Pleospora bjoerlingii in the USA

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ABSTRACT

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Pleospora bjoerlingii, the perfect stage of *Phoma betae*, developed on seedstalks of sugarbeet after inoculation of the flowering plants with conidia of *Phoma betae* following harvest, and exposure of the seedstalks outdoors. Single-ascospore isolates were more virulent than a standard *Phoma* isolate on genotypes developed for storage-rot resistance. The *Pleospora* stage was found on sugarbeet seedstalk stubble in the Salem, Oregon, area where most of the seed used by the U.S. sugarbeet industry is produced.

Phoma betae (Oud.) Frank is an important pathogen of sugarbeet (*Beta vulgaris* L.); it causes seedling disease, leafspot, and storage rot. *Pleospora bjoerlingii* Byford is the perfect stage, which never has been cultured in vitro and has been described only three times in nature (1,2,3). Reports from Europe described *P. bjoerlingii* on sugarbeet seedstalks after an overwintering period. The first successful attempt to produce *P. bjoerlingii*, and its occurrence in the USA under natural conditions, are reported here.

MATERIALS AND METHODS

Flowering greenhouse-grown potted sugarbeet plants were sprayed with a conidial suspension of *P. betae* on 15 May 1977, placed in a lighted moist chamber at 18–20 C for 48 hr, then transferred to the greenhouse and incubated at 21–27 C. About 1 mo later, the diseased stalks were harvested. During the 1st wk in July 1977, the main stems were tied in a bundle and were fastened to a fence outdoors.

Twelve field-grown flowering sugarbeet plants were sprayed with 50 ml of a water suspension containing 5.75×10^6 conidia/ml on 23 August 1977. The inoculum was prepared from a single-spored culture of an isolate of *P. betae* that has been used to evaluate roots for resistance to storage rot. Six weeks later, the stalks were cut and allowed to dry in the field. Infected stalk pieces were tied in a bundle and left exposed outdoors. Stems were examined periodically for pseudothecia and ascospores.

Single ascospores were picked up with a glass needle and placed on green bean agar (GBA). The GBA was prepared by heating 250 g of green beans at 60 C in 1 L of distilled water for 30 min, adding 15 g of agar, and autoclaving the preparation for 20 min at 1.05 kg-force/cm^2 (15 psi).

The pathogenicity of single-ascospore cultures was tested on 1 cm^3 blocks of sugarbeet surface-root tissue. Thirty-six blocks of tissue were prepared from each of five roots from four different sugarbeet genotypes. The 36 blocks were distributed among nine test cultures with four replications. A replicate consisted of a petri-dish culture of the isolate with 20 blocks (five roots and four genotypes). Two of the genotypes were F1001 and F1002, which were released in 1977 as sources of resistance to *P. betae*, *Botrytis cinerea*, and *Penicillium claviforme*. The third genotype was PBP4, the seed of which came from a single plant that had been interpollinated with 13 others. All were selected because they were resistant to all three storage-rot pathogens. The final genotype was a commercial cultivar, American Crystal 2 hybrid B (2B), which is susceptible to all three storage-rot pathogens. Blocks were placed in 1% NaOCl for 1 min and then rinsed twice in sterile distilled water. The blocks were placed on 1-wk-old pure cultures of the fungus in petri dishes and incubated at 20–22 C for 14 days. After incubation, the blocks were sliced, the distance rot had progressed into the block was measured, and a rot rating was assigned

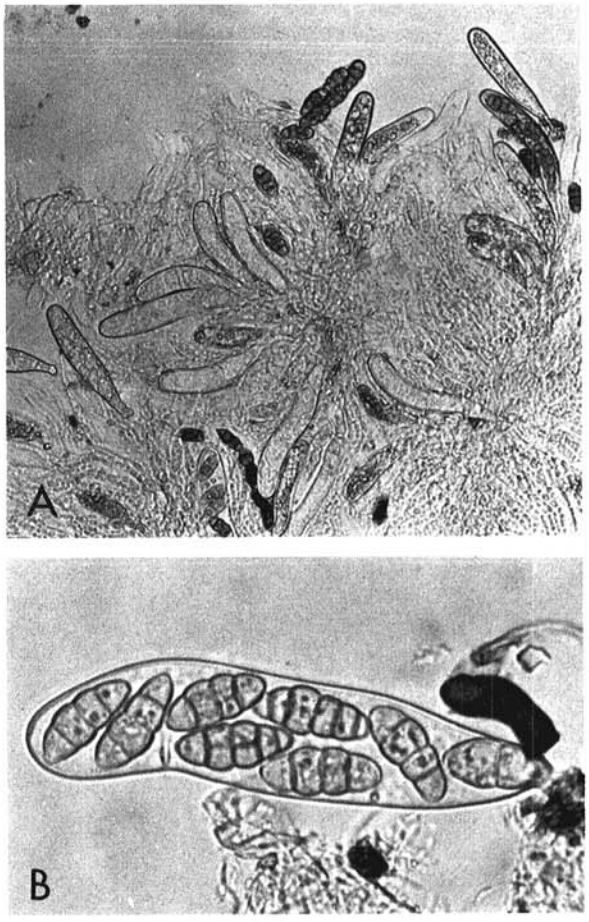

Fig. 1. *Pleospora bjoerlingii*. **A)** Pseudothecium with ascospores and asci in various stages of maturity. **B)** Ascospores with three transverse septa and a single longitudinal septum in some cells (×606).

according to the scale: 0 = 0 mm; 1 = 1–2 mm; 2 = 2–4 mm; 3 = 4–6 mm; 4 = 6–8 mm; and 5 = 8–10 mm.

Sugarbeet seedstalk debris was collected 20–21 March 1978 from seven fields near Salem, Oregon. The stalks were examined microscopically for *P. bjoerlingii* fruiting structures, or by placing stalk pieces in a petri dish with water and examining the inside of the cover several hours later for discharged ascospores.

RESULTS AND DISCUSSION

Pleospora bjoerlingii was discovered on inoculated stem pieces of sugarbeet in mid-November 1977. Attention was drawn to the pseudothecia by discharged ascospores that, under the microscope, resembled shiny projectiles. Gross morphology of the pseudothecia, the eight-spored asci, and the ascospores (Fig. 1) all agreed with the original description for *P. bjoerlingii* (1). Ascospores had three transverse walls and often the second cell had a longitudinal wall. Certain pseudothecia were more deeply embedded than pycnidia in stem tissue. Pycnidia exuded a cream-colored mass of conidia, whereas the pseudothecia exuded brown ascospores. These features were useful for distinguishing the two types of fruiting structures under 70× magnification.

Leach (4) reported the production of pseudothecia and ascospores by *Pleospora herbarum* in culture after exposure to ultraviolet light and specific temperatures. Attempts to produce pseudothecia in *P. betae* by those methods failed three times.

Single-ascospore cultures produced only the *Phoma* stage. Eight ascospore cultures and the standard isolate of *P. betae* were compared for pathogenicity (Table 1). All of the ascospore isolates caused significantly ($P < 0.01$) more rot than the standard isolate on cultivar F1001. Three caused more rot on F1002, and four caused more rot on PBP4 than the standard isolate, but the differences were not statistically significant. The greatest incidence of rot occurred on the susceptible cultivar 2B. There was a low level of pathogenic variation among the ascospore isolates on each host. Isolate 4 in F1001 and isolate 6 in PBP4 were more virulent than the others. The F1002 and PBP4 were more resistant than F1001 to the ascospore isolates but not to the standard *Phoma* isolates. Even though the ascospore stage resulted from inoculation of plants with conidia from the standard *Phoma* isolate, the procedures did not insure sterility; the possibility of contamination with other *Phoma* isolates could not be eliminated.

TABLE 1. Pathogenicity to four sugarbeet cultivars of eight single-ascospore isolates of *Pleospora bjoerlingii* and a standard isolate of *Phoma betae*

Isolate	Rot rating[a] on sugarbeet cultivar:			
	F1001	F1002	PBP4	2B
P. betae (standard)	0.8	1.1	1.4	3.4
Ascospore 1	2.4	1.2	1.4	4.8
2	3.0	1.1	1.4	5.0
3	3.1	1.4	1.3	4.9
4	3.5	1.2	1.9	4.0
5	2.8	1.7	1.4	4.8
6	3.2	1.8	2.3	4.8
7	3.1	1.7	1.8	4.4
8	2.4	1.4	1.7	4.8
LSD ($P = 0.01$) = 0.9				

[a]Rot rating indicates the distance rot progressed through a 1-cm block of root tissue during 2 wk of incubation at 20 C: 0 = 0 mm; 1 = 1–2 mm; 2 = 2–4 mm; 3 = 4–6 mm; 4 = 6–8 mm; 5 = 8–10 mm.

The standard isolate of *P. betae* has been used for 4 yr in our storage-rot program. It was selected as the most virulent isolate from among many collected throughout the USA and from imported seed. Three breeding lines reacted similarly to this isolate (Table 1). The increased virulence found in the small population of isolates studied here suggests that an ascospore stage produced from a broad genetic base would contain many individuals with greater virulence than that seen here. This possibility must be investigated and suitable isolates must be used for screening to insure future selection of roots with the highest levels of resistance.

There was a highly significant ($P < 0.01$) interaction between isolates and host genotypes (Table 1). Genotype F1002 was more resistant than F1001 to the ascospore isolates. Genotype F1002 is the product of three generations of selection against *P. betae*. The original seed was from plants resistant to crown rot caused by *Rhizoctonia solani*. Genotype F1001 is the product of two generations of selection against *P. betae*. This original seed was introduced from the USSR, where it was developed for resistance to storage rot caused by *B. cinerea*. The extra selection cycle in F1002 may account partially for its greater resistance. Genotype PBP4 also is of Soviet origin. It has gone through one cycle of selection in the USA for combined resistance to *P. betae*, *B. cinerea*, and *P. claviforme*. The simultaneous selection for resistance to three pathogens may accelerate the process of developing resistance to *Phoma*.

The *Pleospora* stage, which is identical to the stage produced in Fargo, ND, was found on stubble from each of the seven fields sampled in the Salem, Oregon area. Most of the U.S. sugarbeet seed is produced in the Salem area, along with small amounts of seed for other countries, which raises the possibility that virulent isolates of the pathogen already may have been seed-disseminated throughout the USA and probably to sugarbeet-growing areas in foreign countries. More effective control measures such as seed treatment, preharvest fungicides, and storage rot-resistant cultivars are required before dissemination of the pathogen can be reduced and Phoma storage rot can be controlled.

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