GENETIC SYSTEMS FOR REACTION OF FIELD BEANS (<u>PHASEOLUS VULGARIS</u> L.) TO INFECTION BY THREE RACES OF <u>COLLETOTRICHUM LINDEMUTHIANUM</u>

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By

Francisco A. Cardenas-Ramos

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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ABSTRACT FRANCISCO A. CARDENAS R.

The inheritance reaction of beans (Phaseolus vulgaris) to alpha, beta, and gamma physiological forms of Colletotrichum lindemuthianum was studied in 16 different crosses involving nine parental varieties.

The inoculation technique used, in order to determine the reaction of the same plant to the three forms of the pathogen, was as follows: each one of the three leaflets, of the first incompletely developed leaf, was inoculated with one of the three races of the fungus, rubbing with a camel's hair brush moistened in a spore suspension of the pathogen. The inoculated plants were kept for seven days in a moisture chamber at 70^{o}F temperature, then transferred to the greenhouse bench and three days later the reaction readings were made.

The inheritance of alpha reaction was studied in eight crosses. In the F_1 resistance was always dominant. In crosses between resistant x susceptible 3:1 F_2 ratios were observed, and 15:1 in crosses between resistant x resistant. This indicates at least two different genes conferring resistance. No linkage was observed between the genes conferring resistance to alpha and those producing resistance to either beta or gamma.

The inheritance reaction to beta was studied in ten different crosses. In three of the eight resistant x susceptible crosses, susceptibility was dominant in the F_1. To explain the observed F_2 ratios: 1:3, 7:9, 13:3, 15:1, 57:7 and

249:7, two different kinds of resistance were postulated, one due to the presence of duplicate genes, each having the ability to confer resistance, and the other, due to the complementary action of two genes. A series of four alleles for each one of the four proposed genes, two conferring resistance and two conferring susceptibility, one of which is dominant over one of the dominant alleles, was proposed, to explain the cases in which susceptibility was dominant. A dominant suppressor of the dominant alleles present in Michelite, was postulated to be present in the bean variety Dark Red Kidney.

This hypothesis is not unique; there may be other explanations of the observed results. One of these explanations assumes the presence of nine different gene pairs, five of which interact in order to produce resistance; the other four modify the effect of the recessive genes making them dominant when in the heterozygous condition.

In order to ascertain which one of these hypothesis is correct, the crosses between the susceptible varieties Dark Red Kidney x Emerson 51-2, and Dark Red Kidney x Cornell 64-23 need to be made. If all the F_2 plants are susceptible the former hypothesis is supported; if segregation is observed the second hypothesis will be supported.

Assuming that the first interpretation of the beta results is correct, it was found that one of the duplicate genes present in the variety Algarrobo conferring resistance to beta, is in the same linkage group, about nine crossover units apart, with one gene conferring resistance to gamma. In the variety Emerson 847 one of the complementary genes conferring resistance to beta is in the same chromosome (8.7 crossover units apart) with one gene conferring resistance to gamma. In Dark Red Kidney it was found that the beta-resistance supresor gene is located about 20 crossover units apart from one gene conferring susceptibility to gamma.

The inheritance to gamma was studied in 12 crosses, eight of which were resistant x susceptible, and four resistant x resistant. Resistance was always dominant in the F_1. To explain the observed F_2 ratios: 3:1, 15:1, 9:7, and 249:7, two different kinds of resistance were proposed, one due to the presence of duplicate factors, and the other to the presence of two complementary factors.

The linkage relationship found between the genes conferring resistance to beta and gamma explains the more frequent occurrence of bean varieties either resistant to beta and gamma or susceptible to beta and gamma, than either resistant to beta and susceptible to gamma, or susceptible to beta and resistant to gamma.

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INTRODUCTION

The genetic information in regard to the inheritance of reaction to bean anthracnose, caused by Colletotrichum lindemuthianum (Sacc and Magn.) Briosi and Cov. in beans, Phaseolus vulgaris L., does not show agreement among the results of several workers.

It is important from the genetic, as well as from the plant breeding point of view, that knowledge be obtained with respect to the linkage relationship between the genetic factors which confer resistance in the bean plant to the different physiological races of the pathogen. At present, no such information is available. This lack of information also extends to the linkage relationship of the genes confering anthracnose resistance and genes involved in other physiological, as well as morphological characters of the bean plant.

The inoculation techniques used in the past in genetic studies of disease resistance in beans have not facilitated the collection of data on the linkage relationships of the genetic factors involved.

This work was undertaken with a two-fold aim: one, to attempt to develop a consistent and clarifying genetic interpretation of the reaction pattern of field beans to infection by the alpha, beta and gamma strains of C. lindemuthianum, and two, to develop and use a method of inoculation which would yield unambiguous linkage data with respect to genes conditioning reaction to anthracnose as well as with genes affecting other traits.

The data reported here include the F_1, F_2, some F_3 families, and some backcrosses of 16 crosses involving nine commercial varieties of beans, all of which, except one, are used as dry beans.

REVIEW OF LITERATURE

Bean anthracnose, incited by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Briosi and Cov., is a disease of beans principally affecting varieties of *Phaseolus vulgaris* L. According to York (21) it was first definitively reported and described in 1843 but probably existed at a much earlier date. In 1875 it was identified on beans in Germany; in 1880 the disease was found in Red Kidney beans in England (8) and in 1884 was common in Italy, France and the United States.

Since that time, it has been reported in every continent and nearly every country where beans are grown, except in those areas where the fungus is excluded because of prohibitive climatic conditions.

All parts of the plant, even the roots, are subject to the disease (7). The symptoms on the seed are not always easy to distinguish from those caused by certain other diseases (bacterial blight and halo blight). In anthracnose the causitive organism produces yellowish to brown sunken cankers which can be small or extended over a large part of the seed according to the severity of infection. The lesions in the hypocotyl of the seedlings and in the stems of the plants are rust-colored specks that in the beginning are minute and gradually enlarge along the length of the stem and finally become sunken. Infection may occur in both the petiole and veins of the leaf. The infection in the leaves occurs underside along the veins, producing a dark brick-red to purplish color, that later turns brown or almost black. Anthracnose symptoms are more easily recognized in the pods; the first evidence is noted in small flesh-to rust colored spots, which when fully developed are nearly circular (21, 7, 12).

In 1910, Barrus (4), testing bean varieties for resistance to anthracnose and employing several different isolates, found that most of the varieties were susceptible; he also observed that varieties which had proved to be resistant to one group of isolates, were susceptible when inoculated with other groups of isolates of the fungus, and in other cases, the reverse condition was observed. His results clearly demonstrated that he was working with at least two physiological races. In 1918, Barrus (6) published results of inoculating a large number of varieties of dry and snap beans with ten different isolates; one group of isolates was designated as a single race, "alpha," a second group as "beta." He also presented evidence that resistance to anthracnose was not due to any morphological characters of the host plant which made impossible the penetration of the fungus germ tube, but rather suggested that it was due to the physiological or chemical activities of the plant. Preliminary attempts to break down the resistance of certain varieties to a given race by

means of heavy nitrogen fertilization, by heavy continued watering, by shading, by stunting the plant through withholding water, and by injuring the plant mechanically, scraping the epidermis just before inoculation, resulted in every case in failure.

In New York Burkholder (10) isolated from the white imperial bean a third race that he designated as "gamma."

Leach (12), as a result of inoculating 14 varieties of beans with isolates from several different sources, found eight distinct races, different from the three previously described. In grafting experiments between susceptible and resistant beans, Leach (13) found no indication that resistance or susceptibility were influenced by either stock or scion.

In Germany, Schreiber (17) carried out inoculation experiments on 57 varieties of beans mostly of European origin, with a considerable number of pathogenic isolates including some belonging to alpha, beta and gamma and others isolated from German-grown beans, and determined that he had 34 different strains, and, further concluded that these 34 strains could be put into three main groups: one group resembling very closely the infecting ability and host range of alpha, another of beta, and the third of gamma.

Five different physiological forms distinct from alpha, beta and gamma were reported by Muller (16) in Holland.

In Mexico Yerkes and Ortiz (20) reported at least ten new races different from alpha, beta and gamma.

The actual number of genetically distinct races may not correspond to the number reported because of the possibility of the same race or races having been reported by different workers, because the set of differentials used by one worker has been different from the differentials used by other workers, except for the varieties used to distinguish between alpha, beta, and gamma.

The first to report on the genetics of resistance to a strain of the pathogen was Burkholder (9). He crossed Well's Red Kidney (resistant to alpha and beta) with White Marrow (resistant to beta) and obtained in F_2, out of a total of 473 plants tested, 362 resistant and 111 susceptible. These data indicated a typical monofactorial segregation, three resistant, to one susceptible. He did not test the F_1.

In crosses made between Well's Red Kidney (resistant to alpha and beta) and a selection from Michigan Robust (susceptible to alpha and resistant to beta) McRostie (14, 15) observed that the F_1 was resistant, and in F_2 of 1970 plants 1471 showed resistance to alpha and 499 susceptibility. These results approximate closely a 3:1 ratio between resistance and susceptibility. In crosses between selections B, (resistant to both races) and German Wax (susceptible to both strains), he found that of 1404 F_2 plants inoculated with a spore mixture of both races, 712 were resistant to alpha and beta and 692 susceptible to either alpha or beta. These figures fit very well a 9:7 ratio., the expected ratio for reaction to a two-fold mixture of races where individually the segregation is for single genetic factors.

Schreiber (17) crossed Anthracnose Resistant 22 x Konserva, and Anthracnose Resistant 22 x Wacks Best von Allen, and inoculated portions of the respective F_2 progenies with either: (1) one race, (2) a mixture of two races, or (3) a mixture of three races. From the inoculations with one race he obtained a 3:1 ratio, with resistance as dominant. When F_2 progenies were inoculated with a mixture of two races, a 9:7 ratio of resistance to susceptibility was noted, indicating a two factor difference, one resistant gene for each race. When inoculations were made with the three races together a 27:37 ratio was observed, suggesting a three factor difference, one for each one of the races. Schreiber concluded that each of the three factors for resistance was on a different chromosome.

In a subsequent paper, Schreiber (18) reported a more complecated interaction of genes responsible for antracnose resistance in some F_4's of the cross Anthracnose Resistant x Konserva. In order to explain the observed ratios found in various single-plant progenies, he concluded that at least eight different dominant genes were present.

Separately the inheritance of resistance to three physiological forms of C. lindemuthianum (beta, gamma and delta) in 30 combinations between 15 different parental varieties was studied by Andrus and Wade (2). They state that they did not use alpha because of the lack of varieties highly resistant to it. (Actually, Burkholder had reported resistance to alpha since 1918 in Well's Red Kidney, and subsequently resistance to alpha had been reported in many other varieties). In the case of race beta in crosses of resistant x resistant, resistant x tolerant, and resistant x susceptible, resistance was always dominant if F_1. The ratios 3:1, 15:1, 63:1, 13:3 and 11:5 were observed in the F_2, the first three ratios were supported with F_3 data. In order to explain their results Andrus and Wade postulated three series of multiple alleles, with ten alleles and with the property that some alleles in each series conferred resistance or susceptibility depending on the genes present in the other series. In the gamma investigations the ratios 13:3, 11:5, 57:7, 63:1, 15:1, 9:7, 3:1 and 1:3 were observed. In order to explain these results they proposed that the same system of ten alleles in three series postulated to explain the beta data, would also suffice for gamma. In crosses made between resistant x susceptible and susceptible x susceptible in the case of the delta strain, most of the F_2's gave a 3:1 or 9:7 ratio. In conclusion, they state that a system of ten genes in three allelomorphic series, involving both duplicate and complementary genes for resistance, one dominant gene for susceptibility and "gene interaction at three points" is the simplest Mendelian hypothesis that will explain all the beta and gamma anthracnose data. In the case of delta, three independent pairs of genes explain the data. Furthermore, they conclude that "in spite of probabilities in its favor, linkage is not involved to any important extent in the inheritance of anthracnose reaction."

York (21) studied the inheritance of reaction to anthracnose in the following crosses:

Tendergreen x Emerson 51

Tendergreen x Red Mexican

Red Mexican x Emerson 51

Tendergreen is a bean variety susceptible to the three _C_. _lindemuthianum_ races; Red Mexican is susceptible to alpha and resistant to beta and gamma and, Emerson 51 is resistant to all the races.

He divided the F_2 population in three parts and each of them was inoculated with a particular race. In crosses of Tendergreen x Red Mexican all the F_2 plants were susceptible to alpha; a 3:1 segregation was observed for beta and a 9:7 for gamma. In crosses Tendergreen x Emerson 51 a 3:1 segregation was observed for alpha as well as for beta and gamma. In crosses of Red Mexican x Emerson 51 a 3:1 segregation for alpha, a 15:1 segregation for beta and a 63:1 for gamma were observed. All these ratios were supported with F_3 data.

In all the early reports on the inheritance of reaction to bean anthracnose only monohybrid F_2 ratios were reported, regardless of the race involved in the study. On the contrary, in the work of Andrus and Wade more complicated situations, including duplicate, triplicate, complementary factors, etc., were reported.

From the previously cited works, it may be concluded that the degree of complexity in regard to the interpretation of the inheritance of reaction to a particular race, is proportional to the number of crosses studied; possibly this is intimately related to the origin of the parental varieties used in the studies. When a resistant and susceptible variety are crossed, if they are phylogenetically far apart, a more complex segregation will be expected than when the varieties crossed are closely related.

MATERIALS AND METHODS

Strains of the Pathogen

All the studies reported herein were made with the three races of C. lindemuthianum designated as alpha, beta and gamma. Single spore isolates of these three races were obtained from Dr. Axel Andersen. The racial identity of the pathogen was established from several inoculation experiments with appropriate bean varieties (differentials) carried out under greenhouse conditions.

Bean Varieties--All the nine varieties of beans used in this study belong to the species Phaseolus vulgaris L. The origin, type of seed and reaction to the three races of the pathogen are presented in table 1.

TABLE 1.--Origin, type of seed and reaction to alpha, beta and gamma strains of C. lindemuthianum of nine bean varieties.

Variety	Origin	Seed type	Reaction to Alpha	Reaction to Beta	Reaction to Gamma
Algarrobo	Colombia, S. A.	Kidney	R*	R	R
Emerson 847	United States	Navy	R	R	R
Emerson 51-2	United States	Garden	R	S	R
Michelite	United States	Navy	S	R	R
Red Mexican	United States	"Small Red"	S	R	R
Perry Marrow	United States	Marrow	R	R	S
Mich. Dark Red Kidney	United States	Kidney	R	S	S
Cornell 64-23	United States	Yellow Eye	R	S	S
Brazilian Red	Brazil	Kidney	S	R	S

R*--resistant S--susceptible

Maintenance of the Pathogen.--The cultures of the pathogens have been maintained in bean pod agar medium which was prepared in the following way:

The juice of a "snap bean" (can #303) was poured into a flask and distilled water was added to complete 500cc.; to this solution, 12g. of "Bacto" agar were added. The medium was melted, poured in test tubes and steamed for 20 minutes at 15 pounds pressure. Bean pod agar as well as a mixture of cooked wheat, barley and oats was used as substrate in order to increase the amount of inoculum.

Because cultures cannot stand for long periods without deterioration, transfers of conidia recently sporulated were made every 8-10 days. Periodically the pathogenicity of the cultures was tested on the differential varieties.

Making Inoculations.--Bean pod agar, or wheat, barley and oat media bearing spores were washed with distilled water from the flasks, then the spore suspension was used in the inoculations.

Each one of the crosses of the generations studied was planted in a three-inch pot filled with sand, seeding one seed per pot; during the growing period of the plants, the pots were watered three times weekly with a nutrient solution.

Fourteen to 18 days after planting when the first trifoliate leaf had appeared each one of the three young leaflets was inoculated on the underside with one of the three races of the pathogen, brushing with an artist's No. 4 camel hair brush, previously wetted in the spore suspension. Immediately after the inoculation each one of the pots was placed inside a moist chamber, large enough to accommodate 400 plants. The temperature inside the chamber was kept constantly at 70^{o}F and the relative humidity close to 100%.

The plants were maintained in the chamber for a period of seven days. Two or three days later the readings were made, in order to determine the level of infection. In each one of the crosses studied, the F_1, F_2 and back crosses, or F_3 in some instances, were inoculated. To test the pathogenicity of the strains as well as the completeness of the inoculation itself, there were inoculated in every case ten seedlings of each one of the following varieties: Michelite, Perry Marrow, and Michigan Dark Red Kidney plus the two parental varieties involved in the cross.

Readings in order to determine infection.--Readings were made from 10 to 12 days after the inoculation.

Four classes were used to designate the different degrees of infection.

0 - Immune--no signs of infection

1 - Resistant--very small "flecks" confined mainly to the secondary and tertiary veins.

2 - Susceptible--large "flecks" in primary and secondary leaflet veins.

3 - Highly susceptible--infection is severe and extended all over the leaf blade. Very often the leaf or a part of it dies.

The classes 0 and 1 were combined and considered resistant; classes 2 and 3 combined, were considered susceptible.

Crossing technique.--The flower chosen for pollination was one in the advanced bud stage which was expected to open the following day. The standard petal was split along the suture. The two wings were then folded back in such a way that the keel was easily accessible. Then a small piece of the distal end of the keel was removed, after which the ten stamens were removed.

Due to the fact that pollen is normally deposited in abundance in the terminal part of the stigma of recently opened flowers, such flowers were used as sources of pollen.

To make pollinations the pollen-bearing stigma of the male parent was rubbed over the stigmatic tuft of the emasculated flower. After this, the female flower was closed again with the aid of a forceps. All crosses were made under greenhouse conditions on plants previously tested in regard to anthracnose reaction.

Analysis of the data.--The observed ratios of resistant and susceptible plants were tested against theoretical ratios using the X^2 method as described by Walker and Lev (19).

In crosses in which linkage was suspected the method of maximum likelihood was used to evaluate the per cent of recombination using the formulas developed by Allard (1).

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EXPERIMENTAL RESULTS

Inoculation technique.--Table 2 shows a comparison between the reaction readings obtained when the central and the opposite leaflets of the same leaf were inoculated with the same strain of *C. lindemuthianum* (see figures 1, 2, 3 and 4).

TABLE 2.--Degrees of infection in the "central" and "opposite" leaflets of the same leaf inoculated with alpha, beta and gamma strains of *C. lindemuthianum*.

	C. lindemuthianum Race					
	Alpha		Beta		Gamma	
	Leaflets					
Bean Variety	Central	Opposite	Central	Opposite	Central	Opposite
Algarrobo	0	0	0	0	0	0
Emerson 847	0	0	1	1	0	0
Michèlite	3	3	1	0	1	1
Red Mexican	3	3	0	0	0	0
Emerson 51-2	0	0	3	3	0	0
Perry Marrow	0	0	0	0	3	3
Dark Red Kidney	0	0	3	3	3	3
Cornell 64-23	0	0	3	3	3	3
Brazilian Red	3	3	0	0	3	3

The experimental results of the 16 crosses studied are presented in the first place according to the race (alpha, beta and gamma); in the second place according to the segregating generation (F_1, F_2 backcrosses); and finally each cross will be discussed.

Segregation of one Race Alone

Alpha

F_1 Results.--Of the 16 crosses studied, seven were of the combination resistant x susceptible and nine resistant x resistant; in both cases the F_1 plants tested were resistant as shown in table 3.

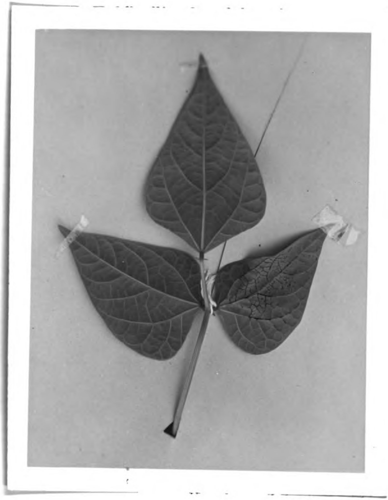

Figure 1.--From left to right the reaction of the individual leaflets to gamma (R), beta (R) and alpha (S) C. lindemuthianum strains respectively, in Michelite.

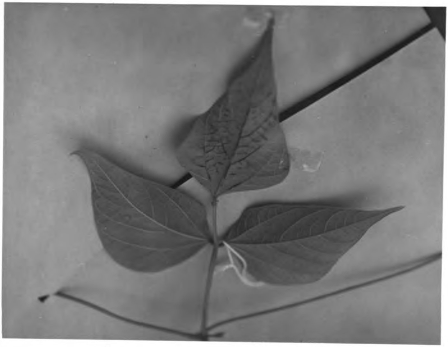

Figure 2.--From left to right the reaction of the individual leaflets to gamma (R), beta (S), and alpha (R), respectively, in Emerson 51-2.

Figure 3.--From left to right the reaction of the individual leaflets to gamma (S) , beta (R), and alpha (R) *C. lindemuthianum* strains, respectively, in Perry Marros

Figure 4.--From left to right the reaction of the individual leaflets to alpha (R), beta (S), and gamma (S), in Dark Red Kidney

TABLE 3.--Degrees of infection of F_1 plants inoculated with alpha, beta and gamma in 16 different crosses.

Cross	Degree of infection C. lindemuthianum race Alpha	Beta	Gamma
Michelite x Algarrobo	0	0	0
Michelite x Emerson 847	0	0	0
Red Mexican x Algarrobo	0	0	0
Red Mexican x Emerson 847	0	0	0
Emerson 51-2 x Algarrobo	0	0	0
Emerson 51-2 x Emerson 847	0	3	0
Perry Marrow x Algarrobo	0	0	0
Perry Morrow x Emerson 847	0	0	0
Dark Red Kidney x Algarrobo	0	0	0
Dark Red Kidney x Emerson 847	0	1	0
Cornell 64-23 x Algarrobo	0	0	0
Cornell 64-23 x Emerson 847	0	1	0
Brazilian Red x Emerson 847	0	0	0
Dark Red Kidney x Brazilian Red	0	3	3
Michelite x Dark Red Kidney	0	3	0
Algarrobo x Emerson 847	0	0	0

F_2 and Backcrosses Results.--The observed number of resistant and susceptible F_2 plants of the cross Michelite x Algarrobo, when 292 plants were inoculated with alpha, is compared with a 3:1 expected ratio in table 4.

Of the backcross (Michelite x Algarrobo) x Michelite 33 plants were tested; 17 were resistant and 16 susceptible, this proportion is very close to a 1:1 ratio, as expected.

When 212 F_2 plants of the cross Michelite x Emerson 847 were inoculated with alpha, the proportion of resistant and susceptible plants fit a 3:1 ratio, as presented in table 4.

From the backcross (Michelite x Emerson 847) x Michelite, 95 plants were inoculated; 47 were resistant and 48 susceptible; this proportion fits very well a 1:1 ratio.

Two-hundred and twenty-two F_2 plants of the cross Red Mexican x Algarrobo were inoculated with alpha. In table 4 a comparison between the observed and expected (3:1) number of resistant and susceptible plants is presented.

TABLE 4.--Observed number and ratio of alpha-resistant and alpha susceptible F_2 plants in nine different crosses.

Cross	Observed Results R*	S	Total	Ratio R : S	Probability between
Michelite x Algarrobo	221	71	292	3 : 1	.75 - .90
Michelite x Emerson 847	153	59	212	3 : 1	.25 - .50
Red Mexican x Algarrobo	171	51	222	3 : 1	.50 - .75
Red Mexican x Emerson 847	191	68	259	3 : 1	.50 - .75
Brazilian Red x Emerson 847	190	52	242	3 : 1	.10 - .25
Michelite x Dark Red Kidney	152	48	200	3 : 1	.50 - .75
Dark Red Kidney x Brazilian Red	169	60	229	3 : 1	.50 - .75
Cornell 64-23 x Emerson 847	214	10	224	15 : 1	.25 - .50
Cornell 64-23 x Algarrobo	231	19	250	15 : 1	.25 - .50

R*--resistant S--susceptible

The observed number of resistant and susceptible F_2 alpha-inoculated plants of the cross Red Mexican x Emerson 847, is compared with the expected number for a 3:1 ratio, in table 4.

Table 4 shows the comparison between the observed and expected (3:1) number of resistant and susceptible plants obtained when 242 F_2 plants of the cross Brazilian Red x Emerson 847 were inoculated with the alpha strain of anthracnose.

A comparison between the observed and expected (3:1) number of resistant and susceptible F_2 alpha-inoculated plants of the cross Michelite x Dark Red Kidney is presented in table 4.

From the backcross (Michelite x Dark Red Kidney) x Michelite 35 plants were inoculated with alpha; 18 were resistant and 17 susceptible; this proportion fits very well a 1:1 ratio.

The ratio between the observed number of resistant and susceptible F_2 alpha-inoculated plants of the cross Dark Red Kidney x Brazilian Red fits very well a 3:1 ratio as shown in table 4.

The difference between the observed and expected, assuming a 15:1 ratio for number of resistant and susceptible F_2 alpha-inoculated plants of the cross Cornell 64-23 x Emerson 847, is not significant at the 1% level (see table 4).

When 250 F_2 plants of the cross Cornell 64-23 x Algarrobo were inoculated with alpha, the proportion of resistant and susceptible plants fits the expected 15:1 ratio, as shown in table 4.

In the following crosses, both parents are alpha resistant, and segregation for susceptibility was not observed in the F_2 generation: Perry Marrow x Algarrobo; Perry Marrow x Emerson 847; Dark Red Kidney x Algarrobo; Dark Red Kidney x Emerson 847; Emerson 51-2 x Emerson 847 and, Emerson 51-2 x Albarrobo.

Beta

F_1 Results.--Eight out of the 16 crosses were of the combination resistant x susceptible and eight of the combination resistant x resistant. Thirteen out of the 16 F_1 crosses inoculated with beta were resistant, and in the crosses Emerson 51-2 x Emerson 847, Michelite x Dark Red Kidney and Brazilian Red x Dark Red Kidney the F_1 plants inoculated were susceptible (see table 3).

A very close fit to a 1 resistant : 3 susceptible was obtained when 200 F_2 plants of the cross Michelite x Dark Red Kidney were inoculated with anthracnose beta strain as presented in table 5.

Out of 35 plants of the backcross (Michelite x Dark Red Kidney) x Michelite inoculated with beta, 18 were resistant and 17 susceptible. These figures fit very well a 1:1 expected ratio.

The observed number of resistant and susceptible F_2 plants of the cross Dark Red Kidney x Brazilian Red when inoculated with beta is compared with the expected number (1:3) in table 5.

TABLE 5.--Observed number and ratio of beta-resistant and beta-susceptible F_2 plants in ten different crosses.

Cross	Observed Results R*	S	Total	Ratio R : S	Probability between
Michelite x Dark Red Kidney	57	143	200	1 : 3	.25 - .50
Dark Red Kidney x Brazilian Red	62	167	229	1 : 3	.25 - .50
Emerson 51-2 x Emerson 847	97	116	213	7 : 9	.50 - .75
Cornell 64-23 x Emerson 847	122	102	224	9 : 7	.50 - .75
Dark Red Kidney X Emerson 847	186	122	308	9 : 7	.10 - .25
Cornell 64-23 x Algarrobo	198	52	250	13 : 3	.50 - .75
Dark Red Kidney x Algarrobo	259	16	275	15 : 1	.50 - .75
Emerson 51-2 x Algarrobo	163	14	177	15 : 1	.25 - .50
Brazilian Red x Emerson 847	210	32	242	57 : 7	.25 - .50
Algarrobo x Emerson 847	255	6	261	249 : 7	.50 - .75

R*--resistant S--susceptible

The observed number of resistant and susceptible F_2 beta-inoculated plants of the cross Emerson 51-2 x Emerson 847 is compared in table 5 with an expected 7:9 ratio. The observed data fits very well the expected ratio.

Table 5 shows the comparison between the observed and expected (9:7) number of resistant and susceptible plants obtained when 224 F_2 plants of the cross Cornell 64-23 x Emerson 847 were inoculated with beta.

Assuming a 9:7 ratio the difference between the observed and expected number of resistant and susceptible F_2 beta-inoculated plants of the cross Dark Red Kidney x Emerson 847 is not significant at the 1% level (see table 5).

When 250 F_2 plants of the cross Cornell 64-23 x Algarrobo were inoculated with beta, the proportion of resistant and susceptible plants fits an expected 13:3 ratio, as shown in table 5.

In table 5 is presented the discrepancy between the observed and expected (15:1) number of resistant and susceptible plants when 275 F_2 plants of the cross Dark Red Kidney x Algarrobo were inoculated with the beta strain of C. lindemuthianum.

The observed number of resistant and susceptible F_2 plants of the cross Emerson 51-2 x Algarrobo, of which 177 plants were inoculated with beta, is compared with the expected number, assuming a 15:1 ratio, in table 5.

A comparison of the observed and expected (57:7) number of resistant and susceptible F_2 plants of the cross Brazilian Red x Emerson 847 when inoculated with beta, is presented in table 5.

The ratio between the observed number of resistant and susceptible F_2 beta-inoculated plants of the cross Emerson 847 x Algarrobo fits very closely a 249:7 ratio (see table 5).

In the following crosses: Perry Marrow x Algarrobo; Perry Marrow x Emerson 847; Michelite x Algarrobo; Michelite x Emerson 847; Red Mexican x Algarrobo, and Red Mexican x Emerson 847, both parents are resistant and no segregation for susceptibility was observed in the F_2's.

Gamma

F_1 Results.--Of the 16 crosses studied, all the F_1 plants were resistant except those of the cross Dark Red Kidney x Brazilian Red in which both parents and the F_1 were susceptible (see table 3).

F_2 and Backcross Results.--In 12 out of the 16 crosses inoculated with C. lindemuthianum gamma strain segregation for resistance and susceptibility was observed in the F_2 populations. Eight of the crosses were of the combination resistant x susceptible and four resistant x resistant.

The observed number of resistant and susceptible F_2 gamma-inoculated plants of the cross Dark Red Kidney x Algarrobo is compared with the expected number (3:1) in table 6.

When 308 F_2 plants of the cross Emerson 847 x Dark Red Kidney were inoculated with gamma, the proportion of resistant and susceptible plants fits an expected 3:1 ratio, as presented in table 6.

TABLE 6.--Observed number and ratio of gamma-resistant and gamma-susceptible F_2 plants in twelve different crosses.

Cross	Observed Results R*	S	Total	Ratio R : S	Probability between
Dark Red Kidney x Algarrobo	204	71	275	3 : 1	.25 - .50
Emerson 847 x Dark Red Kidney	228	80	308	3 : 1	.50 - .75
Cornell 64-23 x Algarrobo	192	58	250	3 : 1	.50 - .75
Cornell 64-23 x Emerson 847	170	54	224	3 : 1	.75 - .90
Brazilian Red x Emerson 847	191	51	242	3 : 1	.10 - .25
Michelite x Dark Red Kidney	142	58	200	3 : 1	.10 - .25
Perry Marrow x Algarrobo	129	116	246	9 : 7	.10 - .25
Perry Marrow x Emerson 847	134	93	227	9 : 7	.25 - .50
Red Mexican x Algarrobo	205	17	222	15 : 1	.50 - .75
Michelite x Algarrobo	271	21	292	15 : 1	.50 - .75
Emerson 51-2 x Algarrobo	170	7	177	249 : 7	.25 - .50
Emerson 847 x Algarrobo	255	6	261	249 : 7	.50 - .75

R*--resistant S--susceptible

Table 6 shows the comparison between the observed and expected (3:1) number of resistant and susceptible plants, when 250 F_2 plants of the cross Cornell 64-23 x Algarrobo were inoculated with gamma.

Nine out of 16 plants of the backcross (Cornell 64-23 x Algarrobo) x Algarrobo inoculated with gamma were resistant and, 7 susceptible, approaching closely a 1:1 ratio.

The observed number of resistant and susceptible F_2 plants of the cross Cornell 64-23 x Emerson 847 inoculated with gamma is compared in table 6 with an expected 3:1 ratio.

A comparison of the observed and expected (3:1) number of resistant and susceptible F_2 gamma-inoculated plants of the cross Brazilian Red x Emerson 847 is presented in table 6.

Assuming a 3:1 ratio, the difference between the observed and expected number of resistant and susceptible F_2 gamma-inoculated plants of the cross Michelite x Dark Red Kidney is not significant at the 1% level (see table 6).

The proportion between the observed number of resistant and susceptible F_2 gamma-inoculated plants of the cross Perry Marrow x Algarrobo fits a 9:7 ratio (see table 6).

A very close fit to a 9 resistant : 7 susceptible ratio was obtained when 227 F_2 plants of the cross Perry Marrow x Emerson 847 were inoculated with C. lindemuthianum gamma strain as shown in table 6.

From the backcross (Perry Marrow x Emerson 847) x Perry Marrow, 70 seedlings were inoculated resulting in 13 resistant and 57 susceptible; this approaches a 1:3 ratio (χ^2 equal to 1.55).

In table 6 is presented the discrepancy between the observed and expected (15:1), number of resistant and susceptible plants, when 222 F_2 plants of the cross Red Mexican x Algarrobo were inoculated with gamma strain.

When 292 F_2 plants of the cross Michelite x Algarrobo were inoculated with gamma, the proportion of resistant and susceptible plants fits an expected 15:1 ratio, as shown in table 6.

The observed number of resistant and susceptible F_2 gamma-inoculated plants of the cross E merson 51-2 x Algarrobo is compared in table 6 with a 249:7 ratio. These data fit well the expected ratio.

The ratio between the observed number of resistant and susceptible F_2 gamma-inoculated plants of the cross Emerson 847 x Algarrobo fits very closely a 249:7 ratio as presented in table 6.

In the following crosses between two resistant parents, all the F_2 individuals were resistant: Michelite x Emerson 847; Red Mexican x Emerson 847; Emerson 51-2 x Emerson 847. In the cross Dark Red Kidney x Brazilian Red, both parents were susceptible, likewise all the F_2 progeny from the cross.

Joint Segregation for Reaction to Two Races

Alpha and Beta

In five of the 16 crosses studied, an analysis for the joint segregation for alpha and beta was carried out in order to see if recombination occurred between the genes conferring resistance to alpha and beta.

In the cross Cornell 64-23 x Algarrobo, segregation for alpha and beta was observed in the F_2 generation. The observed proportion of F_2 plants resistant to alpha and beta, resistant to alpha and susceptible to beta, susceptible to alpha and resistant to beta, and susceptible to both strains of the pathogen is not significantly different from a 195:45:13:3 ratio (see table 7). This ratio was obtained multiplying the F_2 ratios for the segregation of alpha and beta alone, that is, 15:1 for alpha x 13:3 for beta.

When the F_2 data of the cross Cornell 64-23 x Emerson 847, was analyzed for the joint segregation of alpha and beta, the number of plants resistant to both strains of the pathogen, resistant to alpha and susceptible to beta susceptible to

TABLE 7.--Observed and expected ratios for F_2 plants of the cross Cornell 64-23 x Algarrobo, inoculated with alpha and beta strains.

Alpha-Beta		Number of Plants Observed	Expected 195:45:13:3	X^2	Probability between
R*	R	184	190.43	.373	
R	S	47	43.95	.580	
S	R	14	12.70	.133	
S	S	5	2.92	1.482	
Total		250	250.00	2.668	.25 - .50

*R--resistant S--susceptible

alpha and resistant to beta, and the number of plants susceptible to both races of the fungus, was in close agreement with a 135:105:9:7 (15:1 x 9:7) ratio, as shown in table 8.

TABLE 8.--Discrepancy between the observed and expected frequencies for F_2 inoculated plants with alpha and beta strains of the cross Cornell 64-23 x Emerson 847.

Alpha-Beta		Number of Plants Observed	Expected 135:105:9:7	X^2	Probability between
R*	R	118	118.125	.000	
R	S	96	91.875	1.907	
S	R	4	7.875	.185	
S	S	6	6.125	.003	
Total		224	224.000	2.095	.50 - .75

R*--resistant S--susceptible

The observed number of F_2 plants of the cross Brazilian Red x Emerson 847 resistant to alpha and beta, resistant to alpha and susceptible to beta, susceptible to alpha and resistant to beta, and susceptible to both races, fits well a 171:21:57:7 (3:1 x 57:7) ratio as presented in table 9.

TABLE 9.--Observed and expected ratios for F_2 inoculated plants of the cross Brazilian Red x Emerson 847, with alpha and beta strains.

Alpha-Beta		Number of Plants Observed	Expected 171:21:57:7	X^2	Probability between
R*	R	167	161.65	.177	
R	S	23	19.85	.500	
S	R	43	53.88	2.197	
S	S	9	6.62	.856	
Total		242	242.00	3.730	.25 - .50

*R--resistant S--susceptible

In table 10, the observed number of F_2 plants resistant to alpha and beta, resistant to alpha and susceptible to beta, susceptible to alpha and resistant to beta, and susceptible to alpha and beta, of the cross Dark Red Kidney x Brazilian Red is compared with an expected 3:9:1:3 ratio (3:1 for alpha x 1:3 for beta). The observed data for the segregation to both races shows a very good agreement.

TABLE 10.--Comparison between observed and expected frequencies for F_2 inoculated plants of the cross Dark Red Kidney x Brazilian Red.

Alpha-Beta		Number of Plants Observed	Expected 3:9:1:3	X^2	Probability between
R*	R	46	42.94	.262	
R	S	123	128.81	.218	
S	R	16	14.31	.200	
S	S	44	42.94	.026	
Total		229	229.00	.706	.75 - .90

*R--resistant S--susceptible

Segregation for alpha and beta reactions was observed in the cross Michelite x Dark Red Kidney in the F_2 generation. The observed numbers of individuals resistant to both races, resistant to alpha and susceptible to beta, susceptible to alpha and resistant to beta, and susceptible to both strains of the pathogen, are presented in table 11. These figures are not significantly different from a 3:9:1:3 ratio.

Alpha and Gamma

In six of the 16 crosses studied, segregation for alpha and gamma reaction was observed; it offers an opportunity to analyze the data in order to see if recombination has occurred between the genes conferring resistance to these races.

TABLE 11.--Observed and expected ratios from F_2 inoculated plants with alpha and beta strains of the cross Michelite x Dark Red Kidney.

Alpha-Beta		Number of Plants Observed	Expected 3:9:1:3	X^2	Probability between
R*	R	45	37.5	1.500	
R	S	107	112.3	.269	
S	R	12	12.5	.020	
S	S	36	37.5	.060	
Total		200	200.0(	1.849	.50 - .75

*R--resistant S--susceptible

The observed number of F_2 plants of the cross Michelite x Algarrobo resistant to alpha and gamma, resistant to alpha and susceptible to gamma, susceptible to alpha and resistant to gamma, and susceptible to both races fits very well the 45:3:15:1 ratio. This ratio was obtained by combining the

3:1 F_2 ratio for alpha with the 15:1 F_2 ratio for gamma, obtained previously when the F_2 data were analyzed for each one of the races separately (see table 12).

TABLE 12.--Discrepancy between observed and expected frequencies for F_2 inoculated plants with alpha and gamma strains of the cross Michelite x Algarrobo.

Alpha-Beta		Number of Plants Observed	Expected 45:3:15:1	X^2	Probability between
R*	R	206	205.31	.002	
R	S	15	13.69	.125	
S	R	65	68.44	.173	
S	S	6	4.56	.455	
Total		292	292.00	.755	.75 - .90

*R--resistant S--susceptible

When the F_2 data of the cross Red Mexican x Algarrobo were analyzed for the joint segregation of alpha and gamma, the number of plants resistant to both strains of the pathogen, resistant to alpha and susceptible to gamma, and the number of plants susceptible to both races of the fungus was in close agreement with a 45:3:15:1 ratio (3:1 for alpha x 15:1 for gamma) as shown in table 13.

TABLE 13.--Observed and expected ratios for F_2 alpha-gamma inoculated plants of the cross Red Mexican x Algarrobo.

Alpha-Gamma		Number of Plants Observed	Expected 45:3:15:1	X^2	Probability between
R*	R	160	156.03	.101	
R	S	11	10.43	.031	
S	R	45	52.05	.955	
S	S	6	3.49	1.805	
Total		222	222.00	2.892	.50 - .75

*R--resistant S--susceptible

When 242 F_2 plants of the cross Brazilian Red x Emerson 847 were classified according to their reaction to alpha and gamma strains of C. lindemuthianum, the number of plants resistant to both races of the pathogen, resistant to alpha and susceptible to gamma, susceptible to alpha and resistant to gamma, and susceptible to both strains of the pathogen is presented in table 14. These figures are not significantly different from a 9:3:3:1 ratio (3:1 for alpha x 3:1 for gamma).

TABLE 14.--Observed and expected frequencies for F_2 inoculated plants of the cross Brazilian Red x Emerson 847.

Alpha-Gamma		Number of Plants Observed	Expected 9:3:3:1	X^2	Probability between
R*	R	146	136.125	.716	
R	S	44	45.375	.042	
S	R	35	45.375	2.372	
S	S	17	15.125	.235	
Total		242	242.000	3.365	.25 - .50

*R--resistant S--susceptible

Two hundred F_2 plants of the cross Michelite x Dark Red Kidney were classified according to their reaction to alpha and gamma strains, the number of plants resistant to both races of the pathogen, resistant to alpha and susceptible to gamma, susceptible to alpha and resistant to gamma, and susceptible to both strains, approach a 9:3:3:1 ratio as shown in table 15.

In the F_2 generation of the cross Cornell 64-23 x Algarrobo the observed proportion of plants resistant to alpha and gamma, resistant to alpha and susceptible to gamma,

TABLE 15.--Discrepancy between the observed and expected ratios for F_2 inoculated plants of the cross Michelite x Dark Red Kidney.

Alpha-Gamma		Number of Plants Observed	Expected 9:3:3:1	X^2	Probability between
R*	R	105	112.5	.500	
R	S	47	37.5	2.406	
S	R	37	37.5	.007	
S	S	11	12.5	.180	
Total		200	200.0	3.093	.25 - .50

*R--resistant S--susceptible

susceptible to alpha and resistant to gamma, and susceptible to both strains, is not significantly different from a 45:15:3:1 ratio (15:1 for alpha x 3:1 for gamma), as shown in table 16.

TABLE 16.--Comparison between observed and expected proportions for F_2 inoculated plants of the cross Cornell 64-23 x Algarrobo.

Alpha-Gamma		Number of Plants Observed	Expected 45:15:3:1	X^2	Probability between
R*	R	179	175.78	.059	
R	S	52	58.59	.741	
S	R	13	11.72	.140	
S	S	6	3.91	1.117	
Total		250	250.00	2.057	.50 - .75

*R--resistant S--susceptible

In the cross Cornell 64-23 x Emerson 847 the observed number of F_2 plants resistant to alpha and gamma, resistant to alpha and susceptible to gamma, susceptible to alpha and resistant to gamma, and susceptible to both strains of the pathogen, approach a 45:15:3:1 ratio (15:1 for alpha x 3:1 for gamma) as presented in table 17.

TABLE 17.--Observed and expected frequencies between F_2 alpha-gamma inoculated plants of the cross Cornell 64-23 x Emerson 847.

Alpha-Gamma		Number of Plants Observed	Expected 45:15:3:1	X^2	Probability between
R*	R	164	157.5	.269	
R	S	50	52.5	.119	
S	R	6	10.5	1.929	
S	S	4	3.5	.071	
Total		224	224.0	2.388	.50 - .75

*R--resistant S--susceptible

Beta and Gamma

In eight of the 16 crosses studied, segregation for the genes conferring resistance to anthracnose beta and gamma strains was observed, giving opportunity to analyze the data in order to see if the genes conferring resistance to these two races segregate independently.

In table 18 the observed number of F_2 plants of the cross Michelite x Dark Red Kidney resistant to beta and gamma, resistant to beta and susceptible to gamma, susceptible to beta and resistant to gamma, and susceptible to both strains of the fungus is compared with a 3:1:9:3 ratio (1:3 for beta x 3:1 for gamma).

When 247 F_2 plants of the cross Cornell 64-23 x Emerson 847 were classified according to their reaction to antracnose beta and gamma strains the number of plants resistant to both races, resistant to beta and susceptible to gamma, susceptible to beta and resistant to gamma, and susceptible

TABLE 18.--Comparison between observed and expected ratios for F_2 inoculated plants of the cross Michelite Dark Red Kidney.

Beta-Gamma		Number of Plants Observed	Expected 3:1:9:3	X^2	Probability between
R*	R	54	37.5	5.336	
R	S	3	12.5	8.167	
S	R	88	112.5	7.260	
S	S	55	37.5	7.220	
Total		200	200.0	27.983	less than .001

*R--resistant S--susceptible

to beta and gamma was compared with a 27:9:21:7 (9:7 beta x 3:1 gamma) ratio, a significant difference was observed (see table 19).

TABLE 19.--Observed and expected frequencies for F_2 beta-gamma inoculated plants of the cross Cornell 64-23 x Emerson 847.

Beta-Gamma		Number of Plants Observed	Expected 27:9:21:7	X^2	Probability between
R*	R	118	94.5	5.844	
R	S	6	31.5	6.888	
S	R	51	73.5	20.643	
S	S	49	24.5	24.500	
Total		224	224.00	57.875	less than .001

*R--resistant S--susceptible

The observed number of F_2 plants of the cross Cornell 64-23 x Algarrobo resistant to beta and gamma, resistant to beta and susceptible to gamma, susceptible to beta and resistant to gamma, and susceptible to both strains of the fungus, was significantly different from a 39:13:9:3 (13:3 for beta x 3:1 for gamma) ratio, as shown in table 20.

In the cross Dark Red Kidney x Algarrobo, segregation for beta and gamma was observed. The proportion of F_2 plants resistant to both races of the pathogen, resistant to beta and susceptible to gamma, susceptible to beta and resistant

TABLE 20.--Comparison between the observed and expected ratios for F_2 inoculated plants of the cross Cornell 64-23 x Algarrobo.

Beta-Gamma		Number of Plants Observed	Expected 39:13:9:3	X^2	Probability between
R*	R	181	150.52	6.194	
R	S	13	50.17	27.539	
S	R	8	34.73	20.573	
S	S	45	11.52	97.301	
Total		247	247.00	151.607	less than .001

*R--resistant S--susceptible

to gamma, and susceptible to both strains was compared with a 45:15:3:1 (15:1 beta x 3:1 gamma) ratio. A significant departure between the observed and expected data was obtained, as presented in table 21.

TABLE 21.--Observed and expected frequencies for F_2 inoculated plants of the cross Dark Red Kidney x Algarrobo.

Beta-Gamma		Number of Plants Observed	Expected 45:15:3:1	X^2	Probability between
R*	R	201	196.36	.302	
R	S	58	64.45	.646	
S	R	3	12.89	7.588	
S	S	13	4.30	17.602	
Total		275	275.00	26.138	less than .001

*R--resistant S--susceptible

Segregation for beta and gamma reaction was observed in the F_2 generation of the cross Emerson 847 x Dark Red Kidney. A significant difference between the observed and expected number of plants resistant to both races of the fungus, resistant to beta and susceptible to gamma, susceptible to beta and resistant to gamma and susceptible to beta and gamma was observed (see table 22).

TABLE 22.--Difference between observed and expected ratios for F_2 beta-gamma inoculated plants of the cross Emerson 847 x Dark Red Kidney.

Beta-Gamma		Number of Plants Observed	Expected 27:9:21:7	X^2	Probability between
R*	R	173	129.94	14.27	
R	S	13	43.31	21.21	
S	R	55	101.06	20.99	
S	S	67	33.69	32.92	
Total		308	308.00	89.40	less than .001

*R--resistant S--susceptible

When the F_2 data of the cross Brazilian Red x Emerson 847 was analyzed for the joint segregation of beta and gamma, the number of plants resistant to both strains of the pathogen, resistant to beta and susceptible to gamma, susceptible to beta and resistant to gamma, and susceptible to beta and gamma, was not in agreement with an expected 171:57:21:7 (57:7 beta x 3:1 gamma) ratio, as shown in table 23.

TABLE 23.--Observed and expected frequencies for F_2 inoculated plants of the cross Brazilian Red x Emerson 847.

Beta-Gamma		Number of Plants Observed	Expected 171:57:21:7	X^2	Probability between
R*	R	179	161.6	1.87	
R	S	31	19.9	3.14	
S	R	12	53.9	9.73	
S	S	20	6.9	27.21	
Total		242	242.0	41.95	less than .001

*R--resistant S--susceptible

DISCUSSION

The inoculation technique used in this work, in contrast to the common technique, proved to be very effective in antracnose inheritance studies when these were concerned with more than one physiological form of the pathogen, in that it facilitated testing the same plant with more than one strain of the fungus. Because it is a time-consuming technique, its main application will be in inheritance studies which involve more than one C. lindemuthianum strain, when the main object of the study is to discover whether the genes conferring resistance to one of the races are linked with the genes conferring resistance to the other race, or in linkage studies carried out in order to know if the genes conferring resistance to any anthacnose race are located in the same chromosome with other genes affecting some other characteristic (morphological or physiological) of the bean plant. This inoculation technique does not kill the susceptible plants, while the common technique does.

Reaction to Alpha

The information derived from this study in regard to the inheritance of resistance to the alpha strain confirms the general information previously known, that resistance is dominant. In all previous studies reported, only monofactorial F_2 ratios were observed, in the present work, besides the

3:1, 15:1 ratios were observed. The most plausible hypothesis required to explain these results is to assume that the 15:1 ratios are due to the presence of duplicate factors independent in a linkage sense. According to this hypothesis the genotypes of the nine varieties used in this study, with regard to alpha, will be:

Algarrobo	AAbb	Resistant
Emerson 847	AAbb	Resistant
Emerson 51-2	AAbb	Resistant
Perry Marrow	AAbb	Resistant
Dark Red Kidney	AAbb	Resistant
Cornell 64-23	aaBB	Resistant
Michelite	aabb	Susceptible
Red Mexican	aabb	Susceptible
Brazilian Red	aabb	Susceptible

In the five crosses in which we observed segregation for alpha and beta, the statistical analysis for independence of the genes conferring resistance to alpha and beta was carried out; in all cases the data show the genes are located in different linkage groups (see tables 7, 8, 9, 10, 11).

When we carried out the analysis for the joint segregation for alpha and gamma, in the six crosses studied, the data support the thesis that the genes conferring resistance to alpha are located in different linkage groups than those conferring resistance to gamma (see tables 12, 13, 14, 15, 16, 17);

These results are in accord with the casual observation of several bean breeders that there is no tendency to observe, more often than expected by chance, bean varieties or segregation progenies susceptible to both alpha and beta, and/or alpha and gamma.

Reaction to beta

The situation with respect to the inheritance of reaction to the beta strain in more complicated. In the F_1 of some crosses resistance was dominant, while in other crosses susceptibility was dominant, depending on the parents used in the cross (see table 2). In order to explain the F_2 ratios observed, it is necessary to assume two different kinds of resistance, one of them due to the presence of duplicate factors c and d, each one with the capacity of conferring resistance alone; and the other due to the complementary action of two genes, e and f. Besides, as required by the data, in the case of duplicate as well as in the case of the complementary genes, four different alleles are present. The alleles c^1, d^1, e^1 and f^1 are genes conferring susceptibility and are recessive to c^3, d^3, e^3, f^3; and c^4, d^4, e^4, f^4 respectively, which are alleles conferring resistance. The susceptibility alleles c^2, d^2, e^2, f^2, are dominant over the resistant genes c^3, d^3, e^3, f^3, but recessive to the alleles c^4, d^4, e^4, f^4. In the first case there are two different alleles for susceptibility and two for resistance, one of the alleles for susceptibility is dominant over one of the alleles conferring resistance. In the case of the complementary genes a similar situation is observed. According to this reasoning the genotypes used in this study of the nine varieties with respect to reaction to beta will be:

Algarrobo	$c^4\ c^4\ d^4\ d^4\ e^1\ e^1\ f^1\ f^1$	Resistant
Emerson 847	$c^1\ c^1\ d^1\ d^1\ e^3\ e^3\ f^3\ f^3$	Resistant
Perry Marrow	$c^4\ c^4\ d^4\ d^4\ e^4\ e^4\ f^4\ f^4$	Resistant
Red Mexican	$c^4\ c^4\ d^4\ d^4\ e^4\ e^4\ f^4\ f^4$	Resistant
Michelite	$c^3\ c^3\ d^3\ d^3\ e^3\ e^3\ f^3\ f^3$	Resistant
Brazilian Red	$c^1\ c^1\ d^3\ d^3\ e^1\ e^1\ f^1\ f^1$	Resistant
Dark Red Kidney	$c^1\ c^1\ d^2\ d^2\ e^1\ e^1\ f^1\ f^1$	Susceptible
Cornell 64-23	$c^1\ c^1\ d^1\ d^1\ e^1\ e^1\ f^1\ f^1$	Susceptible
Emerson 51-2	$c^1\ c^1\ d^1\ d^1\ e^2\ e^2\ f^2\ f^2$	Susceptible

The simplest hypothesis which explains the F_2 results of the cross Michelite x Dark Red Kidney (1 resistant : 3 susceptible) assumes that Dark Red Kidney possesses a dominant inhibitor of the beta-resistance genes (c^3, d^3, e^3, f^3) present in Michelite.

The 13:3 ratio observed in the cross Cornell 64-23 x Algarrobo can be explained if we assume that Cornell 64-23 carries a dominant suppressor of either the c^4, or d^4 dominant resistance genes present in Algarrobo, and located at different loci.

This hypothesis is not absolute, there are other possible explanations of the results, like one suggested by

Dr. Allen S. Fox. Nine different gene pairs (A, B, C, D, E, F, G, H, J) are required in order to explain the observed F_2 results. In this hypothesis susceptibility results as a consequence of the simultaneous presence of at least two recessive genes of the five (A, B, C, D, E) which interact in order to produce susceptibility. The other four genes (F, G, H, J) are modifiers: ff makes c dominant over C, gg makes d dominant over D, h is a duplicate gene for a and, in absence of dd, for g, and J makes b dominant over B. According to this scheme the susceptible genotypes are as follows:

aabbcc----F---

aabb--dd--F---

aa--cc----ff--

aabbCc----ff--

aa----dd----gg

aabb--Dd----gg

aabb----ee----

According to this hypothesis the genotypes of the nine varieties used in this study are:

Algarrobo	(R)	AAbbCCDDEEFFGGHHjj
Emerson 847	(R)	aaBBccddEEFFGGHHjj
Brazilian Red	(R)	aabbCCDDEEffGGHHjj
Michelite	(R)	aabbCCDDeeFFGGHHjj
Perry Marrow	(R)	AABBCCDDEEFFGGHHjj
Red Mexican	(R)	AABBCCDDEEFFGGHHjj

Dark Red Kidney	(S)	aabbccDDeeffGGHHjj
Emerson 51-2	(S)	aabbCCddEEFFGGHHJJ
Cornell 64-23	(S)	aabbCCddEEFFgghhjj

In order to know which one of these two hypotheses is correct, crosses between Dark Red Kidney x Emerson 51-2, and Dark Red Kidney x Cornell 64-23 have to be made. If the first hypothesis is correct, no segregation will be expected in the F_2, while if segregation is observed, the second hypothesis proposed will be correct.

When the data were analyzed from six of the 16 crosses studied for the joint segregation of beta and gamma (assuming that the first hypothesis is the correct one) , it was found that some of the genes conferring resistance to gamma did not segregate independently; that is, they were linked

The calculation of the recombination values was made according to the method of miximum likelihood, using the formulas given by Allard (1). In the crosses Michelite x Dark Red Kidney, Cornell 64-23 x Emerson 847, Dark Red Kidney x Algarrobo, and Dark Red Kidney x Emerson 847, the recombination values obtained were: $.206 \pm .0671$, $.105 \pm .0513$, $.0938 \pm .0406$, $.807 \pm .0287$, respectively.

According to the linkage values obtained in the crosses Dark Red Kidney x Algarrobo, and Cornell 64-23 x Algarrobo, $8.07 \pm 2.53\%$, and $10.5 \pm 5.13\%$ respectively, it appears that one of the duplicated factors conferring resistance to beta is located about nine crossover units apart from the gene conferring resistance to gamma, present in Algarrobo.

In the crosses Cornell 64-23 x Emerson 847, and Dark Red Kidney x Emerson 847, the linkage values observed were $9.38 \pm 4.06\%$, and $8.05 \pm 2.8\%$, respectively, from which it may be inferred that one of the complementary factors conferring resistance to beta is located about 8.7 crossover units from the gene conferring resistance to gamma.

In the cross Dark Red Kidney x Michelite, $20.6 \pm 6.71\%$ recombination was observed between the beta suppressor gene, and the gene producing susceptibility to gamma. In order to explain these results, it is necessary to assume that the supressor gene is located in the same chromosome of Dark Red Kidney, about 20 crossover units apart from one of the genes conferring susceptibility to gamma.

These linkage relationship results do not support the statement of Andrus and Wade (2) that linkage is not involved to any extent in the inheritance of antracnose resistance.

The linkage between the genes conferring resistance to beta and gamma may explain the results published by the Department of Plant Pathology of Cornell University (3). The report states that out of 62 bean varieties inoculated with beta and gamma races of antracnose, 31 were susceptible to both strains of the pathogen, 22 were resistant to beta and gamma, 5 were resistant to beta and susceptible to gamma, and 4 were susceptible to beta and resistant to gamma.

Reaction to Gamma

The most plausible interpretation of gamma results is to assume, as in the case of beta, two different systems producing resistance, one due to the presence of duplicated genes, (G and H) each one of which is able to produce resistance, and the other due to the joint effect of two complementary genes (J and K). Following this model, the genotypes of the nine varieties used in this work will be:

Algarrobo	ggHHjjKK	Resistant
Emerson 847	GGhhJJkk	Resistant
Emerson 51-2	GGhhJJkk	Resistant
Perry Marrow	gghhjjkk	Susceptible
Dark Red Kidney	gghhjjkk	Susceptible
Cornell 64-23	gghhjjkk	Susceptible
Michelite	GGhhjjkk	Resistant
Red Mexican	GGhhjjkk	Resistant
Brazilian Red	gghhjjkk	Susceptible

In the crosses Perry Marrow x Algarrobo, and Perry Marrow x Emerson 847, the 9:7 F_2 observed ratios can be explained assuming that Perry Marrow possesses a recessive suppressor gene of the duplicated factors (G and H).

SUMMARY AND CONCLUSIONS

During the course of this work, the inheritance of reaction to alpha, beta and gamma races of C. lindemuthianum was investigated, using 16 crosses between nine parental varieties.

In order to determine the reaction to the three forms of the fungus by the same plant, a new inoculation technique was developed. It consists mainly in rubbing with a camel's hair brush wetted in a spore suspension of the pathogen each one of the incompletely developed leaflets, of the first trifoliate leaf, with one of the three physiological forms of the pathogen. For a period of seven days, after inoculation, the plants were kept in a moisture chamber at approximately 70°F and almost 100% relative humidity. After 10 days the reaction readings were made.

The inheritance of reaction to alpha was studied in eight crosses; 3:1 F_2 ratios were observed in crosses between resistant x susceptible varieties, indicating that resistance was due to the presence of a single dominant gene; 15:1 ratios were observed in certain crosses between resistant x resistant varieties, indicating that at least two different genes, each one capable of conferring resistance, were segregating. No linkage was observed between the genes conferring resistance to alpha and those conferring resistance to either beta or gamma.

The inheritance of reaction to beta was studied in ten different crosses, eight of which were between resistant x susceptible varieties, and two resistant x resistant. In three of the eight resistant x susceptible crosses, susceptibility was dominant in the F_1. In order to explain the F_2 observed ratios (1:3, 7:9, 9:7, 13:3, 15:1, 57:7, 249:7), two different kinds of resistance were postulated, one due to the presence of duplicated factors, each one having the capacity of conferring resistance, and the other due to the complementary action of two genes. In order to explain the cases in which susceptibility was dominant, it was necessary to assume four alleles for each one of the four postulated genes, two of the alleles conferring resistance, and two conferring susceptibility, one of these is dominant over one of the alleles conferring resistance. In one variety (Dark Red Kidney), it was found necessary to postulate a dominant suppressor of the dominant resistance genes present in Michelite.

The above interpretation of beta results is not absolute. Another hypothesis that explains the beta observed data; assumes the presence of nine different gene pairs, five of which have a direct effect on the reaction to anthracnose, and the other four when recessive modify the dominance relationship, making the recessive alleles dominant in the heterozygous condition.

The inheritance of reaction to gamma was studied in 12 crosses, eight of which were between resistant x susceptible varieties, and four resistant x resistant. In the F_1 resistance was dominant in all cases. In order to explain the F_2 observed ratios (3:1, 15:1, 9:7, 249:7), it was necessary to assume, as in the case of beta, two different kinds of resistance, one due to the presence of duplicate genes, each one capable of conferring resistance, and the other due to the complementary action of two genes. In the crosses Perry Marrow x Emerson 847, and Perry Marrow x Algarrobo, it was necessary to assume that Perry Marrow possesses a recessive supressor gene of the duplicate dominant factors conferring resistance, in order to yield a 9:7 F_2 ratio.

Linkage was observed between the genes conferring resistance to beta and those conferring resistance to gamma. It was possible to determine that in Algarrobo one of the duplicate genes conferring resistance to beta is located about nine crossover units from one gene conferring resistance to gamma. In the variety Emerson 847 it was possible to determine that one of the complementary genes conferring resistance to beta was located in the same chromosome, about 8.7 crossover units, from one gene conferring resistance to gamma. In the variety Dark Red Kidney, it was possible to determine that the beta resistance suppressor gene is located about 20 crossover units from one of the genes conferring susceptibility to gamma.

These linkage relationships explain, in part, why in nature are found more bean varieties either susceptible or resistant to both beta and gamma, and very few varieties resistant to beta and susceptible to gamma, and/or susceptible to beta and resistant to gamma.

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