A Vacuum Collection and Seed Separation Technique for Enumeration of Sclerotia of *Claviceps purpurea* in Perennial Ryegrass Fields

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ABSTRACT

Alderman, S. C., Churchill, D. B., and Bilsland, D. M. 1993. A vacuum collection and seed separation technique for enumeration of sclerotia of *Claviceps purpurea* in perennial ryegrass fields. Plant Dis. 77:1020-1022.

A portable electric wet-dry shop vacuum run by a gas-powered generator was used to collect sclerotia of *Claviceps purpurea* from 1-m^2 areas in a commercial field of perennial ryegrass. Sclerotia were separated from soil and straw residue using an air-screen (seed separation) machine, in which samples were partitioned based on particle width and thickness, and terminal velocity. The procedure also was used to determine the number of sclerotia in bales of annual ryegrass straw. Efficiency of recovery of sclerotia using the air-screen machine with known numbers of ergot was 98–100%. Recovery by vacuum collection in the field of a known number of sclerotia distributed over surface residue and plant crowns was 83% ± 4% in areas without soil cracks, 73% ± 15% in areas with deep soil cracks, and 79% ± 13% in areas selected at random. In perennial ryegrass with 23% of heads infected (two sclerotia per infected head), 39 sclerotia per square meter of soil surface were recovered.

Ergot, caused by *Claviceps purpurea* (Fr.:Fr.) Tul., is an important disease of forage grasses grown for seed in the Pacific Northwest (1,3). The disease is characterized by elongated black sclerotia (1–2 mm × 3–8 mm) which replace seed in the seed head. Sclerotia are dislodged and fall to the soil surface during mechanical harvest, which includes cutting, combining, and baling.

A method for enumeration of postharvest sclerotial levels could be useful in epidemiological studies and in evaluation of postharvest residue treatments. Various methods have been used for enumerating soil fungi, such as isolation from host tissue, baiting from soil, selective media, wet or dry sieving, and flotation (2,4,6). An alternative, simple, and convenient method for the collection of *C. purpurea* may be vacuuming sclerotia from the soil surface. Extraction of sclerotia from straw residues and soil or from straw bales may be achieved with a laboratory-scale air-screen machine. Air-screen machines are commonly used in seed-conditioning facilities and are similar in operation to equipment used in seed-cleaning plants. The objective of this study was to determine if vacuum collection and air-screen separation would be an acceptable means of enumerating sclerotia of *C. purpurea* in perennial ryegrass.

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Oregon State Agricultural Experiment Station technical paper 10,086.

Accepted for publication 28 June 1993.

MATERIALS AND METHODS

Sample sites and method of collection. A high incidence of ergot was found in a first-year field of perennial ryegrass (185 ha) near Corvallis, Oregon, during July 1992. In a collection of 400 seed heads from the site prior to harvest, 23% contained ergot, with an average of two sclerotia per head. An area 61 × 122 m within the field was selected as a field site because ergot appeared to be uniformly distributed.

A portable wet-dry shop vacuum run by a portable gas generator was used to collect sclerotia and surface residue from each of eight 1-m^2 locations selected at random within the field site. Samples were placed in paper bags and stored at room temperature until processed. Samples were collected 3 August 1992, and the experiment was repeated 20 August 1992.

To determine the efficiency of vacuum collection, an adjacent field in which ergot was not observed was selected as a test site. Three weeks after harvest and baling, 30 sclerotia were distributed on the surface of four 1-m^2 areas selected at random. The sclerotia were distributed by hand and raked across the soil surface and among the crowns to simulate sclerotial deposition during harvest operations.

To determine the effect of large soil cracks on efficiency of vacuum collection, 30 sclerotia were distributed in each of four 1-m^2 areas without cracks and in each of four areas with one to three soil cracks. Crack size and depth were measured for 20 cracks selected at random while traversing the field site.

To determine if sclerotia were present in straw bales, eight bales were selected at random from the infested site immediately after baling. A 5-cm^2 core (0.5–1.0 kg) traversing each bale was removed and stored in a paper bag until processed. Bale samples were threshed in a laboratory-scale brush debearder to separate a seed–sclerotial fraction from long straw. The seed–sclerotial fraction was then processed in an air-screen machine. The experiment was repeated once.

Sample separation and enumeration of sclerotia. Preliminary tests were made using hand screens to determine effective screen opening shapes and sizes. Identical screens were fit to a laboratory-sized air-screen machine (Fig. 1), and the samples were partitioned into seven fractions based on particle width and thickness. For perennial ryegrass, a scalping screen (6.35-mm round opening) and top screen (3.18-mm round opening) were selected to remove large nonseed material, including long straws and particles of soil with widths greater than that of the seed and sclerotia (fractions 1 and 2). A single-bottom sifting screen (woven wire with 0.608 × 0.051 cm rectangular opening)

was selected to remove fine material with thickness less than that of the seed and sclerotia (fraction 3).

After screening, the remaining sized material entered an air column to remove particles with terminal velocity less than that of the seed and sclerotia (fractions 4 and 5). Since it was important that all sclerotia be saved, air velocity in the column was adjusted to remove only unfilled (empty) seeds and other light material. This determination was made by microscopically examining samples of the lifted (light) fraction (fraction 4) from the air column and adjusting air velocity accordingly. In the remaining seed–sclerotia fraction (fraction 6) ergot sclerotia were counted manually. Sclerotia broken in half were paired to estimate whole sclerotia numbers. The mean and standard deviation number of sclerotia in each fraction was determined.

RESULTS

Perennial ryegrass residue samples were partitioned into six fractions. Coarse and fine materials were included in the first three fractions. Air separation partitioned the light (fraction 4) and the heavy-light fraction (fraction 5) from the sample. Ergot was detected only in fraction 6 (Table 1), which included seed, sclerotia, and a small quantity of soil. Fraction 6 was 16.8% ± 3.5% (mean and standard deviation) by weight of the original sample. In a second experiment, 35 ± 21 sclerotia were detected, and all sclerotia were partitioned into fraction 6 (15.7% ± 5.0%, by weight). The distribution of fraction weights was similar to that reported in Table 1. In all cases the air-screen separation took about 10 min per sample for processing.

In each of four samples into which 30 sclerotia were introduced in the lab prior to separation, all sclerotia were recovered in fraction 6. In a second experiment, 98% of the introduced sclerotia were recovered. In both experiments, sclerotia were not broken or visibly damaged.

At each of four locations in which sclerotia were distributed on the soil surface and vacuum collected, 79% ± 13% of the sclerotia were recovered. In areas without soil cracks, 83% ± 4% of the sclerotia were recovered. In areas with soil cracks, one to three cracks were present. The cracks ranged in width and depth from 0.3 to 2.8 cm and from 1.3 to 15 cm, respectively, with mean and standard deviation width 1.5 ± 0.6 cm and depth 7.0 ± 3.3 cm. At sites with soil cracks, 73% ± 15% of the sclerotia were recovered.

Sclerotia of *C. purpurea* were detected in each of eight bales sampled (Table 2). Fraction 6 was 1% by weight of the original sample. Although 10.5 ± 4.56 sclerotia were detected among samples, sclerotial weight was only 4×10^{-3}% of the total sample weight.

DISCUSSION

The objective of the conditioning procedure was to remove as much material as possible without removing sclerotia. Seed-conditioning equipment such as the air-screen machine separates particles based on differences in physical properties such as length, width, thickness, and terminal velocity. However, since seeds, sclerotia, and other particle types may have similar physical properties, some of the extraneous material usually remains after conditioning. However, all of the sclerotia were retained within a single fraction (fraction 6), which included both sclerotia and seed.

The air-screen machine was effective in removing 75–80% of the sample mass. Since the bulk of the sample was straw residue, fraction 6 was sufficiently small to be easily examined for sclerotia.

The vacuum procedure proved effective in the retrieval of sclerotia from the soil surface. After harvesting and baling, the remaining plants are short, sparse, and desiccated, and it is easy to vacuum in and around the crowns. Rain following harvest initiates regrowth in the crowns. Tests of vacuum efficiency were conducted 3 wk after baling, and some regrowth had occurred. Sclerotia falling within a regrown crown may escape the

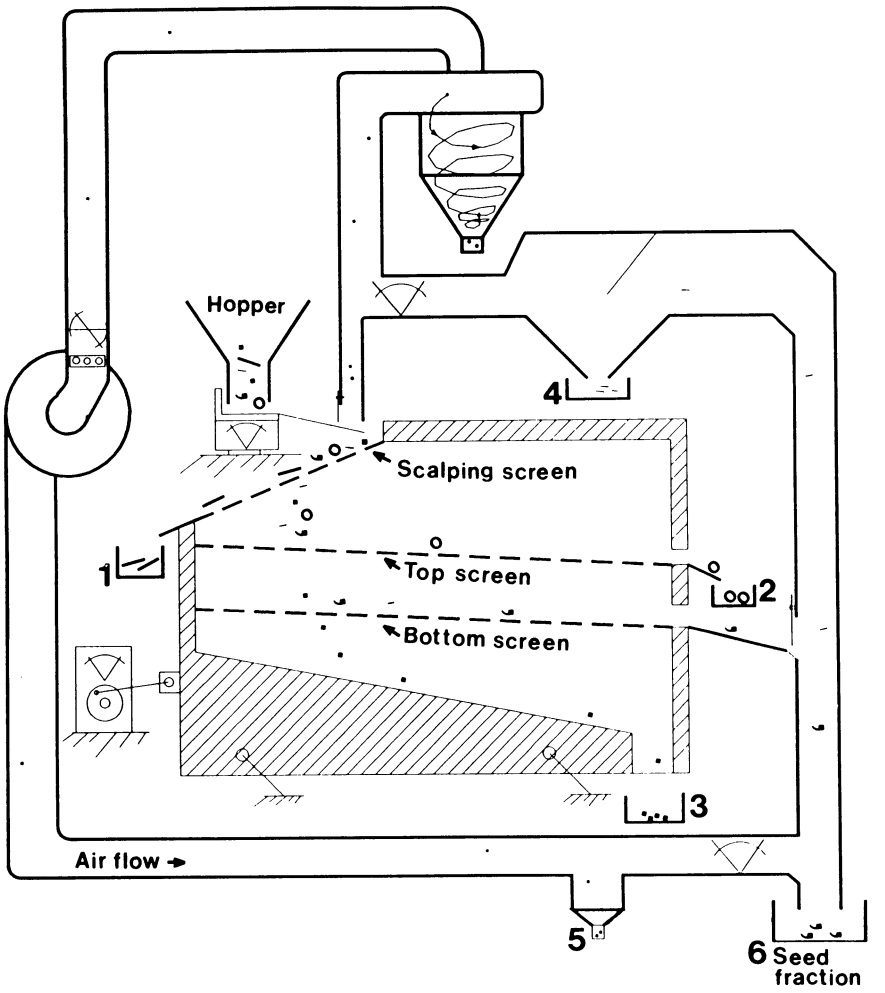

Fig. 1. Diagram of air-screen machine. Numbers refer to collection sites of the seven fractions. Commas represent seed or sclerotia; other symbols are used to illustrate separation of particles (e.g., straw, soil, weed seed, or other organic or inorganic debris) based on size, weight, or shape.

Table 1. Ergot sclerotia recovered from residue samples partitioned into fractions with a laboratory air-screen machine

Fraction	Sclerotia (no.)	Sclerotia (% of fraction weight)	Fraction wt. (% of total wt.)
1	0[a]	0[a]	13.20 ± 4.63[a]
2	0	0	16.39 ± 8.67
3	0	0	33.78 ± 10.81
4	0	0	6.32 ± 2.35
5	0	0	11.49 ± 5.73
6	38.63 ± 15.63	0.10 ± 0.06	16.79 ± 3.46

[a] Mean % and standard deviation based on eight samples.

Table 2. Ergot sclerotia recovery from straw bales of perennial ryegrass, partitioned into fractions with a laboratory air-screen machine

Fraction	Sclerotia (no.)	Sclerotia (% of fraction wt.)	Fraction wt. (% of total wt.)
1	0[a]	0[a]	36.21 ± 1.43[a]
2	0	0	31.76 ± 2.19
3	0	0	19.05 ± 1.74
4	0	0	11.01 ± 1.89
5	0	0	0.55 ± 0.19
6	0	0	0.41 ± 0.08
7	10.5 ± 4.56	0.004 ± 0.003	1.01 ± 0.15

[a] Mean % and standard deviation based on a 0.5–1 kg sample from each of eight bales.

vacuum because of the density of the foliage. Vacuuming should be conducted after harvest and baling but before regrowth.

Although extensive soil cracking may have some effect on sclerotial recovery, significant differences in sclerotial numbers among areas with and without cracks were not observed. Heavy soils are common in the central Willamette Valley of Oregon and are subject to cracking when dry. Sclerotia falling within cracks may be protected from management treatments such as burning, flail chop with vacuum sweep, and post-harvest chemical treatments. The significance of cracks in the protection of weed seeds from chemical treatments has been reported (5).

It is possible that sclerotia of *C. purpurea* on the soil surface could become incorporated into the upper soil horizon through cultivation practices or rainfall. However, in the Willamette Valley, rains are rare when grass seed is harvested in July (average rainfall for July from 1961 through 1990 ranged from 1.31 cm in the south valley to 1.44 cm in the central valley). Thus, collection of sclerotia immediately after harvest should reflect numbers returned to the soil.

Most sclerotia of *C. purpurea* are removed with the seed or fall to the ground during cutting or combining. We found about 10 sclerotia in 0.5–1 kg bale samples. Sclerotia in bales may be a source of inoculum if used as mulch. Additionally, ergot contamination in straw bales should be considered if the straw is used for animal feed, because the sclerotia of *C. purpurea* are widely known to contain alkaloids toxic to animals.

The vacuum recovery and seed-conditioning techniques proved an acceptable means of enumerating sclerotia of *C. purpurea*. The technique may be of use for recovery of seed or propagules distributed on the soil surface. Additionally, seed-conditioning equipment may be useful in partitioning various fractions of crop residues in ecological or pathological studies.

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