The Occurrence and Biology of Botryotinia fuckeliana on Beans in New York

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Thanks are due to Gertrude Catlin and Rose McMillan for preparation of illustrations.

Accepted for publication 11 January 1975.

ABSTRACT

Apothecia of *Botryotinia fuckeliana* were found in bean fields and adjacent areas at four locations in the bean-growing region of central and western New York State. Ten single ascospores obtained from one apothecium produced typical *Botrytis cinerea* cultures and all were pathogenic to bean leaves and pods. However, two of the 10 isolates consistently induced smaller lesions. The optimum temperature for lesion formation was 20 to 25 C with continuous leaf wetness. The rate of colony growth on potato-dextrose agar was also optimum at 20 to 25 C. Crosses made by combining all 10 ascospore cultures resulted in the production of apothecia under laboratory conditions, indicating the presence of both mating types. The occurrence of apothecia of *B. fuckeliana* under field conditions suggests that ascospores may play a major role in the epidemiology of gray mold of beans in New York, and also may be a source of genetic variability in the fungus.

Phytopathology 65:657-660

Additional key words: Phaseolus vulgaris, Botrytis cinerea, gray mold of bean.

The conidial stage *Botrytis cinerea* Pers. ex. Fr. is a ubiquitous fungus which causes the characteristic gray mold diseases of many vegetables throughout the world, especially under cool, humid conditions (1, 7). It is capable of attacking beans at different stages of growth, and also causes an important postharvest disease in most of the bean-growing areas of the world (9).

In New York State, gray mold of beans is an endemic disease which occurs every year. Occasionally, however, gray mold has assumed epiphytotic proportions in rainy seasons in bean fields surrounded by orchards or uncultivated wooded land, where air movement is restricted and high humidity persists for considerable periods of time. Consequently, New York growers have been forced to spray with protectant fungicides to minimize losses inflicted by this disease.

Groves and Drayton (2) and Groves and Loveland (3) demonstrated that *Botryotinia fuckeliana* (deBary) Whetz., is the perfect stage of *B. cinerea*. However, recent attempts to find it under field conditions and to reproduce it in culture generally have failed. Overwintered stromata and sclerotia of the fungus formed on infected tissues of bean, other cultivated hosts, and weeds produce abundant conidia early in the spring and throughout the growing season in and around bean fields in New York. Conidia, therefore, have been considered the primary source of inoculum of gray mold of beans as is assumed to be the case with many other diseases incited by this fungus.

This paper reports on the occurrence of *B. fuckeliana* in and around bean fields in New York, its reproduction in culture, and pathogenicity to beans. A role for ascospores

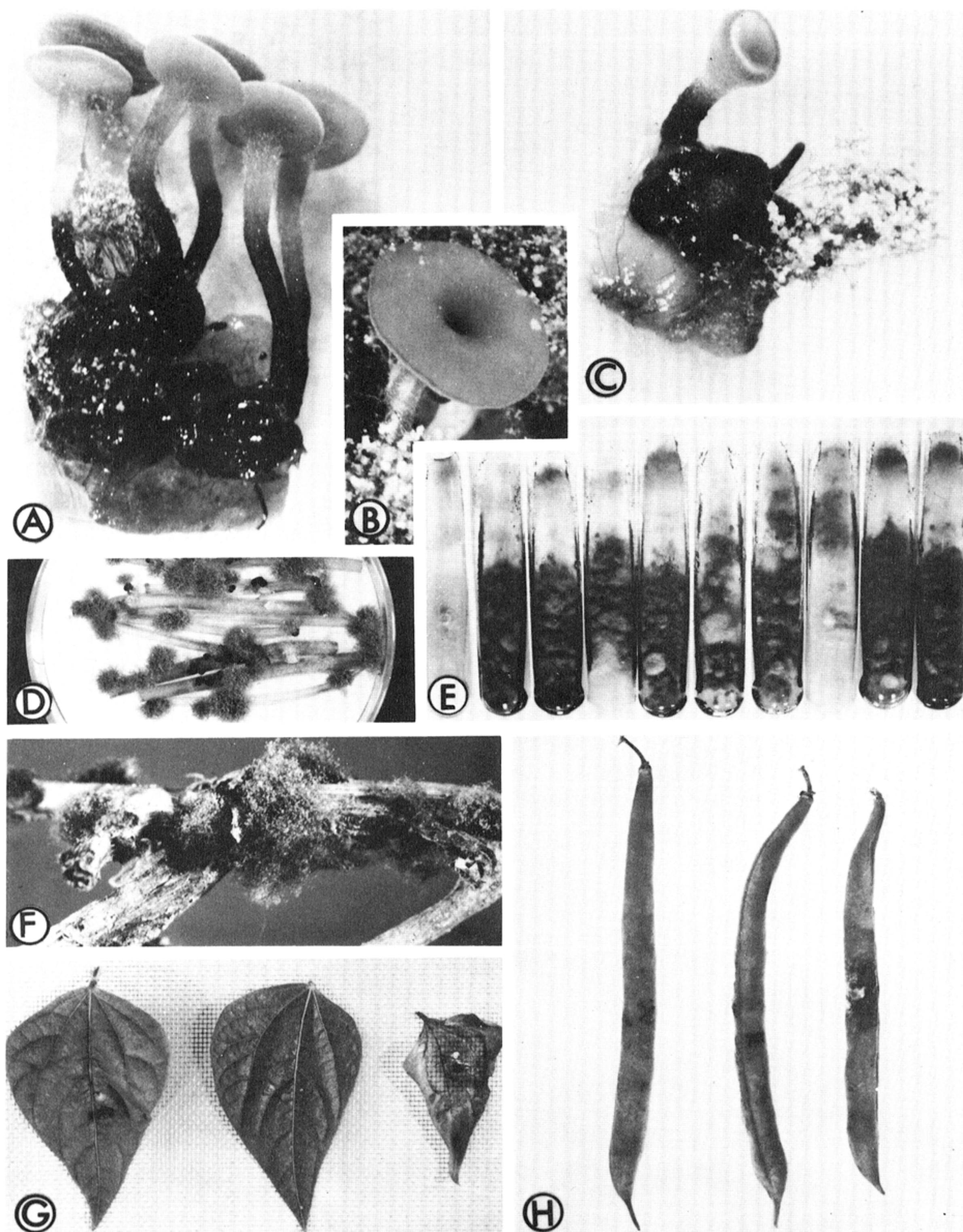

Fig. 1-(A to H). Gross morphology of *Botryotinia fuckeliana* and its pathogenicity to beans. **A-C)** Apothecia and the conidial stage of the fungus produced in culture. **D)** Sclerotia and conidia produced on autoclaved bean stem segments. **E)** 10 single ascospore cultures obtained from a field-collected apothecium. **F)** Conidia produced on overwintered infected bean stems under field conditions. **G, H)** Bean leaves and pods were (left to right) uninoculated check, inoculated with isolate 8, and inoculated with isolate 10 of *B. fuckeliana*, respectively.

in the epidemiology of gray mold of beans in New York, and also in the genetic variability of the fungus, is suggested. A short report of this research has already appeared (6).

MATERIALS AND METHODS.—Apothecia of *B. fuckeliana* were collected from bean fields and adjacent apple plantings in the springs of 1973 and 1974 in central and western New York. From one apothecium collected in 1973 from a bean field where 7.8% of the plants were infected the previous season, 10 single ascospores were isolated and grown on potato dextrose agar (PDA).

In determining the pathogenicity of these isolates, leaves and pods of greenhouse-grown bean plants (*Phaseolus vulgaris* L. 'Cascade') were placed on screen trays approximately 2-cm above the bottom of closed plastic boxes. Humidity was kept high by placing water in the bottom and lining the sides and cover of each box with moist filter paper. Half of the leaves or pods in each box were wounded by pricking with a needle and all were inoculated with a 4-mm plug of the fungus obtained from the margin of a 3-to-5-day-old PDA culture. Leaves and pods were misted with distilled water from an atomizer immediately after inoculation, and twice daily thereafter. The boxes were incubated in the laboratory at 20 to 24 C. Lesion diameter was recorded daily. Pathogenicity of a few isolates also was tested on intact plants under mist chamber conditions.

The effect of temperature on lesion initiation and development on bean leaves and pods was determined as described above at 5, 10, 15, 20, 25, and 30 C. The rate of increase of colony diameter on PDA was measured at these same temperatures. All temperature and pathogenicity tests were repeated at least three times.

Apothecia were produced in culture by combining all 10 single-ascospore cultures using modifications of the technique of Groves and Loveland (3). Autoclaved celery stem segments and bean stems were inoculated with a mixture of the 10 ascospore cultures of *B. fuckeliana*. The plates were incubated in the dark at 15 C for 4 weeks or until sclerotia were well developed. Further incubation was in the dark at 0 and 5 C for 4 and 2 weeks, respectively. Sclerotia were then removed, placed oı moist autoclaved sand in glass petri dishes, and spermatized by adding approximately 10 ml of a conidial suspension of the 10 ascospore cultures to each dish. Spermatized sclerotia were covered with sterilized soil, moistened with distilled water, and incubated in the dark at 15 C. After 4 weeks of incubation, the dishes were transferred to an unheated greenhouse where they were exposed to northern light but were shaded from direct sunlight during the months of Februaıy, March, and April. Temperature varied between −17 to +24.4 C. Sterile distilled water was added as necessary to maintain moistness. Apothecial initials appeared 4-5 weeks after the plates were transferred to the greenhouse. Single ascospores were isolated from mature apothecia and were maintained on PDA slants.

RESULTS.—*Occurrence of B. fuckeliana under field conditions.*—Several apothecia were found in May, 1973, in a bean field near Alton, N.Y. where 7.8% of the plants had gray mold in 1972. However, most of the stromata on infected bean tissues and sclerotia found in this field produced the imperfect stage, *B. cinerea* (Fig. 1-F). Several apothecia were also found in an apple orchard adjacent to a bean field near Geneva, N.Y. Likewise, in late April and early May, 1974, apothecia of *B. fuckeliana* were found in a bean field near Hall, N.Y. and in a hedgerow next to a bean field near Springville, Y. Furthermore, a few field-collected sclerotia of *B. fuckeliana,* which showed no sign of apothecial production, subsequently formed both apothecia and conidia when partially submerged in water and incubated for 2-3 weeks in a growth chamber at 15 C and 4,304 - 5,380 lx fluorescent and incandescent light 14 hours per day. Single ascospore cultures obtained from field-collected apothecia always produced the imperfect stage, *B. cinerea sensu* Hennebert (4) (Fig. 1-D, E).

Pathogenicity of ascospore cultures to bean.—Eight of 10 single-ascospore cultures of *B. fuckeliana* were highly pathogenic to detached bean leaves and pods (Table 1). However, isolates 1 and 8 consistently induced smaller lesions or failed to infect both leaves and pods (Fig. 1-G, H). Both injured and noninjured tissues became infected, but slightly larger lesions were produced on the injured tissues. Similar results were obtained with all isolates on leaves of intact bean plants under mist chamber

TABLE 1. Pathogenicity of 10 single-ascospore cultures of *Botryotinia fuckeliana* to injured and noninjured bean leaves and pods as measured by lesion diameter at 72 hours after inoculation

	Lesion diameter, (mm)[a]			
	Bean leaves[b]		Bean pods[c]	
Isolate	Injured	Noninjured	Injured	Noninjured
Check	0	0	0	0
1	7	7	19	0
2	26	27	40	37
3	18	18	43	35
4	27	26	45	38
5	26	25	43	34
6	28	25	40	29
7	26	22	39	34
8	8	7	23	0
9	18	15	41	35
10	21	21	37	33

[a]Each measurement is the mean of six replicates.
[b]Inoculated leaves were incubated under fluctuating temperature of 20-24 C.
[c]Inoculated pods were incubated at a constant temperature of 24 C.

TABLE 2. Effect of temperature on pathogenicity to beans and growth in culture of two ascosporic isolates of *Botryotinia fuckeliana*

	Isolate 1		Isolate 9	
Temperature (C)	Lesion diameter (mm)	Colony diameter (mm)	Lesion diameter (mm)	Colony diameter (mm)
5	0[a]	0[b]	0[a]	12[b]
10	9	26	12	43
15	8	52	20	71
20	9	73	26	81
25	8	77	22	81
30	2	0	6	19

[a]Each value, in millimeters, is the mean of eight replicates after 72 hours of incubation.
[b]Each value is a mean of eight replicates after 72 hours of incubation on potato-dextrose agar plates.

conditions. In all pathogenicity tests it was necessary to maintain free moisture for successful lesion initiation.

Temperature and lesion formation.—The effect of temperature on growth of the fungus in culture and lesion development on bean leaves was determined with isolates 1 and 9, which represented the extremes of virulence. Isolate 1 induced smaller lesions than isolate 9 at all temperatures used. The optimum temperature for lesion formation was about 20 C (Table 2). No lesions formed at 5 C, and only a few incipient lesions were produced at 30 C. The diameter of colonies of both isolates on PDA was largest at 20 to 25 C. Slight growth of isolate 9 occurred at 5 and 30 C, whereas isolate 1 failed to grow at either temperature.

Production of apothecia in culture.—The apothecia of *B. fuckeliana* produced by sclerotia formed on bean and celery stem segments (Fig. 1-A to C) agreed in morphology and anatomy with those described by others (2, 3, 4, 5, 8). These sclerotia also produced the imperfect stage (*B. cinerea*), and occasionally both stages were produced simultaneously by the same sclerotium (Fig. 1-A, C). About 100 single ascospores were isolated from several apothecia, and all cultures were typical of *B. cinerea* as recently described by Hennebert (4).

DISCUSSION.—Collection of apothecia of *B. fuckeliana* from several bean-growing areas in New York indicates that the prevailing environmental conditions in those areas were conducive to their production. Thus, ascospores are present and can function as a primary source of inoculum for infection of bean or other susceptible hosts. The production of apothecia is also important as a means of increasing the genetic variation of the fungus which may result in new pathotypes and fungicide-resistant strains.

Two of the isolates tested on beans were found to be less virulent than the other eight. These same isolates, however, sporulated less profusely than the others, which may account for the apparent differences in their virulence.

The effects of temperature and moisture reported here support field observations made on the appearance of gray mold on beans in New York. Gray mold is most severe during cool, wet periods. The role of temperature and moisture in the epidemiology of gray mold must be analyzed in more detail so that a forecasting system can be developed.

Further research is needed to identify the mating types of the single-ascospore cultures which were obtained. It is apparent that the 10 cultures used in this study included both mating types of *B. fuckeliana*. This is essential for further genetical studies on the inheritance of pathogenicity, fungicide resistance, morphological variation, and speciation in *Botryotinia*.

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