Techniques

A Qualitative Baiting Technique for Selective Isolation of *Rhizoctonia zeae* from Soil

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ABSTRACT

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A baiting technique was developed for selective isolation of *Rhizoctonia zeae* from naturally infested soil using fungicide-treated stem segments of cotton and a selective medium consisting of 2% water agar and benomyl, metalaxyl, penicillin G, and streptomycin sulfate at 10, 10, 50, and 50 μg a.i./ml, respectively. Cotton stem segments soaked in benomyl at 500 μg a.i./ml and metalaxyl at 100 μg a.i./ml or in benomyl at 1,000 μg a.i./ml were successfully used to isolate *R. zeae* from two naturally infested soils. Fungicide-treated stems were colonized in significantly higher numbers by *R. zeae* than untreated stems. The selective medium also increased recovery of *R. zeae* from coloniżed stems. Untreated stems were colonized by *R. solani*, binucleate *Rhizoctonia*-like fungi, *Pythium* spp., and a number of other common soil-inhabiting fungi.

Rhizoctonia zeae Voorhees is a soil-inhabiting fungus that was first isolated from diseased corn in 1934 (17). Since that time, *R. zeae* has been isolated and identified infrequently (5,6,14,16). It has been isolated with other *Rhizoctonia* spp. when a nonselective or semiselective isolation medium has been used.

Baiting techniques for studying the occurrence and distribution of *R. solani* Kühn have used seeds, paper disks, or excised plant parts as baits (2,3,8–10,13). These techniques are usually semiselective, and several *Rhizoctonia* spp. may be isolated. In this case, characterization of these fungi may be time-consuming because they are not readily distinguishable during initial growth in culture.

The objective of this study was to develop a baiting technique for the selective isolation of *R. zeae* from soil.

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TABLE 1. Effect of selected fungicides and antibodies on radial growth of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi

Chemical	Rate (μg a.i./ml)	Percentage inhibition of growth[z]											
		R. zeae isolate					*R. solani* isolate					Binucleate *Rhizoctonia*	
		RZ 215	RZ 243	RZ 250	RZ 251	RZ 253	RS 16	RS 28	RS 31	RS 41	RS 245	BN 231	BN 236
Benomyl	10	3	2	10	6	6	100	100	100	86	100	100	100
	50	28	36	39	32	32	100	100	100	100	100	100	100
Metalaxyl	10	10	2	4	0	0	0	0	0	0	4	0	0
	50	33	12	16	18	10	2	0	0	0	15	0	0
Streptomycin sulfate	10	0	5	3	4	0	0	0	0	0	3	1	0
	50	0	7	4	5	0	0	14	0	2	3	7	10
Penicillin G	10	0	0	0	2	0	0	0	2	0	0	0	0
	50	0	0	3	0	3	0	0	0	1	0	0	0

[z] Growth at 48 hr compared with growth on potato-dextrose agar.

MATERIALS AND METHODS

Soils. A Cecil clay loam and a Wickham sandy loam were collected from a tall fescue lawn from Wake County and a soybean field from Edgecombe County, North Carolina, respectively. Both soils were passed through a sieve with an opening of 2 mm and used immediately. Percentage moisture at −1/3 bar matric potential was determined for each soil using a pressure plate apparatus (4). In one experiment, seven soils from North Carolina, two soils from Mississippi, and one soil each from Florida, Missouri, and South Carolina were assayed for *R. zeae*, in addition to the Cecil clay loam and the Wickham sandy loam.

Effect of fungicides and antibiotics on radial growth. Two fungicides and two antibiotics were evaluated for their effect on radial growth of five isolates each of *R. zeae* and *R. solani* and two isolates of binucleate *Rhizoctonia*-like fungi. One isolate of *R. zeae*, four isolates of *R. solani*, and both binucleate *Rhizoctonia*-like fungi isolates were provided by S. B. Martin (Box 1106, New Haven, CT 06504). Stock cultures were maintained on potato-dextrose agar (PDA) slants at 22–28 C.

Benomyl (50% WP; E. I. du Pont de Nemours & Co., Wilmington, DE 19898); metalaxyl (2 EC; Ciba-Geigy Corp., Greensboro, NC 27409); penicillin G (1,675 units per milligram; Sigma Chemical Co., St. Louis, MO 63178); and streptomycin sulfate (17 WP; Pfizer Inc., New York, NY 10017) were suspended in sterile distilled water and added at 10 and 50 μg a.i./ml to sterile cool (50 C) PDA before the medium was dispensed into plastic petri dishes. A 10-mm-diameter agar disk was taken from the periphery of a 3-day-old PDA culture of each of the *Rhizoctonia* isolates and placed mycelial-side-down on PDA containing fungicide or antibiotic at 0, 10, or 50 μg a.i./ml. Five replications of plates were arranged in a completely randomized design and incubated at 28 C. The radius of growth on each plate was measured at 24 and 48 hr.

Recovery of *R. zeae* from naturally infested soils. A technique previously used for isolating *R. solani* (7) was modified for this test. Internodal sections of cotton stems were cut into 10-mm segments and autoclaved at 121 C at 1.05 kg/cm pressure for 30 min. Stem segments were soaked for 2 hr in a suspension of benomyl at 500 μg a.i./ml plus metalaxyl at 100 μg a.i./ml or in a benomyl suspension at 1,000 μg a.i./ml and blotted dry. Nontreated stem segments were soaked in sterile distilled water for 2 hr and used as a control.

The Cecil clay loam and Wickham sandy loam soils were used in this experiment. Twenty stem segments were added to an equivalent of 150 g of oven-dry soil in 600-ml beakers with the moisture content adjusted to approximate −1/3 bar matric potential. The beakers were covered with aluminum foil and incubated at room temperature (22–28 C), and at 72 hr the stems were removed and washed in tap water for 20 min. The stems were blotted dry with sterile paper towels and plated onto 2% water agar or the selective medium (2% water agar, benomyl at 10 μg a.i./ml, metalaxyl at 10 μg a.i./ml, penicillin G at 50 μg a.i./ml, and streptomycin sulfate at 50 μg a.i./ml). Plates were observed at 24 and 48 hr for *Rhizoctonia*-like mycelium using a dissecting scope. Hyphal tip transfers were made to PDA from colonies resembling *Rhizoctonia* spp. Isolates were grown on PDA for 2 wk while species determinations were made. *Rhizoctonia* spp. were identified using criteria previously published (1,11,17). Fungi not resembling *Rhizoctonia* spp. were also identified.

TABLE 2. Isolation of *Rhizoctonia zeae* from two naturally infested soils with cotton stems presoaked in benomyl, metalaxyl, or combination and cultured on either selective medium or 2% water agar

Fungicide	Rate (μg a.i./ml)	Medium	Stem segment colonization (%)[x]	
			Cecil clay loam	Wickham sandy loam
Benomyl + metalaxyl	500 100	Selective[y]	74.6 a[z]	9.3 a
Benomyl	1,000	Selective	68.0 a	1.3 bc
Benomyl + metalaxyl	500 100	Water agar	66.6 a	8.0 ab
Benomyl	1,000	Water agar	40.0 b	0.0 c
Control		Selective	26.6 bc	2.6 bc
Control		Water agar	10.6 c	0.0 c

[x] Percentage of 75 cotton stems colonized by *R. zeae*.
[y] Selective medium was 2% water agar with benomyl, metalaxyl, penicillin G, and streptomycin sulfate at 10, 10, 50, and 50 μg a.i./ml, respectively.
[z] Means followed by same letter are not significantly different by Waller-Duncan's *K* ratio *t* test (*K* ratio = 100 ~ $P = 0.05$).

Each stem treatment and isolation agar combination was replicated five times in a completely randomized design and the experiment was run twice. Statistical analysis was accomplished using the Statistical Analysis System (12). Orthogonal contrasts among stem treatments and between isolation media were made (15).

Soil assays for *R. zeae*. Soil samples collected from 14 locations were assayed for the presence of *R. zeae*. Untreated cotton stem segments were used as the bait for all *Rhizoctonia* spp. and 2% water agar for the culturing medium. This method was compared with recovery of *R. zeae* from cotton stem segments soaked in a suspension of metalaxyl at 100 μg a.i./ml plus benomyl at 500 μg a.i./ml in sterile distilled water and this selective medium used for plating. The techniques described previously for isolating *R. zeae* from the Cecil clay loam and the Wickham sandy loam soils were repeated in this experiment.

RESULTS

Effect of fungicides and antibiotics on radial growth. Benomyl completely inhibited growth of *R. solani* and binucleate *Rhizoctonia*-like fungi at 10 and 50 μg a.i./ml (Table 1). Benomyl slowed the radial growth of isolates of *R. zeae* by about 30% at a concentration of 50 μg a.i./ml. *R. zeae*, *R. solani*, and binucleate *Rhizoctonia*-like fungi were not inhibited or only slightly inhibited in radial growth by metalaxyl, streptomycin sulfate, and penicillin G at concentrations of 10 and 50 μg a.i./ml (Table 1).

Recovery of *R. zeae* from naturally infested soils. The use of fungicide-treated baits and the selective medium increased recovery of *R. zeae* from the Cecil clay loam and the Wickham

TABLE 3. Assays for *Rhizoctonia zeae* from soil samples collected at 14 locations

Crop	Location	Stem treatment/ culture media: None/ water agar	Stem treatment/ culture media: Fungicide[y]/ selective medium
Tall fescue	Wake Co., NC	+[z]	+
	Forsythe Co., NC	0	+
	Burke Co., NC	0	+
Bermudagrass	Wake Co., NC	+	+
	Brunswick Co., NC	+	+
	Kiawah, SC	+	+
	Vero Beach, FL	+	+
	Copiah Co., MS	0	0
Creeping bentgrass	Moore Co., NC	+	+
Kentucky bluegrass	Buncombe Co., NC	0	0
Zoysia	St. Louis, MO	0	+
Tobacco	Johnston Co., NC	+	+
Tomato	Copiah Co., MS	0	+
Cotton	Edgecombe Co., NC	0	+

[y] Fungicide-treated stems were presoaked in suspension containing benomyl at 500 μg a.i./ml and metalaxyl at 100 μg a.i./ml before adding to soil sample.

[z] + signifies that *R. zeae* was isolated from soil sample.

sandy loam soils, compared with fungicide-free segments and culture on 2% water agar (Table 2). The fewest number of cotton stems colonized by *R. zeae* occurred when untreated cotton stems were used as the bait and water agar as the culture medium. The inclusion of benomyl in the selective medium and/or in the stem presoak effectively inhibited the recovery of *R. solani* and binucleate *Rhizoctonia*-like fungi and increased recovery of *R. zeae*. Many common soil fungi, such as species of *Alternaria, Aspergillus, Curvularia, Fusarium, Pythium,* and *Trichoderma*, were recovered with the untreated stems plated onto water agar.

There were significant differences in recovery of *R. zeae* as determined by linear contrasts with a single degree of freedom among media, and between stem treatments. There were significantly more isolates of *R. zeae* recovered from soils that included the selective medium as the culture medium compared with water agar ($P = 0.01$). There were also significant increases in the recovery of isolates of *R. zeae* among treatments that used fungicide-treated stems and soils where untreated stems were used ($P = 0.001$). Recovery of *R. zeae* was decreased among treatments that included stems presoaked in benomyl at 1,000 μg a.i./ml compared with treatments that included stems presoaked in metalaxyl at 100 μg a.i./ml and benomyl at 500 μg a.i./ml ($P = 0.05$).

Soil assays for *R. zeae*. When the fungicide-treated stems were used with the selective medium, *R. zeae* was isolated from 12 of the 14 soils assayed (Table 3). When untreated baits were used with water agar, *R. zeae* was isolated from 7 of 11 soils collected from turf areas and from the three soils collected from fields in which tobacco, cotton, or tomatoes had been grown.

DISCUSSION

Use of fungicide-treated baits and the selective medium not only enhanced recovery of *R. zeae* but also increased the selectivity of the technique. Common soil-inhabiting fungi that normally colonize baits were inhibited by the fungicide-treated baits and the selective medium. The recovery of *R. zeae* with baits treated with benomyl concentrations higher than the previously published EC_{50} values for *R. zeae* (7) was surprising. The concentration of benomyl in the segments may have been less than the reported EC_{50} values. In preliminary experiments, *R. zeae, R. solani*, and binucleate *Rhizoctonia*-like fungi colonized stems that had been soaked in benomyl at 5, 50, or 100 μg a.i./ml (*unpublished data*). Only when stems were soaked in suspensions with benomyl at 500 μg a.i./ml or higher were *R. solani* and binucleate *Rhizoctonia*-like fungi no longer recovered from artificially infested soil.

The difference in the number of isolates of *R. zeae* recovered from the Cecil clay loam as compared with the number recovered from the Wickham sandy loam may be explained by the crop history of these soils. *R. zeae* has been shown to be pathogenic to grasses (6), and the Cecil clay loam was collected from a tall fescue turf. The number of propagules of *R. zeae* in the Cecil clay loam and the thatch layer could account for the increased recovery of isolates of *R. zeae*. The Wickham sandy loam was collected from a field in which soybeans had been planted the previous season. Since *R. zeae* has not been reported to be a pathogen of soybeans, the isolates recovered may have been surviving saprophytically.

In assays of the 14 soils, *R. zeae* was isolated from 12 soils using the fungicide-treated baits and the selective medium, whereas *R. zeae* was isolated from only 7 of the 14 soils where untreated baits and water agar were used. It appears that using fungicide-treated baits and the selective medium increased selectivity without sacrificing sensitivity.

The techniques described in this paper would be satisfactory for use in qualitative studies such as distribution studies. Before the technique can be used to estimate populations of *R. zeae*, studies will need to be conducted to evaluate its sensitivity and precision.

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