Elutriation Procedures for Quantitative Assay of Soils for Rhizoctonia solani

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ABSTRACT

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Procedures for the use of a semiautomatic elutriator to assay soils for *Rhizoctonia solani* are described and compared with a wet-sieving technique. The standard procedure involves elutriation of 500-cm^3 samples of soil for 8 min. Debris collected on a 0.425-mm sieve is suspended in 2% water agar and after 18 to 24-hr of incubation colonies of *R. solani* are identified by observation at ×10 to ×400 on the assay plates. Compared to the wet-sieving technique, the elutriation procedure is more rapid and has a lower threshold of detection. It allows identification of colony origin, and can be conducted simultaneously with certain nematode assays. *Rhizoctonia solani* populations from 29 cotton fields in the coastal plain of North Carolina range from 0 to 35 propagules/500 cm^3. Colonized segments of cotton stalks and roots were frequently observed as sources of *R. solani*.

Additional key words: cotton seedling diseases, *Gossypium hirsutum* L.

Quantitative assays previously developed for *Rhizoctonia solani* Kühn have measured three general parameters: (i) the severity and/or incidence of disease produced in a soil (5, 10); (ii) the degree of saprophytic colonization of introduced substrates (5, 7, 9, 10, 11, 13); and (iii) actual numbers of propagative units of *R. solani* in soil (3, 8, 14). Measurements of disease or saprophytic colonization are complicated by factors other than inoculum density of *R. solani*. Such factors include the environment, the suitability of the host or substrate, pathogenicity of the *Rhizoctonia* population, and conduciveness of the soil. Such methods alone cannot be used to differentiate between soils with low inoculum density and soils with moderate to high inoculum densities but with suppressive factors (1). *Rhizoctonia solani* may be present in soil in colonized debris, or as sclerotia, hyphae, or basidiospores (2). Relatively large particles of colonized debris predominate in most soils (3, 14).

Weinhold (14) recently developed a method to directly determine propagule density of *R. solani* in soil by screening soil and incubating the retained debris in water agar (14). A similar assay for microsclerotia of *Cylindrocladium crotalariae* has been developed which employs a semi-automatic elutriator for increased efficiency and convenience (12). This study was conducted to determine whether the Weinhold wet-sieving procedure could be successfully adapted or modified for use with an elutriator.

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MATERIALS AND METHODS

Collection of soil samples.—Samples were collected during June and July from 29 cotton fields in the coastal plain of North Carolina with a 2.5-cm-diameter soil sampling tube. Samples were taken from 20 to 30 spots located systematically in each field. In two fields soil samples were collected from 20 to 30 locations using a shovel. Both types of samples were collected to a depth of 15 to 20 cm. The soil was placed in polyethylene bags, stored at 15 C, and assayed within 3 wk. After mixing, a 500-cm^3 subsample was taken for each assay from a sample consisting of 750-1,000 cm^3 of soil.

Five soils were used to compare the elutriation procedure with the Weinhold screening procedure. All were from fields planted to cotton at the time of sampling. The McArthur and McLeod soils were Marlboro loamy sands. The McNair soil was a Portsmouth loam with approximately 7% organic matter. The Rocky Mount soil was a Norfolk sandy loam and the Everett soil was a Norfolk fine sandy loam.

Elutriation procedures.—Design and use of the North Carolina semi-automatic elutriator have been described (4). Air and water flow was adjusted to 40-50 cm^3/sec and 80 ml/sec, respectively. The sieve arrangement for the standard *R. solani* assay procedure was the same as is routinely used in nematode assays. Four samples were elutriated simultaneously.

For the standard extraction of *R. solani*, debris was retained on a 15-cm-diameter, 0.425-mm sieve. Material which passed the 0.425-mm sieve and the turbinate sample splitter was retained on a 20-cm-diameter, 0.038-

mm sieve. Material retained on the 0.038-mm sieve was collected and processed for nematode enumeration (4). Debris collected on the 0.425-mm sieve was rinsed with a spray of water into a 600-ml plastic beaker for further processing.

When it is necessary to extract *Meloidogyne* eggs or individuals of endoparasitic genera (e.g., *Pratylenchus* or *Hoplolaimus*), the *R. solani* and nematode assays must be conducted as separate procedures.

Enumeration of extracted Rhizoctonia solani propagules.—Debris from the 0.425-mm sieve was collected on 15-cm-diameter fluted filter paper in a conical glass funnel. Molten (50 C) 2% water agar was used to rinse the debris from the filter paper into 10-cm-diameter petri dishes. The amount of debris per dish was controlled by eye to allow better observation of colonies. Incubation was in darkness at 24 C for 18 to 24 hr. Assay plates were scanned under a dissecting microscope at ×10 using a combination of incident and transmitted light to locate colonies and their origin. Colonies suspected of being *R. solani* were examined under a compound microscope at ×100 or ×400 for verification. When required, isolation of *R. solani* from assay plates is best achieved by transferring hyphae to plates of selective medium (6, 8).

The Weinhold wet-sieving procedure was performed as described (14).

RESULTS

During preliminary development of the elutriation methods, several procedural variations were examined. The McLeod soil, which had a high density of *R. solani* was employed. There was no appreciable difference between the numbers of propagules estimated using a 0.425-mm or 0.350-mm sieve to collect the organic debris. A 1.651-mm sieve retained 18% of the propagules. Increasing elutriation time from 4 to 8 min increased population estimates by 30%. Material which passed the 0.425-mm seive was retained on a 0.149-mm or a 0.038-mm sieve and assayed for *R. solani*. No *R. solani* was detected in three samples and in the fourth sample the *R. solani* detected in the 0.038-mm sieve fraction accounted for 10% of the total population.

When the organic debris collected on the 0.425-mm sieve and suspended in water was exposed to 0.25% NaClO for, 1 min, several undesirable effects were observed: population estimates were reduced by 44%; soil aggregates were dispersed causing the suspensions to become cloudy; and hyphal growth on the water agar was abnormal even after thorough rinsing of the debris. Thus, the adapted procedure did not include a surface-sterilization treatment. The use of the selective medium of Ko and Hora (8) as modified with benomyl (6) was compared to water agar with the McLeod, McNair, and Rocky Mount field soils. Counts using the selective medium were 95, 130, and 145% of those obtained using water agar for the three soils, respectively. Because of the added cost and difficulty in preparing the selective medium, water agar was used routinely in the assay procedure.

Examination of assay plates from cotton-field soils indicated that *R. solani* was originating in most cases from pieces of cotton roots, stems, or stalks (Fig. 1). In no case was free sclerotia observed in the samples nor did colonies arise from debris-free soil aggregates. Pieces of cotton stem from 2-mo-old plants grown in the greenhouse were cut into lengths of 1, 2, and 4 cm and five pieces of each length were mixed with 500 cm^3 of soil (two samples) and elutriated. Following elutriation for 8 min, 100, 80, and 90% of the 1, 2, and 4-cm-long stem segments were recovered on the 0.425-mm sieve, respectively.

Organic debris recovered from soil by use of the elutriator was relatively free of the clay and silt material which frequently caused clouding of agar in the Weinhold method. Because of the reduced amount of total material extracted, the number of plates required for a 500-cm^3 sample of soil processed by the elutriation method was roughly the equivalent to the number required to process a 50-cm^3 sample by the Weinhold method. The total

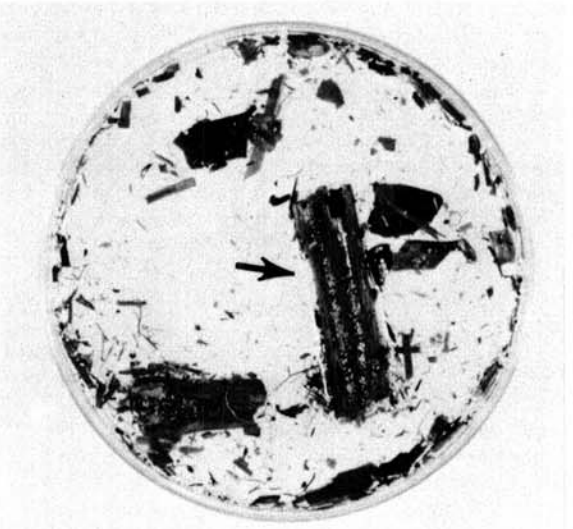

Fig. 1. An assay plate prepared using the elutriation procedure. Arrow is pointed to a segment of cotton stalk from which a colony of *Rhizoctonia solani* developed. The hyphae are not visible in the photograph. (Diameter of plate = 10 cm).

TABLE 1. Comparison of the elutriation method with the Weinhold method for quantitative assay of *Rhizoctonia solani* from five North Carolina cotton soils

Soil	Elutriation method[a,c] (propagules/500 cm^3)	Weinhold method[b,c,d] (propagules/500 cm^3)
McArthur	7.5 (± 4.2, 28%)	10 (± 30.9, 158%)
Everett	9.5 (± 4.2, 22%)	0
McLeod	13.5 (± 9.7, 37%)	33 (± 40.8, 63 %)
Rocky Mount	19.5 (± 12.5, 33%)	20 (± 19.6, 50 %)
McNair	35.0 (± 2.8, 4%)	20 (± 19.6, 50 %)

[a]Averaged from two 500-cm^3 subsamples.
[b]Averaged from three 50-cm^3 subsamples.
[c]Parentheses contain P=0.05 confidence intervals (±) and the coefficient of variation (%).
[d]Estimates were converted to propagules/500 cm^3 based on assays of 50 cm^3 of soil.

number of propagules of *R. solani* estimated from field soils was in the same range for both methods when differences in soil volume were taken into account (Table 1). However, there was one soil in which *R. solani* was not detected by the Weinhold method but was estimated to contain 9.5 propagules/500 cm^3 by the elutriation method.

It is difficult to compare the elutriation and Weinhold methods for removal of extraneous microorganisms since discrete colonies are not formed on the water agar.

Soils collected from 29 cotton fields in various regions of the coastal plain of North Carolina were assayed by the standard elutriation procedure. *Rhizoctonia solani* was not detected in two soils, 13 soils were estimated to contain 1-5 propagules, eight soils had 6-10 propagules, five soils had 11-15 propagules, and one soil had in excess of 30 propagules per 500 cm^3 of soil. One field sampled 26 May 1977 (shortly after a high incidence of post-emergence damping-off was first observed) was estimated to have 10 propagules of *R. solani*/500 cm^3 of soil. Then the field was disked and replanted to soybeans; a subsequent assay on 16 June 1977 detected 35 propagules/500 cm^3.

Two soil samples collected with a shovel rather than the standard 2.5-cm-diameter sampling tube also were processed. A number of colonies of *R. solani* were observed to originate from segments of cotton stalk as large as 1-2 cm in diameter × 5-7 cm in length.

DISCUSSION

The elutriation method described here is a modification of the Weinhold wet-sieving procedure. It combines many of the attributes of the particle debris isolation method (3) and the Weinhold wet-sieving procedure (14). The following comparisons can be made between the elutriation and the Weinhold procedures: (i) the use of a greater volume of soil in the elutriation procedure affords a lower threshold of detection and increased sensitivity using equivalent numbers of assay plates; (ii) greater clarity of the assay plates following elutriation allows direct observation of hyphae at high magnifications; (iii) the greater clarity also facilitates identification of the substrate of origin of the *Rhizoctonia* colonies; (iv) the elutriation method can be used for simultaneous assay for *R. solani* and many species of nematodes; and (v) the elutriation procedure requires less time.

The elutriation and wet-sieving techniques are direct measures of the number of propagules of *R. solani* present in the soil, whereas assays that employ saprophytic colonization or disease indices are complex functions of the number of propagules, the saprophytic activity of the various forms present, the conduciveness of the soil to activity of *R. solani*, and (in some) the pathogenicity of the *R. solani* present in the particular soil (5, 7, 9, 10, 11, 13, 14). In contrast, the various colonization techniques alone cannot be used to distinguish satisfactorily between soils with low populations of *R. solani* and soils with moderate to high populations of *R. solani* but with suppressive properties. Therefore, there is obviously a place for the use of both methods in conjunction with one another.

Our observations and those of Weinhold (14) indicate that in cotton fields, propagules of *R. solani* are sufficiently large to be retained on sieves with mesh sizes of 0.350 to 0.425 mm. For such soils, material collected on the 0.425-mm sieve by the elutriation procedure is a reliable estimate of populations of *R. solani* and nematode assays can be conducted simultaneously. Some consideration also should be given to the means of collecting the soil to be sampled since standard 2.5-cm-diameter sampling tubes cannot be used to pick up large pieces of cotton stalks which may harbor *R. solani*.

The elutriation procedure was used to assay soil from 29 cotton fields in North Carolina. *Rhizoctonia solani* populations fell in the same range as those previously observed by Weinhold (14). The convenient use of larger volumes of soil, however, lowers the threshold of detection and increases sensitivity of the elutriation procedure. This feature should enable greater discrimination between soils in view of the low populations reported.

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