Rapid Separation of *Sclerotinia minor* Sclerotia from Artificially and Naturally Infested Organic Soil

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ABSTRACT

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Sclerotia of *Sclerotinia minor* were separated from artificially and naturally infested organic soils by a new procedure which involves wet-sieving of the soil and flotation of the sclerotia in 70% glycerol. In the procedure, soil samples are air-dried, sifted on 2-mm wire mesh, blended in tap water in a Waring Blendor, and wet-sieved through 2-mm (9-mesh) and 297-μm (48-mesh) sieves. Residue on the 2-mm sieve is discarded and the residue on the 297-μm sieve is transferred (after washing) to a centrifuge tube containing 70% glycerol. After centrifugation for 3 min at 3,000 rpm, the sclerotia in the overlying liquid are removed, counted by using a stereoscopic microscope, surface sterilized, and then plated on acidified potato dextrose agar to determine viability. The average percent recovery of sclerotia in two typical experiments by this procedure from an artificially infested organic soil was 93%. The numbers of viable sclerotia of *S. minor* extracted from an organic soil naturally infested with the fungus, cropped to lettuce, and sampled at the seedling stage and shortly after harvest were 10 and 30 sclerotia, respectively, per 10 g of oven dry soil.

Additional key words: lettuce drop.

Future investigations on the dissemination, survival, and ecology of *Sclerotinia minor* Jagger, which causes the drop disease of lettuce (*Lactuca sativa* L.), will require a reliable and rapid procedure for the extraction of sclerotia from soil. This is particularly true for organic soils in New York in which lettuce is grown commercially and the disease currently is prevalent. A wet-sieving procedure for separation of sclerotia of *S. minor* from soil has been described (1), but, in our experience, reliable and rapid extraction of sclerotia by this technique was precluded in organic soils by the large amount of organic particles of the same color and in the same size range of the sclerotia. During the summer of 1978 a research technician working full time in this laboratory for 3 mo with the wet-sieving technique was able to extract only 1–2 sclerotia at most from 100-g (air-dry basis) samples of organic soil collected in lettuce fields with continuous histories of the drop disease. During the 3 mo period only a limited number of samples of a large series collected were assayed because of the inherent difficulties in detecting the sclerotia and the exorbitant amount of time required to complete the task when the screened residue was examined under the stereoscopic microscope. In later studies in this laboratory,when the wet-sieving procedure was attempted for the extraction of sclerotia of *S. minor* from organic soils, not only was the procedure insensitive, but the time required to complete the visual examination of the residue of each sample again was unrealistically exessive. The present investigation developed a wet-sieving flotation procedure which is rapid and reliable for recovering practically all sclerotia of *S. minor* artificially added to organic soils. The procedure appears to be highly sensitive and rapid for the extraction of sclerotia of *S. minor* from naturally infested organic soils that have been cropped to lettuce and/or onions.

MATERIALS AND METHODS

Sclerotia of *S. minor* used for artificially infesting organic soil were produced from an isolate of *S. minor* obtained from a lettuce plant infected by the fungus in a commercial lettuce field at the Sorbello farm near Fulton, New York. Large numbers of sclerotia were formed when the fungus was grown on potato dextrose agar (PDA) at 20 C in the dark. Sclerotia were separated from 14-day-old cultures with a spatula and air-dried. Known numbers of sclerotia were added to 10-g samples of organic soils and these were assayed for recovery of the sclerotia.

Soil samples (organic soil) were collected during 1978 from a field cropped to lettuce and naturally infested with *S. minor* at the Sorbello farm near Fulton, New York. The samples were collected with a hand spade from the top 10 cm of soil in the crop row when lettuce plants were at the seedling stage and then again after harvest. The samples were placed in individual polyethylene bags, stored at 4 C until they were air-dried for 24 hr at room temperature, sifted on 2-mm wire mesh, and then directly assayed (five 10-g subsamples per sample) by the wet-sieving flotation procedure for sclerotia (Fig. 1). The moisture content of each soil sample was determined by oven-drying two 10-g subsamples at 105 C for 24 hr.

The distribution of sclerotia of *S. minor* in commercial lettuce fields (organic soil) cropped to lettuce (nine farms) and onion (four farms) in Oswego County, New York, was studied in a large scale survey conducted during the 1979 growing season. Soil samples were collected from the top 10 cm of soil in the crop row, placed in individual polyethylene bags, and stored at 4 C until assayed by the same procedures used during the 1978 study. After different periods of storage, the samples were air-dried for 24 hr, sifted, and then directly assayed (three 10-g subsamples per sample) for sclerotia. The soil moisture of each sample was determined by the procedures used in the 1978 samplings.

Sclerotia extracted during 1978 from the naturally infested soil at the Sorbello farm were tested for germination. The sclerotia were surface sterilized in a 0.5% sodium hypochlorite solution for 2–3

TABLE 1. Recovery of *Sclerotinia minor* sclerotia from an artificially infested organic soil by the wet-sieving flotation procedure

Experiment	Sclerotia added (no./10 g soil)	Sclerotia recovered[a,b] (avg. no./10 g of soil)	(%)
I	20	18	90
II	20	19	95
			Avg. 93

[a]The averages were obtained from five subsamples rather than the three now suggested for the wet-sieving flotation procedure.
[b]Sclerotia per gram of soil based on oven-dry soil weight determined by drying soil at 105 C for 24 hr.

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min, rinsed several times with sterile distilled water, and plated on acidified PDA medium. The plates were incubated at 20 C for 2–3 wk and inspected daily for sclerotial germination and typical mycelial growth of *S. minor*.

RESULTS AND DISCUSSION

The separation procedure. After considerable preliminary experimentation with kinds and sizes of sieves, blending organic soil suspensions for different time periods, flotation of sclerotia in solutions of different sugars, salts, and organic chemicals, different concentrations of these materials, and different speeds and periods of centrifugation, a procedure for the separation of sclerotia of *S. minor* from organic soil was adopted (Fig. 1). This procedure adds to the list of the wet-sieving and flotation techniques utilized in recent years to separate sclerotia of plant pathogenic fungi from infested mineral and organic soils (2–5).

In the procedure each soil sample is air-dried for 24 hr at room temperature and sifted on 2-mm wire mesh. Three air-dried 10-g subsamples are placed individually in a Waring blendor containing 100 ml of water and blended at low speed for 5 sec. Each subsample then is washed thoroughly with tap water onto a 2-mm (9-mesh) sieve placed over a 297-μm (48-mesh) sieve. Residue on the 2-mm sieve is discarded. Residue on the 297-μm sieve is washed for 2 min and then transferred to a centrifuge tube containing 70% glycerol. After centrifugation in an International Clinical centrifuge (International Equipment Co., Needham Heights, MA 02194) for 3 min at 3,000 rpm, the sclerotia in the overlying liquid are removed and counted by using a stereoscopic microscope. In developing the wet-sieving flotation procedure, optimal flotation of the sclerotia was achieved with 70% glycerol. In the tests conducted, other concentrations of glycerol appeared to be less effective for flotation of the sclerotia.

TABLE 2. Wet-sieving flotation extraction of *Sclerotinia minor* sclerotia from a naturally infested organic soil collected at the Sorbello farm near Fulton, New York

Soil samples	Average number of sclerotia/10 g soil[a,b]			Percent viability
	Viable	Nonviable	Total	
Seedling stage[c]	10	3	13	77%
After harvest[d]	30	3	33	91%

[a] The average obtained from five subsamples rather than the three now suggested for the wet-sieving flotation procedure.
[b] Sclerotia/g soil based on oven-dry soil weight determined by drying soil at 105 C for 24 hr.
[c] Soil samples collected when the lettuce plants were at the seedling stage.
[d] Soil samples collected shortly after the lettuce plants were harvested.

Recovery of sclerotia of *S. minor* from an artificially infested organic soil. The effectiveness of the wet-sieving flotation procedure for the recovery of sclerotia of *S. minor* from artificially infested organic soil was tested by assay of 10-g samples of noninfested organic soil to which 20 sclerotia per sample had been added to each sample. In two typical experiments, the average number of sclerotia recovered was 18 and 19 sclerotia (Table 1). The average recovery of sclerotia for the two experiments was 93%.

Recovery of sclerotia of *S. minor* from naturally infested organic soils. The extraction of sclerotia from soil samples collected at the

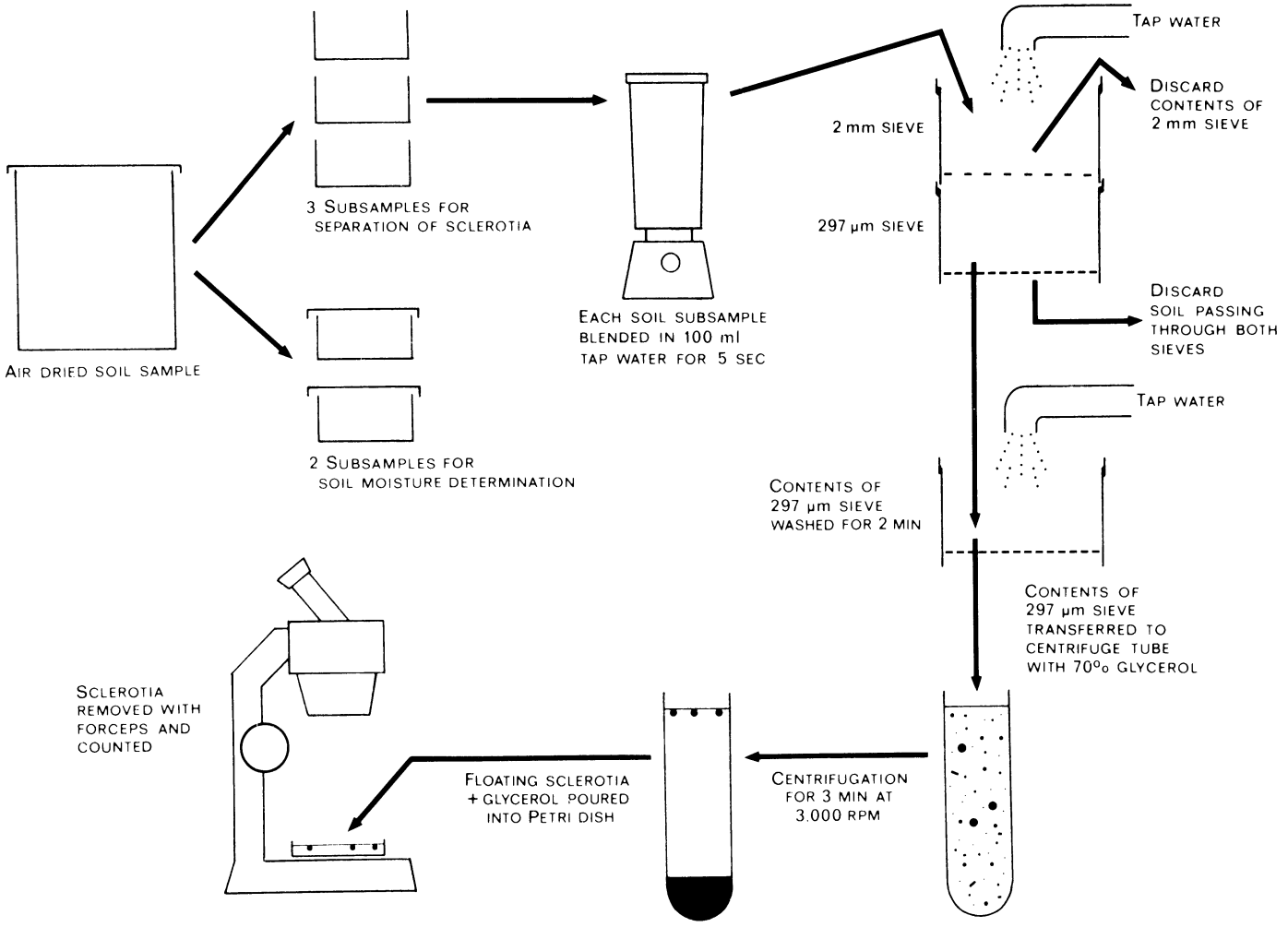

Fig. 1. Schematic diagram of the wet-sieving flotation procedure used for the separation of sclerotia of *Sclerotinia minor* from artificially or naturally infested organic soil.

Sorbello farm at the lettuce seedling stage and shortly after harvest by the wet-sieving flotation procedure resulted in the recovery of 13 and 33 sclerotia per 10 g of soil (Table 2). The viability percentages of the sclerotia in each of these samples were 77 and 91% respectively. The wet-sieving and flotation procedure was successful for the large scale survey conducted during 1979 on the distribution of sclerotia of *S. minor* in organic soils planted to lettuce and onions in Oswego County, New York. The average number of sclerotia per 10 g oven-dry soil ranged 0–23 at a series of sample locations in lettuce fields on the nine farms and 0–3 at a series of sample locations in onion fields on the four farms.

Adams (1) reported inoculum densities of 16–82 sclerotia of *S. minor* per 100 g of organic soil (air-dried) collected from three lettuce fields in Oswego County with histories of the lettuce drop disease. In the present study, sclerotial counts of *S. minor* in two representative extractions (13 and 33 sclerotia per 10 g of organic soil) by the wet-sieving flotation procedure were higher than those detected by Adams (1) who used only the wet-sieving procedure. Since the wet-sieving procedure utilized by Adams (1) was both relatively insensitive and very time consuming in the preliminary studies conducted in this laboratory for extraction of sclerotia of *S. minor* from naturally infested organic soils, the new wet-sieving flotation procedure provides an alternative method that can be used for this task with confidence. It appears that this new procedure provides the techniques needed for the relative complete and rapid extraction of sclerotia of *S. minor* from organic soils in future studies on the ecology and epidemiology of the fungus. The success of the large scale survey conducted during 1979 on the distribution of sclerotia of *S. minor* in a number of organic soils cropped to lettuce in Oswego County provides strong evidence for the utility of wet-sieving flotation procedure.

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