastic can (1,890-cm^3 capacity), 10 seeds of Eagle snap bean (*Phaseoulus vulgaris* L.) were distributed over the surface, and the seeds were covered 3–4 cm deep with 600 ml of soil. The mixing and planting required 6 days in the first experiment and 4 days in the second.

Plants were hand-watered to prevent moisture stress. Soil temperature mimima and maxima ranged from 5–16 to 24–37 C in the first experiment and from 10–18 to 26–32 C in the second experiment. Emergence and postemergence damping-off were recorded 7–8 and 10–14 days after planting. After 21–22 days, the number of deformed plants (bald heads, stunted) and the foliage fresh weight for

each replicate were recorded. Plants were lifted and washed, and the severity of root and hypocotyl disease symptoms was evaluated on a scale of 1–5, where 1 = <2, 2 = 2–10, 3 = 11–50, and 4 = >50% discoloration and decay; 5 = dead or dying plant. The ratings for all plants in a treatment were averaged to get a root and hypocotyl disease index. The types of lesions (reddish brown cankers, black, gray, or tan discoloration), an estimate of root growth (1 = very poor, 5 = excellent), and root fresh weights were determined. The soil from each pot was mixed, and a sample was saved for assay on TABA.

Data were analyzed with least squares analysis of variance and general linear models statistical procedures. Various linear comparisons were tested with the F-test.

RESULTS

Experiment 1. In untreated soil, *R. solani* AG-4 and AG-2 type 2 caused the most severe root and hypocotyl rot, whereas *R. solani* AG-2 type 1 and the RLBF CAG-5 caused moderate root and hypocotyl rot. Both AG-4 and AG-2 type 2 caused reddish brown to grayish black sunken cankers on the hypocotyls, whereas AG-2 type 1 caused tan to light brown water-soaked lesions that did not coalesce into large deep cankers. CAG-3 caused lesions similar to those caused by AG-2 type 1. CAG-4 caused small (<1.5 cm) superficial reddish brown water-soaked lesions. CAG-5 caused hypocotyl lesions similar to those caused by AG-4,

but the lesions were not as deep and did not extend into the stem. CAG-5 caused numerous reddish brown lesions on taproots and fibrous roots. The RLBF CAG-3 and CAG-4 caused only slight root and hypocotyl disease; CAG-2 was not pathogenic. The isolates of fungi tested and the analysis of variance are shown in Table 1. There was a significant interaction among isolates of fungi and dosage of pencycuron but no interaction between inoculum density and dosage of pencycuron. Pencycuron was effective in controlling root and hypocotyl diseases caused by all of the isolates except *R. solani* AG-2 type 1.

Colony-forming units (cfu) of *R. solani* AG-4, AG-2 type 2, and RLBF CAG-5 were not recovered from soil containing either density of inoculum 3 wk after treatment at 10 μg/g of soil. At an inoculum concentration of 1:2,000, the fungi were not recovered at 1.0 μg/g of soil 3 wk after treatment (Table 2). In contrast, pencycuron had no effect on populations of *R. solani* AG-2 type 1 in soil at either inoculum density (Table 2). No cfu of CAG-2 were recovered from soil treated with pencycuron at 10 μg/g, but high populations were found at 1 μg/g (92 and 157 cfu/100 g of oven-dry soil with low and high inoculum densities, respectively). CAG-3 was not detected in soil 3 wk after treatment but was present in all soil treatments with pencycuron immediately after treatment (5–53 cfu/100 g of oven-dry soil). Populations of CAG-4 were present in all soils immediately after treatment with

pencycuron. There was no interaction of levels of inoculum density and dosages of pencycuron. An average of 26, 100, 154, and 231 cfu of CAG-4 per 100 g of oven-dry soil were detected 3 wk after treatment with 10, 1.0, 0.1, and 0 μg of pencycuron per gram of soil, respectively.

Fresh weight of foliage was significantly increased by pencycuron in soils infested with all isolates except CAG-2 and CAG-3, but root growth was only increased by pencycuron in soils infested with AG-4, AG-2 type 2, and CAG-4.

Experiment 2. Populations in soil were lower than in experiment 1 (probably because older inoculum was used), and root and hypocotyl disease was severe only in soil infested with *R. solani* AG-2 types 1 and 2. Pencycuron reduced root disease severity caused by *R. solani* AG-2 types 1 and 2 and the number of hypocotyls with lesions caused by one isolate of *R. solani* AG-4 (from corn) (Table 3). One isolate of AG-4 (from soil) and isolates of CAG-2, CAG-3, CAG-4, and CAG-5 did not cause root and hypocotyl rot. There was no interaction of dosage rates of pencycuron with isolates. None of the isolates showed resistance to pencycuron in soil. Populations of isolates in soil at planting and 3 wk later ranged from undetectable to 25 cfu/100 g of oven-dried soils in soil not treated with pencycuron and averaged 8 cfu/100 g of soil. Populations of isolates in soil treated with 1.0 or 10.0 μg of pencycuron per gram of soil ranged from undetectable to 2 cfu/100 g of soil and averaged 0.4 cfu/100 g of soil.

Table 1. Root disease severity and plant growth in soil infested with isolates of *Rhizoctonia solani* and *Rhizoctonia*-like binucleate fungi (RLBF) and treated with pencycuron in experiment 1

Fungi (F)[u]	Source	Total number of plants	RHDI[v]	Foliage weight (g)	Dead plants	Plants with hypocotyl lesions	Root growth[w]	cfu[x] (21–22 days)	
								EL	L
R. solani									
AG-4	Peanut seed	6.5	2.4 b[y]	10.9	0.5 b	1.4 bcd	3.9 c	8.1 b	25.0 b
AG-2 type 1	Soil, Tift County	6.9	2.9 a	10.8	0.3 c	6.4 a	3.9 bc	12.5 a	61.1 a
AG-2 type 2	Soil, Colquitt County	7.9	2.1 b	14.7	0.9 a	2.5 bc	4.2 abc	0.3 c	2.4 cd
RLBF									
CAG-2	Soil, Tift County	8.4	1.2 c	17.9	0.1 d	0.7 d	4.9 a	0.0 c	14.2 bcd
CAG-3	Soil	7.6	2.0 b	8.9	0.0 e	2.9 b	4.4 abc	0.0 c	0.0 d
CAG-4	Soil	7.3	1.9 b	9.0	0.0 e	1.3 cd	4.4 abc	0.0 c	7.4 bc
CAG-5	Soil	7.1	1.9 b	8.5	0.1 de	1.2 cd	4.6 ab	0.0 c	11.8 bcd
Control	⋯	7.0	1.4 c	10.0	0.1 de	0.2 d	4.9 a	0.0 c	0.0 d
Comparisons[z]									
Fungi (F)		NS	0.01	NS	0.05	0.01	0.05	0.01	0.01
Inoculum density (ID)		NS	NS	NS	NS	0.05	NS	0.05	0.05
Pencycuron (P)		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
F × ID		0.05	NS	NS	NS	0.01	NS	0.05	NS
F × P		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
ID × P		NS	NS	NS	NS	NS	NS	NS	NS
F × ID × P		NS	NS	NS	0.01	NS	NS	NS	NS

[u] One isolate of each AG or CAG. Data are means of four replicates at two inoculum densities and four dosage levels of pencycuron.

[v] Root and hypocotyl disease index: 1 = <2, 2 = 2–10, 3 = 11–50, and 4 = >50% dead or dying plants.

[w] Visual estimate on a scale of 1–5, where 1 = very poor and 5 = excellent.

[x] Colony-forming units per 100 g of oven-dried soil. EL = colonies >4 cm in diameter after 48 hr at 26 C on tannic acid-benomyl agar and L = colonies >2 cm in diameter.

[y] Numbers followed by the same letter are not significantly different according to Duncan's multiple range test.

[z] Various linear comparisons were tested with the F-test. NS = no significant differences, 0.05 and 0.01 = levels of significance.

Table 2. Root and hypocotyl disease severity in snap bean and survival of propagules of *Rhizoctonia solani* and *Rhizoctonia*-like binucleate fungi in soil treated with pencycuron in experiment 1

Inoculum density (ID)	Pencycuron (P) (μg/g)	AG-4 RHDI[a]	cfu[b] (days) 0	21	AG-2 type 1 RHDI	cfu (days) 0	21	AG-2 type 2 RHDI	cfu (days) 0	21	CAG-5 RHDI	cfu (days) 0	21
1:500	10.0	1.0	0	0	3.2	32	346	1.0	0	0	1.6	16	0
	1.0	1.0	32	22	3.4	11	216	1.7	5	0	1.2	43	16
	0.1	3.3	213	367	3.5	102	319	1.5	11	8	2.1	53	86
	0.0	4.3	256	356	2.7	48	351	4.4	59	5	2.4	21	275
Level of significance		0.01		0.01	NS		NS	0.01		NS	0.0.5		0.01
1:2,000	10.0	1.0	0	0	2.6	59	84	1.0	5	0	1.7	0	0
	1.0	1.4	5	0	2.4	16	270	1.5	21	0	1.2	0	0
	0.1	2.6	59	97	3.0	21	408	1.9	5	3	1.6	16	78
	0.0	4.2	26	167	2.5	0	254	3.8	5	5	3.2	5	116
Level of significance[c]		0.01		0.01	NS		NS	0.01		NS	0.05		0.01
ID $\times$ P		NS		0.05	NS		NS	NS		NS	NS		0.05

[a] Root and hypocotyl disease index: 1 = <2, 2 = 2–10, 3 = 11–50, and 4 = >50% discoloration and decay; 5 = dead or dying plant.
[b] Colony-forming units per 100 g of oven-dried soil; cfu at planting represents one sample from each soil batch; therefore, data were not analyzed statistically.
[c] $P = 0.05$ or 0.01; NS = no significant difference.

Table 3. Root and hypocotyl disease severity and foliage weight in snap bean 3 wk after planting and survival of *Rhizoctonia solani* in soil treated with pencycuron in experiment 2

Pencycuron (μg/g)	AG-4[a] Hypocotyls with lesions (%)	RHDI[b]	cfu[c]	Foliage weight (g)	AG-2 type 1 Hypocotyls with lesions (%)	RHDI	cfu	Foliage weight (g)	AG-2 type 2 Hypocotyls with lesions (%)	RHDI	cfu	Foliage weight (g)
10.0	3	1.1	0	19.6	0	1.0	0	20.6	3	1.1	0	16.1
1.0	3	1.1	1	19.7	3	1.1	0	20.4	3	1.1	0	17.5
0.1	30	1.7	6	20.0	15	1.5	2	16.2	12	1.4	1	16.4
0.0	76	1.9	25	17.2	58	2.2	1	14.2	100	4.6	2	6.3
Level of significance[d]	0.05	NS	NS	NS	0.01	0.01	NS	0.05	0.01	0.01	NS	0.01

[a] The AG-4 and AG-2 type 1 isolates were from sorghum, and the AG-2 type 2 isolate was from corn.
[b] Root and hypocotyl disease index: 1 = <2, 2 = 2–10, 3 = 11–50, and 4 = >50% dead or dying plants.
[c] Colony-forming units per 100 g of oven-dried soil 21 days after soil treatment.
[d] $P = 0.05$ or 0.01; NS = no significant differences.

Foliage weight (Table 3) and root growth (data not shown) were increased by pencycuron in soil infested with AG-2 types 1 and 2 but not in soil infested with isolates of AG-4, CAG-3, CAG-4, or CAG-5. There was no interaction of inoculum levels with dosages of pencycuron. Pencycuron increased plant stand in soil infested with CAG-5 but not in other treatments.

DISCUSSION

The *R. solani* most commonly isolated from soils and diseased plants in the Georgia coastal plain is AG-4, and none of the three isolates tested was resistant to pencycuron. Both AG-2 types 1 and 2 are common in soil in Georgia and have been isolated from visibly sound peanut seed, snap bean, rye, sorghum, and corn (1,16,17). Crown and brace root rot of corn is caused by AG-2 type 2 (17), and AG-2 type 1 causes root rot in canola, bean, and cowpea (7,16). The only resistance found to pencycuron was in one isolate of AG-2 type 1 from soil; however, another isolate of AG-2 type 1

from sweet sorghum roots was sensitive to pencycuron. The RLBF are indigenous in soil in the coastal plain and are infrequently isolated from roots of numerous plants. None of the CAG isolates showed resistance to pencycuron, but one of two isolates each of CAG-4 and CAG-5 was detected in soil 3 wk after treatment with 10 μg/g of soil. Isolates of CAG-2 and CAG-4 have shown some activity as biocontrol agents against *R. solani* AG-2 type 2 and AG-4 in greenhouse tests (D. R. Sumner and D. K. Bell, *unpublished*).

With PCNB, disease severity increased as inoculum density increased in radish (2), but in this study, there was no interaction between inoculum density and dosage of pencycuron on root disease severity. Even at extremely high inoculum densities of >50 cfu/g of soil, pencycuron gave complete control of sensitive isolates. However, propagules were detected on TABA more frequently in soil infested with high inoculum densities and treated with 1 μg/g than in soil treated with 10 μg/g.

Based on this study with seven isolates of *R. solani* and seven isolates of RLBF, there is a possibility that resistance to pencycuron could exist in some indigenous pathogenic *R. solani* isolates in fields. Other research with pencycuron has indicated a wide variability in the sensitivity of different *R. solani* isolates (13).

Earlier research with PCNB showed there was a wide range of reaction to the fungicide among *R. solani* isolates in in vitro and in greenhouse tests (15); however, there was no attempt to separate the isolates according to AG, and isolates of RLBF may have been included in the experiments.

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