## Viability of Stored Bromegrass Seed and Seed-borne Spores of a Leaf Spot Pathogen

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## ABSTRACT

The survival of spores of the common leaf spot fungus, Selenophoma bromigena, on seed of Bromus inermis stored at controlled humidities for 16 months appeared directly related to per cent relative humidity (RH). Spore counts steadily declined as per cent RH increased. While spore germination

declined sharply to zero between 50 and 70% RH, seed germination was high and not affected by this change. The differential effect of RH on spore and seed viability may have practical applications in ridding seed of this and other pathogens. Phytopathology 60:1470-1471.

The fungus Selenophoma bromigena (Sacc.) Sprague & A. G. Johnson is the cause of a common leaf and stem spot on mature plants of smooth bromegrass, Bromus inermis Leyss, in the prairies of Canada (8). Although pycnidia and spores are often seed-borne (1, 6), seedling infections have not been observed in the field or after inoculation in growth chambers. Systemic transmission of the pathogen has been suggested, but it has not been found in living roots or crowns of infected plants (1). I have not found it in cleared and stained embryos of heavily-infected seed (5). As part of studies to determine the epidemiological significance of seed-borne inoculum, the effect of various storage conditions on the seed-borne spores was examined. Spores washed from bromegrass seed were not viable after storage for more than 18 months in cotton bags at 18-24 C. Those from seed kept in closed polyethylene bags for 2-7 years at -10 C showed 10-25% germination. A critical examination of the effect of storage at different relative humidities (RH) was made.

MATERIALS AND METHODS.—Two-g samples of seed of the S-7388 and Carlton strains from the 1968 harvest with heavy spore loads were placed in open sample tubes inside duplicate, sealed screw-capped jars. The required humidity was developed and maintained in the jars by sulphuric acid:water mixtures for 16 months. The storage temp was 18 C. A seed-washing and haemocytometer-counting technique were employed to determine numbers of spores present. Spore germination was tested on potato-dextrose agar containing 50 µg/ml each of dihydrostreptomycin sulphate and vancomycin to suppress bacteria. Counts of spores from seed were made in duplicate, and spore germination was determined on four lots of 100 spores from each seed sample. Seed germination was tested on six samples of 100 seeds on filter pads.

RESULTS AND DISCUSSION.—The survival of spores appeared directly related to per cent RH. This is indicated by a steady decline in spore count with increase in RH, the absence of spore germination above 50% RH (Table 1), and the observation that most of the spores in the wash water from seeds stored at 70, 80, and 90% RH were in a shrunken and ungerminated condition. Most spores at these RH levels may have

germinated earlier. The preservation of viable spores at 10 and 50% RH may be ascribed to reduced fungal respiration and absence of exogenous substrate (2, 4). But seed from storage at 70, 80, and 90% RH showed little mold in germination tests as compared with that from 50 and 10%, on which the most common species was an *Alternaria*.

At 100% RH, a heavy mold growth developed on seed a few days after the start of the test. At the end of the test, the endosperm of the caryopses was rotted but the testae and paleae appeared little affected. Selenophoma spores were not found. There was little obvious fungal overgrowth on seeds stored at 90% RH. The most common fungal spores in washings from seed at 90 and 100% RH were those of Penicillium spp. There was no noticeable surface mold on seeds stored at lower humidities. A Pseudomonas sp. was recovered in almost pure culture from seeds stored at 80 and 70% RH, but at 50 and 10%, yeasts were predominant. A similar microflora developed on chaff, leaves, and culms of the two bromegrass strains heavily infected with S. bromigena which were stored under the same conditions as the seed. Spore counts and germination showed similar trends to those on the seed.

The absence of spore germination above 50% RH is of particular practical interest, since there was no significant difference in seed germination at this level and at 70% RH (Table 1). On the other hand, storage at 10 and 80% RH significantly lowered seed germination. It may be possible to use this differential effect to rid seed of this and other pathogens without affecting its germination. In an airtight container, the requisite RH of the atmosphere in the spaces between the seeds could be generated by careful control of their initial moisture content. If seed is stored in a particular atmosphere, it gives off or takes up moisture until equilibrium is reached between the vapor pressure of the atmosphere and the moisture content of the seed. If the atmosphere is of small volume compared with the seed volume, the moisture of the seed is the atmospheric conditioning factor. Isotherms relating per cent RH of the air to the moisture content of the seed of several grasses have been developed by Dexter (3) and Sijbring (7).

TABLE 1. Spore count and spore germination of Selenophoma bromigena and seed germination in two strains of Bromus inermis

% Relative humidity	Spore count $\times$ 10 <sup>3</sup> /g seed		% Spore germination		% Seed germination	
	S-7388	Carlton	S-7388	Carlton	S-7388	Carlton
10	37	18	52	67	86	79
50	24	13	46	58	93	95
70	19	12	0	0	93	93
80	12	9	0	0	23	5
90	10	3	0	0	0	0
100	0	0	No spores	No spores	0	0

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