Sclerotinia sclerotiorum: Viability and Separation of Sclerotia from Soil

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ABSTRACT

A combination of dry- and wet-sieving efficiently separates sclerotia of *Sclerotinia sclerotiorum* from soil. Ten times as many sclerotia occurred in rhizosphere soil (12 sclerotia/500 g) from sunflower with root rot than in control soil. About 50% of the sclerotia were nonviable and contaminated, often by *Coniothyrium minitans*. Viability of sclerotia is best assessed on carrot disks, while contaminants are more easily recognized on potato-dextrose agar.

Additional key words: Helianthus annuus, Whetzelinia sclerotiorum.

Sclerotinia sclerotiorum (Lib.) de Bary [*Whetzelinia sclerotiorum* (Lib.) Korf and Dumont] is an important pathogen of sunflower (*Helianthus annuus* L.) in Manitoba (2, 3). Sclerotia are paramount in survival and epidemiology of the pathogen. Hence, an accurate and simple method of assessing the number of soil-borne sclerotia is basic to studies on survival and ecology of the fungus. Adams and Tate (1) "wet-sieved" sclerotia of *S. sclerotiorum* from soil, but omitted technical details. A wet-sieving technique was successfully used to separate sclerotia of other fungi from soil (4, 5). We describe the application of a similar technique for the sclerotia of *S. sclerotiorum*. Because only viable sclerotia are relevant, the studies also included a method of assessing the sclerotial viability.

MATERIALS AND METHODS.—*Soil samples.*—Rhizosphere and control soil samples were collected in October 1974 from a sunflower field in which severe Sclerotinia root rot had occurred in 1973. "Rhizosphere soil" was collected as follows. Sunflowers with severe root rot were severed at 20 cm stem height, dug to a depth of about 15 cm by spade, pulled up, shaken to remove excess soil, taken to the laboratory, and air dried. Soil and sclerotia adhering to the roots were scraped off and included with sclerotia from dissected tap roots and stem portions. Control soil consisted of 30 pooled spade-fulls from the top 15 cm, collected every 2 m along a main pathway, which had been kept free of vegetation during the season by repeated cultivation.

Separation of sclerotia from soil.—Air-dry soil was crushed with a wooden roller and 500-g aliquots were successively screened through a set of 2.00-, 0.85-, and 0.36-mm (10-, 20-, and 40-mesh) sieves. Preliminary studies indicated that no sclerotium was small enough to pass the 0.36-mm sieve, and that the 0.85-mm sieve would suffice. However, because of the size of the aliquot and the presence of large amounts of debris, it appeared most efficient to place soil in the 2.00-mm sieve with the 0.85-mm sieve underneath and to dry-sieve until each sieve contained unpassable residue only. Following transfer, residues were washed on the 0.36-mm sieve, its smaller pores increasing the accuracy of sclerotial recovery. Washing consisted of immersion of the soil in water and repeated rotating and shaking until only unpassable material remained. Finally, the contents on the sieve were rinsed under running tap water. Sclerotia, which are relatively hard and appear glossy when wet, were easily distinguished from stones, sand particles and other debris by feel and sight. They were readily collected by means of a needle or forceps and a ×10 magnifying glass.

Viability of sclerotia.—Sclerotia were surface-sterilized for 30 seconds in a 1:1 (v/v) mixture of Javex (5.25% Na-hypochlorite) and 95% ethanol, and cut in half. One half was transferred to a 100 mm diameter petri dish containing 20 ml of potato-dextrose agar (PDA);

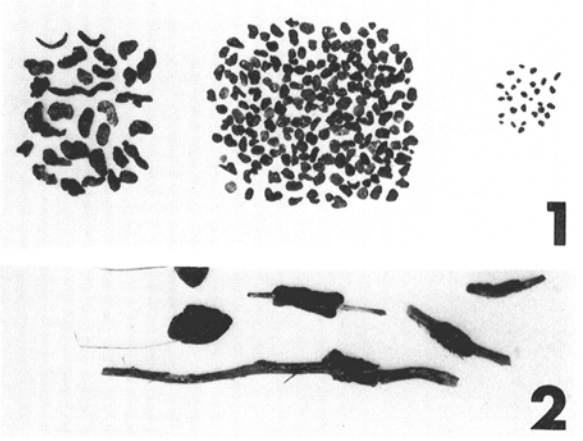

Fig. 1-2. Sclerotia of *Sclerotinia sclerotiorum* recovered from sunflower rhizosphere soil by dry- and wet-sieving. **1)** Large (left), medium (middle) and small (right) sclerotia from 10,000 g of soil (× 0.35). **2)** Sclerotia on rootlets and fibers (× 0.9).

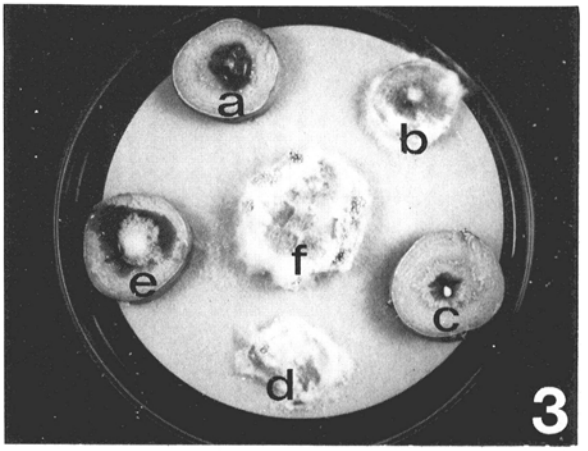

Fig. 3. Viability test of sclerotia of *Sclerotinia sclerotiorum* on carrot disks; a,c - nonviable; b,d,f - viable; e - viable, but with contaminant.

each dish contained four sclerotial pieces. The other half was placed on an aseptically and freshly cut carrot disk on water-moistened filter paper in a petri dish, 150 mm in diameter. Material was incubated for 7 days at room temperature (22 ± 2 C) and inspected for fungal growth. *Sclerotinia sclerotiorum* was easily recognized by its white, fluffy mycelial growth and/or production of sclerotia. Fungi other than *S. sclerotiorum* were purified by transfer to PDA slants and identified.

RESULTS.—*Sclerotia and debris retained by sieving.*—Rhizosphere soil contained more than ten times as many sclerotia as did control soil. One hundred samples of rhizosphere soil contained a total of 1,197 sclerotia while only 115 sclerotia occurred in 100 samples of control soil. Most sclerotia occurred free of other material (Fig. 1) but some were attached to roots or fibers (Fig. 2). The sieving technique proved reliable because identical numbers of sclerotia were counted in samples processed once, reconstituted, and reprocessed.

Sclerotia varied in size (Fig. 1) from large (> 6 mm) to small (< 3 mm) but most were of medium size (3-6 mm). Sclerotia from inside the tap root were mostly small. Sclerotia varied also in shape, with greatest variation occurring in large sclerotia, while other sclerotia appeared more uniform (Fig. 1).

Debris consisted of bits of plant tissue, small stones and sand, seeds, wings of the sunflower leaf beetle *(Zygogramma exclamationis* F.) and pupae of the sunflower maggot (*Strauzia longipennis* Wied.).

Viability of sclerotia.—Of 420 sclerotium halves incubated on PDA, 34% were pure *S. sclerotiorum*, 14% yielded *S. sclerotiorum* that was contaminated while 52% yielded only fungal or bacterial contaminants sometimes accompanied by nematodes. Similar results were obtained with the corresponding halves incubated on carrot disks; the respective percentages were 32%, 11%, and 57%. If the sclerotium was uncontaminated, pure colonies of *S. sclerotiorum* developed on either medium, the colony on carrot covering the entire disk (Fig. 3-b, d, f). If the sclerotium was moderately contaminated, both *S. sclerotiorum* and contaminants developed on PDA while on carrot the disk was only partially covered and the growth of *S. sclerotiorum* was suppressed by contaminants (Fig. 3-e). Nonviable and heavily contaminated sclerotia did not produce any growth of *S. sclerotiorum* on either medium, but growth of contaminants was conspicuous on PDA whereas on carrot only bacteria developed to some extent while fungal growth was sparse and inconspicuous (Fig. 3-a, c).

Of 219 (or 52%) nonviable sclerotia on PDA, 84 were colonized by *Coniothyrium minitans* Campbell alone, 22 by *C. minitans* and other contaminants, three by *Trichoderma viride* Pers. ex Fr. alone and 110 by bacteria and/or fungal contaminants such as *Acremoniella atra* (Cda.) Sacc., *Alternaria alternata* (Fr.) Keissler, *Cylindrocarpon* spp., *Fusarium* spp., *Gliocladium* sp. and *Papulospora* sp. Similar fungi including *C. minitans* contaminated the 59 (or 14%) sclerotia that were still viable. Most fungi isolated from contaminated sclerotia were saprophytes but *C. minitans* and *T. viride* were parasites of *S. sclerotiorum* (H. C. Huang and J. A. Hoes, *unpublished*).

DISCUSSION.—Amount of debris and size of the soil sample are factors which affect the ease of separation of sclerotia of *S. sclerotiorum* from soil. Sclerotia can be collected using a 0.85-mm sieve alone, but a 2.00-mm sieve to screen out coarse debris and larger sclerotia, followed by washing the residue on a 0.36-mm sieve facilitates the collection of sclerotia. The dry- and wet-sieving technique is quantitative, gives reproducible results, and can be used conveniently to index a large number of samples. However, sample size and methods of taking soil samples are factors still to be determined in surveying farm fields for sclerotial inoculum.

Viability of sclerotia can be assessed with equal efficiency on either PDA or carrot disks if the sclerotia are uncontaminated. However, the viability of moderately contaminated sclerotia may be masked on PDA by contaminants that grow faster than *S. sclerotiorum*. Viability per se is best studied on carrot disks because the pathogen, though suppressed in growth, is more conspicuous than on PDA. The PDA medium, however, is preferable if the contaminants are to be identified.

The inoculum potential immediately after harvest of sunflowers with Sclerotinia root rot is considerable. On the average, about 12 sclerotia occurred in 500 g of rhizosphere soil, which is about equivalent to a concentration of one sclerotium per 30 cm^3 of soil. The inoculum potential of *S. sclerotiorum* in rhizosphere soil remained considerable even though sclerotial viability was reduced by about 50%, ascribed mainly to the action of parasites as *C. minitans* and *T. viride*.

Sclerotium size and shape might have a bearing on sclerotial viability. Small sclerotia have less reserve food and are more easily destroyed by soil organisms than large sclerotia. Sclerotia of similar volume but of different shape have the same amount of reserve food but may differ in surface area exposed to drying conditions. Sclerotia in control soil were scarce. It may be that repeated cultivation is an effective control measure for *S. sclerotiorum* in Manitoba.

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