STUDIES ON SPORULATION, GERMINATION, AND INFECTION BY COLLETOTRICHUM LINDEMUTHIANUM (SACC. AND MAGN.) SCRIB. AS INFLUENCED BY TEMPEATURE

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By

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ABSTRACT

STUDIES ON SPORULATION, GERMINATION, AND INFECTION BY COLLETOTRICHUM LINDEMUTHIANUM (SACC. AND MAGN.) SCRIB. AS INFLUENCED BY TEMPERATURE

Bean anthracnose incited by Colletotrichum lindemuthianum (Sacc. and Magn.) Scrib. is an important disease in Michigan. The disease problem is complicated by the presence of three physiologic races of the pathogen, which can be identified by using the varieties Michelite (susceptible to alpha, but resistent to beta and gamma races); Dark Red Kidney (resistant to race alpha and susceptible to beta and gamma races); and Perry Marrow (resistant to alpha and beta but susceptible to the gamma race) as differential hosts. The investigations were designed to aid in clarifying and interpreting observations on disease development.

Among the several media tested, Difco bean pod agar was the most favorable medium upon which abundant sporulation was obtained. Using this medium it was observed that the minimum, optimum and maximum temperatures for sporulation were 16, 26 and 28 C. respectively. With respect to germination, the beta and gamma races germinated over a wider range of temperature conditions than race alpha. Thus, after 24 hours incubation the spores of the alpha race had a germination of 0, 0, 5, 90, and 9 percent respectively at 16, 20, 24, 28 and 32 C. as compared to 19, 37, 32, 93, and 25 percent for beta and 6, 16, 62, 96, and 6 percent for the gamma race at the same temperatures.

In the studies on the relation of temperature and period of exposure to continued wetness to infection, little or no plant infection

was established even after 72 hours of exposure to continued wetness at 10 C. Infection was readily established in 12 hours at 20 and 25 C.

Studies on the relation of host development to infection indicated that bean plants were very susceptible to infection from the time of emergence until they were 3 to 5 weeks old and as they increased in age they become less susceptible to infection. In this respect the three races reacted similarly.

The best pod infection was obtained on 1 to 4-day old pods. Five to 23 day-old pods appeared equally susceptible to infection but less susceptible than the younger pods. Pods older than 24 days resisted infection. Seed infection occurred at any stage of pod development where pod infection had taken place.

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INTRODUCTION

Bean anthracnose incited by Colletotrichum lindemuthianum (Sacc. and Magn.) Scrib. has been and still is an important disease of beans in Michigan. Muncie (26) mentioned that the first authentic report of the disease in Michigan was in 1889. He reported losses of $1,500,000. in 1914 and $3,000,000. in 1915 to beans in Michigan from this disease.

Andersen (1) reported a loss of 200,000,000 pounds of beans valued at $1,400,000. in Sanilac and St. Clair counties of Michigan in 1950. Since Michigan farmers plant approximately 500,000 acres of beans every year valued at nearly $35,000,000. and since the climatical conditions in Michigan tend to favor the development of bean anthracnose, this disease must continue to receive considerable attention in a research program.

The disease problem is further complicated by the presence of more than one physiological race of the pathogen. In Michigan, the variety Michelite and other older varieties of Navy beans are susceptible to the alpha race and resistant to the beta and gamma races. Ordinarily, this disease has been important only in those years of excessively high rainfall. The Dark Red Kidney and Cranberry beans are resistant to the alpha race but susceptible to the beta and gamma races. However, the disease is generally present every year in the latter two varieties but only the beta race has been consistently isolated. Thus several questions arise as to why the disease outbreaks occur as they do and why certain races predominate.

It is important that we try to clarify the reactions of the anthracnose pathogen so that we can carry on a successful disease control and breeding program. These investigations were conducted to enhance this work.

The preliminary investigations which included a study of various media were made in order to select a medium that would support abundant sporulation in the shortest time possible. In addition, the optimum temperatures for sporulation were determined. The principal part of the investigations involved a study on the relation of temperature to spore germination and host infection by the three races of Colletotrichum lindemuthianum. Furthermore, the investigations included a study of symptomatology as related to stage of host development and the relation of age of the pods to susceptibility to infection and penetration by the alpha and beta races of the anthracnose fungus.

REVIEW OF LITERATURE

Geographical distribution

Bean anthracnose incited by Colletotrichum lindemuthianum occurs throughout the world. For example, Saccardo reported serious epiphytotics in parts of Europe, specifically mentioning its occurrence in Germany, Italy, France and England (6).

Barrus (6) in reviewing the occurrence of the disease, reported its presence in many countries including Australia in 1892, Argentina in 1899, Brazil in 1901, Cuba in 1908, Japan in 1911, Russia in 1912, Formosa in 1914, Alaska in 1916 and India in 1918. However, he did mention that the disease is actually of minor importance in some of these countries and in others it may be very serious, depending upon the climatic conditions of the region.

In the United States, Ellis and Everhart (13) first reported the disease in Massachusetts in 1882. Barrus (6) in his review reported that the disease was found in Pennsylvania, Maryland and Louisiana in 1887, in Michigan in 1889, and in New York and Ohio in 1890.

Taxonomy

Several American authors described the early history of this pathogen (6) (11) (20). They agree that Colletotrichum lindemuthianum was first collected by Lindemuth at the Agriculture Institute of Pappelsdorf at Bonn Germany. Three years later Saccardo classified the fungus as Gloeosporium lindemuthianum (Sacc. and Magn.). In 1889 Scribner changed

the name to Colletotrichum lindemuthianum (Sacc. and Magn.) Scrib. because of the presence of setae in the acervulus. Edgerton (11) reported that Briosi and Cavara classified the fungus as Colletotrichum lindemuthianum (Sacc. and Magn.) Bri. and Cav. the same year (1889) and this classification has been used until recently when it was recognized that the original classification given by Scribner had priority. This classification has been challenged several times because the presence of setae in the acervulus is not a constant character and their presence depends upon the medium in which the fungus is grown.

Shear and Wood (29) found asci and perithecia in cultures grown from anthracnose lesions on beans. They claimed that the perithecia were the perfect stage of Colletotrichum lindemuthianum and hence they named the fungus Glomerella lindemuthianum (Shear and Wood). Edgerton (10) (11) (12) made studies on the physiology and development of the fungus and suggested that the ascigerous stage described by Shear and Wood probably belonged to a saprophytic species. Since the perfect stage has not been reported since the observations of Shear and Wood, the name of the imperfect stage given by Scribner is recognized today.

Pathogenicity

The infectious character of this fungus was first demonstrated by Frank in 1883, who also established the important fact that the fungus is seed transmitted (6). Later, in 1892 Halsted (14) corroborated the work of Frank as to the infectious nature of the fungus. Hasselbring (15) made studies on the appresoria and decided that this character may or may not be present at the time of infection.

The first evidence of the presence of physiological races of Colletotrichum lindemuthianum was reported by Barrus (3) (4) (5) in 1911. He noted that Red Kidney varieties differed considerably in their susceptibility to infection by various isolates of the pathogen, some being completely susceptible and others highly resistant. He reported two races and labeled them strains alpha and beta. This is the first instance on record in which distinct physiologic races within a morphologic species of a pathogen were defined on the basis of horticultural varieties of a single species rather than on the basis of differences between a host species or genera (30).

McRostie (22) in 1919 studied the inheritance of anthracnose resistance. He crossed resistant and susceptible varieties and demonstrated that resistance to each race was inherited as a distinct unit character, that is, that resistance was due to single gene effects (33). These results were later corroborated by Burkholder (8) who discovered the gamma race of the same organism and showed that resistance to this particular race was also governed by a single separate gene as was the case with the two races previously described. Rands and Brotherton (27) confirmed the nature of disease resistance using several varieties.

Since the work of Barrus (6) and Burkholder (8) there have been numerous articles published describing new races and biotypes of the anthracnose pathogen. Thus Muller (25) in 1926 reported four new races present in Holland which differed from the three races previously described in the United States. Schreiber (28) reported several different races in Germany. More recently Yerkes (31) reported at least four new groups of races in Mexico, each of which is composed of various biotypes. Some

of these races were different from the three races known in the United States.

Several investigators have studied the physiology of parasitism (2) (7) (16) (20). A good account of the method of penetration of the host by Colletotrichum lindemuthianum was given by Barrus (6) and Dey (9). Leach (20) in his work states that Colletotrichum lindemuthianum does not normally kill the host cell in advance of its growth but penetrates a large number of cells without any immediate lethal effect and obtains its nourishment from the living cells. All his evidence indicates that resistance is due to the inability of the fungus to obtain nourishment from the living protoplast. He tried to change the parasitic capabilities of the fungus and to breakdown host-resistance but failed in both instances.

Environment in relation to infection

Lauritzen (18) (19) studied the relation of air temperature and relative humidity to infection and found that disease developed at temperatures from 7° to 27°C. with the highest percentage of infection occurring at 25°C. Infection was established in 5 days when the plants were exposed at 22°, 25° and 27°C.; in 7 days at 15.5° and 17.5°C.; in 9 days at 12°C.; and in 14 days at 7°C. Melendez (24) obtained good infection at temperatures from 18° to 27°C., with a maximum at 30°C. and a minimum at 13°C. at relative humidities of 60 to 95 percent. She found that symptoms were produced 7 to 8 days after inoculation with the alpha race and 7 to 15 days after inoculation with the beta and gamma races.

Environment in relation to sporulation

Barrus (6) described the mechanism of spore formation and observed that spores germinate at room temperature within 24 hours in nutrient culture media and on bean agar. At 22°C. he noticed that bean pods inoculated with spores produced mycelium very rapidly which in two days began to darken and after three days produced spores in an acervulus. His results indicated a minimum temperature for growth in culture from 0° to 4°C., an optimum near 22°C. and a maximum at 34°C. Leach (20) studied the effect of temperature upon the growth of Colletotrichum lindemuthianum on agar plates. His data showed 0°C, 22.5°C, and 32-24°C. respectively, were the minimum, optimum and maximum temperatures for growth. He stated that "the reaction to temperature, as indicated by the rate of growth, was approximately the same for the three biologic forms."

Mathur et. al. (21) studied the sporulation of Colletotrichum lindemuthianum on synthetic media and found that a medium containing glucose, mineral salts and neo-peptone gave as good sporulation as that occurring on sterilized bean pods or bean agar. They obtained no spore formation below pH 3, some spore production at pH 3.6, and maximum spore development between pH 5.2 and 6.5.

Hopkins (17) studied the effect of lactic acid on spore production. He obtained the best sporulation at a pH of 3.8 which he obtained by adding three drops of 50 percent lactic acid to the medium. He states that "spore production appears to increase with increase in hydrogen-ion concentration and with an accompanying decrease in the amount of growth." Leach (20) attempted to differentiate the three biologic forms by using different nutrient agars but was not able to find any difference

between the races at different pH levels. He obtained optimum growth on the alkaline side.

MATERIALS AND METHODS

Laboratory studies

A representative culture of each of the three principal races of anthracnose (alpha, beta and gamma) was used in each phase of the laboratory investigations. These cultures were initially maintained on natural bean pod agar at room temperature (20-25°C.).

In order to select a medium favorable for abundant spore production, several types of media were compared. The media tested included sterilized green bean pods; natural bean pod agar (200 gm/liter); Difco lima bean, bean pod and corn meal agar; sterilized barley seed and a synthetic medium consisting of 2.8 gm. glucose, 1.23 gm. $MgSO \cdot 7H_2O$, 2.72 gm. KH_2PO_4 and 20 gm. of agar per 1000 ml. of water. Except for the bean pods and barley seed, which were placed in tubes, the media were poured into Petri plates and inoculated by placing one ml. of spore suspension in the center of each plate. Each medium was replicated four times and the cultures were incubated 8 days at 26°C. prior to making spore counts using a hemacytometer. In each case the number of spores from a 20 mm. area surface from each colony was determined. Five drops from each sample were counted. On the basis of this determination the total number of spores per colony were estimated.

In the study on the effect of temperature on sporulation the same method of comparison was used as described in the previous paragraph. In this case only Difco bean pod and lima bean agars were used. After inoculation, four plates of each medium were placed in incubators main-

tained at 12°, 16°, 20°, 24°, 26°, 28°, 30°, and 37°C. Spore counts were made 8 days after inoculation.

The three races of Colletotrichum lindemuthianum were used in the studies on the effect of temperature upon spore germination. Spore suspensions containing 50,000 spores/ml. were placed on 7 mm. agar discs on glass slides and placed in covered Petri dishes in which a high humidity was maintained with water. Eight plates of each race were placed at 16°, 20°, 24°, 28° and 32°C. In a preliminary test, observations were made hourly beginning 4 hours after inoculation in order to determine the time the first visible germination occurred and to establish the time to make readings. On the basis of these observations, readings were made after 9, 24, 33, and 48 hours. A total of 200 spores were observed in each of three fields chosen at random. The percentage spore germination was based upon the averages of the three readings.

Greenhouse investigations

The variety Michelite, susceptible to alpha and resistant to the beta and gamma races of anthracnose, and the variety Dark Red Kidney, resistant to alpha but susceptible to beta and gamma, were used in all the greenhouse infection studies. The plants were grown in pots in sand culture using a standard nutrient solution. All greenhouse studies were made during the period from May 1 to November 1. The same representative cultures of the three races used in the laboratory studies were used in the greenhouse investigations.

A temperature controlled inoculation chamber was used in all infection studies. The humidity in the chamber was maintained at 100 per-

cent by the use of pneumatic atomizing nozzles. The temperature was maintained with a refrigeration system.

Three-week old plants were used in the studies on the relation of temperature and period of exposure to continued wetness to infection. Eight plots of four plants each were inoculated with each race of anthracnose using the Michelite variety for the alpha race and Dark Red Kidney for the beta and gamma races. One pot inoculated with each was removed after 12, 18, 24, 36, 48, 60 and 72 hours after inoculation. These studies were made at 10°, 15°, 20°, 25°, and 30°C. In each case the experiment was repeated. Disease readings were made 15 days after inoculation. The number of lesions served as a basis for comparison. Thus a reading of 0 = no infection, 1 = a trace, 2 = moderate, 3 = moderately heavy and 4 = severe infection with the plants all dying. Readings were made upon both stem and leaf infection.

In order to study stage of host development in relation to infection, beans were planted at weekly intervals until the first planting reached maturity. At this time a plant 3 weeks old had 2 primary leaves; the 4-week old plants were in the 1-2 trifoliate stage; the 5-week old plants were beginning to flower; the 6 and 7-week old plants were in the past flowering stage with small to fully developed pods; and the 10-week old plants had pods that were completely mature. Similar plantings were made in order to obtain pods in all stages of development for studies on the relation of age of pod to infection and penetration. In this case each flower was tagged at the time it opened. The plants were inoculated at the same time and placed in the inoculation chamber at 24°C. for 48 hours. After 10 days of incubation, the readings were made using the disease index mentioned above.

EXPERIMENTAL RESULTS

Laboratory investigations

Comparison of media for sporulation

A comparison was made of several media in order to obtain a medium upon which abundant spores could be produced in as short a time as possible. This knowledge was necessary in order to obtain inoculum for use in these studies as well as for possible use on a field scale in a breeding program. The results presented in Table 1 indicate that Colletotrichum lindemuthianum produced twice as many spores on Difco bean pod agar as on any of the media tested. Furthermore, this medium was readily prepared. Probably there is not a good correlation between the results obtained with the media placed in Petri plates and those on test tubes because of differences in the dimensions of the colonies produced, but as far as the Difco bean pod agar is concerned a large number were produced on the medium in the tubes as well as on the plates and hence it was chosen for the following experiments.

Effect of temperature on sporulation

Difco bean pod and lima bean agars were used to compare the effects of temperature on sporulation of the three races of anthracnose. The results are presented in Table 2. It is significant to note that no sporulation occurred at 28°C. on the lima bean agar and that all the counts on the lima bean agar were considerably lower than those on the

TABLE 1. A comparison of spore production by the three races of *Colletotrichum lindemuthianum* on different culture media.

Culture medium[1/]	Average No. spores/colony (millions)		
	alpha	beta	gamma
Difco bean pod agar	1987	1756	1600
Sterilized barley seed	958	747	945
Sterilized bean pods	947	848	925
Difco lima bean agar	647	540	515
Synthetic agar	547	610	445
Natural bean pod agar	143	145	105
Natural navy bean pod agar	140	127	158
Difco corn meal agar	1	1	1

1/ See Materials and Methods.

TABLE 2. The effect of temperature on sporulation by the three races of Colletotrichum lindemuthianum.

Temperature degrees C.	Lima bean agar			Bean pod agar		
	alpha	beta	gamma	alpha	beta	gamma
	Millions of spores per colony[1]					
12	0	0	0	0	0	0
16	71	175	87	162	131	151
20	147	148	210	415	740	684
24	410	433	414	945	1022	1354
26	566	582	516	1756	2015	1867
28	0	0	0	153	275	284
30	0	0	0	0	0	0
37	0	0	0	0	0	0

[1] Spore counts were made 8 days after inoculation.

bean pod agar. This indicates that the lima bean medium was not as satisfactory as the bean pod medium for spore production. In both cases maximum sporulation occurred at 26°C. and sporulation was inhibited at 12°C. but not at 16°C. On the bean pod agar no sporulation occurred at 30°C. but did at 28°C. All races reacted similarly at the different temperatures. The results obtained indicated a higher maximum temperature for sporulation than that obtained by Mathur et. al. (21) for the gamma race. His results showed that 20°C. was optimum for sporulation.

Since the optimum temperature for sporulation was 26°C., this temperature was used for the production of inoculum for use in the greenhouse experiments.

Influence of temperature on spore germination

Another important objective was to study the influence of temperature upon spore germination, and to determine if the three races of anthracnose would react similarly at the different temperatures. The results obtained are presented in Table 3 and illustrated in figures 1 and 2. The results definitely show that 28°C. was the optimum temperature for spore germination of all three races of anthracnose. But even though the optimum temperature for germination was the same for all races, the rate of germination varied at different temperatures for the different races. For example, no germination had taken place after 24 hours by the alpha race incubated at 16° and 20°C. whereas both the beta and gamma races showed some germination after 9 hours at these two temperatures. Thirty-three hours after the cultures were seeded, all the spores of the gamma and nearly all of the beta race had germinated

TABLE 3. Effect of temperature upon germination of spores of the three races of Colletotrichum lindemuthianum.

Temperature degrees C.	Time in hours: 9	24	33	48
	Percentage germination			
	alpha race			
16	0	0	3	10
20	0	0	19	22
24	5	5	18	16
28	60	90	96	100
32	4	9	33	28
	beta race			
16	13	19	17	50
20	31	37	63	100
24	32	32	94	100
28	75	93	100	100
32	18	25	33	33
	gamma race			
16	5	6	4	7
20	12	16	29	100
24	36	62	100	100
28	54	96	100	100
32	6	6	12	18

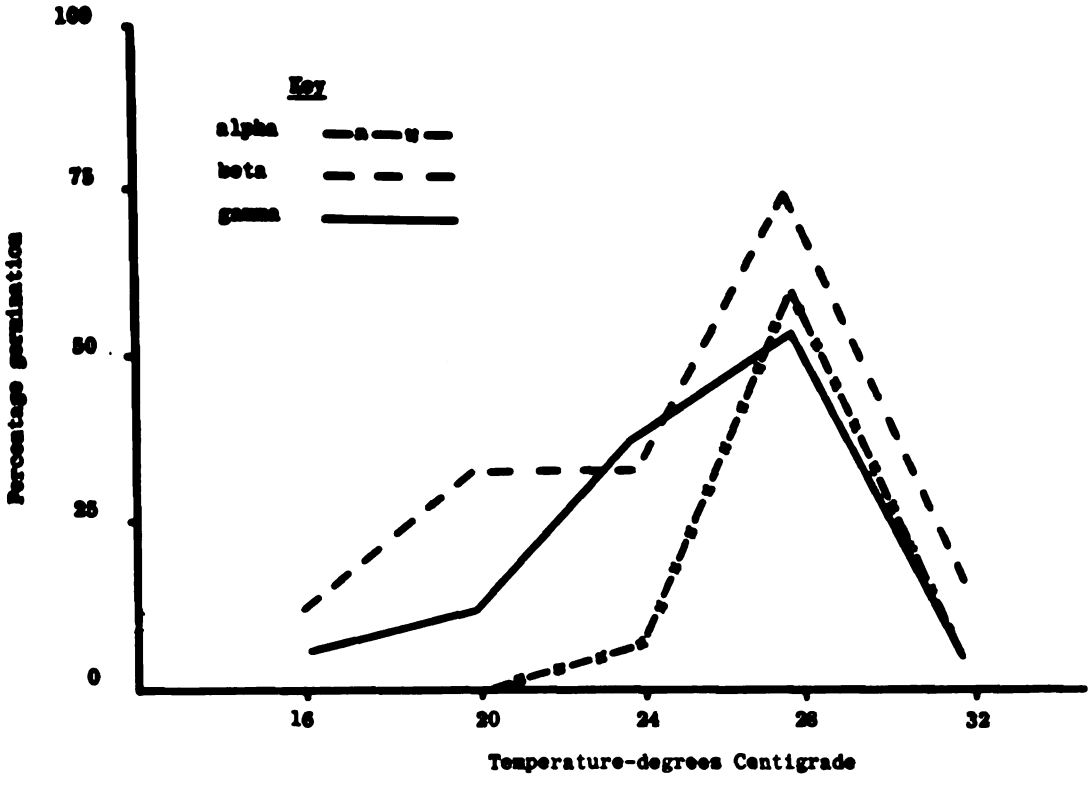

Figure 1. Effect of temperature upon spore germination of alpha, beta and gamma races of Colletotrichum lindemuthianum after 9 hours.

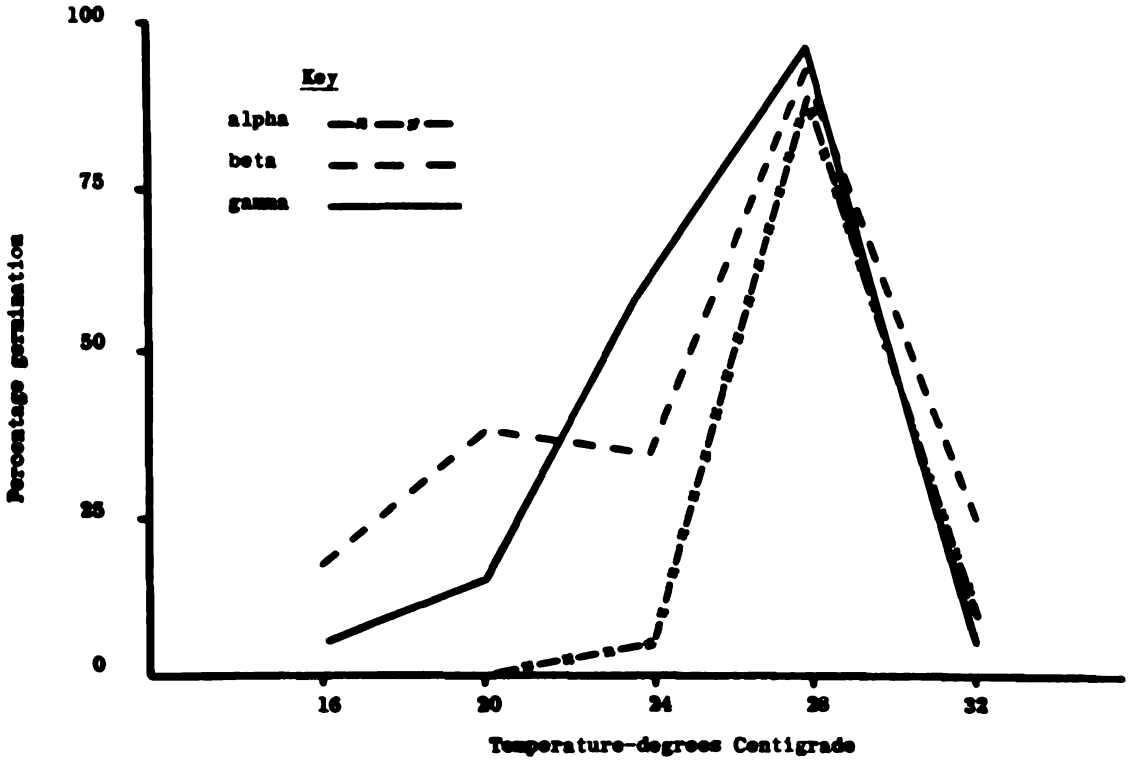

Figure 2. Effect of temperature upon spore germination of alpha, beta and gamma races of Colletotrichum lindemuthianum after 24 hours.

at the end of the same period. There was a similar trend after 48 hours. The results point to a narrow optimum temperature range for the alpha, and a very wide range for the beta and gamma races.

The germ tubes produced at the different temperatures varied somewhat. Those produced at 28°C. and lower were normal in appearance whereas those produced at 32°C. were thick, somewhat distorted and very short. Leach (20) made similar observations when he was studying germination.

Greenhouse investigations.

Relation of air temperature and period of continued wetness to infection.

In order to study the effect of air temperature and period of continued wetness in relation to infection, and to compare the reactions of the three races of anthracnose under these conditions, a series of experiments were conducted in which inoculated plants were removed at regular intervals from the inoculation chamber and placed upon the greenhouse bench for the remainder of the incubation period. A separate experiment was made for each temperature and all precautions were taken so as not to get cross contamination between the beta and gamma races. The results from this series of experiments are tabulated in Table 4.

Very little infection occurred at 10°C. even after 72 hours exposure to continued wetness. In this case the infection occurred only on the leaves of the plants inoculated with the alpha and gamma races. No infection was observed on any of the plants exposed to 60 hours or less in the moist chamber.

More infection occurred on plants exposed at 15° than at 10°C.

TABLE 4. Relation of temperature and period of exposure to continued wetness to infection of the variety Michelite by Colletotrichum lindemuthianum race alpha, and the variety Dark Red Kidney by the races beta and gamma.

Period of exposure to continued wetness. (hours)	Michelite		Dark Red Kidney			
	alpha		beta		gamma	
	Disease index**					
	stem	leaves	stem	leaves	stem	leaves
	at 10°C.					
12	0	0	0	0	0	0
18	0	0	0	0	0	0
24	0	0	0	0	0	0
36	0	0	0	0	0	0
48	0	0	0	0	0	0
60	0	0	0	0	0	0
72	0	1	0	0	0	1
	at 15°C.					
12	0	0	0	0	0	1
18	0	0	0	1	1	2
24	0	1	1	2	1	3
36	0	1	1	2	1	2
48	0	1	1	2	2	2
60	1	2	1	2	2	2

Continuation of Table 4.

Period of exposure to continued wetness. (hours)	Michelite		Dark Red Kidney			
	alpha		beta		gamma	
	Disease index**					
	stem	leaves	stem	leaves	stem	leaves
	at 20°C.					
12	2	2	3	3	2	4
16	3	4	4	4	4	4
18	4	4	4	4	4	4
24	4	4	4	4	4	4
36	4	4	4	4	4	4
48	4	4	4	4	4	4
	at 25°C.					
12	2	2	3	3	2	4
16	3	4	4	4	4	4
18	4	4	4	4	4	4
24	4	4	4	4	4	4
36	4	4	4	4	4	4
48	4	4	4	4	4	4
	at 30°C.					
12	0	0	0	0	0	0
16	0	0	0	0	0	0
18	0	0	0	0	0	0
24	0	0	0	0	1	1
36	0	1	0	0	1	1
48	0	1	2	2	1	1

Continuation of Table 4.

** Disease index rating.

0 = no infection.

1 = trace of infection.

2 = moderate infection.

3 = moderately heavy infection.

4 = severe infection.

At 15°C. the Michelite variety showed a trace of infection on the leaves after 24, 36 and 48 hours and a moderate amount of infection after 60 hours exposure to moisture. No infection occurred on the stems until after 60 hours exposure and then only a trace. There was also a trace of infection in the stems by the beta and gamma races after 24 hours exposure and a moderate amount of infection in the leaves after 18 hours.

The infection at 20° and 25°C. was much more severe than at the two lower temperatures tested. At these temperatures a moderate amount of infection was established after 12 hours exposure to moisture and after 16 hours of exposure severe infection resulted. There was no apparent difference in reaction between the three races.

The highest temperature (30°C.) used in the disease infection studies was above the optimum for infection. At this temperature no infection resulted on the Michelite variety until after 48 hours exposure to continued wetness and that was on the leaves only. In the case of the beta race, no infection was established until after 60 hours of exposure to moisture. A trace of infection was established by the gamma race in 36 hours. Apparently the short thickened germ tubes observed on spores germinated at 30°C. can be correlated with the poor infection established at this temperature.

The relation of stage of host development to infection.

In order to better understand disease development it is well to know the reaction of the host to infection in the various stages of development. To obtain this information plants in all stages of development were inoculated at the same time and then the development of the disease was observed. In Table 5 are listed the disease reactions as

observed on the various portions of the plant at the different stages of development. The reactions are given for all three races.

The results in Table 5 indicate that there is actually little or no difference in reaction between the various races of Colletotrichum lindemuthianum. The leaves appear to be the most sensitive to infection with severe infection occurring on young plants and only a trace or light infection on the 10-week old plants. A similar reaction was noted for stem infection, except that the disease developed less on the stems than on the leaves. The lesions that developed on the pods tended to be larger and more severe in the case of plants inoculated with the alpha and beta races.

Pod infection

In order to clarify the observations made in the preceding section as far as pod infection was concerned, an experiment was designed to compare the infection of pods in different stages of growth. The results from an experiment in which flowers were tagged over a 40 day period and the plants thereafter inoculated using the alpha and beta races of anthracnose, are given in Table 6. Severe pod infection occurred on Michelite pods 1 to 3 days old. A moderate amount of infection developed on pods 3 to 18 days old. No infection occurred on pods 19 days old or older.

The beta race did not react entirely like the alpha race in this respect. Although severe infection occurred on the 1 to 4 day old pods, and the degree of infection decreased as the pods became older, the pods of Dark Red Kidney bean were receptive to infection for 23 days after

initiation as compared to 19 for the Michelite pods. This difference may be because of the differences in fleshiness of the pods, the Michelite type being thinner and maturing faster than the heavier Dark Red Kidney pod.

Seed infection occurred at any stage of pod development where infection had taken place.

TABLE 5. Infection at different stages of plant development by the three races of anthracnose.

Age of plant (weeks)	Stage of plant development	Disease index[1]			
		leaves	stems	pods	general
	Michelite inoculated with alpha race				
3	Primary leaves only	4	2	-	4
4	2 compound leaves	4	3	-	3
5	4 compound leaves	3	2	-	3
6	early flowering	3	2	4	3
7	flowering, small pods	2	1	4	3
8	flowering, many pods	2	1	3	1
9	past flowering, green pods	2	1	3	2
10	past flowering, mature pods	1	1	1	1
	Dark Red Kidney inoculated with beta race				
3	Primary leaves only	4	3	-	3
4	2 compound leaves	3	2	-	2
5	4 compound leaves	3	2	-	2
6	early flowering	3	2	-	2
7	flowering, small pods	3	1	3	2
8	flowering, many pods	2	1	3	2
9	past flowering, green pods	1	1	2	1
10	past flowering, mature pods	1	0	1	1

Continuation of Table 5.

Age of plant (weeks)	Stage of plant development	Disease index[1/]			
		leaves	stems	pods	general
	Dark Red Kidney inoculated with gamma race				
3	Primary leaves	4	4	-	4
4	2 compound leaves	4	3	-	3
5	4 compound leaves	4	3	-	3
6	early flowering	3	3	-	3
7	flowering, small pods	3	3	3	3
8	flowering, many pods	2	3	4	3
9	past flowering, green pods	2	2	3	3
10	past flowering, mature pods	2	1	2	2

1/ See footnote Table 4.

TABLE 6. Relation of age of pod to infection by alpha and beta races of anthracnose.

Age of pods (days)	Variety and race	
	Michelite (alpha)	Dark Red Kidney (beta)
	Infection rating[1/]	
1	4	4
2	4	4
3	4	4
4	3	4
5	-	-
6	-	3
7	3	3
8	3	2
9	3	-
10	1	0
11	2	3
12	2	2
13	1	0
14	2	3
15	-	1
16	2	1
17	2	3
18	3	2
19	0	1

Continuation of Table 6.

Age of pods (days)	Variety and race	
	Michelite (alpha)	Dark Red Kidney (beta)
	Infection rating[1/]	
20	0	0
21	0	3
22	0	2
23	0	2
24	0	0

1/ See footnote Table 4.

DISCUSSION

The maintenance of good sporulating cultures of Colletotrichum lindemuthianum is essential to carrying out a good research and breeding program involving this pathogen. The characteristics of a good medium include, in addition to its sporulating ability, ease of preparation and availability.

Difco bean pod agar appeared to fit the qualifications as compared to Difco nutrient agar, Difco lima bean agar, bean pods, bean pod agar, navy bean agar, corn meal agar, barley grain medium and a synthetic agar medium. Mathur et. al. (21) reported that a medium containing glucose, mineral salts and neo-peptone gave as good sporulation as that produced on bean pods or bean agar. Sterilized bean pods, as shown in Table 1, proved to be a good medium for spore production but it is sometimes difficult to have fresh pods at the time it is necessary to produce inoculum. Difco bean pod agar, prepared in Fernback flasks, was easily made and sterilized. This gave a large surface for spore production.

The use of barley seed also gave good spore production, probably because of the larger surface area as compared to the usual agar media. But this method requires soaking of the grain as well as autoclaving two days in succession in order to be sure the grain is sterilized.

One of the greatest difficulties encountered with Colletotrichum lindemuthianum is the maintenance of sporulating cultures, especially during the summer months when the laboratory temperatures go up above 28°C. Furthermore, at these temperatures it was impossible to produce

any spore inoculum in quantity for field inoculations. It appeared that temperature may have been the main factor causing the discontinuance or inhibition of sporulation.

The results obtained in these investigations support the theory that temperature is a limitating factor in spore production. For example, the data in Table 2 shows that sporulation was inhibited at 30°C. on the bean pod agar and at 28°C. on the lima bean agar. Very good sporulation occurred at temperatures from 20° to 26°C. with the optimum temperature at 26°C. Hence in order to produce spores in any quantity, it is necessary that temperature be maintained below 28°C.

Observations on the presence of bean anthracnose in Michigan the past nine years have followed a pattern that appeared to be associated with moisture. For example, in the colored bean areas extending from Montcalm county west along highway M-46 to Muskegon, Michigan, the beta race of anthracnose has been generally present on Dark Red Kidney and Cranberry beans every year. On the other hand, the alpha race has only appeared in epiphytotic proportions twice in the navy bean area during the same period and that was in 1950-51 and in 1956, these years had heavy rainfall and extended periods of wet weather. The navy bean area extends from Gratiot county and east along M-46 and up into the Thumb area of Michigan. The average daily maximum and minimum temperatures during July and August are nearly the same for these two areas (26°-29°C. maximum and 13°-17°C. minimum). The data in Table 3 for both the alpha and beta races may possibly be correlated with the above observations. For example, the alpha race did not germinate in 24 hours or less at 16° and 20°C. indicating that extended moist periods of 24 hours or

more are essential for establishing the disease in proportions that would tend to make it epiphytotic. On the other hand, the beta race germinated at 16°C. in 9 hours, hence it would be able to establish itself during periods of heavy dews and light showers that would tend to keep the plants moist for a period of more than 9 hours. Such conditions are generally present in the bean growing area. The results in Table 4 on infection also tend to support the above supposition. For example at 15°C., which is the average minimum or night temperature in the bean growing areas, the beta race was able to establish itself in the leaves after 18 hours exposure to continued wetness whereas the alpha race required 24 hours of continued wetness to establish itself at this temperature.

The investigations on the susceptibility of the host at various stages of plant development appear to support observations on the disease made under field conditions. For example, young plants in the seedling and trifoliate stages were the most susceptible to infection and were readily killed in these stages. As the plant increased in age its stem and leaf tissues became less susceptible to infection. After flowering, when pod setting had commenced, only the new leaves and shoots were susceptible (Table 5). The pods at this stage were also very susceptible and as they matured less infection developed. In the investigations presented in this paper it was found that pods on the Dark Red Kidney beans over 24 days old were not attacked by the pathogen.

These results agree with those of Leach (20) who observed that the size of the lesions produced on a susceptible bean plant, when inoculated with Colletotrichum lindemuthianum, was inversely proportional to the age of the tissue inoculated.

SUMMARY

1. Several media were tested in order to select a good media for sporulation. Difco bean pod agar, Difco lima bean agar, barley seeds and bean pods, produced the best results among the media tested. However, because of availability and ease in preparation, bean pod agar was selected for use in the investigations presented in this paper.

2. The optimum temperature for sporulation was 26°C. The minimum temperature tested at which sporulation occurred was 16°C. and the maximum 28°C.

3. In studying the effect of temperature upon the germination of the three races of anthracnose, it was found that the beta and gamma races germinated over a wider range of temperature conditions than race alpha. Thus after 24 hours the spores of the alpha race had a germination of 0, 0, 5, 90, and 9 percent respectively at 16°, 20°, 24°, 28° and 32°C. as compared to 19, 37, 32, 93 and 25 percent for beta and 6, 16, 62, 96 and 6 percent for the gamma race.

4. Little or no plant infection was established even after 72 hours of exposure to continued wetness at 10°C. At 15°C., the ability to establish infection varied with the races, the gamma race establishing itself in the shortest period and the alpha race requiring the longest period. Infection was readily established in 12 hours at 20° and 25°C.

5. Bean plants were very susceptible to infection from the time of emergence until they were 3 to 5 weeks old. They become less susceptible to infection with age. The three races reacted in a similar manner.

6. The best pod infection was obtained on pods from 1 to 4 days old. Five to 23-day old pods appeared equally susceptible but less susceptible than the younger pods. Pods older than 24 days resisted infection under greenhouse conditions.

7. Seed infection may occur at any stage of pod development where infection has taken place.

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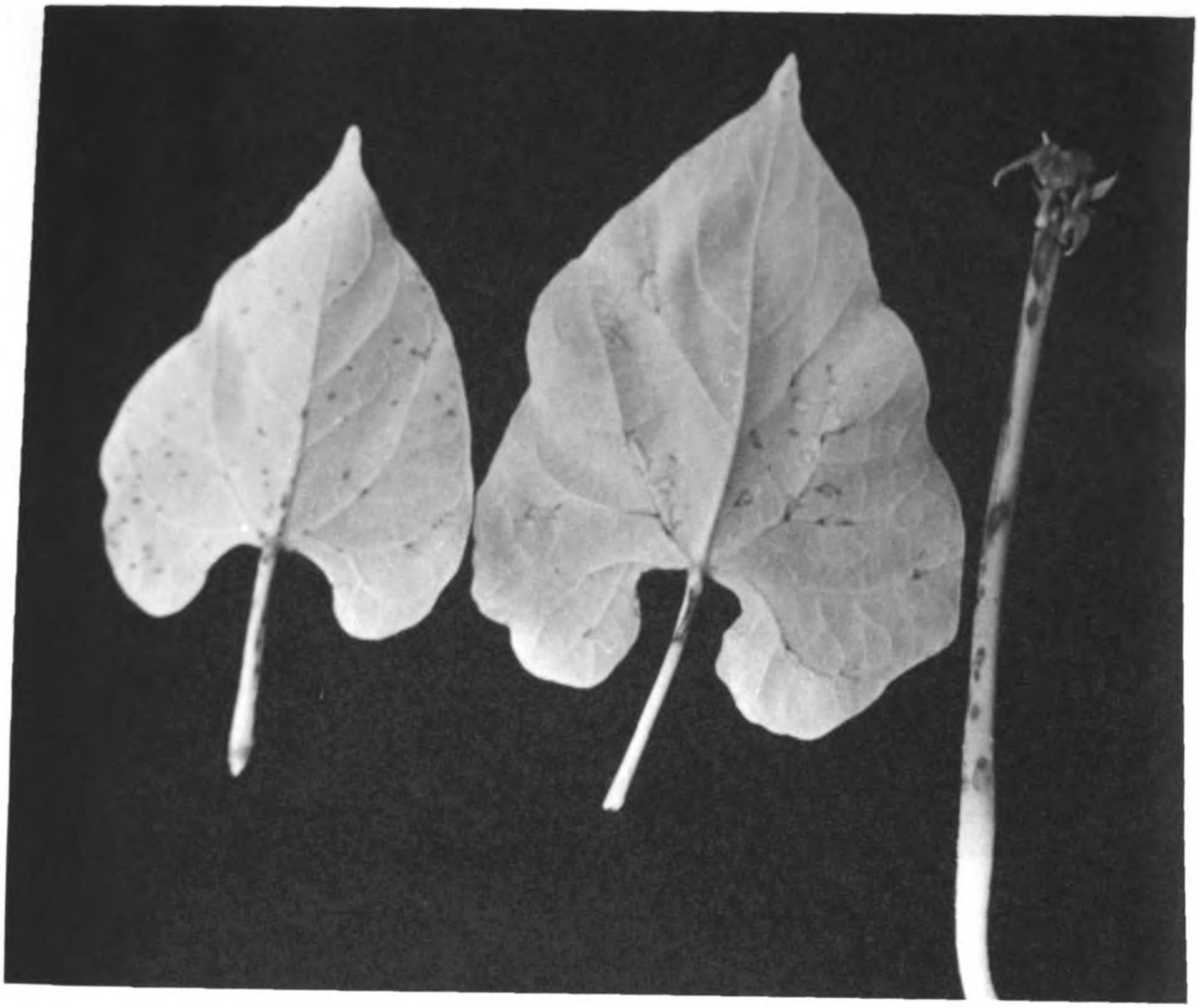

Plate I Anthracnose lesions on leaves and stems of Dark Red Kidney bean incited by the beta race of Colletotrichum lindemuthianum.

Plate II A From left to right, the varieties Michelite, Dark Red Kidney and Perry Marrow beans inoculated with the alpha race of Colletotrichum lindemuthianum.

Plate II B From left to right, the varieties Michelite, Dark Red Kidney and Perry Marrow beans inoculated with the beta race of Colletotrichum lindemuthianum.

Plate II C From left to right, the varieties Michelite, Dark Red Kidney and Perry Marrow beans inoculated with the gamma race of Colletotrichum lindemuthianum.

Date Due
