Epidemiology of Anthracnose Caused by *Colletotrichum lindemuthianum* on White Bean (*Phaseolus vulgaris*) in Southern Ontario: Survival of the Pathogen

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ABSTRACT

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Longevity of *Colletotrichum lindemuthianum* varied greatly depending on environmental conditions. Moisture had a profound effect on its longevity. The fungus survived at least 5 yr in infected pods and seeds of *Phaseolus vulgaris* that were air-dried and kept in storage at 4 C or in dry infected plant materials left in the field in sealed polyethylene envelopes that had no contact with water. In infected materials placed in nylon-mesh pouches and buried in the field in November, *C. lindemuthianum* could not be isolated after mid-May. Laboratory tests showed that an alternating wet-dry cycle was detrimental to survival of the fungus. The fungus in the infected pod segments lost viability after three cycles of 72 hr wet and 72 hr dry. In the field, there was no sign of anthracnose throughout the 1979 and 1980 growing seasons in plots on sites with heavily infected crops the previous year. The degree of transmission by seeds increased with increasing severity of infection and density of conidia in the infested seeds.

An outbreak of bean (*Phaseolus vulgaris* L.) anthracnose in southern Ontario (10) generated renewed interest in the disease. The outbreak was caused by the delta race of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav., which is thought to have been introduced to Canada from Europe (9). By 1977, about 18% of the acreage sown with Select, Foundation, and Certified seed lots was infected (8). Several aspects of the epidemiology of this disease have been investigated, and the climatic conditions of southern Ontario have been found highly conducive to disease development (6,7).

In 1921, Barrus (1) demonstrated the seedborne nature of the bean anthracnose pathogen. Subsequently, a 3-yr rotation was suggested to control the pathogen (11) because it was believed that the fungus could survive 2 yr in dry debris and in the seed (5). Not all infested and infected seeds are capable of transmitting the disease, however, and the incidence of anthracnose attributable to seedborne sources varies greatly among seed lots (1). Variation in seed transmission relates to the degree of infestation as well as the severity and site of infection in the seed.

Even if the majority of currently recommended Ontario white bean cultivars carries the ARE gene that gives resistance to all races (alpha, beta, gamma, delta, and lambda) of this fungus pathogen existing in Ontario, many cultivars of colored beans and snap beans are susceptible to one or more races of the fungus. Other such control measures as seed disinfection and cultural practices will continue to be important. Therefore, the ability of the pathogen to overwinter and its transmission by seed require further investigation in order to formulate rational integrated control measures.

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MATERIALS AND METHODS

Survival of the anthracnose fungus *C. lindemuthianum*, race delta, in the field and in the laboratory was investigated. Three series of experiments were designed to determine the longevity of the pathogen in infected seeds and pods in cool storage conditions or when incorporated with debris in the field and to determine the rate of seed transmission relative to the degree of seed infestation.

Longevity of the pathogen in cold storage. Heavily infected pods were collected from *P. vulgaris* 'Fleetwood' in 1976. The samples were air-dried at room temperature (21 ± 1 C) and put in cold storage at 4 ± 1 C. Attempts to isolate the pathogen were made yearly starting in May 1978. Anthracnose lesions on the pods and seeds were excised and placed on Mathur's agar (MA) (2) supplemented with 40 μg/ml of novobiocin. The basic formula of MA consisted of glucose (2.8 g), $MgSO_4$ (1.23 g), KH_2PO_4 (2.72 g), Bacto peptone (1.0 g), yeast extract (trace), agar (20.0 g), and distilled water (1,000 ml). The inoculated plates were kept at room temperature. Fungal colonies growing from these lesions were identified 5 days later.

Longevity of the pathogen in the field. *Survival of the fungus in plant debris.* Anthracnose-diseased pods and stems were collected in mid-September from an inoculated field plot of Fleetwood at Harrow, Ontario. Samples were taken when bean plants were mature and dry. All samples were air-dried further. Pods and stems with lesions were cut into segments and seeds with lesions were sorted out. These materials were divided into three groups. The first and the second groups were to be buried in the field in nylon-net pouches and in sealed polyethylene pouches, respectively. The third group was subjected to a series of alternating wet-dry conditions in the laboratory.

For the first group, 10 pod segments or seeds were put into a monofilament nylon pouch (0.05 mm^2-mesh). The opening of the pouch was then sealed with a nylon strand. A total of 120 pouches were prepared representing four replicates of 30 pouches for pod segments or seeds. For the second group, 60 more nylon pouches were prepared similarly for pod segments and seeds. Each pouch was then sealed in a polyethylene bag, which permitted gas but no water exchange. All pouches were buried horizontally about 5 cm deep in the field in mid-November 1978. Recovery of the pouches began in early December 1978 and continued into mid-June 1979. One pouch per replicate was recovered at 1- and 2-wk intervals for group 1 and group 2 samples, respectively. At least five tissue samples were retrieved from each pouch, rinsed in sterile water and in 0.5% sodium hypochloride, and plated on MA containing 40 μg/ml of novobiocin. Fungal colonies growing out of the tissues during incubation at room temperature were identified 4–5 days later. This experiment was repeated between November 1979 and June 1980.

For the third group, about 250 pod segments were prepared for five treatments: constantly dry, constantly wet, and one, two, and three wet-dry cycles. Each cycle had a 72-hr wet and a 72-hr dry period. For wet treatment, about 50 pod segments were scattered on a filter paper in a petri plate. The tissues and the paper were sprayed with sterile water and covered. After the wet period, the petri plate cover was removed and the tissues were air-dried at room temperature and about 10–15% RH.

At the end of the treatment, all pod segments were plated on MA containing 40 μg/ml of novobiocin and examined for the presence of *C. lindemuthianum*. The experiment was repeated once.

Infectivity of infested debris. A field plot of Fleetwood heavily affected by anthracnose was lightly disked in the fall and replanted with anthracnose-free

seeds in early June of the following year. The experiment was done in 1979 and again in 1980. Signs of anthracnose symptoms were recorded weekly.

Seed infection and disease transmission. In order to establish the relationship between the severity of seed infection and the degree of seed transmission, Fleetwood seeds were obtained from severely affected pods. The seeds were sorted according to severity of symptoms into five groups: 1) seeds without apparent symptoms, 2) seeds with a brown spot but no acervuli, 3) seeds with brown streaking extending from the hilum but no acervuli, 4) seeds with a brown spot and acervuli, and 5) seeds with pitted lesions extending into the cotyledon.

About 150 seeds of each group (in four replicates) were sown, five seeds per 10-cm pot. The seeds were kept for 3 mo at 4 C before planting. The pots were filled with an autoclaved soil mixture consisting of 2:1:1 (v/v/v) sand, peat, and loam. Seedlings at the late unifoliate stage were misted with water, covered with polyethylene bags, and incubated at 21 ± 1 C for 72 hr with 14 hr of light at 15 klux. Upon removal of the bags, they were maintained in the same growth room for symptom development. Symptoms of anthracnose disease were recorded 7 days after covering. The experiment was repeated once.

Seed infestation and disease transmission. Anthracnose-free Fleetwood seeds were surface-inoculated with various concentrations of a conidial suspension of the delta race of *C. lindemuthianum*. The conidial suspension was prepared from 3-wk-old colonies in MA plates. Five milliliters of sterile distilled water was added to each plate, and the surface of the culture was scraped to dislodge the conidia. The conidial suspensions derived from several plates were adjusted to 10^8 conidia per milliliter. A series of conidial dilutions, 10^1, 10^2, 10^4, 10^6, 10^7, and 10^8 conidia per milliliter, were made. Each dilution was sprayed onto a group of seeds to runoff. A control was sprayed with sterile water. The seeds were then air-dried at room temperature.

For each dilution, more than 400 seeds were sown, five seeds per 10-cm pot, so that four replicates of 100 seedlings were available. At the late unifoliate stage, the seedlings were thoroughly misted with water by using a pressurized hand sprayer, covered with a polyethylene bag, and incubated for 3 days in the 21 ± 1 C growth room. After removal of the bags, all plants were left in the same room for symptom development. The percentage of diseased plants was scored 6 days later after covering.

RESULTS

Longevity of the pathogen in cold storage. *C. lindemuthianum* remained viable for 5 yr or more in diseased tissue air-dried and stored at 4 C. Similarly, the fungus could overwinter in the field in infected plant materials without appreciable loss of viability when the materials were kept dry. *C. lindemuthianum*, however, was not isolated after mid-May from nylon-mesh pouches buried the previous November (Fig. 1).

In both years, the decrease in viability followed a similar pattern that consisted of three phases: 1) a slowly decreasing phase in December (a loss of about 10–15%), 2)a stable phase in January and February (a further loss of about 5–10%), and 3) a rapidly decreasing phase in March, April, and early May resulting in total loss of viability by mid-May.

When the pod segments were subjected to alternating wet-dry cycles, the viability of the fungus decreased drastically after each cycle (Fig. 2). After three cycles or continuous wet, *C. lindemuthianum* could be isolated only rarely from the tissues. In the continuously dry tissue, however, the viability of the fungus remained high (Fig. 2).

Longevity of the pathogen in the field. There was no sign of anthracnose throughout the growing season of 1979 and 1980 in plots on sites that had heavily infected crops the previous year.

Seed infection and disease transmission. Although seed transmission of the bean anthracnose pathogen did occur, it was not invariable. Seeds collected from the diseased pods varied greatly in the severity of symptoms (Fig. 3). The percentage of seedling infection increased with severity of disease symptoms on the seed. Conversely, seeds with less severe disease symptoms resulted in more disease escapes (Fig. 4).

Seed infestation and disease transmission. Figure 5 summarizes the results of contaminating the seeds with conidia. At an inoculum concentration lower than 10^2 conidia per milliliter, contaminated seeds did not give rise to infected seedlings. At inoculum concentrations of 10^4 and 10^6 conidia per milliliter, only 1 and 2% of seedlings became infected,

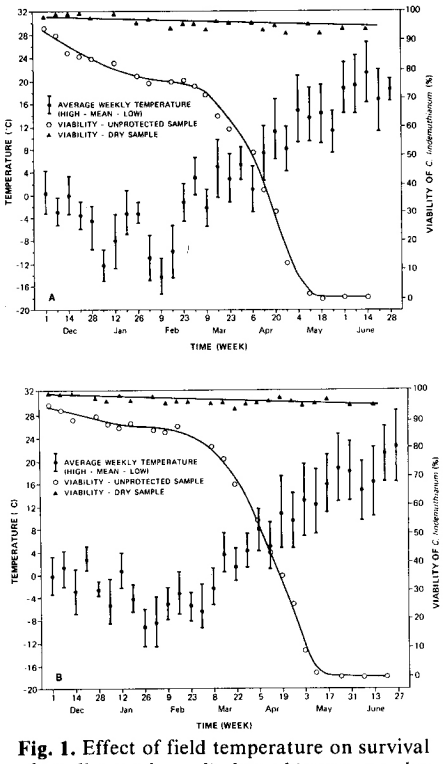

Fig. 1. Effect of field temperature on survival of *Colletotrichum lindemuthianum* on dry bean pod segments or seeds in a nylon-mesh pouch or in a waterproof polyethylene bag buried in the field **(A)** November 1978–June 1979 and **(B)** November 1979–June 1980.

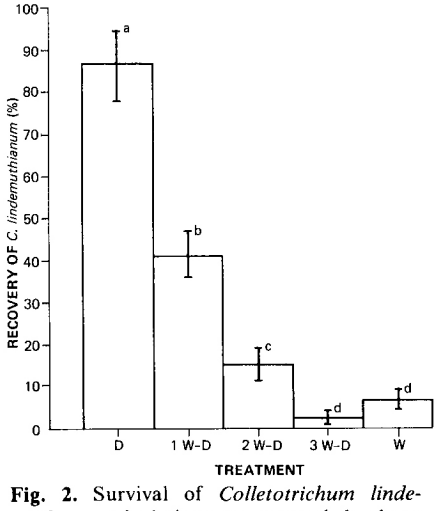

Fig. 2. Survival of *Colletotrichum lindemuthianum* in lesions on matured dry bean pod segments subjected to various wet/dry conditions as follows: constantly dry (D), constantly wet (W), and one, two, and three wet-dry (W-D) cycles with each cycle consisting of a 72-hr wet and a 72-hr dry period. Means bearing the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

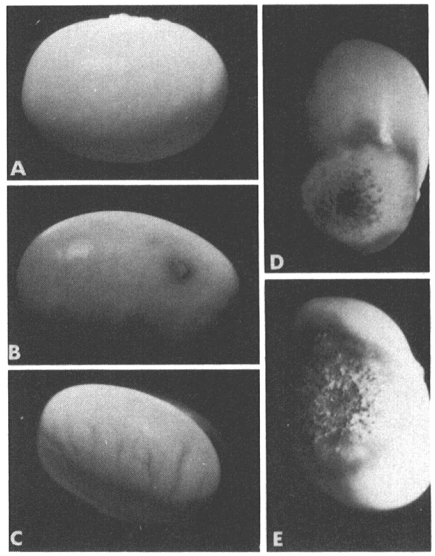

Fig. 3. Seeds recovered from anthracnose-affected bean pods showing different severities of *Colletotrichum lindemuthianum* infection: **(A)** Seed without apparent anthracnose symptoms, **(B)** seed with brown spots but no acervulus, **(C)** seed with brown streaking extending from the hilum, **(D)** seed with brown spots and acervuli, and **(E)** seed with pitted lesions extending into the cotyledon.

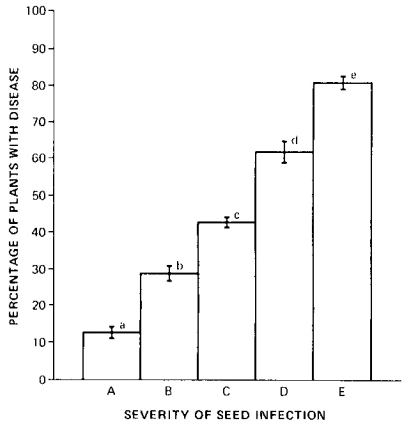

Fig. 4. Relationship between severity of seed infection and seed transmission of bean anthracnose by *Colletotrichum lindemuthianum*. A = bean seed without any apparent symptoms, B = seed with brown spot but no acervulus, C = seed with brown streaks extending from hilum, D = seed with brown spots and acervuli, E = seed with pitted lesion extending into the cotyledon. Each figure is derived from between 109 and 127 plants. Means bearing different letters are significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

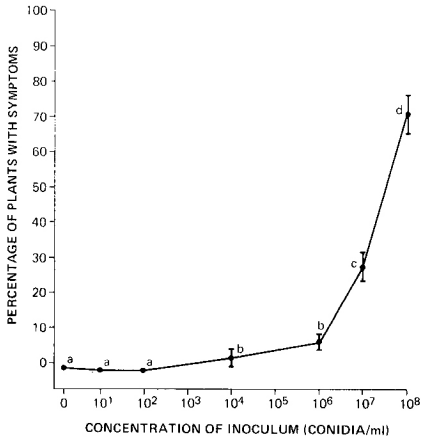

Fig. 5. Incidence of anthracnose in bean seedlings from seeds inoculated with various concentrations of conidia of *Colletotrichum lindemuthianum*. Means bearing different letters are significant at $P \leq 0.01$.

respectively. However, 30 and 71% of seedlings became infected at inoculum concentrations on the seed of 10^7 and 10^8 conidia per milliliter, respectively.

DISCUSSION

Results show that under natural field conditions, conidia of *C. lindemuthianum* failed to overwinter in infected bean straw unless the straw was protected from water. The rapid decrease in viability in wet conditions may be attributed in part to the loss of the mucilaginous water-soluble matrix of the conidia. Nicholson and Moraes (4) demonstrated that removal of the mucilaginous matrix of conidia by water before storage could significantly reduce the conidial viability of *C. graminicola* in a storage period as short as 24 hr.

The probable inability of *C. lindemuthianum* to overwinter was further demonstrated by sowing anthracnose-free seeds in a field that had a heavy infection of anthracnose in the previous year. In both years (1979 and 1980) that this was done, the new crops were free of anthracnose. Such a conclusion is reinforced by observations made in relation to the recent outbreak of anthracnose in southern Ontario in 1976. In 1978, a benomyl formulation (0.3 kg a.i./45 kg of seeds) was used in seed treatment and only isolated cases of anthracnose were detected that year. It is known from laboratory tests that seed treatment is unsuccessful in fewer than 5% of the cases (3). It seems likely, therefore, that the recurrence of anthracnose in growers' fields comes mainly from two sources: reintroduction of anthracnose-diseased seeds and survival of the anthracnose fungus on debris that remains dry, such as that found in large trash piles. Disking down the infected plant debris and avoiding reintroduction of infected seeds should check the spread of this disease.

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