Survival of *Colletotrichum lindemuthianum* in Bean Debris in New York State

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ABSTRACT

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Colletotrichum lindemuthianum, the causal agent of bean anthracnose, overwintered 4 mo in bean (*Phaseolus vulgaris*) debris placed 0, 10, and 20 cm deep in soil on 14 November 1988. A bean plant assay was developed for the detection of low inoculum levels in overwintered debris. The fungus could not be isolated from infested debris on agar medium in April 1989 but could be detected using the host plant assay. The fungus was no longer detectable 22 mo after placement at any depth. Additional field trials were conducted at three locations over a 3-yr period. *C. lindemuthianum* was recovered from naturally infested bean debris after exposure to winter conditions for 3 mo (December through February). The inoculum concentration in overwintered bean debris at the three locations was sufficient to initiate disease on bean plants the following growing season. The relationship between inoculum density and disease severity in the bean plant assay was determined. Disease severity on inoculated leaves increased as inoculum density increased. The relationship can be used as a guideline for estimating the inoculum density in overwintered debris.

Colletotrichum lindemuthianum (Sacc. & Magnus) Lams.-Scrib. causes bean anthracnose, a major disease of common beans (*Phaseolus vulgaris* L.) in many regions of the world (3,13,21). Losses from bean anthracnose are attributed to poor germination of infected seeds, reduced yield and quality, and decreased value of the product (1,3,13,21).

C. lindemuthianum is primarily seedborne (1,3,6,14,21), and control is achieved best through the use of disease-free seeds produced in arid climates (13,21). Other control measures include seed treatment with systemic fungicides and quarantine pedigree inspection (6,18). Several races of the fungus have been described, and cultivars with resistance to one or more races are commercially available (2,8,9,18,19). A 1-2 yr crop rotation commonly is recommended to avoid the possibility of overwintered inoculum (3,12,21).

Research on the survival of *C. lindemuthianum* in bean debris has produced variable results depending on environmental conditions at the test sites. Muncie (12) determined that *C. lindemuthianum* could overwinter in bean debris in Michigan and infect bean crops the following season. However, Barrus (3) considered the level of overwintering inoculum in New York insufficient to cause disease the following year and did not recommend rotation for anthracnose control. In Canada, *C. lindemuthianum* (delta race) could not be isolated on agar media after mid-May from infected plant material that was buried in the field in November (17). Further experiments demonstrated that an alternating wet-dry cycle was detrimental to fungal survival, and that *C. lindemuthianum* survived at least 5 yr on dry, infected plant materials left in the field in sealed polyethylene envelopes that prevented contact with water (17). In Japan, Tochinai and Sawada (14) reported that the fungus was unable to overwinter in the field but was capable of surviving 2 yr in seeds. In subtropical and tropical climates with crop-free periods of 1–3 mo, *C. lindemuthianum* can survive in bean debris in sufficient quantity to mask the expression of seedborne inoculum (1).

In 1987 and 1988, severe outbreaks of anthracnose caused by the beta race occurred in several fields of dry beans grown in New York State. The high incidence in some fields was attributed to the use of noncertified dry bean seeds infected with *C. lindemuthianum.* However, anthracnose also was observed in some fields planted with certified seeds (produced in arid climates) that were not in close proximity to fields planted with noncertified seeds. Identical seed lots of certified seeds planted at other locations did not have anthracnose. Thus, the presence of anthracnose in some fields planted with certified bean seeds implied that *C. lindemuthianum* may have overwintered in infested bean debris from the previous year.

The objective of this study was to determine if *C. lindemuthianum* (beta race) is able to overwinter in bean debris in New York State and initiate anthracnose epidemics the following year. Preliminary results were reported previously (5).

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MATERIALS AND METHODS

Detection of *C. lindemuthianum*. Bean specimens were prepared by surface disinfesting anthracnose lesions on pods and seeds in 0.5% NaOCl for 3 min, rinsing in sterile distilled water (SDW), blotting dry on sterile filter paper, and placing the tissues on Mathur's agar (4) containing 0.10 g/L each of streptomycin sulfate and chloramphenicol to suppress bacterial growth. Five tissue pieces were placed on each petri dish, and five replicate plates were used. *C. lindemuthianum* was identified on agar medium on the basis of morphological characteristics (10) after 9 days of incubation at 20–23 C under cool-white fluorescent lights (12 hr/day).

All isolates in this study were further identified as belonging to the beta race based on disease reactions to a set of differential cultivars (Sanilac, Red Kloud, California Dark, Harofleet, Midnight, Charlevoix). The reactions of the New York isolates were compared to the reaction of a known beta race isolate, ATCC 16989 (American Type Culture Collection, Rockville, MD).

A host plant assay was developed to overcome secondary organisms colonizing the bean debris and interfering with growth and detection of *C. lindemuthianum* on agar media. Certified bean seed (cv. California Dark red kidney) produced in California was planted in potting mix in 9-cm-square plastic pots and grown in a greenhouse at 21–25 C until the first trifoliolate leaves were fully expanded. The infested bean tissues to be tested for *C. lindemuthianum* were incubated for 2 days on elevated wire racks in closed clear plastic boxes at room temperature (20–23 C) under cool-white fluorescent lights (12 hr/day). Sterile distilled water was added to the base of the boxes to promote high relative humidity (RH). After incubation, the specimens were placed in 250-ml Erlenmeyer flasks, to which 10 or 35 ml of SDW was added (depending on specimen size), and placed on a shaker for 1 hr at room temperature (20–23 C). The liquid was distributed on the underside of the trifoliolate leaves using a 1-cm-diameter artist's brush. This method has been used successfully to screen bean breeding lines for resistance to *C. lindemuthianum* (11,19). Positive controls were inoculated with a spore suspension (5×10^5 spores per milliliter) of *C. lindemuthianum,* beta race, ATCC 16989. Control plants were inoculated with SDW. Four pots containing four plants per pot were used for each treatment. The plants were placed in a moist chamber in the greenhouse and incubated for 8 days at 21–26 C in natural

light and supplemental light (5 hr/day) provided by sodium lamps. The moist chamber consisted of a greenhouse bench completely enclosed by clear, 4-mil plastic. Two humidifiers were placed inside the chamber to provide near 100% RH.

Symptoms were rated on a severity scale of 1–5 (11), where 1 = healthy, no disease symptoms; 2 = a few isolated small lesions on mid- and occasionally secondary leaf veins; 3 = many small lesions scattered on mid- and secondary veins, with collapse of surrounding tissue; 4 = few to many large lesions scattered over the leaf blade; and 5 = many large coalesced lesions accompanied by tissue breakdown and chlorotic or abscised leaflets. Isolations were made on Mathur's agar from symptomatic lesions on trifoliolate leaves to confirm infection by *C. lindemuthianum*.

To verify that certified bean seeds were anthracnose free prior to use in the bean assay, isolations for *C. lindemuthianum* were made on Mathur's agar, and seeds were planted in pots in moist chambers in the greenhouse to promote disease development.

Inoculum density and disease severity relationship. Cultures of *C. lindemuthianum* (ATCC 16989) were grown on Mathur's agar. Spore concentrations were adjusted to 1.3×10^1, 10^2, 10^3, 10^4, or 10^5 conidia per milliliter. Certified seeds were grown in the greenhouse as described previously. Four plants in each of five pots were inoculated by distributing the spore suspensions on the underside of the trifoliolate leaves using an artist's brush (11,19). The plants were incubated for 8 days at 21–26 C in a moist chamber in a greenhouse under natural light and supplemental light (5 hr/day) provided by sodium lamps. Disease severity was recorded using the scale described previously. The experiment was repeated once.

Survival in bean debris placed 0, 10, and 20 cm deep in soil. Naturally infected bean pod and seed tissue (cv. Isabella) were collected from a commercial field in Geneva, New York, on 20 October 1988. The isolates were identified as belonging to the beta race based on disease reactions to a set of differential cultivars, and on comparison to the reactions of isolate ATCC 16989 (described previously). Ten pod pieces and 10 seeds were enclosed in separate compartments in nylon pouches made of Nytex fabric, 220 μm mesh. This fabric did not degrade during the study period, and permitted the free exchange of water, gases, and microorganisms (7). On 14 November 1988, 50 pouches containing diseased seeds and pods were placed on the soil surface (0 cm) and held in place with rocks, and 50 pouches each were buried 10 and 20 cm deep in the soil. A total of 150 pouches were placed in the field and marked with flags to show the locations. A set of pouches (one each from 0, 10, and 20 cm depths) was retrieved at regular intervals, and the tissues were tested for viable *C. lindemuthianum*.

Isolations were made on Mathur's agar from 14 November 1988 to 14 February 1989. Pentachloronitrobenzene (PCNB, 0.1 g/L) was added to the medium from 21 February to 4 April 1989 to reduce interference from other organisms. Direct isolation on agar medium was abandoned after 4 April 1989; and thereafter the bean plant assay was used for detection of *C. lindemuthianum*. The pouches were unearthed and washed in tap water for 30 min. Bean debris was removed from the pouches and prepared for use in the plant assay as described previously. A modified procedure, used 30 August, 12 September, and 11 November 1989, consisted of puncture-inoculating detached pods (cv. California Dark red kidney) at four or five sites with a hypodermic needle containing the incubation liquid and incubating for 2 wk at 22–25 C under cool-white fluorescent lights (12 hr/day). Pod inoculations were abandoned in favor of the rapid results obtained with the leaf assay. The only detection procedure used in 1990 was inoculation of trifoliolate leaves of bean plants. Two sets of pouches from each depth were assayed on days 667 and 750 to increase the amount of decomposed material used for detection. At each sampling date, whole plants were inoculated with *C. lindemuthianum* (ATCC 16989), which served as a positive control to ensure appropriate conditions were present in the moist chamber for disease development.

Overwintering in bean debris in infested fields. *Location 1.* Survival of *C. lindemuthianum* was assessed in a commercial field (approximately 10 ha) of light red kidney beans, cv. Isabella, planted 12 June 1988 in Geneva. Anthracnose was severe during the growing season, and the grower was unable to harvest some areas of the field. The field was not disked or plowed in the fall. Whole pod samples with anthracnose symptoms were collected 5 January, 10 March, and 9 May 1989. Isolations from symptomatic pod and seed tissue were made on Mathur's agar as described previously. In addition, pod and seed tissues collected 10 March and 9 May were incubated in plastic boxes as described previously, and 35 ml of slurry were used to inoculate trifoliolate leaves in the bean plant assay. Isolations were made on Mathur's agar from symptomatic lesions. The isolate from this field was characterized as beta race based on disease reactions to a set of differential cultivars and on comparison to the reaction of a known beta race isolate, ATCC 16989, as described previously.

Location 2. Light red kidney bean seeds, cv. Red Kloud, that were naturally infected with *C. lindemuthianum* were planted 27 June 1989 in a 0.1-ha plot at the New York State Agricultural Experiment Station in Geneva. The isolate was characterized as beta race using the methods described previously. Disease incidence of plants with symptoms on leaves or pods was estimated at 25% on 28 October 1989. Following common grower practices, the plot was not tilled in the fall. At regular intervals throughout winter and into the spring of 1990, 10 pods with anthracnose symptoms were randomly collected from the field. Isolations were made from pod and seed tissues with symptoms, and tissues were used in the host plant assay as described previously.

The same location was planted with certified seeds (cv. California Dark) on 31 May 1990. Dark red kidney beans were used to facilitate identification of overwintered volunteer light red kidney beans. A portion of the certified seed was tested for seedborne anthracnose as described previously.

The infested field was divided into four sections, and incidence and severity ratings were recorded from three random locations within each section on all plants and pods in 0.9 m of row. Incidence was assessed as the proportion of diseased plants. Anthracnose severity on individual pods was recorded on a scale of 0 to 9, where 0 = no lesions on a pod; 1, 2, and 3 = (low) 10, 20, and 30% of pod area infected; 4, 5, and 6 = (medium) 40, 50, and 60% of pod area infected; and 7, 8, and 9 = (high) 70, 80, and 90–100% of the pod area infected. Final incidence and severity ratings were recorded 27 September 1990. The field was not irrigated in 1990.

The plot was not fall-tilled. At regular intervals throughout winter and into the spring of 1991, 10 pods with anthracnose symptoms were collected randomly from the field. Isolations were made from pod and seed tissues with symptoms, and tissues were used in the host plant assay as described previously.

The field was mowed and rototilled 10 cm deep in May 1991 and planted with certified Bush Blue Lake 47 snap bean seeds (produced in Idaho) on 23 May 1991. A portion of the seeds was planted in a field in Geneva that was not infested with *C. lindemuthianum* and served as a control plot. The certified seed was tested for seedborne anthracnose as described previously. The field was irrigated as needed to maintain plant growth. Final disease incidence and severity were recorded 20 August 1991.

Location 3. On 1 July 1989, noncertified light red kidney beans (cv. Isabella) were planted in an 11-ha commercial field in Geneva. A portion of the field was harvested in October and the remainder in December. The grower did not observe anthracnose symptoms during the season. Some of the bean

seeds harvested in December were blemished, but it was not determined if those blemishes were caused by *C. lindemuthianum*. The field was disked in May 1990 and planted on 26 June 1990 with certified seed (produced in Idaho) of two processing snap bean cultivars, Bush Blue Lake 47 and True Blue. The first symptoms of anthracnose were observed on 12 August, and by 20 August anthracnose was severe and resulted in rejection of the crop. *C. lindemuthianum* was isolated from symptomatic tissues and characterized as beta race using the methods described previously. A research plot was established in 0.5 ha of the field, and incidence and severity ratings were recorded using the methods described previously. Infested bean debris (cv. True Blue) was left on the soil surface to overwinter in the plot, and winter wheat was planted in the remainder of the field. At regular intervals throughout the winter of 1990 and spring of 1991, pod samples with anthracnose symptoms were randomly collected from the plot. Isolations were made from pod and seed tissues with symptoms, and tissues were used in the host plant assay as previously described.

The plot was chisel plowed and disked 10 May 1991 and planted with certified cv. California Dark bean seed on 23 May 1991. Irrigation was not available at this location. A portion of seed was planted in a field in Geneva not infested with *C. lindemuthianum* to serve as a control. Final disease incidence and severity were recorded on 23 September 1991.

RESULTS

Influence of inoculum density on disease severity. Disease severity on leaves of inoculated bean plants increased with increasing inoculum density from 10^1 to 10^5 conidia per milliliter (Fig. 1), and severity reached its maximum value at 10^5. Plants inoculated with 10^4 or 10^5 conidia per milliliter showed multiple coalesced lesions on the leaves, and the plants wilted and collapsed by the eighth day of incubation.

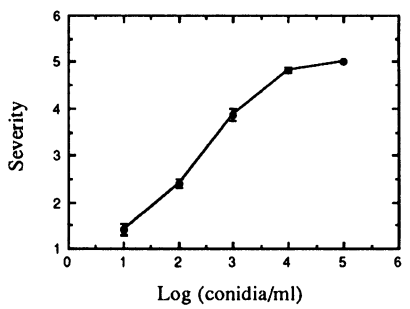

Fig. 1. Effect of inoculum concentration on infection of bean leaves by *Colletotrichum lindemuthianum*, beta race, after 8 days of incubation. Values represent the mean severity rating of whole bean plants inoculated with a spore suspension and incubated at 21–26 C. Disease severity rating scale is defined as 1 = healthy, no disease symptoms, to 5 = many large coalesced lesions accompanied by tissue breakdown and chlorotic or abscised leaflets. Bars represent standard error of the mean.

Survival in bean debris placed 0, 10, and 20 cm deep in soil. Prior to placement at 0, 10, and 20 cm, *C. lindemuthianum* was isolated on agar medium from 46 and 34% of pod and seed tissue pieces respectively. Within 51 days after placing the pouches in their respective locations, *C. lindemuthianum* was difficult to isolate from pod or seed tissues on agar medium regardless of depth (Fig. 2A and B). Common soil inhabitants (*Fusarium, Penicillium,* and *Trichoderma* spp., and nematodes) were frequently isolated. Detection of *C. lindemuthianum* in the pods was enhanced when PCNB was added to the medium. However, *C. lindemuthianum* could not be isolated on PCNB-amended agar medium in April (145 days after burial). Precipitation was below normal, and temperatures were near normal during this period (Table 1).

A whole plant assay was used to recover the fungus from decomposing tissue beginning 238 days after burial. Mild anthracnose symptoms developed on the underside of inoculated bean leaves as linear, dark brick-red to black lesions on leaf veins. *C. lindemuthianum* was isolated on agar medium from symptomatic tissue, with the exception of pod tissue from the 10-cm depth (Table 2). A pod bioassay was used with tissue collected 293, 306, and 364 days after burial. The symptoms that developed on pods were circular, brick-red lesions. Isolations were made from these pod lesions on Mathur's agar, and *C. lindemuthianum* was recovered from symptomatic tissue (Table 2). The decision was made to return to the procedure of inoculating trifoliolate leaves on whole plants for the remainder of the study, because it produced results in less time and the probability of infection was greater due to the larger surface area inoculated. Symptoms developed on leaves inoculated with liquid from decomposing pods at 10 cm and seeds at 10 and 20 cm that were collected 470 days after burial. *C.*

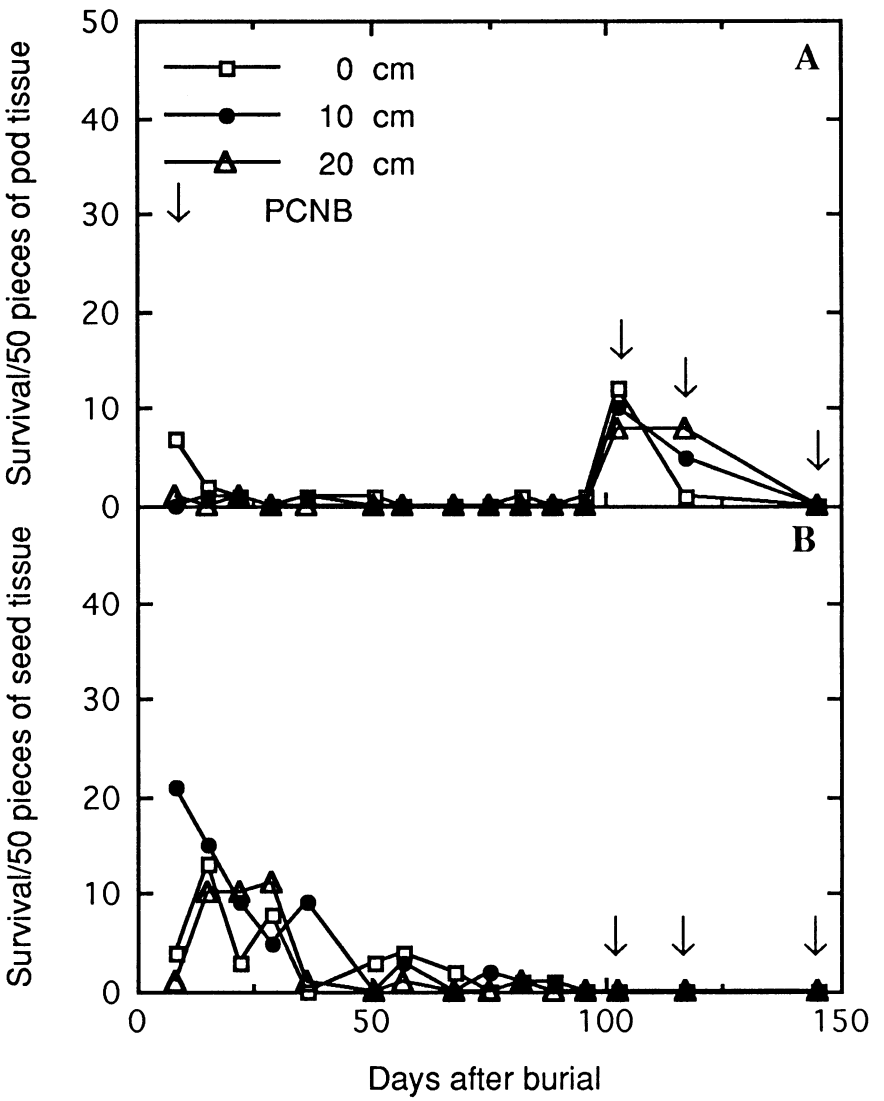

Fig. 2. Overwinter survival of *Colletotrichum lindemuthianum*, beta race, on infested pod and seed tissue of bean placed at 0, 10, or 20 cm depth in soil on 14 November 1988. **(A)** Recovery of *C. lindemuthianum* on agar media from 50 pieces of pod tissue. **(B)** Recovery of *C. lindemuthianum* on agar media from 50 pieces of seed tissue.

lindemuthianum was isolated on Mathur's agar from symptomatic leaf tissue. Mild symptoms developed on one plant inoculated with slurry from pod tissue at 10 cm deep 593 days after burial, and *C. lindemuthianum* was subsequently isolated from the symptomatic tissue. No symptoms developed on trifoliolate leaves of bean plants inoculated with liquid from seeds or pods retrieved 667 or 750 days after placement at any depth.

Control plants inoculated with *C. lindemuthianum* developed severe symptoms (severity = 5) at each sampling date, and the pathogen was readily reisolated from symptomatic tissue. No plants or pods inoculated with SDW developed anthracnose.

Overwintering in bean debris in infested fields. *Location 1. C. lindemuthianum* was isolated on Mathur's agar from symptomatic seeds collected 5 January and 10 March 1989 (Table 3). The fungus was not isolated on agar media from seeds collected 9 May 1989 or pods collected at any sample date. The pods collected 9 May 1989 had bright red-orange halos surrounding the lesions. Microscopic examination revealed spores of several fungal species, including *Alternaria, Colletotrichum,* and *Fusarium.* Precipitation was below normal and temperatures were near normal during this period (Table 1).

A whole plant assay was used to detect *C. lindemuthianum* in pod and seed tissues collected 10 March and 9 May. Symptoms developed on leaves inoculated with liquid from seed and pod tissues collected on either date (Table 3). The fungus was isolated on agar media from symptomatic leaf tissues inoculated with liquid from seed tissues collected 10 March and pod tissues collected 10 March and 9 May.

Location 2. C. lindemuthianum was isolated on Mathur's agar from seeds collected in January, March, and April 1990, and from pods collected in January 1990 (Table 3). Symptoms developed on leaves inoculated with liquid from seed and pod tissues collected on all 1990 sampling dates. The fungus was isolated on agar media from symptomatic leaf tissues. Temperatures were warmer than normal during this period (Table 1).

The field was replanted with dark red kidney beans on 31 May 1990, and the first anthracnose symptoms were observed on 5 July 1990. *C. lindemuthianum* was isolated on Mathur's agar from symptomatic tissue. A few light red kidney bean seeds (cv. Red Kloud) overwintered from the previous growing season, and anthracnose symptoms were observed on seedlings from these seeds. On 27 September 1990, all dark red kidney plants rated were infected with *C. lindemuthianum.* Mean anthracnose severity on the pods was 7.2 (Fig. 3A). Rainfall was above normal in April, May, and July 1990 (Table 1).

C. lindemuthianum was isolated on Mathur's agar from overwintered seeds collected in November 1990 and January, March, and April 1991, but not from pods at any of these dates (Table 3). Symptoms developed on whole plants inoculated with soak solution from seeds and pods collected in January, March, and April 1991. The fungus was subsequently isolated on Mathur's agar from symptomatic tissues on the inoculated plants. Temperatures were warmer than normal during this period (Table 1).

The field was replanted on 23 May 1991 with certified snap bean seed, and the first symptoms were observed on 27 June 1991. On 20 August 1991, 98% of the plants were infected with *C. lindemuthianum.* Because of the hot, dry weather (Table 1), mean anthracnose severity on pods was only 1.1 (Fig. 3B).

All certified seed lots used at this location were free of anthracnose. *C. lindemuthianum* was not isolated from seeds,

Table 1. Monthly precipitation totals and means of air and soil temperatures at Geneva, New York, during 1989–1991[a]

	Precipitation (mm)				Air temperature (C)				Soil temperature (C)[b]		
Month	1989	1990	1991	30-yr av[c]	1989	1990	1991	30-yr av[c]	1989	1990	1991
January	14	42	39	45	−2.1	0.3	−4.7	−5.4	−1.0	−0.5	0.7
February	31	96	19	45	−5.4	−1.7	−1.1	−4.8	−0.7	−0.3	1.4
March	65	47	26	52	−0.2	2.6	1.9	0.7	0.6	3.1	3.3
April	50	113	95	74	5.3	8.5	9.4	7.2	6.4	8.2	9.2
May	166	138	76	76	13.3	11.9	17.0	13.4	13.3	13.0	13.0
June	131	43	36	93	19.1	19.2	20.0	18.4	20.5	21.0	24.4
July	57	122	34	75	21.5	20.9	22.3	21.3	24.6	22.8	26.3
August	50	66	76	80	19.6	20.2	21.3	20.3	22.4	22.7	24.9
September	118	77	54	82	16.0	15.7	15.8	16.3	16.8	17.6	19.1
October	106	175	50	74	10.3	10.8	10.8	10.1	10.4	11.4	11.6
November	54	62	97	78	3.3	5.8	3.3	4.4	4.3	5.7	5.9
December	20	98	56	63	−9.2	0.3	−0.5	−2.2	−1.5	2.4	2.1

[a] Adapted from New York State Agricultural Experiment Station weather data.
[b] Soil temperature at a depth of 10 cm, bare ground.
[c] Data taken from: Climatography of the United States No. 81—Monthly normals of temperature, precipitation, and heating and cooling degree days 1961–1990, New York. U.S. Dep. of Commerce, January 1992.

Table 2. Detection of *Colletotrichum lindemuthianum*, beta race, in overwintering bean debris in New York State

Days after burial[a]	Bean plant assay, mean leaf lesion severity (± SE)[b]						Detached pod inoculation						Isolation from inoculated tissue					
	0 cm		10 cm		20 cm		0 cm		10 cm		20 cm		0 cm		10 cm		20 cm	
	Seed	Pod	Seed	Pod	Seed	Pod	Seed	Pod	Seed	Pod	Seed	Pod	Seed	Pod	Seed	Pod	Seed	Pod
238	1.9 (0.14)	1.5 (0.19)	1.6 (0.20)	1.3 (0.16)	1.9 (0.12)	1.4 (0.18)	...[c]	...	...	...	...	...	+	+	+	−	+	+
293	...	...	...	...	...	...	−	+	−	+	−	−	−	+	−	+	−	−
306	...	...	...	...	...	...	+	−	+	+	−	+	+	−	+	+	−	+
364	...	...	...	...	...	...	−	−	+	−	+	−	−	−	+	−	+	−
470	1.0 (0)	1.0 (0)	1.1 (0.10)	1.2 (0.10)	1.4 (0.16)	1.0 (0)	...	...	...	...	...	...	−	−	+	+	+	−
593	1.0 (0)	1.0 (0)	1.0 (0)	1.1 (0.06)	1.0 (0)	1.0 (0)	...	...	...	...	...	...	−	−	−	+	−	−
667	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)	...	...	...	...	...	...	−	−	−	−	−	−
750	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)	1.0 (0)	...	...	...	...	...	...	−	−	−	−	−	−

[a] Burial date = 14 November 1988.
[b] Bean plant assay: the first trifoliolate leaves of greenhouse-grown plants were inoculated with soak solution from incubated overwintering seed or pod tissue. Severity scale of 1 to 5: 1 = no symptoms, 5 = large coalesced lesions accompanied by tissue breakdown.
[c] ... = Not determined, + = *C. lindemuthianum* detected, − = *C. lindemuthianum* not detected.

greenhouse-grown plants, or plants growing in control fields.

Location 3. On 27 September 1990, all snap bean plants rated were infected with *C. lindemuthianum.* Mean anthracnose severity on pods was 5.8 (Fig. 3C).

C. lindemuthianum was isolated on Mathur's agar from overwintered seeds collected in December 1990, in February and April 1991, and from pods collected in February 1991 (Table 3). Severe symptoms developed on whole plants inoculated with soak solution from seeds and pods collected at all three sample dates. The fungus was isolated on Mathur's agar from symptomatic tissue on inoculated plants. Temperatures were warmer than normal during this period (Table 1).

The field was replanted on 23 May 1991 with dark red kidney beans, and the first symptoms were observed on 27 June 1991. On 23 September 1991, 71% of the plants showed anthracnose symptoms. Mean anthracnose severity on the pods was only 0.7 (Fig. 3D) because of the hot, dry weather (Table 1).

Table 3. Overwinter survival of *Colletotrichum lindemuthianum*, beta race, in bean debris at three locations in New York State

Location / Sampling date	Isolation from debris		Bean plant assay mean leaf lesion severity (± SE)[a]		Isolation from inoculated tissue	
	Seed	Pod	Seed	Pod	Seed	Pod
Location 1						
5 January 1989	+[b]	−	...	...	...	...
10 March 1989	+	−	1.9 (0.118)	1.9 (0.165)	+	+
9 May 1989	−	−	1.3 (0.133)	3.5 (0.340)	−	+
Location 2						
29 January 1990	+	+	1.9 (0.087)	4.7 (0.081)	+	+
19 March 1990	+	−	1.9 (0.105)	4.9 (0.033)	+	+
28 March 1990	+	−	1.4 (0.064)	4.7 (0.080)	+	+
30 April 1990	+	−	3.0 (0.133)	5.0 (0)	+	+
29 May 1990	−	−	3.8 (0.164)	4.8 (0.059)	+	+
(Replanted 31 May 1990)						
29 November 1990	+	−	...	...	...	...
7 January 1991	+	−	1.9 (0.140)	3.5 (0.125)	+	+
4 March 1991	+	−	1.4 (0.068)	1.7 (0.069)	+	+
30 April 1991	+	−	1.4 (0.095)	4.7 (0.064)	+	+
Location 3						
11 December 1990	+	−	5.0 (0)	5.0 (0)	+	+
11 February 1991	+	+	4.9 (0.043)	4.1 (0.047)	+	+
15 April 1991	+	−	5.0 (0)	5.0 (0)	+	+

[a] Bean plant assay: the first trifoliolate leaves of greenhouse-grown plants were inoculated with soak solution from incubated overwintering seed or pod tissue. Severity scale of 1 to 5: 1 = no symptoms, 5 = large coalesced lesions accompanied by tissue breakdown.

[b] ... = Not determined, + = *C. lindemuthianum* detected, − = *C. lindemuthianum* not detected.

DISCUSSION

C. lindemuthianum can overwinter in bean debris in New York State and cause disease in beans the following growing season. Dry beans grown in New York are planted in May and June and harvested in September and October. Because of wet field conditions that occur in the fall, dry bean producers often delay incorporation of bean residue until spring. Bean debris infested with *C. lindemuthianum* remaining on the soil surface may serve as a reservoir of inoculum for the following growing season. *C. lindemuthianum* was also able to survive for 1 yr at low levels in bean

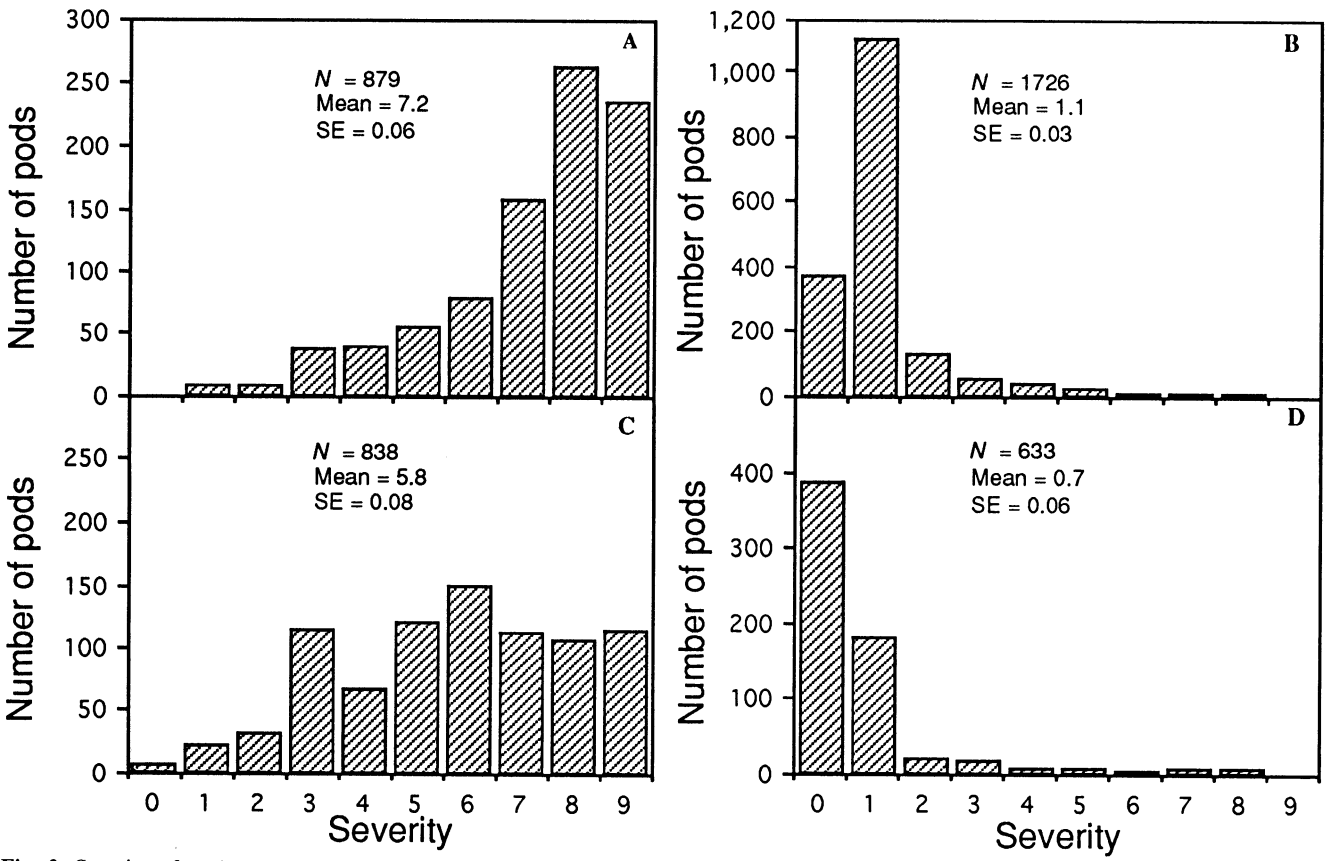

Fig. 3. Severity of anthracnose on bean pods naturally infected with *Colletotrichum lindemuthianum,* beta race. **(A)** Anthracnose severity ratings of bean pods (cv. California Dark) at Location 2 in 1990. **(B)** Anthracnose severity ratings of bean pods (cv. Bush Blue Lake 47) at Location 2 in 1991. **(C)** Anthracnose severity ratings of bean pods (cv. True Blue) at Location 3 in 1990. **(D)** Anthracnose severity ratings of bean pods (cv. California Dark) at Location 3 in 1991.

debris buried 10 and 20 cm deep in the soil.

In previous survival studies conducted in New York, Barrus (3) concluded that *C. lindemuthianum* was capable of surviving more than one season in infected vines and pods, but that spores washed into the soil were not viable after 7 wk. The level of overwintering inoculum was considered insufficient to initiate an epidemic the following year (3). However, field observations in Michigan support the hypothesis that the fungus can overwinter in bean debris and infect the next season's bean crop (12). In more recent studies, Tu (17) was unable to isolate *C. lindemuthianum* on agar medium in mid-May from infected bean debris buried in a field in Ontario in November. The results of this study are similar, in that the fungus could not be isolated on agar medium in April from infected bean debris placed in a field in New York in November. However, *C. lindemuthianum* could be detected in bean debris by using bean plants as the selective medium. In replant studies conducted in Ontario, the fungus was not detected in beans throughout the growing season on sites that had heavily infected crops the previous year (17). In contrast, anthracnose developed in beans (planted with certified seed) grown in fields in New York that had anthracnose the previous year. This inoculum originated from infested overwintered debris and possibly from a few infected volunteer seedlings.

Spread of inoculum and infection by *C. lindemuthianum* are influenced by environmental conditions. Infection is favored by moderate temperatures (13–26 C) and high RH (greater than 92%), and dissemination is most often by wind-driven rain (13,15,16). Anthracnose symptoms do not develop on inoculated plants held at constant temperatures of 28 or 32 C, but do develop if night temperatures are lowered (16). Although hot, dry weather conditions limited disease severity in 1991 in New York, sufficient moisture and cool night temperatures allowed anthracnose to develop on leaves and pods in the field.

The contrasting results of this study and Tu's study (17) may be attributed to differences in races of *C. lindemuthianum,* environmental conditions, and/or cultural practices. The beta race was prevalent in New York, and the delta race was studied in Ontario (17). It is unknown whether there are differences in survival between the two races. Winter temperatures in December, January, and February in Harrow, Ontario, (1978–1980) were lower than temperatures in Geneva (1989–1991) during those months. The colder temperatures may have been detrimental to the survival of *C. lindemuthianum.* Another difference between the two studies relates to cultural practices. In the Canadian study, infested fields were disked in the fall, which would promote microbial decay of the infested debris (17). However, in New York, most dry bean fields are not disked until spring because of wet soil conditions in the fall. Thus, the debris is left on the soil surface and is not subjected to intense microbial activity. Sclerotium-like structures produced by *C. lindemuthianum* have been observed when the fungus is grown in the presence of benomyl in agar media (20). It is not known if other environmental stresses could induce sclerotium production in the field, or whether the sclerotia produced would overwinter.

The source of the primary inoculum for initiation of anthracnose epidemics is most often contaminated seed (1,3,6, 10,12,21). However, based on the results of this study, current recommendations in New York for anthracnose control emphasize a minimum 2-yr rotation from beans, fall incorporation of bean residue whenever possible to hasten decomposition of the debris, and the use of western-grown certified bean seed. Anthracnose has typically been a disease associated with dry bean production in New York, and the disease is only seen on snap beans in fields where dry beans were grown previously. As growers diversify their operations for economic reasons, some are producing both dry beans and snap beans for processing. The role of good rotations and the use of certified bean seed will become of even greater importance to producers of both bean types.

Whole bean plants served as a highly selective medium that facilitated detection of low inoculum concentrations of *C. lindemuthianum* in overwintered bean debris in New York. The technique of inoculating trifoliolate leaves of whole bean plants consumed little time, produced definitive symptoms, and provided a large surface area for inoculation, which increased the chance for infection and thereby detection of *C. lindemuthianum.* Although the concentration of inoculum in overwintering tissues could not be directly determined, an estimate of inoculum density could be made by comparing severity values obtained in the plant assay with inoculum density and disease severity curves determined under controlled conditions. The host plant bioassay is a reliable procedure for determining if the pathogen is present at low but epidemiologically significant levels.

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