Nature and Epidemiological Significance of Infection of Bean Seed by Whetzelinia sclerotiorum

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ABSTRACT

Whetzelinia sclerotiorum was isolated from 48% of seed lots of bean cultivars Great Northern and Pinto harvested from white-mold-infected plants, but from only 6% of seed lots harvested from healthy-appearing plants in western Nebraska. The fungus was recovered from less than 0.5% of normal seed, but from nearly 12% of chalky, discolored, and shrivelled seed. Infected lots of seed planted in sterilized soil in a greenhouse humidity chamber did not produce white-mold-infected bean plants. Seeds infected with *Whetzelinia sclerotiorum* did not germinate in vitro. *Whetzelinia sclerotiorum* can be disseminated in seed, but this is unlikely to be of epidemiological significance.

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White mold disease of bean (*Phaseolus vulgaris* L.) caused by *Whetzelinia sclerotiorum* (Lib.) Korf and Dumont [=*Sclerotinia sclerotiorum* (Lib.) de Bary (5)] is widespread in western Nebraska and has become epidemic in the North Platte Valley in recent years. Production of certified seed of Great Northern types of dry edible beans is a recent commercial venture in the valley. Seed is certified with regard to bacterial diseases such as common blight (caused by *Xanthomonas phaseoli*), but the presence of white mold disease in a field has not influenced certification. Whether *W. sclerotiorum* is transmitted by seed is an important question, especially in bean production areas free of the disease. Nobel and Richardson (7) listed white mold as a seedborne disease, and transmission of the fungus by or with bean and other types of seed has been reported (1, 2, 4, 6). Observations during 3 years, however, gave no indication of seed transmission in Nebraska bean fields. This study was undertaken to determine the significance and nature of *W. sclerotiorum* infection of seed of bean cultivars of Great Northern and Pinto.

MATERIALS AND METHODS.—In September of 1972 and 1973, 92 plant samples were collected from 46 Great Northern or Pinto bean fields where white mold incidence ranged from 1 to 90%. From each field healthy-appearing plants as well as *W. sclerotiorum*-infected plants were harvested. In September, 1974, infected plants were collected from six fields with moderate to severe white mold. Seeds were hulled by hand and passed through an aspirator and over a 5-mm screen to remove debris and broken seeds. Seed was stored at room temperature (22 ± 2 C) for 6-12 months before assay. Seed collected from each sample was considered a seed lot.

Assays for the presence of *W. sclerotiorum* were conducted in the laboratory and greenhouse. Seed was soaked for 15 minutes and washed in flowing tap water for 30 minutes before being plated or planted. Seed placed on 1% water agar was observed for germination and emergence of microorganisms for 1 week. A seed was classified as germinated when the radicle was 3 cm in length. Seeds from the same collections also were planted in sterilized soil in clay pots. Environmental conditions for development of white mold were optimized by holding these pots in humidity chambers at 18-20 C. The relative humidity in the chambers was 97-100% for 16 hours and 50-70% for 8 hours. After 2 weeks, percent germination and white mold infection were recorded. To confirm suspected white mold infection, tissue sections from diseased seedlings were plated on 1% water agar and observed after 1 week for sclerotia of *W. sclerotiorum*.

RESULTS AND DISCUSSION.—*Whetzelinia sclerotiorum* was isolated from 48% of seed lots harvested from white-mold-infected plants but only from 6% of seed lots from healthy-appearing plants. In seed lots from healthy-appearing plants *W. sclerotiorum* was isolated only from abnormal seed (shrivelled, discolored, and/or smaller than normal) (Table 1). Normal-appearing seeds were not infected. In seed lots from diseased plants, *W. sclerotiorum* was isolated in most cases from abnormal seeds, but in three lots 2-6% of the normal-appearing seeds were infected. Overall infection of normal seed was less than 0.5%. This indicates that removal of abnormal seed from certified seed lots harvested from white-mold-diseased fields would not guarantee *W. sclerotiorum*-free seed.

The seed-washing procedure employed in these tests removed a substantial number of external microbial contaminants as determined by washed and nonwashed seed plating comparisons. This procedure did not surface-disinfest the seeds, and bacterial species and fungi such as *Alternaria tenuis* and *Aspergillus* spp. were isolated from some seeds. A total of 500 seeds were tested from samples collected from six different white-mold-infected bean cultivars in 1974. These seeds were washed, immersed in 1.5% NaClO for 1 minute, and rinsed in sterile water before being plated. When compared to washing seed from the same sample, NaClO treatment reduced microbial contamination by more than 85% while *W. sclerotiorum* recovery was reduced 33%. Recovery of *W. sclerotiorum* from disinfested seed indicated that the pathogen was internal. *W. sclerotiorum* was found in 72%, 4%, 2%, and >1% of the moldy- or chalky-appearing light weight seed, discolored seed, slightly shrivelled seed, and normal seed, respectively. No significant difference in this type of seed infection was observed between cultivars.

Seeds planted in greenhouse humidity chambers did not produce white-mold-infected bean seedlings even when chalky- or moldy-appearing seeds were planted. White mold was not observed on over 2,000 bean seedlings, and attempts to isolate *W. sclerotiorum* from

TABLE 1. Percent seed germination and frequency of isolation of *Whetzelinia sclerotiorum* (*W.s.*) from normal and discolored seed of Great Northern and Pinto beans collected in 1972 and 1973 from healthy and *W.s.*-infected plants in western Nebraska

		Seed from healthy plants				Seed from infected plants			
		Normal		Discolored[a]		Normal		Discolored[a]	
Year	Sample unit	Germ. (%)	*W.s.* incidence[b]	Germ. (%)	*W.s.* incidence[b]	Germ. (%)	*W.s.* incidence[b]	Germ. (%)	*W.s.* incidence[b]
1972	Seed lots[c]	. . .	0	. . .	9.0	. . .	8.0	. . .	69.0
	Total seed	92	0	80	2.0	89	0.5	50	35.0
1973	Seed lots[c]	. . .	0	. . .	5.0	. . .	4.6	. . .	30.0
	Total seed	81	0	71	0.9	81	0.3	63	11.0

[a]Discolored seed was often shrivelled and smaller than normal.
[b]Frequency of isolation of *W. s.* from seed expressed as percent of seed lots and of total collected seed.
[c]Total number of seed lots from 1972 and 1973 was 92.

seedlings which exhibited symptoms similar to those of white mold were negative. To verify that conditions favorable for white mold existed in humidity chambers, bean seedlings were successfully inoculated by placing *W. sclerotiorum*-colonized oat seeds at the base of bean stems.

When *W. sclerotiorum* grew from individual seeds on water agar plates, the seeds did not germinate. Similar results also have been reported for soybean (6). In most cases, the fungus consumed the seed in a few days and produced numerous characteristic sclerotia. Seed germination rates in the greenhouse studies were similar to those in the in vitro tests (Table 1) with a 90% average for normal seed and reduced germination for abnormal-appearing seeds. The absence of white mold in the greenhouse test can be explained by the inability of *W. sclerotiorum*-infected seeds to germinate.

W. sclerotiorum was disseminated in a few instances as sclerotia with bean seed. Results from in vitro tests demonstrated dissemination of the fungus as mycelium within seeds. Nongermination of infected seed, however, eliminates the opportunity for direct white mold infection of bean plants by seedborne inoculum.

Infected seed may increase the inoculum potential of *W. sclerotiorum* in soil. Under favorable conditions sclerotia are formed on infected seeds and may be capable of producing apothecia later in the same season (4). *Whetzelinia sclerotiorum* was recovered from overwintered bean seed in Nebraska fields, but the role of infected seed in initiating disease was considered unimportant (3).

Hungerford and Pitts (4) reported that a small number of plants were infected with white mold when seed from *W. sclerotiorum*-infected Pinto bean fields in Idaho was planted in the greenhouse. Results from the experiments reported here do not support their results, although Pinto seed was tested under apparently optimized disease conditions. Precautions apparently were not taken by those workers to avoid proximity to apothecial sources used for other experiments. Thus, it is possible that airborne ascospore inoculum in the greenhouse caused the small number of infected bean plants which Hungerford and Pitts attributed to seedborne infection.

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