

Quantitative Recovery of *Rhizoctonia solani* from Soil

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ABSTRACT

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Only a small percentage (14–61%) of the sclerotia of *R. solani* mixed into soil was recovered with a wet-sieving technique 1 or 2 days after infestation. Most propagules of *Rhizoctonia* spp. retained on a sieve floated in water with the organic matter fraction, but 11% of the propagules sedimented with the sand fraction. The wet-sieving technique was compared with a soil-pelleting technique using an amalgam pistol to produce the pellets. This pelleting method recovered more propagules of *Rhizoctonia* spp. from soil than the wet-sieving method; however, the pelleting technique was more costly and labor-intensive than the wet-sieving technique.

Rhizoctonia solani Kühn is a typical facultative parasite (sensu de Bary) that can survive saprophytically for long periods, almost exclusively in the form of sterile mycelium (hyphae or sclerotia) associated with organic debris in soil (2).

Many enumeration techniques have been developed specifically for *R. solani* during the past 25 yr. The literature on isolation techniques was reviewed by Sneh et al (17), Sinclair (16), and Bouhot (3). As a modification of the categorization proposed by Bouhot (3), Kooistra (11) distinguished three categories for estimating populations of *R. solani* in soil. The first, competitive saprophytic ability, includes immersion and baiting techniques (9,15); the second, inoculum density, includes soil fractionation techniques (15,18,21) and direct plating of soil by weight (10) or in pellets (8); and the third, inoculum potential, includes the use of susceptible host plants (3,4).

The criteria for selecting an isolation technique depend on the objective, which in our case was to monitor inoculum levels established in microplots by infestation with sclerotia. Therefore, minimal bias and high precision were the most important criteria. The precision of different isolation techniques has been addressed by several authors (8,17), but

the bias usually has not been determined because the actual propagule density has been unknown. We developed a method to mass-produce sclerotia for use as a quantifiable source of inoculum (20) that gave us a means of determining the percent recovery from soil with various enumeration techniques.

Henis et al (8) compared a pelleting technique with a screening procedure for quantitative recovery of *R. solani* from soil. The pelleting technique recovered twice as many propagules as the screening procedure. We conducted a similar experiment, comparing a pelleting and screening technique. A dentist's amalgam pistol was used to produce the pellets, because it was readily available and cheaper than Henis's pellet soil-sampler. Our objectives were to determine the effectiveness of a wet-sieving method to retrieve propagules of *Rhizoctonia* spp. from soil infested with known inoculum levels of *R. solani* and to compare this wet-sieving method with a pelleting technique for effectiveness in retrieving propagules of *Rhizoctonia* spp. from soil.

MATERIALS AND METHODS

Soil samples. The soil used in these studies was a Darien gravelly silt loam; its properties were described previously (20). In June 1982 and 1983, eight inoculum levels of *R. solani* were established in microplots, mixing multiples of 110–120 viable sclerotia (300–710 μ m in diameter) per kilogram of dry soil into the upper 10–15 cm of soil. The soil in control plots was mixed without sclerotia (Table 1). The viability of the sclerotia on acidified

water agar (pH 4.8) was 49% in 1982 and 78% in 1983. One isolate of *R. solani* was used (R-2 from G. S. Abawi's collection, N.Y. Agricultural Experiment Station, Geneva), isolated originally from beet roots but highly pathogenic to beans (6). Its anastomosis grouping (AG) was unknown because it failed to anastomose with any of the anastomosis groups tested (AG 1–4) (6). The soil in the microplots had been fumigated with methyl bromide (Dowfume MC-2) 5 wk before infestation. Composite soil samples consisting of three or four subsamples of about 200 ml were collected from each microplot at the beginning of the growing season 1 or 2 days after infesting soil with sclerotia of *R. solani* and at the end of the growing season just after harvesting a dry bean crop. At the end of the growing season, samples were taken from the rhizosphere area (in the bean rows, 60 cm apart) and from the bulk soil (between the rows). Stones were hand-picked from the samples in the beginning of 1982. Subsequent samples were sieved through a 6-mm screen to remove stones. Samples were air-dried for 5 days, and it took another 12 days to process all samples.

Isolation methods. A wet-sieving method modified from Weinhold's method (21) was compared with a pelleting method for recovery of *R. solani* from soil. For the wet-sieving method, subsamples of dry soil used were 109 (\pm 6) g in 1982 and 53 (\pm 2) g in 1983. Soil was sieved under running tap water on a 50-mesh (300- μ m) sieve, and clay particles were gently rubbed through the sieve. The remaining organic matter and sand were transferred to a beaker with tap water, and the floating organic matter was transferred back onto the 50-mesh screen. The nonfloating residue in the beaker was rinsed four or five times with tap water onto the 50-mesh screen until this residue consisted of sand particles only (no organic matter visible with the naked eye). The organic matter was dried on paper towels overnight and then placed in 12–15 small heaps per plate onto five to eight plates, depending on the amount of organic matter in the sample. A medium

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selective for the recovery of *Rhizoctonia* spp. developed by Ko and Hora (10) was used in all experiments. Because the percent recovery of *R. solani* from infested soil appeared low with the wet-sieving method, we hypothesized that part of the sclerotia might be discarded with the sand fraction. Therefore, growth of *R. solani* from the organic debris and sand fractions on a selective medium was compared in two experiments. The sand fraction from each sample remaining in the beaker was sieved through a 25-mesh (710- μ m) sieve onto a 50-mesh sieve. The fine sand on the 50-mesh sieve was spread on paper towels to dry and placed in small heaps onto three or four plates of Ko and Hora's medium. The plates were incubated at 26 C in the dark for 20–24 hr.

An amalgam pistol (Straight No. 940, Hawe-Neos Dental, CH 6925 Gentilino, Switzerland) was used to produce the pellets (Fig. 1). The diameter of the pistol opening was 2 mm and its height was adjustable, but only the longest height (5 mm) was employed. The moisture of a subsample of soil was adjusted to 18–20% (–0.7 to –0.2 bar) in a petri dish, and the pistol was pressed into the soil three times to produce a compact, uniform pellet. The mean oven-dry weights per pellet were 20.3 ± 2.8 , 21.4 ± 4.0 , and 22.6 ± 7.6 mg in June 1982, September 1982, and June 1983, respectively. One hundred, 300, and 200 pellets were made per sample in June 1982, September 1982, and June 1983, respectively. Twenty pellets were placed on a plate of a selective medium (10) with their flat sides touching the medium (Fig. 2). The plates were incubated at 26 C for 20–24 hr.

Identification of *R. solani*. Growth of species of *Rhizoctonia* on assay plates was identified with the naked eye or with a dissecting microscope (smaller colonies). Colonies identified by each technique were recorded separately. With the pelleting method, only one or two colonies of *Rhizoctonia* spp. grew out of 20 pellets per plate. With the wet-sieving method, the organic matter was spread

out sufficiently to distinguish individual colonies in a heap of organic debris (Fig. 3). Thus, for both methods, corrections for multiple propagules per colony (7) were deemed unnecessary.

Isolates were checked for multinucleate condition to determine the proportion of *R. solani* among the *Rhizoctonia*-like isolates recovered. Isolates of *Rhizoctonia* spp. were transferred to potato-dextrose agar (PDA) or acidified PDA (10 g of PDA and 10 g of Bacto agar, pH 4.8) in the summer and fall of 1982 (72 and 103 isolates, respectively). When the cultures were 3–4 days old, mycelial plugs were taken from an area about 2 cm behind the hyphal tips, placed on microscope slides, and stained with a drop of 0.05% Trypan blue in lactophenol. Each plug was covered by a coverslip, and each slide was passed over a flame a few times. The mycelium was checked for multinucleate or binucleate condition under the compound microscope ($\times 40$ –100 objective) (12).

RESULTS

Recovery of *Rhizoctonia* with the wet-sieving method. The numbers of viable sclerotia per kilogram of air-dry soil were calculated for each microplot from the

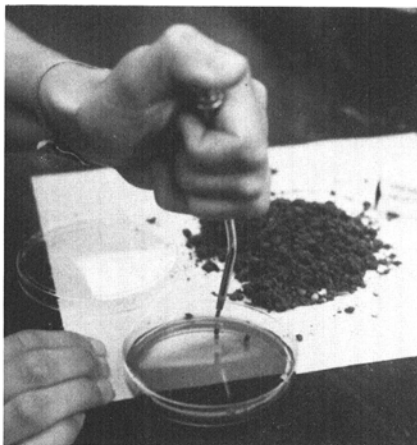


Fig. 1. Production of soil pellets with an amalgam pistol on an agar plate.

numbers of sclerotia added per plot, the mean percent viability of sclerotia on acidified water agar, the measured depth of infestation, and the dry weight per unit volume of soil for each sample (Table 1). The standard deviations of numbers of sclerotia per kilogram of air-dry soil reflect the plot-to-plot variability in the depth of infestation and dry weight per

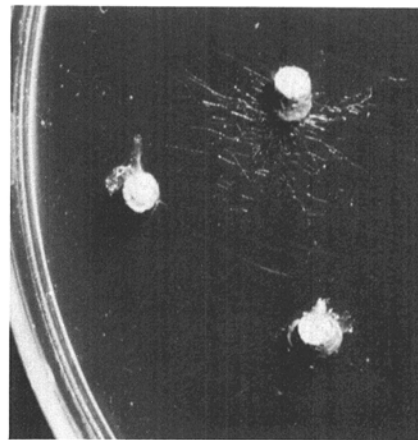


Fig. 2. Hyphae of *Rhizoctonia* sp. growing from a soil pellet produced with an amalgam pistol onto a selective agar medium.

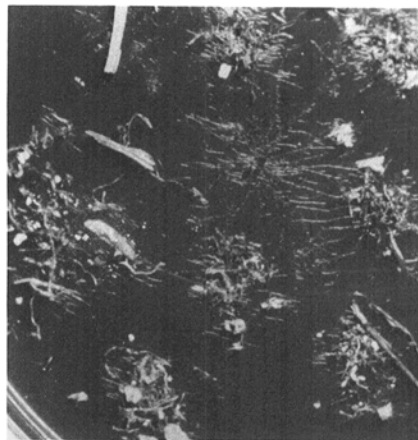


Fig. 3. Colonies of *Rhizoctonia* sp. growing out of organic debris onto a selective agar medium.

Table 1. Numbers of viable sclerotia added and propagules of *Rhizoctonia* spp. retrieved per kilogram of dry soil using a wet-sieving technique in 1982 and 1983

Inoculum level ^a	1982			1983		
	Sclerotia added (no.)	Propagules isolated (no.)	<i>Rhizoctonia</i> spp. retrieved ^b (%)	Sclerotia added (no.)	Propagules isolated (no.)	<i>Rhizoctonia</i> spp. retrieved (%)
0	0 (0) ^c	5 (4)	...	0 (0)	0 (0)	...
1	110 (18)	32 (23)	25 (22)	131 (12)	62 (38)	47 (27)
2	242 (44)	34 (27)	13 (12)	272 (16)	149 (40)	55 (13)
3	366 (132)	68 (23)	19 (10)	348 (0)	305 (118)	88 (34)
4	447 (88)	66 (20)	14 (3)	479 (50)	298 (141)	61 (25)
5	566 (55)	72 (14)	12 (3)	602 (35)	298 (64)	50 (10)
6	747 (109)	91 (27)	12 (4)	711 (34)	429 (49)	60 (9)
7	769 (142)	107 (31)	14 (5)	823 (40)	524 (116)	64 (15)
Total	3,247	470	14	3,366	2,065	61

^aInoculum level in increments of 110–120 sclerotia per kilogram of soil.

^bPercentages calculated after deduction of five sclerotia per kilogram of soil (to adjust for sclerotia isolated from uninfested soil).

^cStandard deviations are given in parentheses.

unit volume of soil. The numbers and percentages of propagules retrieved from soil by wet-sieving were much higher in 1983 than in 1982 (Table 1). The numbers of propagules of *Rhizoctonia* spp. retrieved increased linearly with increasing numbers of sclerotia added to soil ($Y = 12.4 + 0.12X$, $R^2 = 94\%$ in 1982; $Y = 4.1 + 0.61X$, $R^2 = 95\%$ in 1983). Regression of the percent recovery in 1982 on sclerotia added to soil resulted in a line with a significant, negative slope ($b = -0.02 \pm 0.007$, $P = <0.01$), but in 1983, there was no relation between percent recovery and sclerotia added.

Distinguishing *R. solani* from other species of *Rhizoctonia*. Colonies with hyphae typical of *Rhizoctonia* spp. were transferred from the selective medium to PDA to be checked for multinucleate or binucleate condition. However, the *Rhizoctonia* spp. appeared to be parasitized by *Trichoderma* spp., which tended to overgrow the *Rhizoctonia* spp. on PDA. Seventy-two *Rhizoctonia*-like colonies were transferred from the selective medium to PDA in the summer of 1982, but only 23 isolates were identified as *Rhizoctonia* spp. (The rest were mainly overgrown by *Trichoderma* spp.) Twenty-one of the 23 isolates were checked for nuclear condition, and all were multinucleate. In the fall of 1982, 103 *Rhizoctonia*-like colonies were transferred from the selective medium to PDA, and 60 isolates were identified as *Rhizoctonia* spp. (The rest were mainly overgrown by *Trichoderma* spp.) Of the 60 isolates, 29 were checked for nuclear condition, and four were binucleate. On the average, the colonies of *Rhizoctonia* spp. consisted of 92% *R. solani* and 8% binucleate *Rhizoctonia* spp.

Distribution of *Rhizoctonia* spp. between organic matter and sand fractions. The numbers of colonies obtained from the organic matter fraction were compared with those from the sand fraction in a paired *t* test. The mean number of colonies from organic matter was significantly greater than those from sand (Table 2). Because only one sample was taken per plot (three or five plots per inoculum level), the standard deviations reflected differences between plots and sampling error rather than variability of the screening procedure. On the average, 11% of the colonies were associated with the sand fraction.

Table 2. Means (and standard deviations) of *Rhizoctonia* colonies per kilogram of soil isolated from organic matter and sand fractions plated onto Ko and Hora's medium and percentage of colonies isolated from sand

Data set	Samples (no.)	Organic matter	Sand	Paired <i>t</i> test	Significance level	From sand (%)
1	12	35.8 (34.0) ^a	5.3 (8.7)	<i>t</i> = 3.3	0.007	12.9
2	40	104.5 (69.8)	13.0 (20.3)	<i>t</i> = 8.3	<0.001	11.1
3	40	58.0 (29.1)	7.6 (10.7)	<i>t</i> = 10.7	<0.001	11.6
Mean		75.3 (57.5)	9.6 (15.7)	<i>t</i> = 11.3	<0.001	11.3

^aStandard deviations are given in parentheses.

Comparison of pelleting and sieving methods. The mean numbers of colonies of *Rhizoctonia* spp. retrieved per kilogram of dry soil were consistently higher with the pelleting technique than with the wet-sieving method (Table 3). A paired *t* test of all data indicated that the pelleting method recovered significantly more colonies than the wet-sieving method ($t = 3.12$, $P = 0.006$). In June 1982, *Rhizoctonia* spp. were not recovered at inoculum levels lower than 7 (± 800 sclerotia per kilogram of dry soil) because of the relatively low number of pellets per sample (100 pellets).

DISCUSSION

There have been only few reports of a quantitative estimation of recovery of *R. solani* from soil (5,10). Ko and Hora (10) added a few sclerotia to soil, all of which was plated onto a selective medium so that there was no sampling error involved. The percent recovery was 90–100%. Castro et al (5) infested soil with mycelial fragments and sclerotia and, using a pelleting technique, recovered 17 and 33% on Ko and Hora's medium (for AG 3 and AG 4, respectively).

Although we were unable to ascertain if the *Rhizoctonia* spp. retrieved from soil were the same species and anastomosis grouping as the *R. solani* added to soil, very few colonies of *Rhizoctonia* spp. were obtained from control soil, and very few binucleate *Rhizoctonia* spp. were found among all *Rhizoctonia* spp. Therefore, calculation of percent recovery of *R. solani* from soil was based on numbers of sclerotia added and numbers of *Rhizoctonia* spp. retrieved, adjusted for those obtained from control plots.

Percent recovery, however, would actually imply that the same propagules were retrieved from soil as the ones added to soil. If other propagules of the same species (or anastomosis grouping) might be present, the propagules added to soil would have to be labeled to be sure that the same propagules were retrieved. We intended to use propagules of *R. solani* with a fungicide-resistance label and produced isolates resistant to tolclofos-methyl, but these isolates had lower growth rates (and thus probably lower fitness) than their sensitive counterparts (19).

The percent recovery of *R. solani* 1 or 2 days after infestation with *R. solani* was

low with the wet-sieving method (14% in the first year and 61% in the second year). The increase in recovery may be due partly to improved sieving techniques and partly to a better survival of the propagules in soil, because the viability of the sclerotia added to soil was lower in the first year than in the second. In 1982, percent recovery decreased with increasing inoculum level, but percent recovery was not related to inoculum level in 1983.

At medium to high inoculum levels of *R. solani*, recovery of *Rhizoctonia* spp. from soil was much higher with the pelleting technique than with the wet-sieving technique. These results support those of Henis et al (8). At low inoculum levels, however, the numbers of pellets per sample need to be fairly high to be able to detect *R. solani*. The pelleting technique required 15 agar plates per sample when 300 pellets were made per sample, whereas the wet-sieving method required only five plates per sample of 55 g of soil. This last method was therefore less costly than our pelleting method, taking only dispensable material into consideration. Wet-sieving and plating of organic debris took about 15 min per sample, whereas pelleting required at least half an hour per sample when 300 pellets were made per sample. Henis's multiple-pellet soil sampler produces larger pellets (50–500 mg each) and requires fewer agar plates and less labor (8) than the amalgam pistol, but the initial cost of the sampling device is higher. The amalgam pistol may therefore be useful where labor costs are low.

The lower effectiveness of the wet-sieving method appeared to be due partly to loss of propagules in the process of separation of the organic matter from the sand fraction. Eleven percent of the propagules stayed behind in the sand fraction. Papavizas (14) also found some *R. solani* in the heavy sediment with his sieving and sedimentation procedure, especially in artificially infested soil. However, the distribution of *R. solani* over the different fractions was not given in quantitative terms (14). Flotation of

Table 3. Recovery of *Rhizoctonia* spp. from soil using the pelleting or wet-sieving method

Date	Inoc. level ^a	No. colonies of <i>Rhizoctonia</i> spp./kg dry soil	
		Pelleting	Sieving
June 1982	7	500 (500) ^b	107 (31)
Sept. 1982	R ^c	390 (330)	81 (33)
	S	78 (110)	24 (33)
June 1983	1	74 (128)	67 (38)
	4	442 (383)	297 (100)
	7	1180 (338)	564 (83)

^aInoculum levels established in the beginning of June in increments of 110–120 sclerotia per kilogram of soil.

^bStandard deviations are given in parentheses.

^cR = rhizosphere, S = bulk soil.

debris in 2% hydrogen peroxide left only a few propagules of *R. solani* in the sediment (18); this might be a more effective method than flotation in water. Weinhold (21) and Kooistra (11) plated the total fraction (including sand) retained on a sieve onto an agar medium and thus avoided the problem of discarding settled *R. solani* particles. This may be suitable for soil types with relatively little sand, but with our soil, the fraction retained on the sieve would be too large to be plated as a whole.

Another explanation for the reduced effectiveness of the wet-sieving method compared with the pelleting method could be that some of the propagules passed through the 0.3-mm sieve with the silt and clay fraction. However, the soil had been artificially infested with sclerotia ranging from 0.3 to 0.7 mm in diameter, although the sclerotia may have undergone changes during the time from infestation to processing of the soil (1). Naiki and Ui (13) found that only a small proportion of *R. solani* propagules (mostly AG 6) passed through a 0.5-mm sieve, and none passed through a 0.25-mm sieve. Similarly, Ui et al (18) found very few propagules of *Rhizoctonia* spp. among the fine soil particles that passed through a 0.3-mm sieve, and Kooistra (11) found that 2–3% of the propagules of *R. solani* (AG 1, AG 2, and AG 4) passed through a 0.4-mm sieve. Because we used a similar sieve size, it is unlikely that a sizable proportion of the propagules was discarded with the silt/clay fraction.

The effectiveness of a wet-sieving method to retrieve propagules of *R. solani* was relatively low compared with that of a pelleting method. Nevertheless,

for samples with low densities of *R. solani*, the wet-sieving method was preferred because of the large number of pellets and agar plates needed for the pelleting method. The effectiveness of wet-sieving could probably be improved by using hydrogen peroxide rather than water for flotation of the organic debris (18).

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