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A Method for Recovery and Counting of Nematode Cysts

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Abstract: A technique was developed in the early 1980's for recovery and quantification of *Heterodera glycines* (soybean cyst nematode) cysts from soil and soybean roots. Cysts were collected on sieves and counted on lined filter paper. This technique could be applied to other particles of similar dimension and density.

Key words: cyst counts, extraction, *Heterodera glycines*, nematode, sampling, soybean cyst nematode, technique.

The following technique was developed for rapid collection and counting of soybean cyst nematode, *Heterodera glycines*, cysts from soil and soybean roots. Each sample of soil infested with soybean cyst nematode was thoroughly mixed, and 250 cm^3 soil from each sample was collected in a beaker. The soil was not packed tightly into the allotted volume. The soil (and plant roots when collected) was placed into a 10-liter bucket, and the bucket was filled 3/4 full with water. Roots were rubbed in the water to remove cysts, and the water was stirred to suspend the soil per cyst mixture. Immediately after stirring, the soil per cyst suspension was poured through nested 850-μm-pore (20 mesh) over 250-μm-pore (60 mesh) sieves. The material collected on the sieves was rinsed with a spray of water, and the debris on the 850-μm-pore sieve was discarded.

The cysts on the 250-μm-pore sieve were washed from the sieve into a beaker with water from a wash bottle. The water level in the beaker was brought to 100 ml. During stirring to suspend the cysts in the water, 1/10 of the sample (10 ml) was poured into a smaller beaker. Alternatively, accuracy of cyst counts was improved when the sample was stirred with a magnetic stirrer and 10 ml of suspension was removed in 2-ml increments with an automatic pipetter. The pipet tip diameter was widened with a razor blade so that cysts did not lodge in the opening.

Ruled, Number 8, 9-cm-d Schleicher and Schuell filter paper (VWR Scientific) was moistened, and the filter paper was molded using a glass rod to the bottom and sides of a Buchner funnel, or similar equipment, under vacuum. Before ruled filter paper was available, we drew parallel pencil lines about 4 mm apart on plain filter paper circles. The 10-ml sample (or a portion of it if there was much debris or many cysts) was slowly poured and washed with a stream of water onto the filter paper under vacuum. Sufficient sample was poured to make a thin layer of material evenly distributed over the filter paper. The filter paper was removed from the funnel with forceps or a dissecting needle and placed into a 100-mm-d petri dish lid containing a few drops of water. Additional water was added as needed to keep the filter paper and the cysts moist, yet prevent cysts from floating while being counted under a dissecting microscope. The new technique not only increased accuracy, but also required only 25% or less time to recover and count cysts from samples compared to a method modified from Jenkins (1). In the latter procedure, cysts were recovered by centrifugation in sugar solution, collected on sieves, and counted in water. Consequently, the new technique substantially reduced the time required for processing large numbers of greenhouse and field samples for cysts.

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