Population of Rhizoctonia solani in Agricultural Soils Determined by a Screening Procedure

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ABSTRACT

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A rapid, reliable procedure for the quantitative determination of the population of *Rhizoctonia solani* in agricultural soils is presented. The procedure is based on wet-screening soil through a 0.35-mm mesh sieve to remove the organic particles that contain the pathogen. The material retained on the sieve is dispersed in 1% water agar and, after 18 to 24 hr, incubation, suspected *R. solani* colonies are transferred to potato-dextrose agar for identification. Soil from 60 fields in the San Joaquin Valley of California were assayed. Most of the fields, when sampled, were planted to either cotton or potatoes. The population of *R. solani* ranged from 0 to 15 propagules/100 g of soil and was distributed as follows: 22, 24, 6, and 8 fields had populations within the ranges of 0 to 0.5, 0.6 to 2.0, 2.1 to 5.0, and greater than 5.0 propagules/100 g, respectively. Therefore 77% of the soils sampled contained less than 2.0 propagules of *R. solani*/100 g of soil.

Rhizoctonia solani Kühn has been extensively investigated for many years, and yet significant progress in understanding its behavior in natural soil has been made only recently. In 1959, Boosalis and Scharen (1) reported that *R. solani* was associated with particles of organic matter in soil, an observation confirmed by others (2, 6, 10). Also in 1959, Papavizas and Davey (7) reported that *R. solani* colonized plant stem pieces when they were incorporated into soil, and that the pathogen was readily recovered from the colonized tissue. *Rhizoctonia solani* has been isolated from soil and the relative inoculum density determined by organic particle recovery (1, 10), stem segment colonization (2, 7, 8, 10), immersion tubes (2, 5, 10), disk-plates (3), and bioassays with susceptible plants (2, 10). Recently, Ko and Hora (4) published a procedure for quantitatively assaying soil for *R. solani* and presented some information on populations in natural soil.

Detailed studies on the ecology of soilborne plant pathogens are greatly facilitated by the quantitative determination of naturally-occurring populations. This report presents a procedure for the quantitative assay of *R. solani* in field soil, and information on the population of this pathogen in the San Joaquin Valley of California.

MATERIALS AND METHODS

Description of the assay procedure and evaluation of reliability.—To do an assay, the soil sample is mixed thoroughly, a subsample is removed for moisture determination, and three 50-g portions are processed separately. Each portion is wet-screened through a 0.35-mm mesh sieve (U.S. Standard Sieve Series, Sieve No. 45). The material retained on the sieve is washed (without rubbing) into a beaker of water. The particles are evenly distributed on filter papers by filtering with a Büchner filter apparatus. Usually, six to eight papers are used for 9-cm diameter plates, or three to four papers for 15-cm diameter plates. Cooled (52 C) liquid water agar (1.0%) is dispensed into plates to give a layer about 2.5 mm deep, and then immediately the filter paper is inverted in the agar and agitated to dislodge and disperse the particles. The plates are incubated for 18 to 24 hr, and suspect colonies transferred to PDA for identification. An important factor in the success of this procedure is the rapid growth rate of *R. solani*. Suspect colonies are therefore selected on the basis of size and characteristic growth pattern. The colonies are best observed by holding the plates above a black background in front of a fluorescent light. By screening known amounts of soil, it is possible to determine the number of propagules quantitatively.

A selective medium, described by Ko and Hora (4), may be used in place of water agar. For the soils we have assayed, however, water agar appears to be satisfactory and is easy to prepare. If necessary, bacterial growth can be checked by acidifying the medium with lactic acid.

Two approaches were used to evaluate the reliability of the procedure. The first involved screening 500 to 3,000 g of soil, previously determined to have a high population of *R. solani,* through the 0.35-mm screen. All the soil that passed through the screen was collected and dried. The portion that passed through the screen, and that retained, each were brought to the original weight with soil that did not contain *R. solani.* These reconstituted soils were assayed for *R. solani* by a slightly modified version of the plant stem colonization technique of Papavizas and Davey (7), and by growing cotton seedlings in them.

The stem colonization procedure involved incorporation of 3 g of mature cotton stem segments 5 mm long and 3-5 mm in diameter into 500 g of soil. Clay pots 15 cm in diameter were filled to within 6 cm of the top with

R. solani-free field soil, and 250 g of the test soil, plus cotten segments, was layered on the surface and covered by an additional 3 cm of soil. Two pots were used for each 500-g sample. The soil was moistened to capacity by subirrigation and held in a greenhouse for 4 days at 27 C. The tissue pieces were then recovered, washed, and plated on 2% water agar to determine the percentage of segments colonized by *R. solani*.

Determination of field population of Rhizoctonia solani.—Soil was collected from 26 fields in the San Joaquin Valley of California. The population of *R. solani* was determined by both the screening and stem-colonization procedures. In addition, soil from several fields was placed in pots in the greenhouse and used to grow cotton seedlings. As a further check on the effectiveness of the screening assay to detect natural populations, soil was collected from fields at the time they were planted to cotton and assayed for *R. solani*. Nontreated seed was planted in these commercial fields, and then at 4- to 7-day intervals for about 6 wk, seed and seedlings were removed, observed for damage, and isolations made from nongerminated seed and from suspect hypocotyl lesions.

Establishing known populations of Rhizoctonia solani.—Another approach to assessing the validity of the data on natural populations of *R. solani* was to determine disease occurrence in relation to known populations of *R. solani*. Propagules of the pathogen were obtained for incorporation into soil by growing the fungus for 5 days on a liquid medium in petri dishes. The medium contained 20 g glucose, 0.5 g asparagine, and necessary macro- and micronutrients in 1 liter of water (11). Disks (2 mm in diameter) were cut from the mycelial mat and incorporated into soil free of *R. solani* to give the desired population. Each disk was considered to be a propagule. The soil was from the San Joaquin Valley. Cotton was grown in the infested soil in the greenhouse, and disease incidence was determined 9 days after planting. The water potential of the soil, measured with a tensiometer, ranged from −0.15 to −0.65 bars during the test.

RESULTS

Reliability of the quantitative assay procedure.—A primary consideration in evaluating the procedure was whether the 0.35-mm screen retained essentially all the particles that contain *R. solani*. Seven soils that contained high populations of *R. solani* were screened, and the portion that passed through the screen was tested for the presence of *R. solani* by the cotton stem colonization procedure. The pathogen was detected only in one case in soil that passed through the 0.35-mm screen (Table 1), and no disease occurred when cotton seedlings were grown in the screened portions of two of the soils. In contrast, severe lesions developed on seedlings grown in the original nonscreened soil. Apparently, the 0.35-mm screen is adequate to remove inoculum of *R. solani*.

Procedures to determine the population of a soilborne pathogen generally necessitate the assaying of a large quantity of soil. It was found that 50-g samples could be processed conveniently. To evaluate the precision of the method, four soils were selected and nine 50-g subsamples of each soil were assayed. The mean population/100 g (at confidence limits, $P = 0.05$), and the coefficient of variation (%) for the four soils were as follows: 2.5 ± 1.1, 71.2%; 5.0 ± 1.4, 37%; 6.1 ± 1.7, 43%; and 7.3 ± 2.2, 42%. For routine sample analysis, three 50-g subsamples were used. This appeared to be satisfactory for most purposes, and the variation between duplicate assays seldom exceeded 50%.

Disease occurrence in relation to known populations of Rhizoctonia solani.—A population of 5.0 propagules/100 g was established by incorporating 2-mm diameter mycelial disks into the soil. The mean survival of cotton seedlings grown in this soil in the greenhouse was 50% compared with 100% in the noninfested check soil. These data are based on three replications with 10 seeds/replication. The test was done three times with similar results each time.

To determine the relationship between natural propagule number and disease, soil with a high population of *R. solani* (15 propagules/100 g) was diluted with noninfested soil to give a population of five propagules/100 g. The mean survival of cotton seedlings grown in this soil was 42%. This value is based on three tests with four pots, with seven seeds per pot.

Soil samples were collected from 14 commercial cotton fields and assayed for *R. solani*. Nontreated seed was planted at the sample location in each field. The pathogen was not detected in nine of these soils and there was no

TABLE 1. Effectiveness of a 0.35-mm screen in removing propagules of *Rhizoctonia solani* from field soils

Soil source	Population (propagules/100 g soil)[a] (no.)	Cotton stem colonization (%)[b]		
		Original soil (%)	Separated by 0.35-mm screen	
			Retained (%)	Passed (%)
Kimberlina-1	5.5	18.2	23.6	0
Kimberlina-2	6.7	38.0	55.0	0
HW32-McGrass	11.8	58.0	60.0	0
710-46	0.8	8.0	10.5	0
626-23	6.0	40.0	. . .[c]	0
626-25	5.6	44.0	. . .	0
626-49	9.2	36.0	. . .	3

[a]Each value is based on three 50-g samples.
[b]Pieces of cotton stem were incubated in soil in the greenhouse for 4 days. Each value is based on 100 pieces.
[c]Material retained on screen was not tested.

evidence of disease due to *R. solani* on the emerging seedlings. In five soils, however, the pathogen was detected and there was a low incidence of hypocotyl lesions from which *R. solani* was isolated.

Natural populations of Rhizoctonia solani.—Sixty fields were sampled to determine the population of *R. solani* in agricultural soils. All of the fields, except two alfalfa fields, were planted to either cotton or potatoes at the time of sampling. An area of approximately 250 m^2 was sampled with either a soil tube or a trowel. The samples were composited and assayed by both the screening technique and the tissue-colonization procedure.

No special precautions were taken in handling the samples because repeated assays revealed that the population did not change during 1 mo of storage at room temperature. The population of *R. solani* in 26 fields varied from not detectable to 15 propagules/100 g of soil. There was a close agreement between the quantitative data obtained with the screening technique and the percentage of cotton stem pieces colonized by *R. solani* (Fig. 1). The correlation coefficient for data obtained by the two procedures was 0.90, which was significant at $P = 0.001$. Soils from an additional 34 fields were assayed only by the screening technique. When the data from the 60 fields were used, the following distribution was obtained: 22, 24, 6, and 8 fields had populations within the ranges of 0 to 0.5, 0.6 to 2.0, 2.1 to 5.0, and greater than 5.0 propagules/100 g soil, respectively. Therefore, 76.7% of the areas sampled in cotton- and potato-growing soils contained less than 2.0 propagules of *R. solani*/100 g of soil.

Isolates recovered from soil by the screening assay were identified as *R. solani* on the basis of colony morphology. To further verify their identity, over 50 representative isolates were tested for pathogenicity to cotton. A laboratory technique was used in which 5-day-old seedlings were inoculated with mycelium (11). There was variability in virulence, but all were pathogenic to cotton. Four typical isolates were tested for anastomosis affinities and found to belong to anastomosis group (AG) 4. The hyphal cells of the four isolates were multinucleate.

Soils from other areas in California also were assayed for *R. solani*. Potato field soils from Santa Barbara County and bean field soils from Sutter County contained populations up to 6.0 propagules/100 g. All isolates of *R. solani* recovered from these soils belonged to AG 4. Potatoes are grown in the Tulelake area on peat soils. When this soil was assyed, isolates of both AG 3 and AG 4 were recovered. Assay of this soil was facilitated by soaking the material retained on the screen for 1 min in a 0.5% solution of sodium hypochlorite before plating. This treatment increased the recovery rate and reduced variability. The recovery of *R. solani* from the San Joaquin Valley soils was not influenced by treating with sodium hypochlorite.

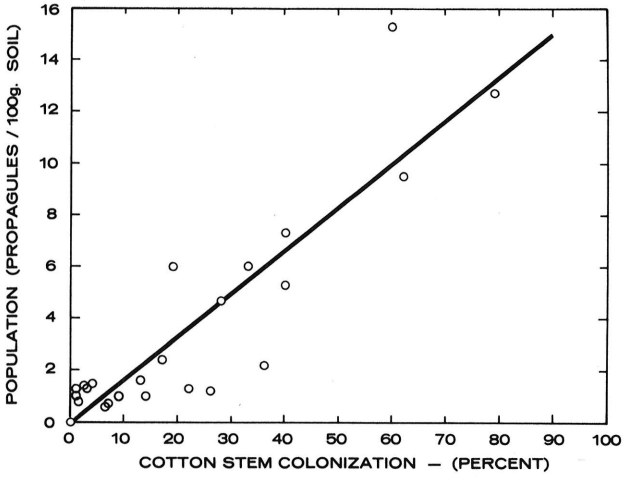

Fig. 1. Relationship between population of *Rhizoctonia solani* in field soil, as determined by the screening assay, and the percentage of cotton stem segments colonized by the pathogen. Each population value is based on at least three 50-g samples. Pieces of cotton stem were incubated in soil in the greenhouse for 4 days; each value is based on 100 pieces. Twenty-six soils were assayed. Correlation coefficient for data from the two procedures is 0.90; significant at $P = 0.001$.

DISCUSSION

This report presents a rapid and reliable quantitative assay for the population of *R. solani* in agricultural soils. The accuracy of this procedure is verified by several types of data. The fact that *R. solani* was rarely detected in soil that had passed the 0.35-mm mesh screen, by either the cotton stem colonization procedure or a bioassay involving cotton seedlings, indicates that this screen removes essentially all of the propagules of *R. solani*. Furthermore, no infection of cotton seedlings grown from nontreated seed was observed in commercial fields that were determined to be free of *R. solani* by the screening assay.

Rhizoctonia solani is a broad species comprised of at least four anastomosis groups (9). Only isolates that belong to AG 4 were recovered from the soils assayed, except those from Tulelake. Therefore, the value of this procedure in determining populations of other anastomosis groups is not known. The fact that isolates of both AG 3 and AG 4 were recovered from the Tulelake soils suggests the procedure may be useful for assaying populations of AG 3.

Among the several procedures that previously have been used to determine the inoculum density of *R. solani* in soil, the plant stem colonization technique appears to be the most satisfactory (2, 10). The high correlation (0.90) between inoculum density obtained by stem colonization and the population obtained by the screening assay, strongly supports the conclusion that both procedures accurately determine *R. solani* levels in soil. Advantages of the screening assay are that it is rapid, easy to accomplish and, most important, provides quantitative data on propagule numbers. Both procedures should be useful in studies on the ecology of *R. solani,* and used together they would increase the reliability of data on population.

Based on the data obtained in this study, the population of *R. solani* in natural soils is relatively low. Seventy-seven percent of the soils sampled had a population less than 2.0 propagules/100 g of soil, and 15.3 propagules/100 g was the highest population detected. These values appear realistic based on the fact

that, in greenhouse tests with artificially infested field soil, cotton seedling survival was reduced 50% by *R. solani* at a population of 5.0 propagules/100 g.

The quantitative assay described above should be a valuable tool in answering the many important questions on the biology of *R. solani,* including the relationship between population and disease, the extent that this relationship is influenced by pathogen nutrition, the effect of population on efficacy of seed treatment, and the effect of cultural practices, such as crop rotation, on population.

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