A New Pellet Soil-Sampler and Its Use for the Study of Population Dynamics of Rhizoctonia solani in Soil

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The appartus described in this paper is under a U.S. Patent Pending. Scientists wishing to receive information regarding purchase of the apparatus may address correspondence to the third or fourth author.

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ABSTRACT

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A new soil sampler is described and its use for quantitative estimation of propagules of *Rhizoctonia solani* in soil is reported. The sampler produces soil pellets of predetermined weight and deposits them on any smooth surface, including an agar medium. It has been used either in the form of a multiple-pellet or as a single-pellet soil sampler. When used as a multiple sampler, 15 soil pellets, each weighing 50 to 500 mg (adjustable capacity), are produced and placed at a fixed position on an agar medium. With 10 successive samples, standard error of the mean weights is less than 2%. Increasing dilutions of *Rhizoctonia* inoculum in soil related linearly to the number of *Rhizoctonia*-containing soil pellets when corrected for multiple colonization. Typical mycelium of *R. solani* growing from a pellet is easily recognized, and both presence and vigor of the propagules can be determined. The sampler is especially useful in counting soil microorganisms at levels ranging between 1-10 propagules/g but lower levels can be measured by increasing sample size whereas for higher levels appropriate soil dilutions should be made. The use of this sampler in the study of soil microorganisms other than *R. solani* is possible.

Additional key words: soil microbiology, soil ecology, soilborne plant pathogens, soil fungi.

Population studies of specific soil microorganisms usually involve dilution plates using appropriate media. When low populations are to be determined, however, enrichment techniques, followed by cultivation on selective media, commonly are used. For some soilborne plant pathogenic fungi, baits such as perforated test tubes containing a specific medium, seeds, or plant tissue segments may be buried in soil for a given period of time. These can be examined for the presence of the particular organism either directly or indirectly after further incubation on a specific medium.

Because of the relatively low levels of *Rhizoctonia* solani Kühn found in naturally infested soil (11, 17, 18), most of the methods used for quantitative estimates of this pathogen are indirect. Such methods include the segment colonization method (4), the immersion tube method (12), and the seedling infection method (13). These methods were evaluated by Sneh et al. (16), who found the bean segment colonization method the most reliable for quantitative estimation of relative abundance of *R. solani* in soil. Recently, the plant debris particle method (3) was adapted for quantitative tests and found to correlate linearly with the segment colonization method (17, 18).

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Many workers have been, and are still using chloramphenicol-supplemented water agar as a selective medium for *R. solani* (16). A more selective medium developed by Ko and Hora (11) was favorably accepted (1, 2, 14, 15). Recently, several modifications of this medium have been suggested (6, 7).

During our studies of populations of *R. solani* we found that neither the soil paste method used by Ko and Hora (11) nor the soil dilution (soil slurry) method (1, 15) could be used in large-scale experiments for counting low populations of *R. solani*. Whereas the method of Ko and Hora was tedious, and required many precisely and individually-weighed samples to be placed separately on agar media, the water-soil dilution method could be used only for soil samples containing relatively high numbers (approximately 100/g) of propagules, well above those that occur in naturally infested soil. Similarly, the use of Andersen's soil sampler commonly employed for quantitative estimation of specific organisms by direct plating of soil on agar plates seemed inadequate for *Rhizoctonia* due to its small capacity (100 mg), which allows counting of only higher inoculum densities of such organisms as *Verticillium* sp. (8). In this paper, the use of a new pellet soil-sampler for counting propagules of *R. solani* in soils is reported, and its precision and advantages for counting soil microorganisms in general and *R. solani* in particular is discussed.

MATERIALS AND METHODS

Soil.—The soil used in experiments involving artificial inoculation was a Fort Collins loamy sand. It was sifted through a 2-mm screen and stored in galvanized cans. Moisture was determined by drying samples for 24 hr at 105 C. Soil moisture potential was determined by the filter paper method of Fawcett and Collis-George (5). The relationship between percent moisture (w/w) and matric potential was similar to that obtained by Rouse (14). Soil moisture was adjusted to 15%, which resulted in −0.7 bar matric potential. *Rhizoctonia solani* was not detected by the water-soil dilution method (1, 15), by Ko and Hora's method (11), or by the seedling infection method (13) using radish seedlings.

Samples of field soils containing *R. solani* were collected from three sugar beet testing plots of U.S. Department of Agriculture, Crops Research Laboratory. These plots were infested with *R. solani* R-9 in 1974, 1975, and 1976, respectively, and are presently planted to barley.

Inoculum.—Isolate R-3 of *R. solani* Kühn (1, 2, 14, 15) was used. Chopped potato-soil inoculum was prepared by the method of Ko and Hora (11) as modified by Benson and Baker (1). After incubation for 3 wk at 25 C, the chopped-potato-soil (CPS) culture was dried in air for 24 hr, screened through a 1-mm sieve, and stored in a gauze-covered flask at room temperature. Its moisture content was 6%.

Determination of inoculum level.—The selective medium developed by Ko and Hora (11) supported growth of *R. solani* from soil pellets. Soil samples were placed on the agar medium by a multiple- or single-pellet soil-sampler, as described below. In some experiments, soil samples were air-dried for approximately 1 hr, sifted through a 1-mm sieve, and spread on the agar medium. Plates were incubated for 18 to 20 hr at 25 C. Typical growth of *R. solani* was recorded, using a stereomicroscope at ×20 to ×40 magnification.

The presence of propagules of *R. solani* in soil samples taken from the field was examined with the soil sampler and with the screening procedure recently developed by Weinhold (18).

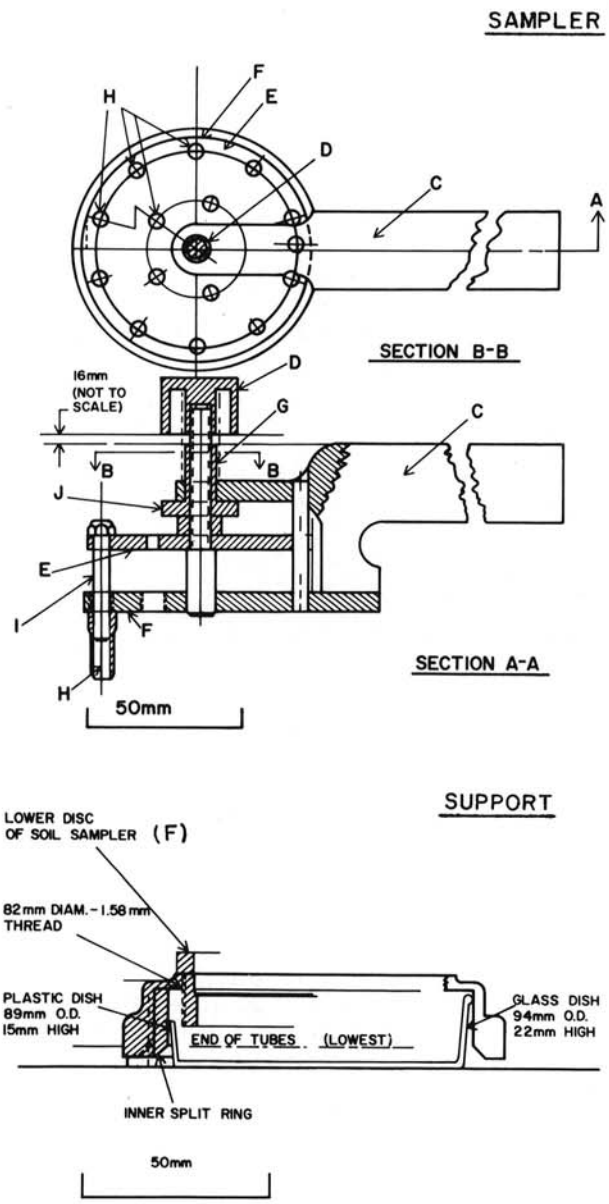

Fig. 1. Diagrammatic representation of the multiple pellet soil-sampler and its support. Abbreviations used: C = handle; D = control knob; E = upper plate (piston holder); F = lower plate (tubes holder); G = spring; H = sampling tubes; I = piston; J = adjusting screw.

RESULTS

Description and use of the multiple-pellet soil-sampler.—The multiple sampler is composed of 15 stainless steel tubes, 5 mm in diameter and 28 mm in length, into which stainless steel pistons are fitted [Fig. 1-A; Fig. 2-(A-C)]. The pistons are held in an upright position by a spring. Their position inside the tubes can be adjusted with a screw. Soil is pressed into the tubes by lightly tapping the sampler several times in a soil sample (i.e. 30 g in a petri dish) with a moisture content of 12-15%. The soil surface at the tube tips is then leveled off by rubbing the sampler tubes lightly on a glass surface (e.g., the cover of a petri dish). The loaded sampler then is placed on its support (Fig. 1-B; Fig. 2-D, E) fitted for either plastic (with inner ring) or glass (without the inner ring) dishes. The knob then is pressed two to four times. The resulting soil pellets are uniform in shape (Fig. 3). To avoid disintegration of the pellets upon collision with agar plate surface, sampler height over the plate is controlled by an adjustable ring (AR Fig. 2-E).

Kershaw (10) suggested a simple subjective analysis to determine the effect of the size of sample on variation in the value of the mean. The method consists of increasing the number of samples several times, calculating a new mean each time, and plotting the values obtained against number of samples taken. The point at which the mean value ceases to fluctuate then is easily determined. Fairly stable mean values of soil pellet weights were obtained with four to five samplings (Fig. 4). When operated by

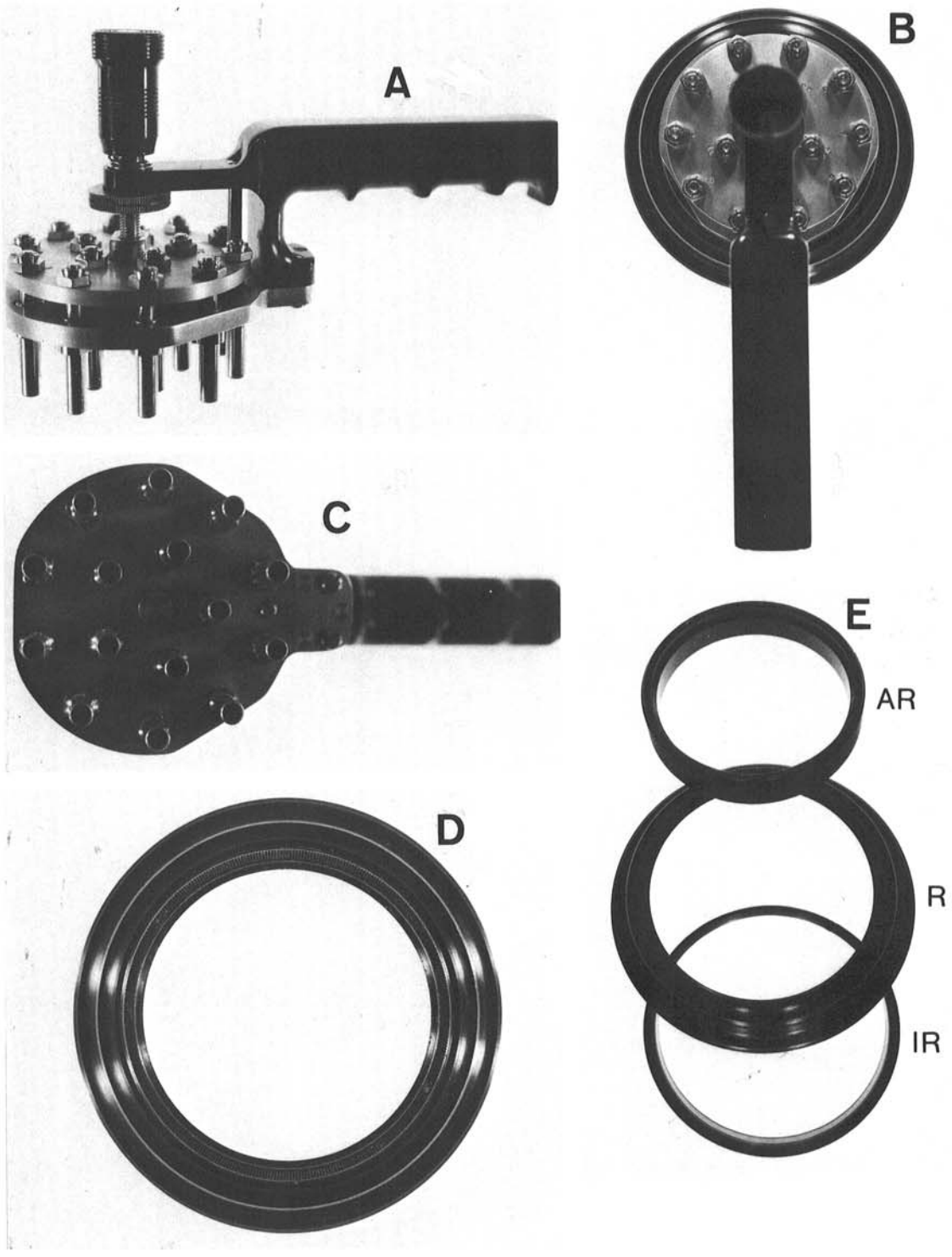

Fig. 2-(A to E). Close-ups of multiple-pellet soil-sampler and its support. **A)** Side view, ×0.71. **B)** Upper view of loaded sampler placed on its support, ×0.620. **C)** Lower view, ×0.654. **D)** Assembled support, ×0.800. **E)** Disassembled support, showing central ring (R), upper adjusting ring (AR) and inner ring used for plastic petri dishes (IR).

two different individuals, average weights of fifteen pellets obtained in one sampling were 112 and 120 mg, with a standard error of the mean ($S_{\bar{x}}$) of ± 1.8 and ± 1.6, respectively. Ten pellets obtained from the same tube over ten successive samplings yielded values of 108 mg (± 1.5) and 117 mg (± 1.2), respectively. Similar results were obtained with samples of sand, loam,and clay containing 10% moisture, and with silt loam and clay loam containing 15% and 20% moisture, respectively.

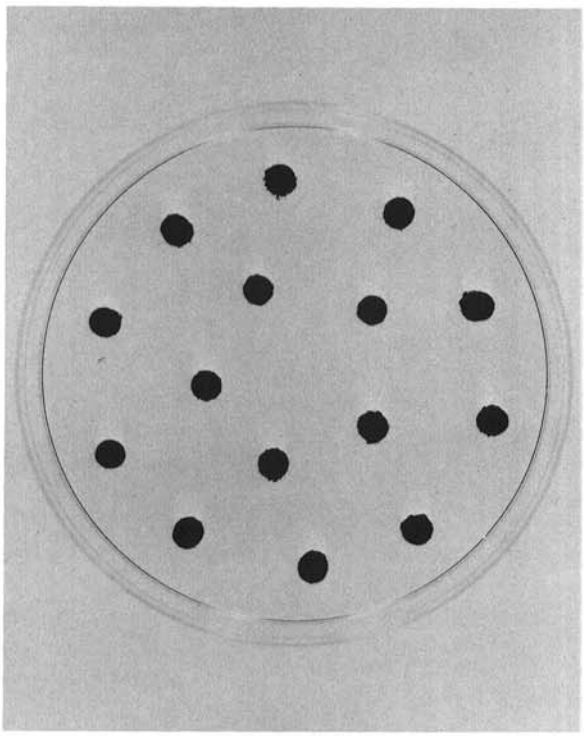

Fig. 3. Plastic petri dish loaded with soil pellets using the multiple sampler, ×1.0.

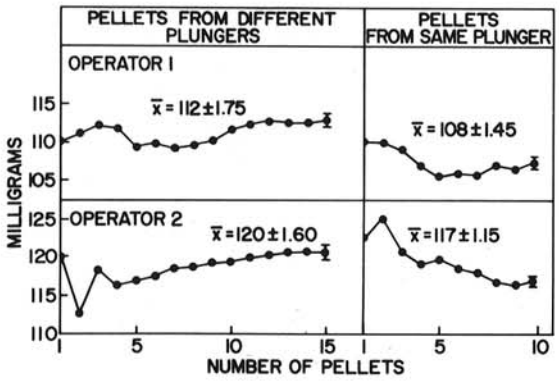

Fig. 4. Effect of increasing number of soil pellets (obtained by successive sampling with the multiple sampler) on the variation in the value of the mean weights of the soil pellets. Limits show standard error of the mean.

Quantitative estimation of Rhizoctonia solani in soil.—Two grams of CPS inoculum were thoroughly mixed by hand with 198 g or raw, air-dried, *Rhizoctonia*-free soil, to give a dilution of 1:100. An aliquot of this sample (100 g) was further diluted by mixing with an equal amount of noninfested raw soil. This double dilution process was repeated four times to obtain CPS dilutions of 1:200, 1:400, 1:800, and 1:1,600. The moisture level of the soil dilutions was adjusted to 15%, and 60 soil samples (four plates, 15 pellets in each) were then taken from each, beginning with the highest, and placed on an agar medium. The sampler tubes were dipped in ethyl alcohol and flamed between each dilution. After 18 hr of incubation, the pellets were examined for the presence of typical *Rhizoctonia* mycelium (Fig. 5, 6). A positive relationship between soil dilution and proportion of colonized pellets was obtained (Fig. 5-A). This relationship deviates from linearity at the lower dilutions, probably because of multiple colonization, as shown by the straight line obtained when plotting inoculum concentration against $\log_e 1/(1-y)$, where y signifies the proportion of *Rhizoctonia*-positive pellets (16). Regression analysis of the values corrected for Poisson distribution (Fig. 5-B) gives an average value of 4,500 propagules/g in the original CPS inoculum.

The soil pellet method not only simplifies detection of *R. solani* mycelium, but for the first time opens a possibility to evaluate the vigor of *Rhizoctonia* propagules in either naturally or artificially infested soil, by recording the number of hyphae emerging from one center in each pellet. A typical growth originating from a presumably large propagule is seen in Fig. 6. Assuming a random distribution of the propagules in the soil, it is highly unlikely that so many hyphae emerged incidentally from the same point. In the present work, propagules of *Rhizoctonia* capable of producing 10 or more hyphae (Fig. 5, 6), emerging from the same center were considered as being large propagules and were counted separately. The possibility of measuring both propagule number and vigor is of great importance in view of the relationship between propagule size, nutrition, and its aggressiveness (9, 17).

Similar results were obtained with the single-pellet soil-sampler (Fig. 7), using sample sizes of 50 to 200 mg. When soil pellets weighing 300 mg or more were used, however, a deviation from linearity was observed.

Comparison between the soil-pellet method and spreading sieved soil on agar medium.—CPS inoculum of *Rhizoctonia* was diluted 200-fold and its propagule content was estimated both by the soil-pellet method (using sixty 100-mg soil pellets, 15/plate in four plates) and by evenly spraying 1 mm sieved soil on four agar plates (using 1 g of sieved soil per plate), counts obtained were 4,500 ($S_{\bar{x}} = \pm 400$) and 1,480 ($S_{\bar{x}} = \pm 100$) per gram CPS, respectively.

Estimation of Rhizoctonia solani in infested field plots: comparison between the soil-pellet method and the screening procedure.—The efficiency of the pellet soil-sampler in the quantitative estimation of *R. solani* in field plots was compared with that of the screening procedure (18). Each test was done in four replicates, using 50 g (dry weight) soil per replicate. For the soil-pellet method, 250 pellets, 200 mg each, in 17 plates, were used for each

subsample. The three soils examined were infested with *R. solani* in 1976, 1975, and 1974, respectively. Mean counts/50 g (± S.E.) of *R. solani* propagules obtained for these soils were 12 ± 2.5 (S.E.), 6 ± 1.1, and 5 ± 1.2 with the soil sampler, compared with 5 ± 1.1, 3 ± 0.91, and 2 ± 0.58, with the screening procedure, respectively.

DISCUSSION

The new pellet soil-sampler described in this report has proven to be both accurate and convenient for quantitative estimation of *R. solani* in the soil. Even though size of pellets is based on volume, the actual average weight of samples is very uniform so that time-consuming weighing operations are eliminated. No other method available so far, is as accurate and convenient. Its principle is based on the plating of a known volume of soil on a selective medium. The stainless steel sampling tubes of the multiple soil sampler can be dipped in alcohol and flamed between samplings. The sampler places the soil pellets on an agar medium in a predetermined pattern, at distances of 18-20 mm apart allowing for the uninterrupted growth of the fungal mycelium during the incubation period. Whenever necessary, single soil pellets

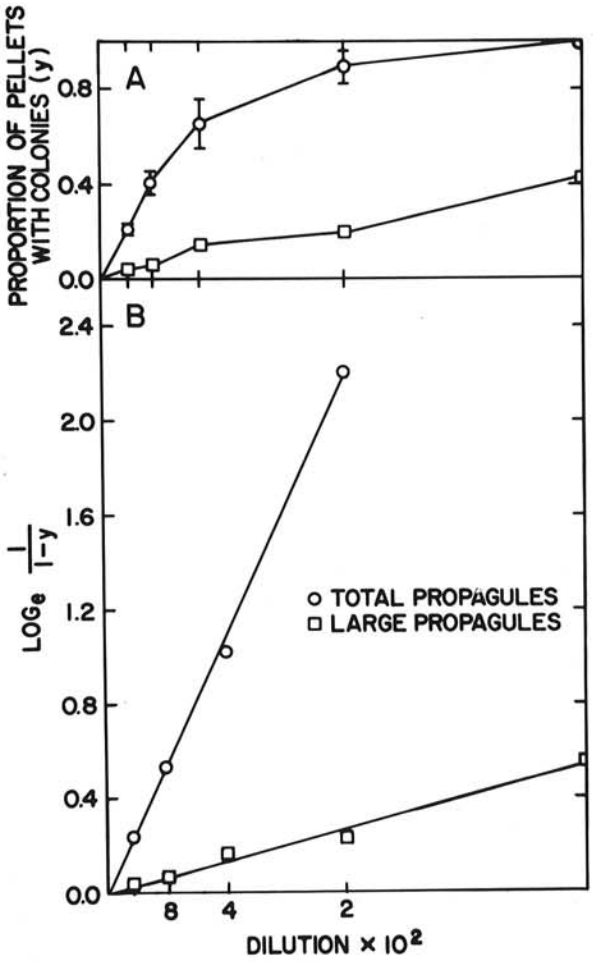

Fig. 5-(A, B). Counting propagules of *Rhizoctonia solani* in soil using the multiple-pellet soil-sampler. **A)** Relationship between soil dilutions of chopped potato soil culture of *Rhizoctonia solani* and counts of soil pellets yielding colonies of the fungus. Each point is the average of five plates, each containing 15 soil pellets of 100 mg (dry weight) each. **B)** Diagrammatic presentation of data of Fig. 5-A plotting $\log_e 1/(1-y)$ instead of y (proportion of colonies from soil pellets). The transformed values represent the number of propagules per gram in the infested soil.

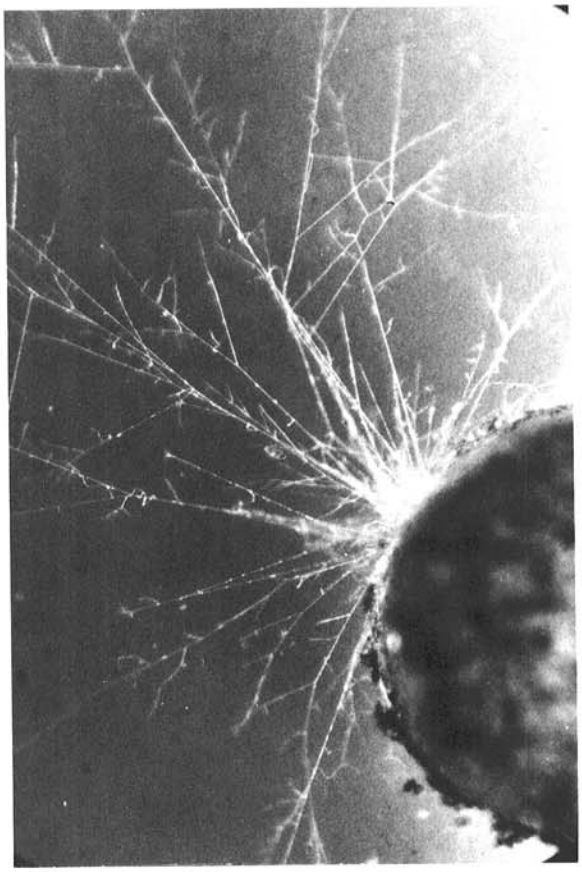

Fig. 6. Typical growth from a large propagule of *Rhizoctonia solani* hyphae emerging from a soil pellet after 18 hr of incubation at 25 C, as observed under the steroscope at ×40 magnification (×100 in photograph).

Fig. 7. Close-up of the single-pellet soil-sampler, ×0.70.

can be prepared with the single-pellet soil-sampler. One advantage of this sampler is its ability to use small quantities of soil (5-10 g) for easy sampling. It can also be used for in situ sampling of soil in pots, plates, etc., provided that soil moisture is not so high that the soil adhers to the tube or so low that pellet formation is not possible.

An important advantage of both the single- and multiple-pellet soil samplers is the uniformity and compactness of the pellets. This allows for a rapid stereoscopic screening for *Rhizoctonia* and possibly for an estimation of the vigor of *Rhizoctonia* propagules in soil, as reflected by the number and length of hyphae emerging from each pellet. The pellet soil-sampler is especially suitable for the quantitative estimation of soil microorganisms at relatively low levels, provided a selective medium is available for the target organism.

The detectable level of propagules of *R. solani* in the soil, using the pellet soil-sampler, is limited only by the number of replicates and by the size of the pellets, which can be modified according to the specific experimental conditions. Thus, field soils containing two-to-five propagules/50 g as determined by Weinhold's technique (18) were easily assayed by the soil sampler by using 250 pellets of 200 mg each per sample replicate. The lower counts of propagules of *R. solani* obtained by the screening procedure, as compared with the soil sampler, could be attributed to some loss of propagules during the wet soil sieving. Furthermore, it is clear that when higher counts (e.g., 1-10/g) are to be determined, the soil sampler method is more convenient than the screening procedure.

Increased soil compaction from sampling and pellet formation had no adverse effect on the emergence of *Rhizoctonia* hyphae. On the contrary, spreading equal amounts of infested air-dried soil over the surface of a selective agar medium yielded counts three to four times lower than those obtained with the pellet soil-sampler. Possibly, single hyphae and small propagules in the spread soil sample either do not grow as fast or are more difficult to detect under the stereoscope than the larger propagules from which hyphae are capable of growing at a rate of 1 mm/hr (1).

Being less active, small propagules would be more subject to suppression by antagonistic microorganisms than larger ones. Indeed, growth of microorganisms other than *R. solani* on the medium of Ko and Hora (11) cannot be completely eliminated, and delaying the recording of *R. solani* growth in 24 hr results in covering of *R. solani* by other microorganisms, making its quantitative recording difficult. It is perhaps easier for *R. solani* to avoid antagonism by growing from a soil pellet into a sterile medium than by overcoming surrounding antagonists on the soil-dusted plate.

Besides *Rhizoctonia*, the pellet soil-sampler may be adapted for screening and counting of any other soil microorganism either plant pathogens such as *Verticillium*, *Pythium*, and *Fusarium* or saprophytes, provided they are able to grow out of the soil pellets, and provided suitable soil dilutions and selective media are used.

LITERATURE CITED

1. BENSON, D. M., and R. BAKER. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: inoculum potential and disease potential interaction. Phytopathology 64:957-962.
2. BENSON, D. M., and R. BAKER. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: Survival. Phytopathology 64:1163-1168.
3. BOOSALIS, M. G., and A. L. SCHAREN. 1959. Methods for microscopic detection of Aphanomyces euteiches and Rhizoctonia solani and for isolation of Rhizoctonia solani associated with plant debris. Phytopathology 49:192-198.
4. DAVEY, C. B., and G. C. PAPAVIZAS. 1962. Comparison of methods for isolating Rhizoctonia from soil. Can. J. Microbiol. 8:847-853.
5. FAWCETT, R. G., and N. COLLIS-GEORGE. 1967. A filter paper method for determining the moisture characteristics of soil. Austr. J. Exp. Agric. Anim. Husb. 7:162-167.
6. FERRIS, R. S., and D. S. MITCHELL. 1977. Evaluation of three selective media for the recovery of Rhizoctonia solani from soil. Proc. Am. Phytopathol. Soc. 3:335.
7. FLOWERS, R. A. 1976. A selective medium for isolation of Rhizoctonia from soil and plant tissue. Proc. Am. Phytopathol. Soc. 3:219.
8. HARRISON, M. D., and C. H. LIVINGSTON. 1966. A method for isolating Verticillium from field soil. Plant Dis. Rep. 50:897-899.
9. HENIS, Y., and Y. BEN-YEPHET. 1970. Effect of propagule size of Rhizoctonia solani on saprophytic growth, infectivity and virulence on bean seedlings. Phytopathology 60:1351-1356.
10. KERSHAW, K. A. 1964. Quantitative and dynamic ecology. American Elsevier, New York. 183 p.
11. KO, W., and F. K. HORA. 1971. A selective medium for the quantitative determination of Rhizoctonia solani in soil. Phytopathology 61:707-710.
12. MARTINSON, C. A. 1963. Inoculum potential relationships of Rhizoctonia solani measured with soil microbiological sampling tubes. Phytopathology 53:634-638.
13. MENZIES, J. D. 1963. The direct assay of plant pathogen populations in soil. Annu. Rev. Phytopathol. 1:127-142.
14. ROUSE, D. I. 1976. Model for biological control: Rhizoctonia solani. M.S. Thesis. Colorado State University, Fort Collins. 66 p.
15. ROUSE, D., and R. BAKER. 1976. Verification of a model for biological control of Rhizoctonia solani preemergence damping off. Proc. Am. Phytopathol. Soc. 3:220.
16. SNEH, B., J. KATAN, Y. HENIS, and I. WAHL. 1966. Methods for evaluating inoculum density of Rhizoctonia in naturally infested soil. Phytopathology 56:74-78.
17. WEINHOLD, A. R. 1976. Population of Rhizoctonia solani in Agricultural soils. Proc. Am. Phytopathol. Soc. 3:219.
18. WEINHOLD, A. R. 1977. Population of Rhizoctonia solani in agricultural soils determined by a screening procedure. Phytopathology 67:566-569.