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Genetic System for Reaction in Field Beans (Phaseolus vulgaris L.) to Three Races of Colletotrichum lindemuthianum (Sacc. and Magn.) Brio, et Cav.

presented by

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Major professor

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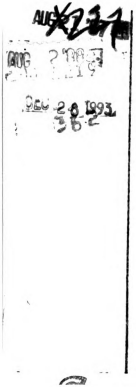

GENETIC SYSTEM FOR REACTION IN FIELD BEANS (*PHASEOLUS VULGARIS* L.) TO THREE RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* (SACC. AND MAGN.) BRIO. *ET* CAV.

By

Catherine S. Muhalet

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ABSTRACT

GENETIC SYSTEM FOR REACTION IN FIELD BEANS (*PHASEOLUS VULGARIS* L.) TO THREE RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* (SACC. AND MAGN.) BRIO. *ET* CAV.

By

Catherine S. Muhalet

Genetics of resistance for reaction of beans to the beta, gamma and delta races of *Colletotrichum lindemuthianum* was studied in ten crosses involving six cultivars. Reactions of individual plants to each race were studied in the parental, F_1 and F_2 generations.

Genetic resistance to race beta is explained by dominant alleles, except in crosses involving Tuscola with Montcalm and Swedish Brown where susceptibility was dominant. Tuscola transmits dominant genes for resistance to beta in other genetic backgrounds. A system of multiple alleles was proposed as governing reaction to race beta except for the "Are" gene. The genetic pattern of segregation revealed a distinct system of single dominant genes, duplicate and complementary factor loci conferring reaction to race beta.

A system of single dominant genes, duplicate and complementary factors also governs reaction to races gamma

and delta, but unlike the beta race, there was no evidence for multiple allelism.

There was a close association in reaction to races beta, gamma and delta in crosses involving C49242 as a common parent. It was, therefore, assumed that the association was between the factors conferring resistance in C49242 to each of the three races. Hence, the "Are" gene from C49242 was postulated to be a complex locus.

To my parents . . . .

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I. INTRODUCTION

Anthracnose of common beans (Phaseolus vulgaris L.) caused by Colletotrichum lindemuthianum (Sacc. and Magn.) Brio. et Cav. is a major disease in many dry edible bean growing areas of the world. The bean anthracnose fungus is seed borne and economical losses caused by the disease can be very high when badly contaminated seeds are planted under conditions favorable for disease development. Losses are due to poor germination of infected seeds, destruction of seedlings and low yield of infected plants. Spotted pods are unsaleable for table use or canning and seed value is lowered when the fungus penetrates into the seed. Breeding for disease resistance is believed to be one of the most appropriate measures of controlling this disease. C. lindemuthianum exists as many different physiological races. Various sources of resistance to different races are known and several of the sources have been utilized in breeding programs.

A race of the bean anthracnose fungus, identified as delta, was detected in Ontario, Canada in the growing season of 1976. This race could easily be introduced into Michigan through importation of infected seeds. Delta

race attacks cultivars with resistance to races alpha, beta and gamma. Bean lines, Cornell 49242 and Kaboon, are resistant to this race. The resistance in C49242 is controlled by a single dominant gene, "Are," which also confers resistance to other races of C. lindemuthianum except races kappa and iota. Anthracnose resistance in most European beans is controlled by the "Are" gene. Inheritance of resistance due to "Are" gene has not been studied in other genetic backgrounds. The black seeded nature of C49242 could be undesirable in some breeding programs.

A second source of resistance to delta race, Kaboon, was included in this study. This cultivar is a large white seeded bean which is resistant to most other races except gamma and iota. Kaboon has been extensively used as a differential variety in Europe but little is known of the inheritance of its resistance to races of C. lindemuthianum.

The objectives of this study were to investigate:

1. The inheritance of resistance to the delta race in crosses involving C49242 and Kaboon with other cultivars.
2. The pattern of inheritance to races beta and gamma of C. lindemuthianum and their linkage relationships to that of delta race.

In the course of this study some lethals and semi-lethals resulting from different crosses were observed. Notes were taken on the pattern of the segregation ratios of normal to semi-lethal plants.

II. LITERATURE REVIEW

1.0 Causal Organism, Disease Symptoms and Host Plants

Anthracnose of common beans (Phaseolus vulgaris L.) is a disease caused by the fungus Colletotrichum lindemuthianum (Sacc. and Magn.) Brio. et Cav. This disease is known wherever common beans are grown (Butler and Jones, 1949). It was first discovered in Germany in 1875 and appeared in England five years later.

The causal organism is a member of the Fungi Imperfecti. The perfect stage of the fungus exists, but rarely, and was originally termed Glomerella lindemuthianum (Shear and Wood, 1913). It has recently been renamed G. cingullata (Kimati and Gali, 1970).

The disease affects all plant parts above the soil level. Characteristic symptoms consist of dark brown to black rather sunken lesions surrounded by reddish or yellow and slightly raised margins. The lesions become darker in color as they enlarge. As the infected tissue becomes dry and collapses a depression develops at the center which, under moist condition, becomes pink and oily in appearance due to liberation of great quantities of spores. Lesions are very conspicuous on the pods and when pods are opened

the lesions on them are often found to have penetrated through the seed coat. On the foliage, the lesions are mostly on the veins and midrib on the underside of the leaves. Sometimes, a petiole is so badly affected that it fails to support the leaf.

This disease occurs mostly in common beans but has also been reported on other hosts, including small lima beans (Phaseolus lunatus L.), large lima beans (P. limensis Macf.), scarlet runner beans (P. coccineus Willd.), tepary bean (P. acutifolius Gray var. latifolius Freeman), mung bean (P. aureus Roxb.), cowpea (Vigna unguiculata Walp.), kudzu bean (Dolichos biflorus L.) and broad bean (Vicia faba L.) (8, 33, 43 and 45).

2.0 Environmental Conditions for Disease Development and Fungal Growth

Moderately cool, humid or rainy weather during the early part of growing season is essential for development of anthracnose in serious levels (Butler and Jones, 1949; Ransey and Wiant, 1941; Rao et al., 1976). Infection does not occur in the field during hot weather and the highest temperature during which undiminished infection occurred on etiolated seedlings was 27°C (Rahe and Kuc, 1970). The authors also found that lesions decreased in size with increasing temperature of incubation between 28-32°C.

Lauritzen (1919) determined that a temperature of 14°C was the lowest limit for the infection to occur during a 24 hour period of incubation. The highest temperature

was found to be 32°C at R. H. of 95% and above. Andrus and Wade (1942) pointed out that initial excess moisture and falling temperature were important for disease development in the mist chamber. Burkholder (1923) noticed that very little infection occurred at a temperature of 27°C and excellent infection occurred at 17°C. Kruger and Hoffman (1978) pointed out that at temperatures exceeding 21°C and at 15°C the intensity of infection decreased using 16 hours of light but at 12 hours of light and 15°C there was more infection than at higher temperatures.

C. lindemuthianum does not develop at temperatures above 31-32°C in culture and growth of the fungus on bean pod agar was near maximal at 18-26°C (Rahe and Kuc, 1970). According to Leakey and Simbwa-Bunnya (1972) the optimum growth of the fungus in culture is at 22-25°C.

3.0 Physiological Races of Colletotrichum lindemuthianum

Isolates of pathogens of the same species with similar or identical morphology but differing in pathogenicity on different varieties of the same host species are termed physiological races. Colletotrichum lindemuthianum is known to occur in numerous different physiological races. Barrus (1911) was the first to report that different cultures of the anthracnose-causing organism has different pathogenicity on different bean cultivars. He also observed that the races were regional in distribution. In 1918, Barrus reported results of inoculating a large collection of

dry and snap beans with ten different cultures of C. lindemuthianum. There appeared to be at least two distinct races of the organism differing in the ability to cause the disease which he designated as alpha and beta races.

Burkholder (1923) isolated a third race from the Imperial white bean variety which was distinct from the alpha and beta races. This race, which Burkholder thought had arisen from beta by mutation, was named gamma. By inoculating fourteen different varieties with various isolates from different sources, Leach (1923) found eight distinct races which were different from the three previously described races.

Schreiber (1932) characterized thirty-four different anthracnose races from inoculations of fifty-seven different bean varieties. Schreiber categorized the races into A, B and C which roughly correspond to the alpha, beta and gamma races of the American system.

Muller (1926) isolated five different physiological races distinct from alpha, beta and gamma in Holland. Andrus and Wade (1942) reported the occurrence of race delta in North Carolina. Waterhouse (1955) and Yerkes and Ortiz (1956) independently described many new races which essentially could be classed into four (alpha, beta, gamma and delta) already existing groups at that time (Hubbeling, 1961). Blondet (1962) described a new race, epsilon, in France. Oliari et al. (1973) identified seven physiological races of C. lindemuthianum in Minas Gerais state in Brazil

which, except for alpha, were different from the American races.

In the U.S.A. the differential varieties, Michelite, Dark Red Kidney, Perry Marrow, and Emerson 847 or Emerson 51-2 were used in identifying races alpha, beta, gamma and delta (Oliari et al., 1973). After the discovery of race epsilon, a new set of differential varieties (Widusa, Dark Red Kidney, Kaboon and Aiguilles Vert) was established in which fifteen physiological races belonging to races alpha, beta, gamma, delta and epsilon could be differentiated (29).

Hubbeling (1961, 1974) isolated a deviant mutant alpha which broke the resistance to alpha, beta, gamma, delta and epsilon; the new race was named lambda. Resistance to lambda also controls resistance to races mentioned so far. This source of resistance was found in the black seeded bean line Cornell 49242, which Hubbeling (1957) referred to as Phaseolus vulgaris nanus viridis, originating from Venezuela. The resistance in C49242 is conditioned by a single dominant gene, "Are" (Mastenbroek, 1960). The "Are" gene had a good run until 1972 at which time Leakey (1979) and Leakey and Simbwa-Bunnya (1972) reported that it failed to confer resistance against Ugandan isolates. According to Goth and Zaumeyer (1965) and Zaumeyer and Meiners (1975), bean line C49242 has undesirable genetic linkages. Fouilloux (1976) and Fouilloux and Bannerot (1977) developed four pairs of isogenic lines, which were

identical except for "Are" gene, with no apparent unfavorable pleiotropic effects.

A new race was detected at Ebnet in Germany in 1973 which broke the resistance of the "Are" gene derived from C49242. The new race was described by Hoffman et al. (1974, 1975) and Schnock (1975) and was named kappa because it was a killer of previously resistant beans. Hubbeling (1976) and Kruger et al. (1977) identified sources of resistance to race kappa which included the European bean cultivars, Kaboon, Coco a la creme, Coco rose selections, Evolutie and B022, and Asiatic cultivars such as Benishibori and Kiuzwa. All of these cultivars carry genes for resistance to alpha, delta and kappa but they are susceptible to lambda.

Hubbeling (1977) reported that in an experiment with the kappa race, several kappa resistant cultivars became infected. Anthracnose isolates from kappa-infected seedlings broke down the resistance of Kaboon, coco a la creme, coco rose, B022 and Evolutie as well as the resistance of "Are" gene to delta. This new race was named iota. The race does not occur under natural conditions (Hubbeling, 1977). Resistance to the iota race was found in the black, small seeded Mexican bean PI 165 422 and small light tan Colombian bean PI 207 262, both of which exhibit a high degree of resistance to races iota and kappa. Fouilloux (1976) described a new race, alpha Brazil, which broke down the resistance from "Are" gene. Fouilloux also in 1978 described another race which was isolated by Hubbeling and

was designated lambda mutant. Table 1 gives a summary of physiological races of C. lindemuthianum to date.

4.0 Genetic Basis for Resistance to Races of Colletotrichum lindemuthianum

Burkholder (1918) first provided evidence of the genetics of resistance of a host to a strain of pathogen. From a cross between bean varieties, Wells' Red Kidney (resistant to alpha and beta races) and White Marrow (susceptible to beta), Burkholder (1918) obtained a 3R:1S ratio in F_2 with race beta. McRostie (1918, 1921) obtained similar results. In a cross between Wells' Red Kidney and Michigan Robust (susceptible to alpha) he obtained a 3R:1S ratio in F_2 with resistance being dominant. McRostie (1921) made crosses between Selection B, a variety resistant to both alpha and beta races and a variety, German Wax, susceptible to both alpha and beta races respectively. He obtained a segregation ratio of 9R:7S in the F_2 population when beans were inoculated with a mixture of spores from the two races. In both races the resistance was governed by a single dominant gene in each case.

Mixed inoculations, as far as genetic studies with more than one race are concerned, can give information about resistance to an individual race. However, mixed inoculations cannot provide information about linkage relationship between the genes conferring resistance to races being studied. Cardenas (1960) provided a technique of inoculating each individual leaflet with one race.

Table 1.--Physiological races of Colletotrichum lindemuthianum to date, year reported and reactions of bean cultivars Kaboon and C49242.

Races	Year Reported	Varieties and their Reaction	
		Kaboon	C49242
Alpha	1918	R	R
Beta	1918	R	R
Gamma	1923	S	R
Delta	1942	R	R
Epsilon	1962	R	R
Lambda	1961 & 1974	S	R
Kappa	1973 & 1975	R	S
Alpha Brazil	1976	?	S
Iota	1977	S	S
Lambda mutant	1978	?	?

R = Resistant; S = Susceptible; ? = No information available.

Schreiber (1932) studied genetics of resistance to races alpha, beta and gamma separately in crosses between Anthracnose Resistant 22 (resistant to all three races) and Conserva (susceptible). Segregation ratios obtained in F_2 were 3R:1S with a single race; 9R:7S with a mixture of two races and 27R:37S with a mixture of three races. He concluded each of the three factors conferring resistance was on separate chromosomes.

York (1950) studied inheritance to the alpha, beta and gamma races of C. lindemuthianum in crosses involving three bean varieties. Variety Tendergreen was susceptible to all three races, Emerson 51 was resistant to all three races and Red Mexican was susceptible to alpha but resistant to beta and gamma. The F_2 populations in each of the crosses was divided into three parts in order to test for each of the races separately. In the Tendergreen X Red Mexican cross all F_2 plants were susceptible to race alpha. Ratios of 3R:1S for race beta and 9R:7S for race gamma were observed. F_2 population from Tendergreen X Emerson 51 segregated into 3R:1S for alpha, 15:1 for beta and 63:1 for race gamma.

Rudorf (1958) reported on resistance to alpha, beta, gamma and delta races in Phaseolus aborigineus, a presumed ancestral bean type. He hybridized P. aborigineus with a fully susceptible bean variety and tested F_2 against all the races. From P. aborigineus, a single dominant gene conferred resistance to race beta, and two complementary

dominant genes were required for resistance to alpha, gamma and delta.

Data from investigations by Andrus and Wade (1942), Cardenas (1960) and Cardenas et al. (1964), suggest that both simple and complex genetic backgrounds condition reactions to races alpha, beta and gamma. The genetic systems include both duplicate and complementary genes (2, 9 and 10), with some linkage between duplicate genes which condition reaction to races beta and gamma (9 and 10).

Andrus and Wade (1942) studied inheritance of resistance to races beta, gamma and delta separately in thirty intervarietal crosses. In crosses between resistant X resistant (R X R), all F_1 and F_2 progenies were resistant except in one case where a segregation ratio of 15R:1S was obtained in F_2 against the beta race. For the same race beta, in crosses of resistant X susceptible (R X S) and tolerance X susceptible (T X S) resistance was dominant except in one case where susceptibility was dominant. Segregation ratios of 3:1, 1:3, 15:1, 13:3 and 63:1 were obtained. Unusual segregation ratios like 11:5, 14:2 and 62:2 were also observed. These ratios were explained by the possibility of partial dominance, gene interaction with the environment, and contribution from lethal factors.

For races beta and gamma, Andrus and Wade (1942) postulated that there are three series of multiple alleles with ten alleles such that some alleles in each series

conferred resistance or susceptibility depending on the genes present in the other series.

In the case of delta race, crosses of R X S resulted in segregation ratio of 3:1 and 9:7 in F_2 with resistance being dominant. F_1 and F_2 progenies of S X S parents were found to be susceptible.

For the overall genetic interpretation of reaction to races beta, gamma and delta, Andrus and Wade (1942) made a complex hypothesis. They postulated that a system of ten genes in three allelomorphic series involving both duplicate and complementary gene interaction at three points as a simple Mendelian hypothesis that could explain data for races beta and gamma. A system of three independent pairs of genes was assumed to explain data for delta race. Also, there was no strong indication of linkage in their results.

Cardenas (1960), Cardenas et al. (1964) extensively studied genetics of reaction to races alpha, beta and gamma races in nine bean cultivars. They observed simple segregation ratios of 3:1 and 15:1 for reaction to race alpha, which was explained on the assumption that there are two loci, the dominant alleles of which are individually capable of conferring resistance. They encountered much more complex genetic systems conferring resistance to races beta and gamma. For beta race, F_1 progenies in three of the R X S crosses were susceptible, where segregation ratios in F_2 were 1:3 for one of the crosses and 7:9 for the second cross and 13:3 for the third. These results were explained

by assuming that two loci were involved where one locus segregated into 3:1 and the other into 1:3 ratios. For beta race, there were crosses in which both sets of complementary and duplicate genes were segregating simultaneously giving ratios of R:S on complete independence in transmission of loci involved. Bean variety Michigan Dark Red Kidney transmits dominant genes for susceptibility only when crossed with Michelite and Brazilian Dark Red varieties which otherwise express dominant genes for resistance.

Cardenas (1960) and Cardenas et al. (1964) also obtained similar genetic patterns for races gamma and beta. In the case of race gamma, crosses of Algarrobo and Emerson 847 with Perry Marrow suggested the presence of a third gene complementary to the two other genes conferring resistance to gamma.

The authors offered proposed genotypes of the nine bean varieties used in their study relative to their reactions to races alpha, beta and gamma of C. lindemuthianum. In addition, three different linkages were detected in crosses which involved genes conditioning reaction to races beta and gamma.

Cardenas (1960) and Cardenas et al. (1964) concluded that there are five main genetic aparati in P. vulgaris conditioning reaction to alpha, beta and gamma as follows:

1. A duplicate-factor set of loci, with resistance usually but not always dominant.

2. A complementary factor set of loci.

3. The genes belonging to the set(s) pertaining to any one race are not linked to each other.

4. There exist strong linkages between genes of the duplicate factor set conditioning reaction to gamma and genes of the duplicate-factor set and complementary factor sets conditioning reactions to beta.

5. Multiple allelism at the duplicate- and complementary-factor loci conditioning reaction to beta.

Hubbeling and Dijke (1979) reported on the genetics of resistance to races iota, kappa and lambda. In their study, the only source of resistance to lambda race was "Are" gene from C49242. The sources of resistance to iota were a single dominant gene from PI 150414 and a second dominant gene from PI 165435. A set of single dominant genes common to both Uberabinha and PI 165422 condition resistance to race iota. Three duplicate pairs of genes in cross of Uberabinha and PI 165422 controlled resistance to race kappa. PI 150414 had two dominant complementary genes and Kaboon had a single dominant gene conferring resistance to race kappa.

All the studies so far have dealt with vertical resistance. I have not encountered any report suggesting the presence of horizontal resistance to bean anthracnose.

III. MATERIALS AND METHODS

1.0 Cultures of the Pathogen

Cultures of the races beta, gamma and delta of C. lindemuthianum were obtained from the American Type Culture Collection as numbers 16989 (beta), 16990 (gamma) and 18989 (delta). Cultures were maintained on commercial bean pod agar (BPA).

Bean pod agar plates were prepared by dissolving 22.5 gm of commercial BPA (Difco) in 1 litre of distilled water. The suspension was steamed for about 20 minutes until dissolved, transferred in 250 ml lots to prescription bottles, and autoclaved for 20 minutes at 250°F and 15 psi. Plates containing about 25 ml of BPA were prepared and allowed to stand for two days after which any contaminated plates were discarded. Occasionally, to promote sporulation a 10 gm of navy bean seeds were steamed in 200 ml of distilled water for 30 minutes, ground in a mortar and paste, and added to BPA.

Cultures were transferred at 7-10 day intervals to maintain sporulation. Small discs of BPA from a culture plate were transferred to 1.5 ml of sterile distilled water in small test tubes. The test tubes were vigorously shaken

to dislodge spores and the spore suspensions were uniformly spread onto BPA plates. Plates were incubated in a dark drawer, and cultures were ready about one week later.

2.0 Preparation of Inoculum

Plants were inoculated by either a brushing or spraying method; there was only a slight difference in the method of preparing spore suspensions. Phosphate buffer, 0.01M, pH 7.2, was used for preparing spore suspensions. Tween 80 (Polyoxythelene Sorbitan Monooleate), a wetting agent, was added to the buffer at 0.1% v/v. For the brushing method of inoculation, about 25 ml of buffered, sterile, distilled water containing Tween 80 was added into a plate culture and the spores dislodged by scraping with a sterile microscope slide. Spore suspensions were then delivered to the underside of the leaflets by using a No. 4 camel's hair brush.

For the spraying method of inoculation, 25 ml of sterile distilled water containing 0.1% v/v Tween 80 was added to the plate culture and the spores were dislodged with a microscope slide as above. Spore suspensions were filtered through a double layer of cheese cloth. Spore concentrations were determined with a haemocytometer and diluted to contain about 10^6 spores/ml. Spore suspensions were atomized in such a way as to deposit a uniform and fine pattern on the underside of the leaves with just enough pressure not to damage the leaf tissue.

3.0 Standardization of Spore Concentration

A preliminary study was performed to determine the optimum spore concentration necessary to yield consistent disease readings in a split plot design with three replications in a randomized complete block. Three different spore concentrations were used as whole units and six bean varieties, used as parent plants, as subunits. Each race was run in a separate experiment. The three different spore concentrations used were high (10^6 spores/ml), medium (10^5 spores/ml) and low (10^4 spores/ml).

Young fully expanded trifoliates were excised from plants, transferred into labelled test tubes and sprayed with spore suspensions. Test tubes were filled with dilute solution of a complete plant nutrient. Inoculated leaves were quickly transferred to a mist chamber maintained near 100% R.H. at ambient greenhouse temperature. After 8-10 days in a chamber, leaves were assessed for disease development.

4.0 Bean Cultivars and Crosses

Six bean varieties all belonging to the Phaseolus vulgaris L. specie were used in this study. Seed type and reaction of the varieties to each of the three pathogen races are given in Table 2.

The following crosses were made in the greenhouse in Spring and Summer of 1978:

Table 2.--Bean varieties used, their seed types and reactions to races beta, gamma and delta of C. lindemuthianum.

Varieties	Seed Types	Reaction to Races		
		Beta	Gamma	Delta
C49242	Small black	R	R	R
Kaboon	Large white	R	S	R
Montcalm	Kidney	S	MS	S
Swedish Brown	Medium brown	S	S	S
Tuscola	Navy	R	R	S
61294	Navy	MR	MR	MR

R = Resistant; S = Susceptible; MR = Moderately resistant, very small lesions on the midrib, petioles and stems; MS = Moderately susceptible, small lesions on the midrib, veins and few large lesions on petioles and stems.

Female/Male	Female/Male
Kaboon X C49242	C49242 X Kaboon
C49242 X Montcalm	Montcalm X Kaboon
C49242 X Swedish Brown	Swedish Brown X Kaboon
C49242 X Tuscola	Tuscola X Kaboon
61294 X C49242	61294 X Kaboon
Swedish Brown X Tuscola	
Montcalm X Tuscola	

The six parents and F_1 plants from the above crosses were tested against each of the physiological races, beta,

gamma and delta. The F_2 populations tested are given in Table 3.

5.0 Inoculation of Plants

Parental plants were tested by using three methods:

1. Young, newly opened trifoliolates were excised and transferred into labeled test tubes filled with dilute solution of complete plant nutrient. The leaves were then sprayed with spore suspensions. Separate leaves for each variety were used for each race. Spore concentrations of about 10^6 spores/ml were used.
2. Entire plants possessing fully expanded first trifoliolate leaves (14-18 days after planting) were thoroughly sprayed with spore suspension separately for each race.
3. One individual leaflet of a newly opened trifoliolate each per variety was brushed with spore suspension of a single race. Plants were inoculated 14-18 days after planting.

Four plants for each variety were used with all three methods. Immediately after inoculation plants or excised leaves were transferred into a mist chamber maintained at near 100% R.H. at ambient greenhouse temperature. Inoculations were performed on cloudy days and early in the morning when temperatures were low. Plants were kept in the mist chamber for 8-10 days.

Table 3.--Crosses in which F_2 populations were tested, the races used and method of inoculation.

Crosses Female/Male	Method of Inoculation	Races Used		
Kaboon X C49242	SP, BR	Beta	Gamma	Delta
C49242 X Montcalm	SP, BR	Beta	Gamma	Delta
C49242 X Swedish Brown	SP	Beta	Gamma	Delta
Tuscola X C49242	SP, BR	Beta	Gamma	Delta
61294 X C49242	BR	Beta	Gamma	Delta
C49242 X Kaboon	SP, BR	Beta	Gamma	Delta
Montcalm X Kaboon	BR	Beta	-	Delta
Swedish Brown X Kaboon	BR	Beta	-	Delta
Tuscola X Kaboon	SP, BR	Beta	-	Delta
Swedish Brown X Tuscola	BR	Beta	Gamma	-
Montcalm X Tuscola	BR	Beta	Gamma	-

SP = Excised leaves were sprayed with spore suspension from individual race.

BR = Each leaflet was brushed with spore suspension from individual race.

- = Not tested.

All F_1 progenies were tested together with their parents and corresponding F_2 populations. All genotypes were tested separately by brushing individual leaflets with spores of a single race. Inoculated plants were transferred into mist chamber as described previously.

Each individual plant in each F_2 population was tested against each of the three races. This was accomplished by inoculating excised or intact leaves with a spore suspension from a single race, by either brushing or spraying as previously explained. Testing each individual plant in F_2 populations with each of the races facilitated a test for linkage relationships between host genes controlling the reaction to the races of the pathogen.

Leaves were usually assessed for disease development 8-10 days after inoculation. Occasionally, plants were left on greenhouse benches for one week, after they had been removed from the mist chamber, before assessing the symptoms.

Plants were assessed according to the infection rate of the disease in a 1-5 scale as follows:

1. Clean, no disease symptoms.
2. A few scattered, small lesions on the midrib and occasionally on main veins. Lesions were corky in appearance.
3. Many small lesions scattered on the midrib and veins with collapse of the tissue.
4. Few large lesions scattered over the leaf blade or many large lesions over the leaf blade.

5. Many large coalesced lesions accompanied by tissue breakdown and sometimes leaves became chlorotic and abscised.

If one assumes that each of the above categories or classes represents a different genetic grouping, it would be necessary to attempt to fit the observed data to genetic models appropriate to five genotypic classes.

An obvious model for five genotypic classes is one involving two loci, where the following states would exist in an F_2:

4 "+" alleles, respectively AABB

3 "+" alleles, respectively AABb, AaBB

2 "+" alleles, respectively AAbb, aaBB, AaBb

1 "+" alleles, respectively Aabb aaBb

0 "+" alleles, respectively aabb

These classes would occur, in the F_2, in proportions of 1/16, 4/16, 6/16, 4/16 and 1/16 for classes of 4, 3, 2, 1 and 0 "+" alleles, respectively. If this kind of model were actually to prevail in regulating reaction to anthracnose, the distribution of phenotypes in F_2 generations should be symmetrical about the intermediate reaction class; parental reaction classes should be recovered in only minimal frequencies, depending on number of genes involved.

Preliminary examination of F_2 data indicates clearly that this was never the case in any cross.

Modifications of the "polygenic" model could include mixed gene effects, that is some genes showing dominance, and some showing additivity; some with greater effects and

some with lesser effects and so forth. These models, for testing purposes and fitting of observational data can become quite arbitrary and in present circumstances cannot be justified. I have chosen, instead, to fit a series of possible classical Mendelian gene-number, gene-action models to the observational data, realizing that ultimately it would be necessary to conduct F_3 progeny tests of F_2 plants classed as 1, 2, 3, 4 and 5.

Therefore, classes 1 and 2 were considered as resistant reactions, while 3, 4 and 5 were considered as susceptible reactions.

6.0 Analysis of Data

The observed numbers of resistant and susceptible plants in F_2 populations were tested against the theoretical ratios of resistant to susceptible plants by using a X^2 (chi-square) test.

Data from F_2 populations was also analyzed for joint segregation to simultaneous reaction to two races of the pathogen. The theoretical joint segregation ratios for two races are obtained by multiplying together the segregation ratios to each of the races. Plants are classified as resistant to both races, resistant to the first race but susceptible to the second race, susceptible to the first race but resistant to the second race and susceptible to both races. The observed and expected numbers of plants in their respective categories compared by using a X^2 test.

Some seedlings from F_2 populations were observed to be segregating for semilethal at about two seeks after emergence. The observed and expected number of normal and semilethal plants was also tested by using a X^2 test.

7.0 Data Reliability

The following points were considered for the reliability of results:

1. In every generation that was tested, parents that were considered as susceptible checks were included; that is, Montcalm was included when plants were being tested against beta race, Montcalm or Tuscola for delta race and Kaboon for gamma race. Montcalm is very susceptible to beta race, Montcalm and Tuscola are very susceptible to delta and Kaboon is very susceptible to gamma race.
2. For every F_2 population tested, its F_1 (four plants) and parents (four plants for each parent) together with Montcalm, Tuscola and Kaboon were tested also. In experiments where the susceptible checks and parents did not show clear results the experiment was discarded and the test was repeated with a new set of plants.
3. Inoculations were done on cloudy days and in the morning when the temperatures were low. The mist chamber was covered with newspapers on the outside to cut off direct sunlight which may otherwise

create a local temperature rise inside the chamber.

4. Since spores were deposited on individual leaflets or on whole plants the chances for "misses" would be very minimal.
5. Plants were inoculated when the trifoliolates were just fully opened. Leaves that are too young or too old do not show proper disease symptoms.
6. Sometimes when no clear symptoms are observed 8-10 days after inoculation, plants are left on the greenhouse benches and assessed again for disease symptoms a week later.

IV. RESULTS

1.0 Standardization of Spore Concentration

1.1 Beta Race

The analysis of variance (Table 4) for disease reaction indicates that there was no significant difference between spore concentrations but there was a significant difference between cultivars. Consequently, there was no significant interaction between the spore concentration and the cultivars.

The results for the treatment means in terms of disease reaction for six cultivars are given in Table 5. Montcalm showed a significantly higher degree of susceptibility to beta race. Swedish Brown was moderately susceptible while 61294, Tuscola, C49242 and Kaboon had similar reactions and were resistant. There was no significant difference between the spore concentration although as the spore concentration was decreased there was a decrease in infection of the cultivars.

1.2 Gamma Race

Results indicate that different spore concentrations had a significant effect on disease reactions of the bean

Table 4.--Effect of spore concentration on disease reaction of six bean cultivars to the beta race using a symptom rating scale of 1-5 (resistant-susceptible).

Source of Variation	Degrees of Freedom	Mean Squares	F
Blocks	2	0.13	
Spore concentration	2	0.91	1.85 NS
Error (a)	4	0.49	
Cultivars	5	8.64	34.56**
Spore concentration X cultivars	10	0.42	1.68 NS
Error (b)	30	0.25	

**$p \leq 0.01$. NS = None Significant.

cultivars (Table 6). There was a significant difference in response of bean cultivars to spore concentrations. Interaction between spore concentrations and cultivars was also significant.

The means of the cultivars in terms of disease reaction showed a significant differential effect of different spore concentrations (Table 7). The concentration of 1.6×10^6 spores/ml showed the highest significant effect, according to Tukey's LSD .05. Concentrations of 1.6×10^5 and 1.6×10^4 spores/ml intermediate and low effects.

Table 5.--Mean reaction of six bean cultivars to spore concentration using a 1-5 (resistant-susceptible) scale for the beta race.

Concentrations (spores/ml)	Cultivar						Means
	C49242	Kaboon	Montcalm	Sw.Br.	Tuscola	61294	
6.7×10^5	1.0	1.0	3.7	2.7	1.0	1.3	1.8
6.7×10^4	1.0	1.0	4.0	1.7	1.0	1.0	1.6
6.7×10^3	1.0	1.0	2.7	1.3	1.0	1.0	1.3
Means	1.0	1.0	3.5	1.9	1.0	1.1	1.6

LSD .05 (cultivars) = 0.48.

Table 6.--Effect of spore concentration on disease reaction of six bean cultivars to the gamma race using a symptom rating scale of 1-5 (resistant-susceptible).

Source of Variation	Degrees of Freedom	Mean Squares	F
Blocks	2	0.575	
Spore concentration	2	6.240	83.20**
Error (a)	4	0.075	
Cultivars	5	10.686	82.84**
Spore concentration X Cultivar	10	1.752	13.58**
Error (b)	30	0.129	

**$p \leq .01$.

Kaboon had a significantly higher rate of infection and Montcalm and Swedish Brown had intermediate infection rate (Table 7). C49242, Tuscola and 61294 were all resistant.

1.3 Delta Race

The analysis of variance indicates that there was a significant difference between spore concentrations and the difference between the cultivars was also highly significant (Table 8). There was also a high interaction between spore concentration and cultivars.

The mean reaction of bean cultivars to spore concentrations is summarized in Table 9. The spore concentration of 2.33×10^6 spores/ml had a significantly higher

Table 7.--Mean reaction of six bean cultivars to spore concentration using a 1-5 (resistant-susceptible) scale for the gamma race.

Concentrations (spores/ml)	Cultivar						Means
	C49242	Kaboon	Montcalm	Sw.Br.	Tuscola	61294	
1.6×10^6	1.0	5.0	4.0	4.0	1.7	1.3	2.8
1.6×10^5	1.0	3.7	2.7	3.0	1.3	1.0	2.1
1.6×10^4	1.0	3.0	1.7	1.7	1.0	1.7	1.6
Means	1.0	3.9	2.8	2.9	1.3	1.3	2.2

LSD .05 (spore concentration) = 0.21.

LSD .05 (cultivars) = .35.

Table 8.--Effect of spore concentration on disease reaction of six bean cultivars to the delta race, using a symptom rating scale of 1-5 (resistant-susceptible).

Source of Variation	Degrees of Freedom	Mean Squares	F
Blocks	2	0.019	
Spore concentration	2	3.574	11.87*
Error (a)	4	0.301	
Cultivars	5	14.196	69.59**
Spore concentrations X Cultivars	10	1.241	6.24**
Error (b)	30	0.204	

*$p \leq .05$. **$p \leq .01$.

effect than the concentration of 2.33×10^4 spores/ml. The spore concentration of 2.33×10^5 spores/ml had an intermediate effect and was significantly different from the highest and lowest concentrations.

There was no significant difference between C49242, Kaboon and 61294 although 61294 showed a mild infection (Table 9). Tuscola and Montcalm had significantly higher infection rates. Swedish Brown had a significantly lower infection rate than Tuscola but was not significantly different from Montcalm.

Table 9.--Mean reaction of six bean cultivars to spore concentration using a 1-5 (resistant-susceptible) scale for the delta race.

Concentrations (spores/ml)	Cultivar						Means
	C49242	Kaboon	Montcalm	Sw.Br.	Tuscola	61294	
2.33×10^6	1.0	1.0	4.0	3.3	5.0	1.7	2.7
2.33×10^5	1.0	1.0	4.0	3.0	3.7	1.0	2.2
2.33×10^4	1.0	1.0	2.0	3.0	2.3	1.3	1.7
Means	1.0	1.0	3.3	3.1	3.7	1.3	2.2

LSD .05 (spore concentrations) = 0.41.

LSD .05 (cultivars) = 0.60.

2.0 Reaction of Parent Bean Cultivars to Each of the Three Races

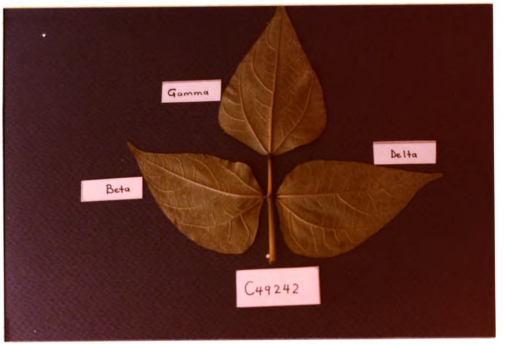
From the results of inoculation with races beta, gamma and delta, the six bean cultivars showed differential reactions to the three races of the pathogen. The results are summarized in Table 2.

Bean cultivar Cornell 49242 is resistant to the races beta, gamma and delta, and it is completely free of symptoms (Figure 1). Kaboon is resistant to races beta and delta but very susceptible to race gamma (Figure 2). Under extremely good conditions for disease development Kaboon has often been given a score of 2 in the rating scale for race delta. Montcalm is very susceptible to races beta and delta but only moderately susceptible to race gamma (Figure 3) which often posed difficulties in classifying plants in the F_2 population. Variety Swedish Brown is susceptible to races beta, gamma and delta (Figure 4). Tuscola is resistant to race beta and gamma but very susceptible to the delta race (Figure 5) and breeding line #61294 is moderately resistant to races beta, gamma and delta (Figure 6).

The F_1 plants in all the twelve crosses made were tested against each of the three races beta, gamma and delta, and the results are given in Table 10. Twelve F_1 plants (four for each race) for each cross were sprayed with spore suspension and twelve F_1 plants for each cross were used for brushing method.

Fig. 1.--Trifoliolate leaf of C49242 showing resistant reaction to the beta, gamma and delta races of C. lindemuthianum.

Fig. 2.--Trifoliolate leaf of Kaboon showing resistant reaction to the beta and delta and susceptible reaction to gamma races of C. lindemuthianum.


Gamma
Beta
Delta
C49242

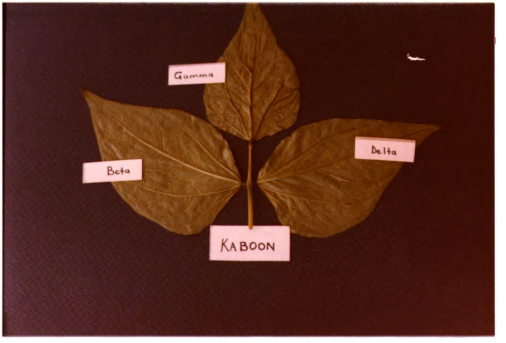
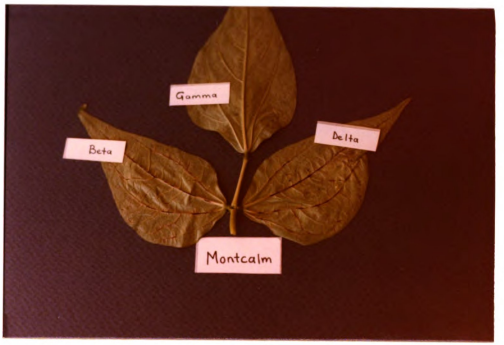

Gamma
Beta
Delta
KABOON

Fig. 3.--Trifoliolate leaf of Montcalm showing susceptible reaction to the beta and delta and moderately susceptible reaction to gamma races of C. lindemuthianum.

Fig. 4.--Trifoliolate leaf of Swedish Brown showing susceptible reaction to the beta, gamma and delta races of C. lindemuthianum.


Gamma
Beta
Delta
Montcalm

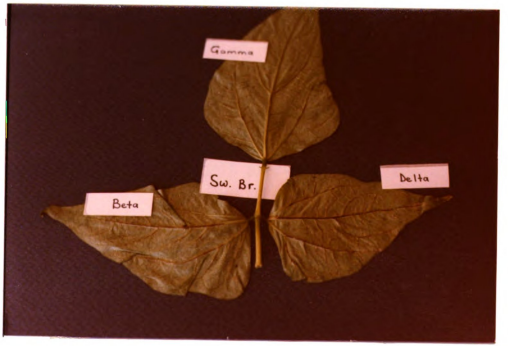
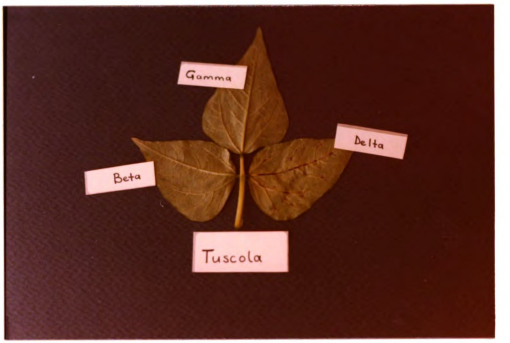

Gamma
Sw. Br.
Delta
Beta

Fig. 5.--Trifoliolate leaf of Tuscola showing resistant reaction to the beta and gamma and susceptible reaction to delta races of C. lindemuthianum.

Fig. 6.--Trifoliolate of 61294 showing moderately resistant reaction to the beta, gamma and delta races of C. lindemuthianum.


Gamma
Delta
Beta
Tuscola


61294
Delta

Table 10.--Reaction of F_1 plants to races beta, gamma and delta.

Cross Female/Male	Race		
	Beta	Gamma	Delta
C49242 X Montcalm	R*	R	R
Swedish Brown X C49242	R	R	R
C49242 X Tuscola	R	R	R
61294 X C49242	R	R	R
C49242 X Kaboon	R	R	R
Montcalm X Kaboon	R	S	R
Swedish Brown X Kaboon	R	S	R
Tuscola X Kaboon	R	R	R
61294 X Kaboon	R	R	R
Swedish Brown X Tuscola	S	R	S
Tuscola X Montcalm	S	R	S
61294 X Montcalm	R	R	R

*R = Resistant; S = Susceptible.

All the F_1 plants in crosses between C49242 and the rest of the five bean cultivars used in this study were resistant to races beta, gamma and delta. F_1 plants from Montcalm X Kaboon and Swedish Brown X Kaboon crosses were resistant to races beta and delta but susceptible to race gamma. The F_1 plants from 61294 X Kaboon cross were resistant to races beta, gamma and delta, which showed that the gene(s) conferring moderate resistance in the breeding line 61294 were dominant over the genes conferring susceptibility to race gamma in Kaboon.

The F_1 plants in the crosses between Swedish Brown X Tuscola and Tuscola X Montcalm were susceptible to races beta and delta but they were resistant to race gamma. In this case, the gene(s) for susceptibility to race beta in varieties Swedish Brown and Montcalm were expressed as dominant over the gene(s) conferring resistance in Tuscola to beta. However, Tuscola had dominant gene(s) for resistance to gamma race in these crosses.

F_1 plants of 61294 X Montcalm were resistant to races beta, gamma and delta. Gene(s) for moderate resistance seemed to be dominant over the genes for susceptibility. However, it was not possible to distinguish whether F_1 plants from the crosses of 61294 X Montcalm and 61294 X Kaboon were resistant or just moderately resistant. These F_1 plants were lethal, leaves turned yellow and abscised before disease symptoms are fully developed.

3.0 Segregations in F_2 Populations to Individual Races

3.1 Race Beta

Nine F_2 populations were observed for segretation of resistant and susceptible plants to race beta (Table 11). All the crosses between resistant X resistant and resistant X susceptible parents were resistant in F_1 except Swedish Brown X Tuscola and Tuscola X Montcalm whose F_1 plants were susceptible to race beta.

In crosses between C49242 X Montcalm and Swedish Brown X C49242 the ratio of resistant to susceptible plants clearly fitted 3:1. This means that a single dominant gene, possibly "Are" gene from C49242 conferred resistance to race beta in these two crosses. The "Are" gene was first reported by Mastenbroek in 1960 (28).

F_2 plants from C49242 X Tuscola cross segregated clearly in a 15R:1S. In this case, there seemed to be two duplicate genes conferring resistance to race beta. One could be the "Are" gene from C49242 and the other must have been from Tuscola since Tuscola is resistant to beta. Both genes are dominant in this cross.

The observed number of resistant and susceptible F_2 plants in the cross between 61294 X C49242 had a close fit to a theoretical ratio of 57:7. This ratio can possibly be explained by three pairs of dominant genes conditioning resistance in 61294 X C49242. One gene pair, presumably "Are" from C49242, had a segregation ratio of 3:1 and two

Table 11.--Observed number and expected ratios of resistant and susceptible plants to beta race in nine F_2 populations.

Cross Female/Male	Number of Plants			Expected Ratio	Probability Between
	R*	S	Total		
C49242 X Montcalm	122	33	155	3:1	.100 - .250
Swedish Brown X C49242	123	54	177	3:1	.100 - .250
C49242 X Tuscola	199	15	214	15:1	.500 - .750
61294 X C49242	195	22	217	57:7	.500 - .750
C49242 X Kaboon	168	13	181	57:7	.100 - .250
Montcalm X Kaboon	67	47	114	9:7	.500 - .750
Swedish Brown X Kaboon	123	74	197	9:7	.050 - .100
Swedish Brown X Tuscola	40	88	128	1:3	.100 - .250
Tuscola X Montcalm	25	90	115	1:3	.250 - .500

*R = Resistant; S = Susceptible.

complementary pairs of genes segregating in a 9:7 ratio. The two complementary pairs of genes can both be from 61294 or one pair from C49242 and the other pair from 61294.

The F_2 plants from the C49242 X Kaboon cross segregated in a 57:7 ratio of resistant to susceptible plants. This case, as above, revealed that there are three pairs of genes involved, one a single dominant pair of genes segregating in a 3:1 ratio and the other two being complementary genes segregating in a 9:7 ratio.

The observed resistant and susceptible plants in the F_2 crosses between both Montcalm X Kaboon and Swedish Brown X Kaboon segregated in a 9:7. The segregation pattern in these two populations seem to suggest that there were two dominant pairs of complementary genes conferring resistance to race beta.

In crosses between Swedish Brown X Tuscola and Tuscola X Montcalm the F_2 plants segregated in a 1:3 ratio of resistant to susceptible plants. The segregation ratio obtained indicate a possibility that a single dominant gene pair conferring susceptibility was expressed over the genes conferring resistance in Tuscola.

3.2 Gamma Race

Segregation patterns of the eight F_2 populations tested against gamma race are given in Table 12. One F_2 population, C49242 X Tuscola, was of a combination of resistant X resistant and 61294 X C49242 was between

Table 12.--Observed number and expected ratios of resistant and susceptible plants to gamma race in eight F_2 populations.

Cross Female/Male	Number of Plants			Expected Ratio	Probability Between
	R*	S	Total		
C49242 X Montcalm	122	33	155	3:1	.100 - .250
Swedish Brown X C49242	131	44	175	3:1	.900 - .950
C49242 X Tuscola	214	0	214	AR	- -
61294 X C49242	196	18	216	15:1	.100 - .250
C49242 X Kaboon	168	13	181	57:7	.100 - .250
Tuscola X Kaboon	163	24	187	57:7	.250 - .500
Tuscola X Swedish Brown	113	15	128	57:7	.750 - .900
Montcalm X Tuscola	87	28	115	3:1	.750 - .900

*R = Resistant; S = Susceptible; AR = All Resistant.

moderately resistant X resistant. Six of the F_2 populations were between resistant and susceptible parents.

The observed ratio of F_2 plants in the C49242 X Montcalm and Swedish Brown X C49242 crosses, closely segregated into a 3:1 theoretical ratio. The segregation ratios observed in these two populations seem to suggest that there is a single dominant gene, possibly the "Are" gene from C49242 conditioning resistance to the gamma race.

In the cross between C49242 X Tuscola, where both parents were resistant to race gamma, all 214 F_2 plants were resistant. This can be accounted for by assuming that the genes conferring resistance to gamma race in the two parents were very closely linked.

The F_2 plants in a cross between C49242 X Kaboon and its reciprocal, Kaboon X C49242 segregated in a 57:7 ratio of resistant to susceptible plants to race gamma. A single dominant gene, possibly the "Are" gene contributed by C49242, confers resistance to race gamma. Also, two additional dominant pairs of complementary genes condition resistance to the race gamma.

In the crosses Tuscola X Kaboon and Tuscola X Swedish Brown, there is a clear fit of 57:7 segregation ratio for resistant to susceptible plants in F_2. This showed that there could be three gene pairs conditioning resistance, one pair being a single dominant gene segregating in a 3:1 ratio and the other two being complementary genes segregating in a 9:7 ratio.

In the cross between 61294 X C49242, 216 F_2 plants inoculated with gamma race segregared in a 15:1 ratio of resistant to susceptible plants. Possibly two duplicate gene pairs conferred resistance, one pair presumably being "Are" gene from C49242 and the other one from 61294.

The observed number of resistant and susceptible plants in 115 plants in Montcalm X Tuscola cross clearly fitted a segregating ratio of 3:1. In this case, Tuscola seemed to have a single dominant gene conferring resistance.

3.3 Delta Race

In the F_2 populations studied for segregation for reaction to race delta, one cross (C49242 X Kaboon) and its reciprocal was between resistant X resistant parents and 61294 X C49242 was between resistant and moderately resistant parents. The rest of the F_2 populations were derived from resistant and susceptible parents. The eight populations segregating for reaction to race delta are presented in Table 13.

The F_2 plants from crosses between Swedish Brown X C49242, C49242 X Montcalm and C49242 X Tuscola segregated in a 3:1 ratio for resistant and susceptible reactions. A single dominant gene, probably the "Are" seems to confer resistance to delta race in these two crosses.

The observed number of resistant and susceptible plants in a total of 217 F_2 plants from 61294 X C49242 clearly fitted the expected segregation ratio of 15:1. It

Table 13.--Observed number and theoretical ratios of F_2 populations segregating for resistance and susceptibility to the delta race.

Cross Female/Male	Observed Results			Ratio R:S	Probability Between
	R*	S	Total		
Swedish Brown X C49242	134	46	180	3:1	.750 - .900
C49242 X Montcalm	122	33	155	3:1	.250 - .500
C49242 X Tuscola	172	42	214	3:1	.100 - .250
61294 X C49242	199	18	217	15:1	.250 - .500
Montcalm X Kaboon	64	49	113	9:7	.900 - .950
Swedish Brown X Kaboon	120	77	197	9:7	.100 - .250
Tuscola X Kaboon	121	66	187	9:7	.010 - .025
C49242 X Kaboon	169	12	181	57:7	.050 - .100

*R = Resistant; S = Susceptible.

appears that there is a possibility of a system of two dominant pairs of duplicate genes conferring resistance. One of the pairs could be the "Are" gene from C49242 and the other pair from 61294.

In a cross between C49242 X Kaboon, the observed proportion of resistant and susceptible plants in F_2 fitted the theoretical ratio of 57:7. Possibly there were three gene pairs conferring resistance to delta race, that is a single dominant gene pair segregating into a 3:1 ratio and the two dominant complementary gene pairs segregating into a 9:7 ratio.

The observed proportion of resistant and susceptible F_2 plants from each of the Montcalm X Kaboon, Tuscola X Kaboon and Swedish Brown X Kaboon populations could be explained by a theoretical ratio of 9:7. In these two populations there seem to be two complementary pairs of genes conferring resistance to race delta.

Some of the F_2 populations studied for the reaction to races beta, gamma and delta were segregating for lethal plants. As a result, in most cases the number of F_2 plants inoculated per population may not have been large enough. The lethal plants were not included in the study for segregation for disease reaction.

4.0 Joint Segregation in F_2 Populations

4.1 Beta and Gamma Races

Five F_2 populations were studied for joint segregation for reaction to races beta and gamma. From the analysis of X^2, three populations showed a degree of association in factors conditioning reaction to races beta and gamma, and two populations showed independent association. The data for joint segregation for reaction to these two races is summarized in Table 14.

In the cross 61294 X C49242 the segregation ratio of resistant and susceptible F_2 plants for race beta was 57:7 and 15:1 for race gamma. The theoretical joint segregation which is the product of the two ratios, was 855:57:105:7. The observed number of plants classified in reaction classes did not fit the theoretical ratio. The observed number of F_2 plants segregating for joint reaction in C49242 X Montcalm population significantly deviated from the theoretical ratio of 9:3:3:1.

In the cross C49242 X Kaboon, the observed number of F_2 plants for joint segregation for reaction to races beta and gamma, significantly deviated from theoretical ratio of 3249:399:399:49. The observed number of F_2 plants in Tuscola X Swedish Brown population segregating for reaction to races beta and gamma fitted the theoretical segregation ratio of 57:7:171:21 at a probability of .005-.010. The proportions of plants observed in the F_2 population of

Table 14.--Observed and expected frequencies in reaction of F_2 populations of five crosses segregating simultaneously for reaction to the beta and gamma races.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Beta	Gamma		Observed	Expected		
61294	R*	R	855	176	172.84	0.06	
X	R	S	57	9	11.52	0.55	
C49242	S	R	105	13	21.23	3.19	
(57:7)(15:1)	S	S	7	9	1.42	40.46	
			1,024	207	207.03	44.26	<.001
C49242	R	R	9	98	87.19	1.34	
X	R	S	3	8	29.06	15.26	
Montcalm	S	R	3	25	29.06	0.57	
(3:1)(3:1)	S	S	1	24	9.69	21.13	
			16	155	155.00	38.30	<.001

Table 14.--Continued.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Beta	Gamma		Observed	Expected		
C49242	R	R	3,249	160	143.57	1.88	
X	R	S	399	8	17.63	5.26	
Kaboon	S	R	399	8	17.63	5.26	
(57:7)(57:7)	S	S	49	5	2.17	3.69	
			4,096	181	181.00	16.09	<.001
Tuscola	R	R	57	40	28.50	4.64	
X	R	S	7	0	3.50	3.50	
Swedish Brown	S	R	171	73	85.50	1.83	
(1:3)(57:7)	S	S	21	15	10.50	1.93	
			256	128	128.00	11.90	.005-.01

Table 14.--Continued.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Beta	Gamma		Observed	Expected		
Montcalm	R	R	3	25	21.56	0.55	
X	R	S	1	1	7.19	5.33	
Tuscola	S	R	9	62	64.69	0.11	
(1:3)(3:1)	S	S	3	27	21.56	1.37	
				115	115.00	7.36	.05-.01

*R = Resistant; S = Susceptible.

Montcalm X Tuscola segregating for reaction to races beta and gamma fitted the theoretical ratio of 3:1:9:3 at a probability of 0.05-0.10.

4.2 Beta and Delta Races

Joint segregation to races beta and delta was studied in six F_2 populations. From the X^2 analysis, four populations showed a degree of association in reaction to races beta and delta and two populations showed independent segregation. The data for the joint segregation for reaction to races beta and gamma is summarized in Table 15.

The observed F_2 plants in C49242 X Tuscola population segregating for joint reaction to races beta and delta significantly deviated from theoretical ratio of 45:15:3:1. The F_2 plants from 61294 X C49242 observed for the joint segregation for reaction to races beta and delta deviated significantly from the theoretical ratio of 855:57:105:7.

The observed F_2 plants segregating for joint reaction to races beta and delta in C49242 X Montcalm significantly deviated from the theoretical ratio of 9:3:3:1.

The observed F_2 plants segregating for joint reaction to races beta and delta in C49242 X Kaboon deviated significantly from the theoretical ratio of 3249:399:399:49. The observed number of plants segregating for joint reaction to races beta and delta in Montcalm X Kaboon fitted the theoretical ratio of 81:63:63:49 at a probability of 0.05-0.10. Finally, the observed number of F_2 plants segregating

Table 15.--Observed and expected frequencies in reaction of F_2 populations of six crosses segregating simultaneously for reaction to the beta and delta races.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Beta	Delta		Observed	Expected		
C49242	R*	R	45	169	150.46	2.28	
X	R	S	15	31	50.16	7.32	
Tuscola	S	R	3	2	10.03	6.43	
(15:1)(3:1)	S	S	1	12	3.34	22.45	
			64	214	214.00	38.48	<.001
61294	R	R	855	184	181.19	0.04	
X	R	S	57	11	12.08	0.10	
C49242	S	R	105	13	22.25	3.85	
(57:7)(15:1)	S	S	7	9	1.48	38.21	
			1,024	217	217.00	42.20	<.001

Table 15.--Continued.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Beta	Delta		Observed	Expected		
C49242	R	R	9	105	87.19	3.64	
X	R	S	3	1	29.06	27.09	
Montcalm	S	R	3	14	29.06	7.80	
(3:1)(3:1)	S	S	1	35	9.69	66.11	
			16	155	155.00	104.64	<.001
C49242	R	R	3,249	158	143.57	1.88	
X	R	S	399	8	17.63	5.26	
Kaboon	S	R	399	9	17.63	4.22	
(57:7)(57:7)	S	S	49	6	2.17	1.54	
			4,096	181	181.00	17.60	<.001

Table 15.--Continued.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Beta	Delta		Observed	Expected		
Montcalm	R	R	81	45	35.75	2.39	
X	R	S	63	22	27.81	1.21	
Kaboon	S	R	63	19	27.81	2.79	
(9:7)(9:7)	S	S	49	27	21.62	1.34	
			256	113	113.00	7.72	.05 - .1
Swedish Brown	R	R	81	81	62.33	5.59	
X	R	S	63	42	48.48	0.87	
Kaboon	S	R	63	39	48.48	1.85	
(9:7)(9:7)	S	S	49	35	37.70	0.19	
			256	197	197.00	8.50	.025-.050

*R = Resistant; S = Susceptible.

for joint reaction to races beta and delta in Swedish Brown X Kaboon significantly fitted the theoretical ratio of 81:63:63:49 at a probability of .025-.050.

4.3 Gamma and Delta Races

F_2 populations from four crosses were studied for joint segregation for reaction to races gamma and delta. The X^2 test for independent segregation showed association in three populations and independent segregation in one population (Table 16).

The observed number of F_2 plants from 61294 X C49242 population for joint reaction to races gamma and delta significantly deviated from the theoretical ratio of 225:15:15:1.

The observed segregation ratio in F_2 plants of C49242 X Montcalm for joint segregation significantly deviated from the theoretical ratio of 9:3:3:1. The observed proportions of F_2 plants from C49242 X Kaboon segregating for reaction to races gamma and delta significantly deviated from the ratio of 3249:399:399:49. The observed proportion of F_2 plants from Tuscola X Kaboon segregating for reaction to races gamma and delta fitted the segregation ratio of 513:399:63:49 at a probability of 0.025-0.050.

Table 16.--Observed and expected frequencies in reaction of F_2 populations of four crosses segregating simultaneously for reaction to the gamma and delta races.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Gamma	Delta		Observed	Expected		
61294	R*	R	225	178	181.93	0.09	
X	R	S	15	11	12.13	0.11	
C49242	S	R	15	10	12.13	0.37	
(15:1)(15:1)	S	S	1	8	0.81	63.82	
			256	207	207.00	64.39	<.001
C49242	R	R	9	106	87.19	4.06	
X	R	S	3	17	29.06	5.00	
Montcalm	S	R	3	13	29.06	8.88	
(3:1)(3:1)	S	S	1	19	9.69	8.94	
			16	155	155.00	26.88	<.001

Table 16.--Continued.

Cross and F_2 Ratios	Reaction Class		Theoretical Ratios	Number of Plants		X^2	P
	Gamma	Delta		Observed	Expected		
C49242	R	R	3,249	162	143.57	2.37	
X	R	S	399	6	17.63	7.67	
Kaboon	S	R	399	7	17.63	6.41	
(57:7)(57:1)	S	S	49	6	2.17	6.76	
			4,096	181	181.00	23.21	<.001
Tuscola	R	R	513	104	93.68	1.14	
X	R	S	399	59	72.86	2.64	
Kaboon	S	R	63	17	11.50	2.60	
(57:7)(9:7)	S	S	49	7	8.95	0.43	
			1,024	187	186.99	6.83	.025-.05

*R = Resistant; S = Susceptible.

5.0 Lethal Plants in F_1 and F_2 Populations

Lethal plants were observed in F_1 of Kaboon X 61294 and Montcalm X 61294 and six F_2 populations were observed segregating for lethal plants. Symptoms for the lethal plants were characterized by chlorosis of primary leaves and general stunting of the plants was observed two weeks after germination. Severe yellowing occurred, leaves dropped and plants died (Figures 7, 8 and 9).

Six F_2 populations from ten populations studied were observed segregating for lethal plants and are presented in Table 17. The parents and their F_1 plants were normal but F_2 plants segregated into normal and lethals two weeks after germination. The observed number of normal plants and lethals was compared with the expected ratios by using X^2 test and the results are given in Table 17.

Fig. 7.--Semi-lethal F_1 plants from the Kaboon X 61294 cross (three weeks old).

Fig. 8.--Semi-lethal F_1 plants from the Montcalm X 61294 cross (three weeks old).

Fig. 9.--Kaboon X C49242 F_2 plants segregating for normal (back line) and semi-lethals (front line) in two weeks old population.


KABOON
LETHAL
CH9242
F2 pop

Table 17.--Observed number and expected ratio of normal and lethal plants in six F_2 populations.

Cross Female/Male	N*	n	Total	Ratio N:n	Probability Between
C49242 X Kaboon	181	61	242	3:1	.90 - .95
Tuscola X Swedish Brown	133	2	135	63:1	.90 - .95
Swedish Brown X C49242	180	40	220	13:3	.90 - .95
C49242 X Montcalm	169	39	208	13:3	1.00
Tuscola X Montcalm	128	22	150	13:3	.25 - .50
Tuscola X Kaboon	187	23	210	57:7	1.00

*N = Normal; n = Lethal.

V. DISCUSSION

1.0 Spore Concentration and Bean Cultivar Reactions

Before screening F_2 plants it seemed desirable to determine spore concentration that would give a roughly clear classification of plants into resistant and susceptible in situations where spraying method was used. A spore concentration of 10^6 spores/ml was found to be about satisfactory for all three races. Reaction of each bean cultivar, except C49242, was different for different spore concentrations for each race. Concentration of 10^4 spores/ml appeared to be too low for inciting disease reaction while concentration of 10^5 spores/ml gave an intermediate reaction.

2.0 Reaction to Race Beta

Data obtained in this study regarding the reaction of six bean cultivars to race beta revealed that genes conferring resistance were generally dominant. However, resistance was recessive in crosses involving Tuscola with Swedish Brown and Montcalm. In other crosses, Tuscola transmits dominant genes for resistance to beta race.

The ratio of 3:1 of resistant to susceptible F_2 plants in crosses involving C49242 with Montcalm and Swedish Brown can best be explained by the presence of a single dominant gene conferring resistance to race beta. The segregation ratio of 15:1 in crosses of C49242 X Tuscola can best be explained on the assumption of two dominant independent, duplicate loci either of which alleles are capable of conferring full resistance to race beta. Since C49242 and Tuscola are both resistant parents, each of them is likely to be contributing one dominant gene pair.

In the crosses involving Kaboon with Montcalm and Swedish Brown, a segregation ratio of 9:7 was observed. These results can be explained by the action of two dominant gene pairs at two separate loci which are interdependent in a complementary way in function.

The segregation ratio of 57:7 observed in crosses involving C49242 with Kaboon and 61294 is assumed to be explained by the action of both complementary gene pairs and a single dominant gene pair segregating simultaneously. These genes are completely independent of each other in conferring resistance.

Finally, the crosses involving Tuscola with Swedish Brown and Montcalm had a segregation ratio of 1:3 and all F_1 plants were susceptible. Tuscola transmits dominant genes for resistance to race beta in other crosses; its dominance for susceptibility is specific to Montcalm and Swedish Brown genetic backgrounds. The dominant genes

transmitted by Montcalm and Swedish Brown could be an allele at d-locus when the genes for resistance in Tuscola to race beta are assigned at d-locus (see Table 18). The results obtained for race beta are similar to those of Cardenas (1960) and Cardenas et al. (1964).

Table 18.--Proposed genotypes of six parental bean cultivars with respect to their reaction to the beta race of C. lindemuthianum.

Cultivar or Line	Duplicate Genes	Complementary Genes
C49242	Are^{β} Are^{β} $c^1c^1d^1d^1$	$e^1e^1f^1f^1$
Kaboon	are^{β} are^{β} $c^1c^1d^1d^1$	$e^2e^2f^2f^2$
Tuscola	are^{β} are^{β} $c^1c^1d^2d^2$	$e^1e^1f^1f^1$
61294	are^{β} are^{β} $c^1c^1d^1d^1$	$e^4e^4f^4f^4$
Montcalm	are^{β} are^{β} $c^3c^3d^3d^3$	$e^1e^1f^1f^1$
Swedish Brown	are^{β} are^{β} $c^1c^1d^3d^3$	$e^1e^1f^1f^1$

Generally the system of genes for reaction to race beta appeared to be difficult to explain, but a system of multiple alleles was assumed to be a suitable one for explaining this genetic pattern. This system was adopted from Cardenas (1960) and Cardenas et al. (1964). In this system it is assumed that there are duplicate factors c and d and complementary factors e and f. According to this system, alleles c^1 d^1 e^1 f^1 and c^3 d^3 e^3 f^3 confer susceptibility; alleles c^2 d^2 e^2 f^2 and c^4 d^4 e^4 f^4 confer resistance. The higher numbered alleles are dominant over the

lower numbered alleles. The genetic pattern proposed for the six parental cultivars in respect to their reaction to race beta is presented in Table 18. No evidence of multiple alleles was observed in complementary factors but it was decided to be appropriate to maintain the system postulated by Cardenas et al. (1960).

The reaction pattern and order of dominance is as follows:

$c^4d^4e^4f^4$ > $c^3d^3e^3f^3$ > $c^2d^2e^2f^2$ > $c^1d^1e^1f^1$

Resistant Susceptible Resistant Susceptible

Where > indicates "dominant over."

3.0 Reaction to Race Gamma

The data explaining reaction to race gamma looks more simple than for race beta. The concept of multiple allelism is not needed in this case. These results are also similar to those of Cardenas (1960) and Cardenas et al. (1964).

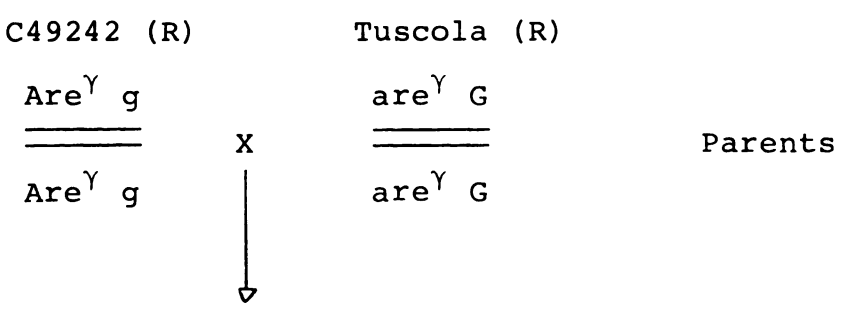
The segregation ratio of 3:1 in crosses involving C49242 with Montcalm and Swedish Brown is assumed to be accounted for by "Are" gene from C49242 conferring resistance to gamma race. A single dominant gene, presumably from Tuscola, can account for the segregation ratio of 3:1 in Montcalm X Tuscola. The segregation ratio of 15:1 in 61294 X C49242 cross can be explained by the action of independent dominant pairs of duplicate genes. One of the

gene pairs being the "Are" from C49242 and the other pair being from 61294.

The segregation ratio of 57:7 in C49242 X Kaboon is assumed to be accounted for by a system of three dominant gene pairs each of which are segregating independently. Under this assumption one gene pair segregating at 3:1 ratio is the "Are" contributed by C49242, and two dominant pairs of complementary genes are segregating in a 9:7 ratio. One pair of complementary genes had to come from C49242 and the other from Kaboon.

A system of three pairs of dominant genes is assumed to be operating in cross of Tuscola X Swedish Brown, giving a segregation ratio of 57:7. This is a similar case as that of C49242 X Kaboon but different gene pairs, I and J confer resistance.

All the F_2 plants from C49242 X Tuscola were resistant to gamma race. It is proposed that the genes conferring resistance to gamma in C49242 and Tuscola are tightly linked and that the 214 F_2 plants was too small a number to reveal any recombinants. See the following sketch:



$$\frac{Are^{Y}\ g}{are^{Y}\ G}$$ F_1 All resistant

$$\frac{Are^{Y}\ g}{Are^{Y}\ g} + \frac{are^{Y}\ G}{are^{Y}\ G}$$ F_2 All resistant, no recombinants observed

The proposed genetic pattern of six parental bean cultivars for reaction to the gamma race of C. lindemuthianum is presented in Table 19.

Table 19.--Proposed genotypes of six parental bean cultivars with respect to their reaction to the gamma race of C. lindemuthianum.

Cultivar or Line	Duplicate Genes	Complementary Genes
C49242	Are^{Y} Are^{Y} gg hh	II jj kk ll
Kaboon	are^{Y} are^{Y} gg hh	ii JJ kk ll
Tuscola	are^{Y} are^{Y} GG hh	II jj kk LL
Montcalm	are^{Y} are^{Y} gg hh	ii jj kk ll
Swedish Brown	are^{Y} are^{Y} gg hh	ii jj KK ll
61294	are^{Y} are^{Y} gg HH	ii jj kk ll

I is complementary to J, K is complementary to L.

4.0 Reaction to Race Delta

The reasonable interpretation for the data of reaction to delta race, as in the case of beta and gamma races, is that systems of both duplicate and complementary genes were operating. Dominant genes were found to be conferring resistance to race delta in all populations tested. The data for the genetic segregation to race delta in crosses involving C49242 with Swedish Brown, Montcalm, Tuscola and 61294 is assumed to be due to the action of two dominant independent loci. Each of the alleles is assumed capable of conditioning full dominance.

In crosses involving Kaboon with Montcalm, Swedish Brown and Tuscola, the segregation pattern of 9:7 obtained was assumed to be due to the action of dominant complementary genes. Finally, the segregation pattern of 57:7 is explained by the system of two dominant complementary genes and one single dominant gene acting simultaneously but independently.

The proposed genotypes of the six bean cultivars studied for the reaction to race delta are given in Table 20.

In the material studied in this work, genes controlling resistance to beta, gamma and delta in C49242 segregated in a 3:1 ratio. This is the single dominant "Are" gene reported by Mastenbroek in 1960. However, from the linkage data to be discussed later, the "Are" gene does not look to be a single dominant gene.

It has been observed in this material that Kaboon has two sets of complementary dominant gene pairs, one

Table 20.--Proposed genotypes of six parental bean cultivars with respect to their reaction to the delta race of C. lindemuthianum.

Cultivar or Line	Duplicate Genes	Complementary Genes
C49242	Are^{δ} Are^{δ} mm	nn pp
Kaboon	are^{δ} are^{δ} mm	NN PP
Tuscola	are^{δ} are^{δ} mm	nn pp
Montcalm	are^{δ} are^{δ} mm	nn pp
Swedish Brown	are^{δ} are^{δ} mm	nn pp
61294	are^{δ} are^{δ} MM	nn pp

which confers resistance to beta and the other confers resistance to delta. It has also one dominant pair of genes which complements with another pair of genes in C49242 and Tuscola (see Tables 18, 19 and 20). From this study also, the breeding line 61294 seems to possess horizontal resistance.

5.0 Linkage Study

When the data from F_2 populations was analyzed for joint segregation to race beta, gamma and delta, it was found that some populations segregated independently and others had associated segregations. Parental phenotypes in populations with associated segregations were observed in greater frequency than expected. Recombination values were calculated according to the method derived by Fisher and others (Strickberger, 1976).

The data from five F_2 populations was analyzed for joint segregations to races beta and gamma whereby three populations had associated joint segregation and the other two had random segregations. The three F_2 populations that had associated simultaneous segregations to races beta and gamma were 61294 X C49242, C49242 X Montcalm and C49242 X Kaboon, and their recombination fractions were 19.2%, 20.9% and 20.3%. These populations involved C49242 as their common parent which contributed the "Are" gene conferring resistance to races beta and gamma. In the cross between 61294 X C49242 there is a possibility that linkage could be between the complementary genes conditioning resistance to beta and the duplicate genes conditioning resistance to gamma in 61294. This possibility can be checked in cross of 61294 X Montcalm but the F_1 progenies of this cross were lethals.

There is also a possibility that one of the complementary genes, the J-locus, conditioning reaction to gamma race in Kaboon is linked to one of the complementary genes conditioning reaction to beta race. It is reasonable to propose that the genes that show linkage behavior are in C49242 because of the behavior of crosses where C49242 was a common parent, including a cross of C49242 X Montcalm. Montcalm has no genes conferring resistance to either of the races.

A significant association was obtained in four out of six populations analyzed for joint segregation to races

beta and delta. The four populations that had associated joint segregations were C49242 X Tuscola, 61294 X C49242, C49242 X Montcalm and C49242 X Kaboon and their recombination fractions were 11.1%, 21.4%, 5.2% and 19.8% respectively. As in the case of beta and gamma, C49242 was involved as a common parent. In this situation again, it seems that the genes conditioning reaction in C49242 to races beta and delta are linked. The recombination fraction is lower in crosses involving C49242 X Tuscola and C49242 X Montcalm than in the crosses of 61294 and C49242 X Kaboon. In the latter crosses there are other dominant genes conditioning resistance to races beta and delta. The segregation ratios contributed by these genes could influence the recombination fraction in the linkage system. There is no evidence of linkage between the genes conditioning resistance to beta and the genes conditioning resistance to delta race in the cross involving Swedish Brown X Kaboon.

Three F_2 populations tested for joint segregation to races gamma and delta had associated segregation and one population had an independent segregation. The crosses that had associated joint segregation to races gamma and delta are C49242 X 61294, C49242 X Montcalm and C49242 X Kaboon, and their recombination fractions are 20.3%, 24.0% and 15.1%. In this case, like in the other two previous cases, C49242 was also involved as a common parent. It is again proposed that the genes conferring resistance in C49242 to races gamma and delta are linked.

The recombination fractions in the linkage data seem inconsistent. Several factors are proposed to be contributing to the inconsistency of recombination fraction: In some of the crosses, except C49242 X Tuscola and C49242 X Montcalm, there are also other genes conferring resistance that are segregating simultaneously; in some crosses the number of F_2 plants is too small; some of the F_2 populations were also segregating for lethal plants. If the factors for formation of lethal plants are associated with the genes conferring resistance to these races, the segregation rations could have been altered.

From the linkage data it seems likely that the factors linked are in C49242. From the previous study (Mastenbroek, 1960), C49242 has a single dominant gene "Are" which confers resistance to these races. From this study it is unlikely that the "Are" is just one gene controlling reaction to these races but a system of genes, that are linked, each of which act as a single dominant gene.

The "Are" gene is therefore postulated to be a complex "tri-part" locus as follows: Are^{β} Are^{γ} Are^{δ} and a single cross-over produces an array of gametes such that both R-S and S-R combinations for joint segregation occur (see sketch below using case of the C49242 X Montcalm cross).

C49242 Montcalm

$$\frac{Are^{\gamma}\quad Are^{\beta}\ Are^{\delta}}{Are^{\gamma}\quad Are^{\beta}\ Are^{\delta}} \times \frac{are^{\gamma}\quad are^{\beta}\ are^{\delta}}{are^{\gamma}\quad are^{\beta}\ are^{\delta}} \quad \text{(Parents)}$$

$$\frac{Are^{\gamma}\quad Are^{\beta}\ Are^{\delta}}{are^{\gamma}\quad are^{\beta}\ are^{\delta}} \quad (F_1)$$

Gametes in joint combinations will be as follows:

Beta-Gamma: $Are^{\gamma}\ Are^{\beta}$, $are^{\gamma}\ are^{\beta}$, $Are^{\gamma}\ are^{\beta}$ and $are^{\gamma}\ Are^{\beta}$

Beta-Delta: $Are^{\beta}\ Are^{\delta}$, $are^{\beta}\ are^{\delta}$, $Are^{\beta}\ are^{\delta}$ and $are^{\beta}\ Are^{\delta}$

Gamma-Delta: $Are^{\gamma}\ Are^{\delta}$, $are^{\gamma}\ are^{\delta}$, $Are^{\gamma}\ are^{\delta}$ and $are^{\gamma}\ Are^{\delta}$

It is suggested that, for linkage study in crosses involving C49242, the other parents be bean cultivars that are fully susceptible to races beta, gamma and delta. F_2 populations from such crosses should not be segregating for lethal plants, as well as the higher numbers of F_2 plants than those used in this study be used.

6.0 Occurrances of Semi-Lethals in F_1 and F_2 Plants

F_1 plants from 61294 X Kaboon and 61294 X Montcalm were lethals. This suggests that there might be a recessive factor acting in a complementary way, where one factor in one parent complements a second factor in the second parent. Van Rheenen (1979) also observed similar results.

Six out of ten F_2 populations from normal parents and normal F_1 plants, in this study, segregated for semi-lethal plants. The genetic pattern for segregation is summarized in Table 18. The segregation ratio of normal to semi-lethal plants of 3:1, 63:1, 13:3 and 57:7, indicate that factors controlling the formation of semi-lethal plants are recessive. There is also a tendency that these factors act in a duplicate and complementary way. Innes (1979) also has observed cases of seedling deaths in segregating populations of crosses between Michigan-bred navy beans and cold tolerant colored beans.

VI. SUMMARY AND CONCLUSION

In this work, the inheritance of reaction to races beta, gamma and delta of C. lindemuthianum was studied in six bean cultivars using ten crosses.

Spore concentrations used in the case of spraying method of inoculation were determined to be between 10^5-10^6 spores/ml, with the latter concentration allowing better assessment of disease symptoms.

Parent plants and their F_1 progeny were inoculated by both spraying and brushing methods. All F_2 populations were inoculated by brushing each individual leaflet of the first young trifoliolate with spore suspension of one race. Individual leaves of some of the F_2 population were excised, transferred into labelled test tube containing nutrient solution, and sprayed with spore suspension of one race. Inoculated plants or excised leaves were transferred into a mist chamber kept at near 100% R.H. and ambient greenhouse temperature.

Inheritance of reaction to beta race was studied in nine crosses. Data obtained from these crosses revealed that genes conferring resistance were generally dominant but resistance was observed to be recessive in crosses

involving Tuscola with Montcalm and Swedish Brown. F_1 plants in these crosses were susceptible. Tuscola transmits dominant genes for resistance in other genetic backgrounds of this material. The segregation ratios obtained for resistance to susceptible F_2 plants were 3:1, 15:1, 9:7, 57:7 and 3:1. The segregation ratio of 3:1 is due to action of a single dominant gene conferring resistance, while the segregation ratio of 15:1 is due to the duplicate pairs of genes each of which is capable of conferring resistance. The segregation ratio of 9:7 is explained by the action of two pairs of complementary genes, and the segregation ratio of 57:7 is explained by the action of both duplicate and complementary genes segregating simultaneously but independently. In the crosses where susceptibility was conditioned by a single dominant gene the segregation of 1:3 was observed.

It became necessary to assume that a system of multiple alleles was operating in the inheritance of reaction to race beta because of dominance of alleles conferring susceptibility in crosses involving Tuscola with Montcalm and Swedish Brown. A system of four alleles was assigned to each of the four genes proposed except the "Are" gene. Two of the four genes assigned are duplicate factors and the other two are complementary factors. Two of the four alleles confer resistance and the other two confer susceptibility. The high numbered alleles were assigned to be dominant over the low numbered alleles.

Eight crosses were studied for the inheritance of reaction to race gamma in six bean cultivars. Genes conferring resistance to race gamma were dominant in this study. The segregation ratios of 3:1, 15:1 and 57:7, which were similar to those for beta race were observed. In this case also a system of duplicate and complementary factor sets are operating. The segregation pattern for reaction to gamma does not call for a multiple allelic system.

In the cross between C49242 X Tuscola all 214 F_2 plants were resistant to gamma. It seems likely that the genes conferring resistance to race gamma in Tuscola are closely linked to the "Are" gene from C49242; also that 214 F_2 plants is too small a number to reveal possible recombination.

The inheritance of reaction to race delta was studied in eight crosses and segregation ratios of 3:1, 15:1, 9:7 and 57:7 were observed. The pattern of genetic segregation is similar to that for beta and gamma, in that there are duplicate factor sets of loci and complementary factor sets of loci. There is, however, no evidence for the multiple allelic system from the segregation pattern of reaction to delta race.

In the F_2 population tested for joint segregation to beta and gamma, beta and delta and gamma and delta a close association of reaction was observed in crosses where C49242 was a common parent. A recombination fraction of about 20% in 61294 X C49242, C49242 X Montcalm and C49242 X

Kaboon was obtained for joint segregation to the beta and gamma races.

Four F_2 populations segregating simultaneously to beta and delta had recombination fractions of 11.1% (C49242 X Tuscola), 21.4% (61294 X C49242), 5.2% (C49242 X Montcalm) and 19.8% (C49242 X Kaboon). The recombination fractions for populations segregating simultaneously to gamma and delta were 20.3% (C49242 X 61294), 24.0% (C49242 X Montcalm) and 15.1% (C49242 X Kaboon).

The recombination fractions observed in these crosses seem inconsistent and it becomes difficult to determine map distance for the factors that are linked. Several factors that might contribute to the inconsistent recombination fraction are: in these crosses, except C49242 X Tuscola and C49242 X Montcalm, there are also other genes conferring resistance that are also segregating, a factor that may interfere with the calculated recombination fraction; the number of F_2 plants is too small in some crosses; and there were also lethal plants segregating in some of these populations. If the factors controlling the formation of lethal plants are associated with the genes conferring resistance to beta, gamma or delta, segregation ratios could be distorted as well as the information about the linkages studied.

From the F_2 populations observed to have associated simultaneous segregation to beta, gamma and delta where C49242 is a common parent, it seems that the factors that

are linked are in fact in C49242. C49242 being the donor of "Are" gene which confers resistance to beta, gamma and delta, it is likely that the "Are" gene is not one gene controlling reaction to these races but a system of genes that are linked, each of which act as a single dominant gene.

Six out of ten F_2 populations (from normal parents and F_1 progeny) studied segregated for lethal plants and the segregation ratio of normal to lethal plants of 3:1, 63:1, 13:3 and 57:7 were observed. The F_1 plants from 61294 X Kaboon and 61294 X Montcalm were lethals. From the segregation pattern and the lethal F_1 plants observed it is likely that the factors controlling the formation of lethal plants are recessive.

APPENDIX

APPENDIX

Table A.--Summary of results of inoculating ten F_2 populations, their F_1 and parents, with beta, gamma and delta races of Colletotrichum lindemuthianum using a 1-5 (resistant-susceptible) scale.

Generation	1-5 (R-S) Scale	Race: Beta Number of Plants Observed	Beta %	Gamma Number of Plants Observed	Gamma %	Delta Number of Plants Observed	Delta %
		A1: Swedish Brown X C49242 Cross					
F_2 Population	1	91	51.1	93	53.1	67	37.2
	2	32	18.0	38	21.7	65	36.1
	3	54	30.3	44	25.1	34	18.9
	4	1	0.6	0	0	7	3.9
	5	0	0	0	0	7	3.9
	Total	178		175		180	
F_1		1*(Beta); 1 (Gamma); 1 (Delta)					
C49242 (P)		1 (Beta); 1 (Gamma); 1 (Delta)					
Swedish Brown (P)**		3 (Beta); 3 (Gamma); 3 (Delta)					

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A2: C49242 X Montcalm Cross					
F_2 Population	1	73	47.1	89	57.4	106	68.4
	2	33	21.3	34	21.9	13	8.4
	3	17	11.0	30	19.4	3	1.9
	4	4	2.6	2	1.3	1	0.6
	5	28	18.1	0	0	32	20.6
	Total	155		155		155	
F_1	1 (Beta); 1 (Gamma); 1 (Delta)						
C49242 (P)	1 (Beta); 1 (Gamma); 1 (Delta)						
Montcalm (P)	5 (Beta); 3 (Gamma); 5 (Delta)						

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A3: C49242 X Tuscola Cross					
F_2 Population	1	177	82.7			171	79.9
	2	22	10.3			0	0
	3	5	2.3			0	0
	4	4	1.9			15	7.0
	5	6	2.8			28	13.1
	Total	214				214	
F_1	1 (Beta); 1 (Delta)						
C49242 (P)	1 (Beta); 1 (Delta)						
Tuscola (P)	1 (Beta); 5 (Delta)						

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A4: 61294 X C49242 Cross					
F_2 Population	1	168	77.4	168	81.2	187	86.2
	2	27	12.4	21	10.1	13	6.2
	3	19	8.8	17	8.2	6	2.8
	4	3	1.4	1	0.5	10	4.6
	5	0	0	0	0	1	0.5
	Total	217		207		217	
F_1	1 (Beta); 1 (Gamma); 1 (Delta)						
61294 (P)	2 (Beta); 2 (Gamma); 2 (Delta)						
C49242 (P)	1 (Beta); 1 (Gamma); 1 (Delta)						

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A5: C49242 X Kaboon Cross					
F_2 Population	1	151	83.4	151	83.4	153	84.5
	2	14	7.7	13	7.2	10	5.5
	3	11	6.1	10	5.5	11	6.1
	4	1	0.6	2	1.1	1	0.6
	5	4	2.2	5	2.8	6	3.3
	Total	181		181		181	
F_1		1 (Beta); 1 (Gamma); 1 (Delta)					
C49242 (P)		1 (Beta); 1 (Gamma); 1 (Delta)					
Kaboon (P)		1 (Beta); 5 (Gamma); 1 (Delta)					

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A6: Montcalm X Kaboon Cross					
F_2 Population	1	24	21.2			29	25.7
	2	43	38.1			35	31.0
	3	24	21.2			21	18.6
	4	8	7.1			9	8.0
	5	14	12.4			19	16.8
	Total	113				113	
F_1	1 (Beta); 1 (Delta)						
Montcalm (P)	5 (Beta); 5 (Delta)						
Kaboon (P)	1 (Beta); 1 (Delta)						

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A7: Swedish Brown X Kaboon					
F_2 Population	1	69	35.0			74	37.6
	2	54	27.4			46	23.4
	3	50	25.4			8	4.1
	4	18	9.1			35	17.8
	5	6	3.0			34	17.3
	Total	197				197	
F_1		1 (Beta); 1 (Delta)					
Swedish Brown (P)		4 (Beta); 5 (Delta)					
Kaboon (P)		1 (Beta); 1 (Delta)					

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A8: Tuscola X Kaboon Cross					
F_2 Population	1			126	67.4	100	53.5
	2			37	19.8	22	11.8
	3			21	11.2	35	18.7
	4			3	1.6	20	10.7
	5			0	0	10	5.3
	Total			187		187	
F_1	1 (Gamma); 1 (Delta)						
Tuscola (P)	1 (Gamma); 5 (Delta)						
Kaboon (P)	5 (Gamma); 1 (Delta)						

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A9: Swedish Brown X Tuscola Cross					
F_2 Population	1	23	18.0	83	64.8		
	2	17	13.3	30	23.4		
	3	43	33.6	14	10.9		
	4	17	13.3	1	0.8		
	5	28	21.9	0	0		
	Total	128		128			
F_1		5 (Beta); 1 (Gamma)					
Swedish Brown (P)		5 (Beta); 4 (Gamma)					
Tuscola (P)		1 (Beta); 1 (Gamma)					

Table A.--Continued.

Generation	1-5 (R-S) Scale	Race					
		Beta		Gamma		Delta	
		Number of Plants Observed	%	Number of Plants Observed	%	Number of Plants Observed	%
		A10: Tuscola X Montcalm Cross					
F_2 Population	1	16	13.9	67	58.3		
	2	10	8.7	20	17.4		
	3	15	13.0	22	19.1		
	4	17	14.8	6	5.2		
	5	57	49.6	0	0		
	Total	115		115			
F_1	5 (Beta); 1 (Gamma)						
Tuscola (P)	1 (Beta); 1 (Gamma)						
Montcalm (P)	5 (Beta); 3 (Gamma)						

*1-5 (R-S) scale for F_1 and parents.

**Inoculation was done on a hot day, so Swedish Brown did not express full susceptibility.

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