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GENETIC SYSTEM FOR REACTION IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) TO FOUR ISOLATES OF *PHAEOISARIOPSIS GRISEOLA*.

By

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ABSTRACT

GENETIC SYSTEM FOR REACTION IN COMMON BEAN (PHASEOLUS VULGARIS L). TO FOUR ISOLATES OF PHAEOISARIOPSIS GRISEOLA

By

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The inheritance of resistance in common bean to four isolates of Phaeoisariopsis griseola Sacc., namely, Michigan 5, Colombia 10, Puerto Rico 2 and Malawi 1, was studied in five crosses. Screening materials for each cross consisted of parental, F_1, F_2, F_3, and backcross populations.

Resistance in G 05686 to the Michigan 5 isolate was inherited as a single major recessive gene with minor modifying gene effects, in the cross involving Montcalm dark red kidney and G 05686. In two resistant by resistant crosses, resistance was conferred by two duplicate dominant genes at either locus. No segregation was observed in the F_2 generation of the other two resistant by resistant crosses. The results of these resistant by resistant crosses revealed that C-20 and G 05686 possess identical genes conferring resistance ($c_2 c_2 d_1 d_1$) whereas Pompadour Checa and BAT-332 possess resistance genes, $c_1 c_1 d_2 d_2$ and $c_2 c_2 d_2 d_2$ respectively. The inheritance of resistance in small-seeded (Meso-American source) lines and medium-large

seeded (Andean source) cultivars was also found to be identical, at least with respect to the Michigan 5 isolate.

Two crosses involving BAT-332 as the resistant parent indicated that different genes conferred resistance in BAT-332 to the Colombia 10 isolate. In one cross, resistance was conferred by two complementary recessive genes, whereas in the other, a dominant inhibitor allele for susceptibility was epistatic to the dominant allele for resistance present in BAT-332.

The resistance in C-20 to the Malawi 1 isolate was inherited through a single recessive gene with a possible influence of minor modifers.

Dwarf lethality observed in some of the crosses was inherited as a consequence of the presence of two complementary dominant genes, and occurred when small-seeded parents (Meso- American source) with an "S" phaseolin were crossed with medium-large seeded ones (Andean source) with a "T" phaseolin. The genetic barrier existing between the parents is indicative of the two distinct gene pools postulated in cultivated common bean germplasm.

To George, my beloved husband and
our children Parry and Paa Kwasi
with love

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CHAPTER ONE

INTRODUCTION

Angular leaf spot (ALS) disease, caused by the fungus Phaeoisariopsis griseola Sacc., is considered primarily a disease in the tropics and sub-tropics. Although ALS has been reported in temperate regions, in the United States it is considered a minor disease but sometimes occurs sporadically in epidemic proportions (Cardona- Alvarez, 1956). ALS can be a widespread and economically important fungal disease in other areas. Under favourable conditions of relative humidity and temperature, ALS can be responsible for severe yield losses in many bean producing areas. Yield losses of 40-60% were observed in fields in the Cauca Valley of Colombia (Barros et al., 1957) and losses of 80% were reported in Mexico (Crispin et al., cited by Schwartz et al 1981). In most bean producing areas of the United States, ALS is considered of minor importance. However, the disease caused losses of 50% or more in several commercial snap bean plantings in central Wisconsin in 1954 (Cardona-Alaverz and Walker, 1956). A similar severe outbreak of ALS was observed during 1982-1983 in the north- eastern lower peninsula of Michigan (Saettler and Correa, 1983). ALS also infects seed and is transmitted within the seeds (Orozco and Cardona-Alvarez, 1959, Sohi

and Sharma, 1974, and Correa, 1984). Various control strategies, such as the use of pathogen free seed and chemicals, can be employed. However, seed programs and chemical control are expensive, and not fully effective. Moreover, chemical control may be environmentally hazardous. A preferred control measure, therefore, would be the use of genetically regulated disease resistance.

Breeding for ALS resistance in common beans requires: 1) the identification of resistant bean genotypes; 2) the determination of the variability of the pathogen; 3) knowledge about the inheritance of resistance; 4) knowledge of the relationship of genes in different resistance sources to a particular ALS pathogen. While there is sufficient literature about the first two steps, there is little and contradictory literature concerning the third step. The literature reviewed provided no information as to whether different sources of resistance to a particular pathogen isolate are controlled by the same or different genes.

Thus, the objectives for the present study were:

1) to study the inheritance of resistance of common bean (Phaseolus vulgaris L) to four ALS isolates from different sources.
2) to determine if different sources of resistance to a particular ALS isolate are characterized by the same or

different genes.

3) to determine if genes for resistance in large-seeded (Andean source) beans are identical in inheritance to resistance genes found in small-seeded (Meso-American sources) lines.

LITERATURE REVIEW

2.1 The pathogen

Phaeoisariopsis griseola (Sacc.) Ferrais, also widely known as Isariopsis griseola Sacc., is an imperfect fungus which belongs to the family Stibaceae. The pathogen was first described by Saccardo in 1877 and is found to be synonymous with Isariopsis laxa (Ell) Sacc., Graphium laxum Ell., Cercospora columnare Ell and Ev., Lindaumyces griseola Gonz. Frag, Arthrobotryum puttemansi Henn, and Cercospora sthulmanni Henn, (Ferraz in Schwartz and Galvez, 1980). Hocking (1967) reported that the only common characteristic with Isariopsis griseola Sacc, is the formation of synnemata.

The reproductive spores, conidiospores, are borne on groups of 8 to 40 conidiophores, which are joined loosely to form dark brownish columnar coremia or synnemata (Miles, 1917, and Ferraz in Schwartz and Galvez, 1980). The condiophores tend to separate, especially with age (Miles 1917, and Chupp and Sherf, 1960). The average thickness of the coremium is 20 to 40u (Miles, 1917, and Ferraz in Schwartz and Galvez, 1980), while different figures are reported for the average length. Miles (1917) reported an average length of 200u, 94 to 163u by Hocking (1967) and 500u by Ferraz (In Schwartz and Galvez,1980).

A detailed description of the conidia (conidiospores)

was provided by Miles (1917). The conidia are borne on the smooth tips of the hyphae, are light gray and cylindrical to spindle form. They are slightly curved without any constriction, measure 50u to 60u in length, 7u to 8u in thickness, and are 1- to 3- septate. The end cells of these septate spores form mycelial hyphae upon germination (Miles, 1917).

2.2 Pathogenic Variability

Knowledge regarding variability of the angular leaf spot (ALS) pathogen is very important in achieving durable control of angular leaf spot disease through genetic resistance. It is also important to know how variability in the pathogen "evolves" in order to develop the broadest possible spectrum of resistance. However, little has been done in these areas, possibly, because in the past the disease was considered to be of minor consequence. Alvarez-Ayala and Schwartz (1979) differentiated a number of physiological races of Phaeoisariopsis griseola in a collection of the pathogen from different regions in Colombia, by inoculating Brazil 260 (Caraota). Brazil 260, a variety reported to be resistant to angular leaf spot in Brazil, was found to be susceptible to 3 of the 4 Colombian isolates used, indicating the existence of variability in the pathogen. They also confirmed the existence of

virulence differences in various isolates of the pathogen. Brock (1951) compared 13 field isolates of Phaeoisaropsis griseola on bean varieties Brown Beauty and Red Mexican and observed pathogenic differences between the isolates. Correa (1984) indicated no pathogenic variation among Michigan isolates of P. griseola but differences were observed among isolates from different countries. Correa (1987), in his extensive studies on pathogenic variability in P. griseola, observed that pathogenicity varied among isolates from different countries as well as among isolates within a country.

In Tanzania, a highly virulent form of P. griseola was identified by Hocking (1967). The symptoms consisted of circular spots but the fungus isolates were morphologically indistinguishable from typical P. griseola. However, the synnemata were found to be somewhat longer and developed on both the upper and lower leaf surfaces. Hocking observed that spore concentrations as low as 10^2 spores/ml of the new virulent form were sufficient for infection, whereas 10^5 spores/ml were needed for infection with the common form, and he surmised that the highly virulent form of P. griseola was possibly the result of a single mutation.

2.3 Inoculation techniques

Several methods of inoculation have been employed in screening germplasm for angular leaf spot disease but the most common has been the spraying of conidial suspensions.

Alvarez-Ayala and Schwartz (1979) utilized three inoculation techniques: (1) rubbing the leaf surface with a conidial suspension of 2.0×10^4 spores/ml; (2) immersing leaf blades in a conidial suspension of 2.0×10^4 spores/ml; and (3) spraying the leaf surface with a conidial suspension of 2.0×10^4 spores/ml. The authors obtained most consistent results with the latter technique. Alvarez-Ayala (1979) and Correa (1984) indicted that conidial suspensions containing 2.0×10^4 spores/ml gave the best results in greenhouse tests. A combination of spores and mycelial suspensions of 10^3 -10^4 pieces/ml caused typical angular lesions and heavy defoliation in susceptible varieties (Brock,1951).

Inglis et al. (1984) investigated the possiblity of using dry inoculum of P. griseola. Dry inoculum containing 3.0×10^6 conidia/g and 4.0×10^7 conidia/g were dusted onto field plants after previously applying a fungicide sticker. Statistically, treatments with dry inoculum had higher leaf lesion ratings, greater defoliation and yielded significantly less than treatments inoculated with conidial suspensions containing 5.8×10^4 spores/ml.

2.4 Modes of survival in P. griseola.

Studies on survival of P. griseola have been carried out mainly with infected plant debris, contaminated seeds and soil. Sohi and Sharma (1974) observed that about 16% of conidia were viable after 224 days of storage under aboratory conditions, whereas 50% of conidia were viable after 142 days when stored in soil under field conditions. However, they did not state the field conditions during which the diseased leaves were buried in the soil. They also identified spores of P. griseola on old infected stem pieces three days after incubation, and concluded that the fungus could overwinter as stromatic tissues on infected plant residues . However, Cardona-Alvarez and Walker (1956) proved that the pathogen can survive two successive winters in the plant debris under Wisconsin conditions, but could not establish the evidence of residual soil transmission. Under Michigan conditions, the pathogen survived at least two winters in buried plant debris and infected standing plants (Correa and Saettler, 1987). Sindhan and Bose (1978) reported that P. griseola conidia remained viable in plant debris for 6 and 8 months under laboratory and field conditions, respectively. The authors, however, did not specify the laboratory and field conditions under which the experiment was conducted.

Reports on the ability of P. griseola to survive or

overwinter as fungal tissue in contaminated seed are contradictory. Sohi and Sharma (1974) showed that the fungus is seed borne, which could constitute a primary means for pathogen dispersal to new localities. Similar results were obtained by Sindhan and Bose (1978) who reported that the fungus remained as dormant mycelium viable in the seed for more than one year. Seeds from both infected and healthy plants were sown in pots containing sterilised soil and kept in the glass house to avoid contamination. The results indicated that germination of infected seeds was poor and that plants arising from infected seeds became heavily diseased when they reached the susceptible stage. However, the developmental state at which plants were susceptible was not reported. Ryan (1965), Bose and Sindhan (1972) and Sattler and Correa (1984) also reported that the disease is transmitted through infected seeds. Orozco- Sarria and Cardona-Alvarez (1959), however, obtained dissimilar results. They found that about 50% of seed harvested from infected plants produced P. griseola when cultured on agar plates, whereas similar tests with seeds of several other varieties were negative. Cardona-Alvarez (1956) did not observe symptoms on plants grown from seeds which were obtained from a field of infected plants, even when conditions favourable for disease development were provided.

2.5 Host range

Cardona-Alvarez (1956) found that the only species susceptible to the pathogen other than Phaseolus vulgaris L. was Phaseolus lunatus L. He showed that none of several soybean (Glycine max) varieties studied were susceptible, though this species was reported as a susceptible host in Russia in 1931 (Abramanof, cited by Cardona-Alvarez, 1956). Pisum sativum (Chupp,1925), P. multiflorus Willd (Brock, 1951), P. mungo (Golato and Meossi, 1973) and Vigna unguiculata (Diaz et al., cited by Correa 1984), have also been reported as susceptible hosts.

Campos (cited by Correa 1984) reported that P. lunatus, P.acutifolius L. P. angularis, P. coccineus and P. calcaratus were susceptible, whereas Vigna unguiculata, Cajanus cajan, Glycine max, Vicia faba., Medicago sativa, Pisum sativum and Lupinus sp. were resistant.

2.6 Host-parasite relations

Cardona-Alvarez (1956) investigated the host-parasite relations by inoculating leaves of Idaho Refugee bean and providing conditions favourable for disease development. He observed that host penetration by the fungus occurs through stomata, and that necrosis of the guard cells and adjoining mesophyll cells occurred after 3 days. At this point chloroplasts showed signs of disintegration. The

spongy parenchyma, palisade mesophyll cells and, finally, the upper epidermal cells disintegrated as the fungus grew intercellularly. At 9 days the fungus became intracellular and stromata, consisting of coarse mycelial strands, developed in the sub-stomatal cavity. At 12 days necrosis became limited by the vascular bundles of the leaf, followed by general collapse of invaded tissue as the fungal stromata developed fully. Symptoms were visible at this time, and heavy sporulation occurred when lesions were exposed to continuous moisture for 48 hours.

2.7 Symptoms

Cardona-Alvarez (1956) gave a detailed description of the typical symptoms of angular leaf spot disease. Other researchers have reported similar symptoms (Miles, 1917, Hater and Zaumeyer, 1957, Chupp, 1980, and Ferraz, in Schwartz and Galvez, 1980). The disease is confined to the aerial parts of the plant, affecting leaves, stems, branches and pods. However, leaf infection is the most common. On leaves, lesions are gray, the colour changing to light-brown with age. The lesions are restricted by the veins and veinlets, resulting in the typical angular shape. In severely diseased plants, lesions increase in size, coalesce and cause partial necrosis and yellowing of leaves, followed by premature defoliation. On stems and

branches, brown elongated lesions occur (Cardona-Alvarez, 1956). Lesions on pods appear as oval to circular spots with reddish-brown centers surrounded by darker coloured borders(Cardona-Alvarez and Walker, 1956, Harter and Zaumeyer, 1957, Cardona and Skiles, 1958, and Correa, 1984). During long periods of high humidity, dark gray to black synnemata and conidia develop in lesions on lower leaf surfaces, stems, branches and pods.

Hocking (1967) observed a different form of symptoms caused by a new virulent form of the fungus on P. vulgaris. He reported that leaves exhibited regular circular brown lesions up to 2 cm in diameter, with abundant robust synnemata on the under surfaces and a few on the upper surfaces. These lesions readily crossed veins to remain symmetrical instead of displaying the typical angular lesions.

2.8 Disease rating scales

The scales used for rating angular leaf spot are as follows.

A) Correa (1984) adopted a 5-point scale based on percent leaf infection.

1 = immune, no infection present.

2 = lesions covering 1 to 15% of leaf area.

3 = lesions covering 16 to 30% of leaf area.

4 = lesions covering 31 to 50% leaf area, lesions increasing in size, presence of chlorosis and partial defoliation.

5 = more than 50% leaflet area infected, with lesions surrounded by chlorosis, and defoliation being common.

B) Schwartz et al (1981) rated infected plants based on actual leaf area infected:

1 = immune, 0%.

2 = light infection, 1-2%.

3 = moderate infection, 3-10%.

4 = heavy infection, 11-25%.

5 = severe infection, more than 26%.

C) Rating in use at CIAT (Correa, 1987) has a 9- point scale as follows:

1 = No visible symptoms of the disease or presence of small lesions on leaves or pods affecting up to 1% of the tissue area.

3 = Approximately 5% of the tissue area affected by the lesions. Small lesions with low or no sporulation on the pods.

5 = Approximately 10% of the tissue area affected by the lesion. Well-defined lesions, some with restricted sporulation on the pods.

7 = Approximately 25% of the tissue area affected by the

lesions. Large lesions with abundant sporulatlion on pods.

9 = Approximately 50% or more of the tissue area affected by lesions causing severe defoliation. Numerous large sporulating lesions on the pods causing pod malformation, empty pods and death.

2.9 Effects of enviromental conditions on disease development

2.9.1 Temperature

Cardona-Alvarez and Walker (1956) investigated the effect of temperature on disease development in a series of experiments in which inoculated plants were exposed to temperatures at 4 °C intervals from 16 °C to 32 °C. They observed that disease development occurred over a wide range of temperature (16 to 28 °C) with an optimum at 24 °C. Disease development was slow at 16 °C, whereas no infection occurred at 32 °C. In a similar study, Inglis and Hagedorn (1984) also noted that 24 °C was optimum for initiation and development of disease. The smallest lesions developed in plants incubated at 28 °C or 16 °C incubation, followed by a 16 °C post-incubation period. Leaf chlorosis was greatest at the 20 °C, 24 °C and 28 °C incubation temperatures coupled with 20 and/or 24 °C post inoculation temperatures. Little or no chlorosis developed

at the 16°C incubation temperature regardless of post-inoculation temperature. Similar results were reported by Sindhan and Bose (1980), who observed that 24°C was optimum for infection and disease development, with infection decreasing greatly below and above this optimum temperature.

Defoliation was also observed to be most rapid at 24°C (Cardona-Alvarez, 1956, Inglis and Hagedorn, 1984).

2.9.2 Relative humidity

Under both field and laboratory conditions, high relative humidity has been found necessary for disease development. Cardona-Alvarez and Walker (1956) observed that a minimum three hour exposure to moisture was required for normal infection, though severity of infection increased with longer exposure to moisture (24 hrs). Pre-inoculation moisture had little or no effect on infection and disease severity under greenhouse conditions. These results differ from those of Sindhan and Bose (1980) who noted that a minimum 24 hrs post- inoculation period of 100% relative humidity was necessary for infection with maximum infection levels following a 96 hour moisture period. No disease developed with 0, 6, and 12 hours of post- inoculation moisture. Maximum infection occurred with 24 hours pre-and 96 hours of post-inoculation moisture. The

authors observed an optimum range of 90.2 and 100% relative humidity for disease initiation and development, however, no infection occured below 85.7% relative humidity.

Srinivasan (1953), Hocking (1967) and Sindhan and Bose (1980), while studying disease development in the field, observed that the fungus requires high humidity and frequent rains for initiation of the disease.

Though moderate temperatures are required for infection and disease development, it appears moisture is the most critical factor in the development of disease epidemics. Sindhan and Bose (1980) observed that relative humidity and precipitation were more important than temperature for disease developments in the field . Cardona-Alvarez and Walker (1956) noted that though rain, dew, or high humidity were important for infection, extended periods of moist conditions were essential for coremial formation and abundant sporulation. However, once penetration has occurred, disease development and stromata formation proceed even in relatively dry atmospheres. Low humidity was also favourable to the release and dissemination of spores. The authors concluded that the most favourable climatic conditions for epidemic disease development included: moderate temperatures and high humidity for 48 hours or more, alternating with periods of

low humidity and wind action.

2.10 Effect of plant age on disease developmnet

Plant and leaf age influence the susceptibility of specific cultivars. Cardona-Alvarez and Walker (1956) studied the effect of plant age on expression of typical symptoms by inoculating plants ranging in age from 10 to 60 days. They observed that all plants exhibited the usual sequence of spotting, necrosis, chlorosis, and defoliation regardless of plant age at the time of inoculation. In a similar experiment, Santos-Filho (cited by Correa 1983) inoculated plants ranging from 30 and 75 days old, followed by incubation at 20-24 °C and 100% relative humidity for 48 hours. Disease was most intense on 30 and 45 day old plants, resulting in severe yield reductions. Plants inoculated at 60 days of age yielded less than those inoculated at all other times, because of severe leaf defoliation during seed maturation.

Cole (1966) reported from Pennsylvania that under field conditions lesions first appeared in late July on the lower foliage. Defoliation and death of the plants occurred at the time when beans would normally be increasing rapidly in size and dry matter content. In Colombia, Barros et al. (1958) observed the first disease symptoms on field plants on the primary leaves after plant emergence; symptoms became more evident during late flowering or

early pod set. Similar field symptoms were observed by Hocking (1967), who indicated that lesions were initially most abundunt on primary leaves, and spread during the season to trifoliate leaves. Sindhan and Bose (1979), on the other hand, observed that two-week old plants of French bean variety Black Queen were completely disease- free, whereas three weeks old plants were the least susceptible in comparison to 4, 5, and 6 weeks old plants.

Weaver and Zaumeyer (1956), in Maryland, observed no pod infection but serious leaf infection in early July. When planting was delayed, heavy infections on both primary and trifoliate leaves as well as pods developed, resulting in considerable defoliation and yield reduction.

2.11 Yield losses

Until relatively recently, angular leaf spot of common bean, although widespread, was not believed to cause serious damage to the crop. However, under conditions favourable for disease initiation and development, ALS can be a very destructive disease.

Cardona-Alvarez and Walker (1956) reported that in 1954 angular leaf spot caused losses of 50% or more in several commercial snap bean fields in central Wisconsin. According to grower reports, the disease caused a high

incidence of small and shrivelled seeds resulting in yield reduction in Red Kidney beans by 10 to 50% (Cole, 1966). In Mexico, Crispin et al. (cited by Schwartz et al., 1981) reported 80% yield losses caused by angular leaf spot infection. A similar magnitude of yield loss was also observed in breeding line BAT 394 in experiments conducted in Colombia (Schwartz et al., 1981). Barros et al. (1958) reported a yield reduction of between 40 to 60% in bean fields in the Cauca Valley of Colombia .

2.12 Disease control strategies

Measures to control angular leaf spot include cultural practices, application of chemicals, and the development of resistant cultivars.

Cultural practices recommended include crop rotation for at least two years, removal of previously infected crop debris, plowing down old bean debris, planting pathogen-free seed and planting in well-drained soils (Cardona-Alvarez and Walker, 1956, Barros et al, 1958, and Chupp, 1960).

The use of chemicals for control of angular leaf spot has been recommended and practiced to some extent; however, some results have been inconsistent . Barros et al (1958) reported a good control of the disease with a mixture of fungicides (Zineb + Thiram + Copper). Costa (cited by

Ferraz In Schwartz and Galvez, 1980) recommended the use of Maneb, Ziram, Copper oxychloride and Bordeaux Mixture. Above 60 °F, Maneb, Ferbam, Captan and Ziram gave fair control when applied before infection, followed by additional sprays on a weekly basis (Chupp, 1960). Treatment of seeds with chemicals may be useful if the seed is contaminated. Araya (Cited by Feraz In Schwartz and Galvez, 1980) observed that seed dressing with Benomyl reduced subsequent leaf infection significantly. In addition to Benomyl, mancozeb was reported to be effective for control of the disease in the field (Schwartz et al. 1981 and Correa, 1984).

The use of genetic resistance to control angular leaf spot is more promising than the other control measures from the standpoint of cost and durability. However. in situations where resistance does not confer immunity to infection, an intergrated control strategy comprising cultural practices, pathogen free seed production, chemicals, and genetic resistance should be utilized when possible. In order to fully exploit host resistance, the identification of resistance sources, knowledge of the inheritance of resistance and an appropriate breeding strategy are required.

2.13 Sources of plant resistance

Various workers have identified sources of resistance to angular leaf spot in P. vulgaris L. Schwartz et al. (1982) at CIAT evaluated more than 13,000 germplasm lines and observed that 56 lines exhibited either resistant or intermediate disease reactions. The authors noted that efforts to obtain sources of stable resistance by traditional screening and breeding methods are complicated by pathogenic variability inherent within populations of P. griseola. In Australia, Brock (1951) inoculated 164 bean cultivars with one isolate of P. griseola. The cultivars found resistant were re-tested using 13 different isolates of the pathogen. He found that no line was immune, and only a few were highly resistant as indicated by absence of spore-bearing lesions or defoliation. These highly resistant varieties were either climbing or field bean types and included Alabama No. 1, Cafe, Epicure, McCaslan, Negro Costa Rica, Scotia and Rojo Chico. The seed classes, California Small White, Mexico Black and Navy Bean were also found to be highly resistant. Varieties reported as resistant were Blue Lake, Blue Podded Pole, Doppette, Feijao, Golden Harvest, G. 150, Grigajy, Ideal Market, Long White Mardrow, Kentucky Wonder (Brown and White seed), Mulatinho, Ousara, Poroto, Arrozchileno, and

Preto Brillante. Olave (cited by Zaumeyer and Meiners, 1975) found that the most resistant varieties included Mexico 11, Mexico 12 and Cauca 27a. Gardner and Mains (1929) observed that Kentucky Wonder was the most resistant of the forty common bean varieties they tested. In screening bean lines for resistance to angular leaf spot, Singh and Sharma (1975) evaluated forty lines during 1972 and 1973 and observed that EC 38921, EC44621, PLB'48 and Kentucky Wonder were immune; EC 10037, EC10039, EC44781, and EC 77007 showed high levels of resistance. Other reported sources of resistance include Carota 260 (Santos-Filho, cited by Ferraz in Schwartz and Galvez, 1980), Cuva 168N, and Manteigao Preto 20 (Costa, cited by Ferraz, in Schwartz and Galvez, 1980), and a group of Guatemalan accessions identified as 2465, 2503-12, 2504 and 2809 (Schieber, cited by Ferraz in Schwartz and Galvez, 1980). These varieties could be utilized as sources of resistance for breeding resistant bean lines to angular leaf spot. However, it is important to note that in the various studies, different isolates of P. griseola were utilized and that a line found resistant in one country may not be resistant to other isolates of the pathogen.

2.14 Inheritance of resistance

The knowledge of how resistance to a pathogen is

genetically controlled is essential to a disease resistance breeding program. A few genetic studies on the inheritance of resistance to angular leaf spot in common bean have been conducted. Santos-Filjo et al. (1984) crossed resistant variety Carota 260 with the susceptible black bean Venezuela 350. F_1, F_2, and backcross populations involving both parents were tested against one isolate in the greenhouse. All F_1 plants exhibited a susceptible reaction while F_2 plants exhibited a 3:1 segregation ratio of susceptible to resistant. A 1:1 segregation ratio of susceptible to resistant plants was exhibited for progenies of F_1 plants backcrossed to Carota 260, the resistant variety. Progenies of F_1s backcrossed to Venezuela 350, the susceptible parent, were all susceptible. These data indicate that resistance in Carota 260 was controlled by a single recessive gene. In another study, Singh and Saini (1980) crossed resistant PLB 257 (*P. coccineus*) with susceptible Contender (*P. vulgaris* L) variety. Parental, F_1, F_2, and F_3 plants were then testd for their reaction to the ALS pathogen. All 60 F_1 plants were susceptible. In the F_2 populations, a 3:1 segregation of susceptible to resistant plants was obtained, indicating that resistance in PLB 257 is governed by a single recessive gene. From the F_2 populations, 8 resistant and 20 susceptible plants were selfed to generate F_3 families. All

F_3 families raised from the 8 resistant F_2 plants produced only resistant plants, indicating homozygosity for resistance of the resistant F_2 plants. Eight of the twenty susceptible F_2 plants produced 834 susceptible and no resistant plants indicating homozygosity for susceptiblity in these F_2 plants. The remaining 12 progenies yielded a 3:1 segregation ratio of susceptible to resistant plants, thus substantiating the F_2 results.

On the other hand, Cardona-Alvarez (1962) reported that resistance to angular leaf spot in breeding line 258 was controlled by a single dominant gene. Barros et al. (1957) found that, in most crosses, resistance was recessive and controlled by two or three independent factors; only in a few crosses was resistance dominant. The resistance in Decal, Maravilta and Huila 14 was attributed to three recessive genes (Zaumeyer and Meiners, 1975). The variation in results from these inheritance studies could be explained by different resistant genes existing in different bean lines and these genes behaving distinctly when challenged by different races or isolates of the ALS pathogen.

MATERIALS AND METHODS

3.1 Isolates of *Phaeoisariopsis griseola* Sacc. and their maintenance

Four different isolates of *P. griseola* from distinct geographic regions were used in this study, namely: Colombia 10, Puerto Rico 2, Michigan 5 and Malawi 1. These isolates were generously provided by Dr. Fernando Correa, former graduate student in the Department of Botany and Plant Pathology, Michigan State University).

All four pathogen isolates were maintained on V-8 juice agar. V-8 medium was prepared by mixing together 3g $CaCO_3$, 18g Bacto-agar, 200ml V-8 juice and 800 ml distilled water. The suspension was steamed for about 20 minutes to ensure complete melting of the agar, transferred into 250 ml prescription bottles and then autoclaved for an additional 20 minutes . Sterile petri plates were prepared to contain about 25-30 ml of V-8 medium and allowed to stand for a few days before use. Maintenance of isolates was achieved by periodically transferring highly concentrated drops of spore suspensions to fresh plates and incubating at 25 ^{o}C for about 8 days. Isolates that were not needed for immediate use were stored in incubators at 5-8 ^{o}C.

3.2 Inoculum preparation and inoculation techniques

Spores for inoculation were harvested in either distilled or distilled-deionised water from petri plates that had been incubated for 6-10 days at 24°C. Spores were dislodged by rubbing the surface of the colony with a thin transfer loop. Spore concentrations were adjusted to 2×10^4 conidia/ml distilled water using a haemocytometer; 0.05% (v/v) Tween 80, (Polyoxyetheylene sorbitan mono-oleate) a wetting agent, was added to reduce the surface tension of water, thereby allowing the spores to adhere to the leaf surfaces.

Two different inoculation techniques were employed. With the exception of one genetic cross that was tested only against one isolate, all crosses were tested against two different isolates (Table 2). Preliminary greenhouse studies showed that two different isolates could be used on a single trifoliate leaf without any cross contamination (Fig.1) by using a No.4 camel's hair brush. This technique of brushing both the upper and lower leaf surfaces on each side of the middle leaflet of the first two-well developed trifoliate leaves with conidia was used on those crosses requiring two isolates. However, during the course of this study it was observed that the F_1's of certain crosses were semi-lethal. In the F_2 populations of these crosses, it was observed that some of the plants died after the formation

of the second or third trifoliate leaves, and as a result of this it was only possible to inoculate the primary leaves of these crosses. In order to avoid possible contamination , a single plant per pot was employed for all plants tested against two isolates. The second inoculation technique employed an atomiser to spray a fine mist of inoculum onto the upper and lower surfaces of the first and second trifoliate leaves of plants between 20-22 days old. Spore suspensisons for this method were passed through a four- layered cheese cloth filter before use. This method was not used for plants that were tested against two isolates in order to avoid possible contamination.

3.3 Age of plants at inoculation

Preliminary studies were conducted to determine the effect of plant age on expression of typical symptoms . Plants ranging in age from 12-24 days (plants with well developed primary leaves to plants with two well developed trifoliates) were inoculated and screened for angular leaf spot.

3.4 Bean cultivars and crosses

Five bean (P. vulgaris L.) genotypes with different seed sizes and reactions to four ALS-isolates from distinct

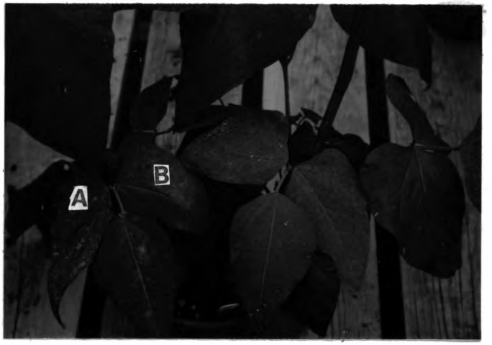

Fig. 1. Single trifoliate leaf of Pompadour Checa inoculated with two different ALS isolates: A = Puerto Rico 2 and B = Michigan 5 isolates respectively.

geographic regions were used in this study (Table 1). This information influenced how the different cultivars were crossed in the different combinations to produce F_1 s during the Spring and Summer of 1985. The crosses made and ALS isolates utilised are listed in Table 2.

Difficulties were encountered in the following crosses: a cross between C-20 and G 05686 produced only a few seeds. Further attempts at obtaining more F_1 seeds proved impossible because all pollinated flowers aborted. Only one F_1 seed was obtained from the cross between Bat-332 and G 05686. Although successful crosses between Mulanje VI and G 05686 were obtained, phenotypic variability was detected in F_1 seeds, indicating that the parents were mixtures, so these were not included in the study. F_1 plants of all crosses included in the study were also backcrossed to their respective parents.

Table 1.

Characteristics of five bean cultivars and their reactions to four isolates of *P. griseola*

Cultivar	Seed size	Reaction to *P. griseola* isolate			
		Michigan 5	Puerto Rico 2	Malawi 1	Colombia 10
G 05686	Large	R	S	R	R
Montcalm	Large	S	S	S	S
C-20	Small	R	S	R	S
BAT-332	Small	R	S	R	R
Pompadour	Medium	R	S	S	S

R = Resistant (immune) S = Susceptible (rating between 7-9)

Table 2:Bean crosses and the *P. griseola* isolates tested

Bean cross	Reaction to *P. griseola* isolate			
	Michigan 5	Puerto Rico 2	Malawi 1	Colombia 10
Montcalm x G 05686	S x R	-	-	-
Pompadour x G 05686	R x R	S x S	-	-
C-20 x BAT-332	R x R	-	-	S x R
Pompadour x BAT-332	R x R	-	-	S x R
C-20 x Pompadour	R x R	-	R x S	-

R = Resistant (immune)S = Susceptible (between 7 and 9)

3.5 Screening of plants

With the exception of the cross Montcalm x G 05686, the parents, susceptible check, F_1 and F_2 plants were inoculated using the brushing method. The F_3 and backcross plants of these R x R crosses in which segregation occurred in the F_2 were also inoculated by the same procedure. The parents, susceptible check, F_1, F_2, F_3, and backcross plants of Montcalm x G 05686 were inoculated by the spraying method. F_3 and backcross plants of R x R crosses showing no segregation in F_2 were also inoculated by the spraying method, since only one isolate was needed for testing. All crosses were tested separately.

After inoculation, plants were incubated in a saturated mist chamber, arranged in a complete randomized design, for 4 days at near 100% relative humidity and 20 to 28 ^{o}C. However, during the summer months of 1986 a maximum day temperature of 30 ^{o}C was observed while a minimum temperature of 16 ^{o}C was recorded in the winter of 1986. Pots were then removed to greenhouse benches and rated perodically for disease reactions. Ratings were terminated when reaction of parents and susceptible checks did not change.

3.6 Disease evaluation

The following evaluation scale, developed at CIAT (Correa, 1987) was used to establish infection grades based on percentage of leaf affected (Fig. 2).

1 = No visible symptom of the disease or presence of small lesions on leaves affecting up to 1% of tissue area.

3 = Approximately 5% of the tissue area affected by the lesions.

5 = Approximately 10% of the tissue area affected by the lesions.

7 = Approximately 25% of the tissue affected by the lesions.

9 = Approximately 50% or more of the tissue area affected by lesions, and severe defoliation.

Fig. 2 Scale used for evaluating disease reaction caused by P. griseola

1 = No visible symptom of the disease or presence of small lesions on leaves affecting up to 1% of tissue area.

3 = Approximately 5% of the tissue area affected by the lesions.

5 = Approximately 10% of the tissue area affected by the lesions.

7 = Approximately 25% of the tissue area affected by the lesions.

9 = Approximately 50% or more of the tissue area affected by lesions, and severe defoliation.

3.7 Data reliability

The following precautions were taken to assure reliability of results:

1. Parents and susceptible checks were included in screening of all F_1, F_2, F_3, and backcross-derived plants. Montcalm was used as susceptible check for all crosses that were tested against Michigan 5, because it was the only common cultivar found susceptible to this isolate in the present study.
2. In three experiments where the parents and susceptible checks did not show clear results the experiments were discarded and repeated with a new set of plants.
3. During the late Spring and Summer months of 1987 numerous plants which showed lethal and semi-lethal traits died before rating for disease reaction. The experiment was discarded and repeated during mid- Fall 1987 when conditions were more favourable for growth of these abnormal plants.
4. The first two well developed trifoliate leaves of plants aged between 20-22 days were inoculated to increase the precision of the results.

3.7 Analysis of data

The observed numbers of resistant and susceptible plants in F_2, F_3, and backcross generations were tested against the theoretical ratios of resistant to susceptible plants using a Chi-Square (X^2) test.

RESULTS AND DISCUSSION

Effect of plant age on angular leaf spot (ALS) development.

The experiment on effect of plant age on disease development showed that all plants exhibited typical angular leaf spot symptoms regardless of plant age at the time of inoculation (Figure 3). These symptoms were; angular lesions, necrosis, chlorosis, and defoliation. Similar results were observed by Brock (1951), Cardona-Alvarez and Walker(1956), and Barros *et al*. (1958). Inoculation of primary leaves could, therefore, be a more efficient way of screening for ALS disease as compared with the traditional greenhouse screening procedure of spraying conidial suspension unto trifoliate leaves, the former being time and space-saving.

These results, however, are different from those obtained by Sindhan and Bose (1979). The apparent juvenile resistance of the two week old plants in their study may have been due to the low inoculum concentration of 400 to 800 spores/ml as compared to the 2×10^4 spores/ml used in this study.

Fig. 3. Primary leaves of Montcalm red dark kidney bean showing ALS symptoms.

4.2 Reaction of parental common bean cultivars to four ALS isolates.

The five parental common bean cultivars utilized in this study showed differential reactions to four isolates of Phaeoisariopsis griseola (Table 2). G 05686, a kidney bean, was immune to Michigan 5, Malawi 1 and Colombia 10 isolates, but susceptible to the Puerto Rico 2 isolate (Fig. 4). Previous studies by Correa (1983), however, had indicated that G 05686 was immune to Puerto Rico 2. The conflict in these results may be due to a possible contamination of the Puerto Rican isolate, or impurity of the G 05686 cultivar. G 05686 was immune to all isolates used in this study except Puerto Rico 2, therefore it is not possible that the difference in results could be attributed to contamination of this pathogen isolate. The susceptible reaction of G 05686 to Puerto Rico 2 was consistent throughout the whole genetic study, and therefore the apparent immunity observed by Correa (1984) was probably due to a lack of symptom expression in the early post-inoculation stage at which scoring was done. Montcalm was highly susceptible to the Michigan 5 isolate (Fig. 5), susceptible to Malawi 1 and Colombia 10 isolates, but exhibited an intermediate reaction to the Puerto Rico 2 isolate. However, over time it eventually became susceptible to the Puerto Rico 2 isolate. C-20 was

susceptible to Puerto Rico 2 isolate but immune to the other three isolates. BAT-332 was immune to Michigan 5 and Malawi 1 isolates and though intermediate to Puerto Rico 2 at the time of rating, it eventually developed a susceptible reaction.

The reaction of BAT-332 to Colombia 10 isolate was complicated, with most of the plants being immune, while a few showed the typical angular leaf spot lesions. However, at the time of rating these lesions were not numerous enough to warrant categorizing them as susceptible plants. The difference in reaction can not be attributed to contamination of the isolate because then the symptoms would have been uniform over all plants, not just a few. The variation in reaction of BAT-332 to Colombia 10 isolate may indicate that either immunity to this isolate did not have 100% penetrance, or the cultivar was impure. A possible way of ruling out impurity of the cultivar would be the use of seeds from single plants but this was not done in this study. Pompadour Checa was immune to the Michigan 5 isolate but susceptible to Puerto Rico 2, Malawi 1, and Colombia 10 isolates (Fig. 6). Conflicting results were again reported by Correa (1984), who observed that Pompadour Checa was immune to the Colombia 10 isolate. A single spore isolation technique of Colombia 10 isolate was employed in this study, therefore the variation in results

could possibly be due to the fact that the cultivar was either impure, or that the reaction was not true resistance but rather due to escapes.

4.3 Production of F_2 populations

All F_1's were selfed to produce F_2 populations. Certain genetic markers, namely flower colour and plant growth habits, were utilized to identify and discard selfed plants which would have otherwise resulted in misinterpretation of data.

The flower colour of the parents Montcalm and G 05686 was white. However, all F_1's of the cross Montcalm x G 05686 had light purple flowers, and therefore those with white flowers were discarded. In the cross C-20 x Pompadour, flowers of C-20 were white, whereas those of Pompadour were purple. C-20 was the female parent and hence, all F_1's with white flowers instead of light purple flowers were discarded.

The growth habit of plants was also used to detect self fertilization. In the cross Pompadour x BAT-332 in which the two parents had purple and pinkish-purple flowers respectively, the viny nature of F_1 plants made it possible for all self fertilized plants to be identified. Pompadour with a type 1 growth habit was the female parent while Bat-332, the viny parent, was the pollen source.

Fig. 4. Suceptible reaction of G 05686 to the Puerto Rico 2 isolate.

Fig. 5. Severe ALS symptoms on Montcalm red dark kidney inoculated with the Michigan 5 isolate.

Fig. 6. Susceptible reaction of Pompadour Checa to the Colombia 10 isolate.

4.4 Segregation for reaction to individual isolates

4.4.1 Michigan 5

With the exception of the cross Montcalm x G 05686, which represented a susceptible by resistant (S x R) cross, all other crosses tested against the Michigan 5 isolate were of the combination resistant by resistant (R x R) reaction (Table 2).

All 25 F_1 plants of the cross Montcalm x G 05686 were susceptible and ranged in rating from 3 to 5 on a 1-9 scale. The grouping of F_2 plants into resistant and susceptible categories was based on F_3 results in which plants rated 1 or 2 were homozygous for resistance, 7-9 were homozygous for susceptibility, whereas plants scored as 3-5 segregated into resistant and susceptible plants. Plants rated as 6 were border line and were sometimes included in either the 3-5 or 7-9 classes based on the behaviour of the F_3 families. The F_2 populations segregated into susceptible and resistant plants in the ratio of 3:1. Though the X^2 value was high no other ratio seemed to offer a better fit (Table 3). The results of the F_1 and F_2 generation tests indicated that the resistance in G 05686 is governed by a single recessive gene. The F_2 segregants, however, were found to fall into different classes of ratings instead of discrete forms associated with a single major gene displaying complete dominance. The combined

results of the F_1 and F_2 populations suggest that possibly minor modifying genes are involved in disease resistance or there is incomplete (partial) dominance for susceptibility. The F_3 and backcross data confirm F_2 results (Table 3). The results of the F_3 families are presented in Appendix 1. From the F_3 population, 10 resistant and 20 susceptible F_2 plants were selfed to generate F_3 families. All 10 F_3 families resulting from resistant plants produced only resistant reactions, suggesting homozygosity for resistance of the F_2 resistant plants. Within the 20 susceptible F_2 plants, 10 F_3 families produced only susceptible reactions with no resistant plants, indicating homozygosity for susceptibility of the F_2 plants. The remaining 10 progenies segregated into susceptible and resistant plants in a ratio of 3:1. Backcrosses were made to both Montcalm and G 05686, with F_1 plants as female parents. Progenies of F_1's backcrossed to Montcalm, the susceptible parent, were all susceptible. A 1:1 segregation of susceptible to resistant plants was exhibited by progenies of F_1's backcrossed to G 05686. These results are in agreement with those of Singh and Saini (1975) and Santos-Filho (1984), but contrary to that of Cardona-Alvarez (1962), who reported that resistance in line 258 was controlled by a single dominant gene. This discrepancy in results is not unexpected since different bean lines probably carry different gene(s) for

resistance. Secondly, different pathogenic races exist in the ALS organism and could also account for the differences in the results.

Table 3.

Observed number and expected ratio of susceptible and resistant plants in F_2 and backcross populations of Montcalm x G 05686 to the Michigan 5 isolate of ALS.

Populations	Number of plants			Expected	Chi-Square	P
	S	R	Total	ratio	(X^2)	
F_2	166	70	236	3:1	2.49	.10-.25
B.C.$_1$	10	0	10	1:0	0.00	1.0
B.C.2	2	4	6	1:1	0.16	.50-.75

S = Susceptible

R = Resistant

$B.C._1 = F_1 \times$ Montcalm

$B.C._2 = F_2 \times$ G 05686

In crosses Pompadour x G 05686, C-20 x BAT-332, Pompadour x BAT-332, and C-20 x Pompadour, all involving a resistant by resistant (R x R) type reaction, all 25 F_1 plants of each cross were immune to the Michigan 5 isolate. In the F_2 populations no segregation for susceptibility was observed in crosses C-20 x BAT-332 and Pompadour x BAT-332 (Table 4).

Table 4

Reaction of F_2 populations of Pompadour x BAT-332 and C-20 x BAT-332 to the Michigan 5 isolate.

Cross	Number of plants		
	R	S	Total
Pompadour x BAT-332	189	0	189
C-20 x BAT-332	179	0	179

R = Resistant S = Susceptible

However, a 15:1 ratio of resistant to susceptible plants was observed in both Pompadour x G 05686 and C-20 x Pompadour (Table 5 and 6). The genetic segregation of these crosses for reaction to the Michigan 5 isolate may be explained on the assumption that two loci which are independent in transmission are involved. In this case, a duplicate dominant epistasis is implied, where the dominant allele at either locus overrides the homozygous recessive at the other locus. The F_3 and backcross results confirm the F_2 segregation pattern.

In the cross Pompadour x G 05686, 24 F_2 plants were selfed to produce F_3 families. Of these, five were susceptible plants, four of which yielded only susceptible progenies in the F_3 (Appendix 2). The fifth F_2 susceptible plant produced 18 offspring 16 of which were susceptible

and two resistant. These two resistant plants were probably escapes and not truly resistant since all F_2 susceptible plants were expected to produce only susceptible progenies. Fourteen of the 19 resistant F_2 plants yielded only resistant F_3 plants. Two of the remaining five resistant F_2 plants produced F_3 plants which segregated into resistant and susceptible plants in a ratio of 15:1, whereas the other three exhibited resistant and susceptible plants in a ratio of 3:1. Theoretically, of every 19 resistant F_2 plants, progenies of nine of them should be resistant, five segregating in a 15 resistant to 1 susceptible ratio,and the remaining five in a 3 resistant to 1 susceptible ratio. Two reasons may be advanced to explain the deviation of these results from the theoretical expectation. Possibly, there was a poor selection in the F_2 plants, selecting within a narrow instead of a wide range, and secondly, the number of F_3 plants per family was small. Where two genes are involved in disease reaction a large number of plants should be evaluated in order to realize a reliable segregation ratio. However, F_3 plants in the families of this cross ranged from 11 to 22. It is therefore possible that some of those F_2 resistant plants which produced only F_3 resistant plants could have given rise to resistant and susceptible plants either in a ratio of 15:1 or 3:1, but due to small numbers, susceptible plants were not

recovered.

F_1 plants in this cross were backcrossed to both parents and their progenies tested against the Michigan 5 isolate. All 12 backcrossed plants in both populations were resistant (Table 6). This is expected if duplicate dominant epistasis is involved in the resistance in both Pompadour and G 05686 to the Michigan 5 isolate.

Table 5.

Observed number and expected ratio of resistant and susceptible plants in F_2 and backcross populations of the cross Pompadour x G 05686 to Michigan 5 isolate of ALS.

Population	Number of plants			Expected ratio	Chi-Square (X^2)	P
	R	S	Total			
F_2	178	8	186	15:1	.659	.25-.50
B.C.$_1$	12	0	12	1:0	0.000	1.00
B.C.2	12	0	12	1:0	0.000	1.00

R = Resistant
S = Susceptible
B.C.$_1$ = F_1 x G 05686
B.C.$_2$ = F_1 x Pompadour

Similarly, in the case of the cross C-20 x Pompadour, 36 F_2 plants consisting of six susceptible and 30 resistant plants were selected to produce F_3 families (Appendix 3). All six susceptible F_2 plants produced only susceptibile F_3

progenies, indicating homozygosity for susceptibility. This reaction presumably resulted from the homozygous double recessive genes recovered in susceptible F_2 plants. Of the remaining 30 resistant F_2 plants, 20 produced only resistant F_3 families, six segregated to give resistant and susceptible plants in the ratio of 15:1, whereas the remaining four generated resistant and susceptible plants in a 3:1 ratio. The high incidence of F_2 resistant plants producing only resistant F_3 plants as was the case in the cross Pompadour x G 05686, could be attributed to either poor selection in the F_2 population, or plant number being so small in F_3 families that plants that would have been susceptible were not recovered. F_1s were backcrossed to both parents. All plants generated from both backcrosses were resistant (Table 6).

Table 6

Observed and expected ratio of resistant and susceptible plants in F_2 and backcross population of the cross C-20 x Pompadour to the Michigan 5 isolate of ALS.

Population	Number of plants			Expected ratio	Chi-Square (X^2)	P
	R	S	Total			
F_2	192	8	200	15:1	1.365	.10-.25
B.C.$_1$	7	0	7	1:0	0.000	1.00
B.C.$_2$	10	0	10	1:0	0.000	1.00

R = Resistant

S = Susceptible

B.C.$_1$ = F_1 x C-20

B.C.$_2$ = F_1 x Pompadour

Though in both the Pompadour x G 05686 and C-20 x Pompadour crosses two duplicate dominant genes are postulated to confer resistance to the Michigan 5 isolate, it is not conclusive that these resistance genes of the different parents are identical. The system of multiple alleles adopted by Cardenas (1960) and Cardenas *et al* (1964) for anthracnose (*Colletotrichum lindemuthianum*) could account for the F_2 results of the crosses tested against the Michigan 5 isolate. This system assumes that there are duplicate factors c and d, with multiple alternative allelic states. The alleles c^1 and d^1 confer susceptibility and are recessive to the alleles c^2 and d^2

which confer resistance. However, the other susceptibility alleles c^3 and d^3 are dominant over the resistant c^2 and d^2 alleles. The duplicate factors and order of dominance are as follows:

c3d3 >	c2d2 >	c1d1
Susceptible	Resistant	Susceptible

Based on this system, the proposed genotypes of the five parental cultivars with respect to their reaction to the Michhigan 5 isolate are as follows:

Montcalm	$c^3 c^3 d^1 d^1$	Susceptible
G 05686	$c^2 c^2 d^1 d^1$	Resistant
Pompadour Checa	$c^1 c^1 d^2 d^2$	Resistant
C-20	$c^2 c^2 d^1 d^1$	Resistant
BAT-332	$c^2 c^2 d^2 d^2$	Resistant

Thus, in the cross Montcalm x G 05686, it was only the factors c^3 and c^2 that segregated in the F_1 population since the two parents share a common d^2 gene, and therefore result in a 3S:1R ratio. With respect to the crosses Pompadour x G 05686 and C-20 x Pompadour, the two susceptible factors $c^1 c^1 d^1 d^1$ are recovered in the F_2 generation, thus giving a ratio of 15R:1S. The combined results of the resistant by resistant crosses tested against the Michigan 5 isolate indicate that Pompadour Checa and BAT-332 have different resistance alleles, at least at the c locus, whereas C-20 (a small-seeded Meso-

American line) and G 05686 (a large-seeded Andean source) have identically acting resistance genes.

4.4.2 Colombia 10 isolate

The two crosses that were tested against Colombia 10 isolate were susceptible by resistant (S x R) types (Table 2).

With respect to the cross C-20 x BAT-332, all 25 F_1 plants were intermediate in susceptibility, rating between 5 and 6 on a 1-9 scale. The F_1 reaction reflects either additivity or partial dominance of genes for susceptibility. Based on the segregation pattern in the F_3, all plants rating between 1-3 were classified as resistant and those plants rating 4-9 as susceptible. An F_2 ratio of 13 susceptible to 3 resistant was obtained in this cross. Data from F_3 progenies as well as backcrosses of F_1s to both parents support the F_2 interpretation (Table 7).

Among the 30 F_2 randomly selected plants, four demonstrated a resistant reaction with a rating of 1 and 2. The F_3 progenies of these plants were all resistant (Appendix 4). Theoretically, of every 30 F_2 plants, two plants are supposed to generate only resistant F_3 families, and therefore, the results obtained do not deviate significantly from the expected. Six resistant plants of the remaining 27 F_2s with a rating of 2 and 3 segregated,

producing resistant and susceptible plants in a ratio of 3:1. However, another F_2 plant with a rating of 6 and therefore considered susceptible, produced resistant and susceptible F_3 plants in a ratio of 3:1. Since only resistant plants are expected to segregate in this manner, this F_2 plant must have been misclassified. In theory, four out of every 30 F_2 plants showing resistance should segregate in this pattern, and therefore plants with a rating of 3 were considered resistant. Nine F_2 susceptible plants with a rating of 4 and 5 produced F_3 families that segregated to give susceptible and resistant plants in a ratio of 3:1. F_3 plants generated from two susceptible F_2 plants with a rating of 5 and 6 segregated in a ratio of 13 susceptible to 3 resistant. The remaining nine F_2 susceptible plants produced only susceptible F_3 families. Plant number in the F_3 families ranged from 26 to 60, and therefore the results obtained were considered reliable.

In the backcross populations, all 20 offspring of F_1 s backcrossed to C-20, the susceptible parent, were all susceptible. However, eight offspring of F_1 s backcrossed to BAT-332, the resistant parent, segregated in a ratio of 1 resistant to 1 susceptible (Table 7).

The F_2, F_3, and backcross results indicate that resistance is controlled by two independent loci, with a dominant inhibitor allele for susceptibility being

epistatic to the dominant allele which confers resistance in BAT-332. This type of allelic interaction indicates that the presence of the dominant allele conferring susceptibilty inhibits any resistant reaction regardless of the allelic condition at the resistance locus, thus giving a ratio of 13 susceptible to 3 resistant in the F_2 population. Though Barros *et al.* (1957) found that in most crosses resistance was recessive and controlled by two or three independent factors, no specific conclusions were made concerning the type of gene action. However, Zaumeyer and Meiners (1975) reported that resistance in Decal, Maravilta and Huila 14 was controlled by three recessive genes. These results can be attributed to the fact that different resistance genes exist in different bean lines and these genes behave distinctively when challenged by different isolates of the ALS pathogen.

All 20 F_1 plants of the cross Pompadour x BAT-332 were susceptible to Colombia 10 . In the F_2 a ratio of 9 susceptible to 7 resistant plants was obtained. Even though the chi square (X^2) value was high, a ratio of 9:7 was accepted since no other ratio presented a better fit. Twenty F_3 families were tested for their reaction to the Colombia 10 isolate (Appendix 5). Seven of the 20 F_2 plants were resistant and produced only resistant F_3 plants. Of the remaining 13 susceptible F_2 plants, five produced only

susceptible F_3 progenies, five segregated into susceptible and resistant plants in a ratio of 9:7, and the remaining three gave rise to F_3 plants in the ratio of 3 susceptible

Table 7

Observed number and expected ratio of resistant and susceptible plants in F_2 and backcross populations of the cross C-20 x BAT-332 to the Colombia 10 isolate of ALS.

Population	Number of plants			Expected ratio	Chi-Square (X^2)	P
	S	R	Total			
F_2	147	32	179	13:3	0.041	.75-.90
B.C.$_1$	20	0	20	1:0	0.000	1.00
B.C.$_2$	3	5	8	1:1	0.031	.75-90

R = Resistant
S = Susceptible

$B.C._1 = F_1 \times C\text{-}20$

$B.C._2 = F_1 \times BAT\text{-}332$

to 1 resistant. Theoretically, of every 20 F_2 plants, one is expected to produce only susceptible F_3 plants, five generating progenies segregating into 9 susceptible to 7 resistant, five in a 3 susceptible to 1 resistant, and all nine resistant plants producing only resistant F_3 families. However, a high number of susceptible F_2 plants generated only susceptible plants in the F_3 generation, possibly due to inadequate sampling. The reaction of the backcrosses

confirm the F_2 and F_3 results. F_1s backcrossed to Pompadour, the susceptible parent, produced only susceptible plants, whereas those backcrossed to BAT-332 (resistant parent) segregated into resistant and susceptible plants in the ratio of 3:1 (Table 8).

These results suggest that two independent complementary dominant genes are involved in the expression of susceptibility to the Colombia 10 isolate. Hence, homozygous recessive genes at either locus regardless of a dominant gene at the other locus confer resistance to the Colombia 10 isolate. It can be concluded, therefore, that double recessive homozygous genes confer resistance in BAT-332 to the Colombia 10 isolate.

The inference here is that probably different resistance genes present in the same cultivar (BAT-332) confer resistance to the same race of the pathogen (Colombia 10 isolate). In the case of the cross C-20 x BAT-332, resistance is controlled by a dominant inhibitor factor, whereas in Pompadour x BAT-332 homozygous recessive genes at the two independent loci result in a resistant reaction. The system of multiple alleles adopted by Cardenas et al (1964) could account for the results of these two crosses tested against the Colombia 10 isolate. However, in this case, in addition to the complementary alleles e, and f, there is a dominant inhibitor allele Inh

which is epistatic to the dominant C allele for resistance.

Table 8

Observed number and expected ratio of susceptible and resistant plants in F_2 and backcross populations of the cross Pompadour x BAT-332 to the Colombia 10 isolate of ALS.

Population	Number of plants			Expected ratio	Chi-Square (X^2)	P
	S	R	Total			
F_2	97	92	189	9:7	1.667	.10-.25
B.C.$_1$	5	0	5	1:0	0.000	1.00
B.C.$_2$	9	4	13	3:1	0.026	.75-.90

R = Resistant

S = Susceptible

B.C.1 = F1 x Pompadour

B.C.$_2$ = F_1 x BAT-332

The susceptibility alleles $e_3 e_3 f_3 f_3$ are dominant over the resistance alleles $e_2 e_2 f_2 f_2$. Based on this reasoning, the proposed genotypes of the three bean cultivars with respect to their reaction to the Colombia 10 isolate are as follows:

BAT-332	inhinhCC	$e_2 e_2 f_2 f_2$	Resistant
C-20	InhInhcc	$e_2 e_2 f_2 f_2$	Susceptible
Pompdour Checa	inhinhcc	$e_3 e_3 f_3 f_3$	Susceptible

4.4.3 Malawi 1

The cross C-20 (Resistant) x Pompadour (Susceptible)

was the only one that was tested against the Malawi 1 isolate (Table 2). All 25 F_1 plants were susceptible. The F_2 plants segregated in a ratio of 3 susceptible to 1 resistant plants (Table 9). The results of F_3 and backcrosses of F_1s to both parents confirm the F_2 results. Within 30 F_2 plants chosen to generate F_3 families, 10 were resistant and 20 susceptible. All progenies of the 10 resistant F_2 plants were resistant to this isolate, indicating homozygosity for resistance. Of the 20 susceptible F_2 plants, 10 produced only susceptile F_3 families, indicating homozygosity for susceptibility. The remaining 10 produced F_3 progenies segregated into susceptible and resistant plants in a ratio of 3:1. The progenies of F_1s backcrossed to Pompadour, the susceptible parent, were all susceptible, whereas those backcrossed to C-20 segregated into resistant and susceptible plant in the ratio of 1:1. (Table 9). The results of F_2,F_3 and backcross populations indicate that resistance in this cross is controlled by a single recessive gene.

Puerto Rico 2

The only cross tested against the Puerto Rico 2 isolate was a susceptible by susceptible type (Table 2). All 25 F_1 plants of the cross Pompadour x G 05686 were susceptible. Within the 186 F_2 plants four were scored as

resistant. These "resistant" plants were raised to generate F_3 families and re-tested against this isolate to determine if the reaction was true resistance or that of escape.

Table 9

Observed number and expected ratio of susceptible and resistant plants in F_2 and backcross populations of the cross C-20 x Pompadour to the Malawi 1 isolate of ALS.

Population	Number of plants			Expected ratio	Chi-Square (X^2)	P
	S	R	Total			
F_2	146	54	200	3:1	0.327	.50-.70
B.C.$_1$	3	2	5	1:1	0.000	1.00
B.C.$_2$	10	0	10	0	0.000	1.00

R = Resistant
S = Susceptible

B.C.$_1$ = F_1 x C-20
B.C.$_2$ = F_1 x Pompadour

All the "resistant" F_2 plants produced only highly susceptible F_3 families and were, therefore, considered as escapes. Based on F_1 and F_2 results, it could be inferred that these results are consistent with a hypothesis of common identity of allele(s) for susceptibility in Pompadour and G 05686 to the Puerto Rico 2 isolate.

Joint Segregation for Reaction to Two Isolates

The cross C-20 x Pompadour was the only one that segregated for reaction to two isolates, namely Michigan 5 and Malawi 1. An analysis for joint segregation, was therefore carried out to determine if recombination occurred between genes conferring resistance to both the Michigan 5 and Malawi 1 isolates.

In the cross C-20 x Pompadour, the segregation ratios of resistant to susceptible F_2 plants were 15:1 and 1:3 for the Michigan 5 and Malawi1 isolates, respectively. the theoretical ratio for joint segregation which is the product of the two ratios, was 15:45:1:3(RR:RS:SR:SS). The observed number of F_2 plants resistant to both the Michigan 5 and Malawi 1, resistant to Michigan 5 but susceptible to Malawi 1, susceptible to Michigan 5 but resistant to Malawi 1, and susceptible to both isolates of the pathogen fitted the theoretical segregation ratio (Table 10). This result indicates that genes affecting ALS reaction in the F_2 plants segregated independently to the Michigan 5 and Malawi 1 isolates. It is premature, however, to conclude that linkage is not involved in the inheritance of angular leaf spot resistance, since in this study it was possible to analyse the joint segregation in only one cross.

Table 10

Observed and expected ratios for F_2 population of the cross C-20 x Pompadour inoculated with the Michigan 5 and Malawi 1 isolates.

Reaction Class		Theoretical	Number of Plants		X^2	P
Michigan 5	Malawi 1	Ratios	Observed	Expected		
R	R	15	47	46.88	0.00	
R	S	45	145	140.88	0.14	
S	R	1	1	3.13	1.45	
S	S	3	7	9.38	0.60	
Total		64	200	200.02	2.19	.50-.75

R = Resistant S = Susceptible

SUMMARY AND CONCLUSIONS

This study was conducted to determine : (1) the inheritance of resistance to angular leaf spot (ALS) disease in five crosses of the common bean, (2) the relationship between resistance genes found in large-seeded and small-seeded lines, and (3) the relationship between resistance gene(s) sources to particular ALS isolates.

Five common bean cultivars used in this study were crossed in different combinations to produce F_1's during the Spring and Summer of 1985. Parental, F_1, F_2, and F_3 populations and progenies of reciprocal backcrosses were inoculated by either the brushing or spraying method depending on the number of isolates tested on each cross. Based on reactions of F_3 families, plants rated 1 and 2 were considered resistant (R), whereas those ranging from 3-9 were susceptible for all crosses except the cross C-20 x Bat-332 in which a rating of 3 was considered as resistant as well. All plants rated as 3 in the cross C-20 x Bat-332 segregated in a manner expected of some of the F_2 resistant plants.

The inheritance of resistance to the Michigan 5 isolate was studied in all five crosses, four consisting of an R x R reaction; the cross Montcalm x G 05686 was a susceptible by resistant (S x R) type. Results from F_1's revealed that

the resistance in G 05686, in this cross, to Michigan 5 was controlled by recessive gene. The segregation ratio of susceptible to resistant reaction in the F_2 was 3:1, which indicated that resistance in G 05686 is controlled by a single major recessive gene. Minor modifying genes seemed to be involved, since disease reaction was not discrete. F_3 and backcross data confirmed F_2 findings. In the cross Pompadour x Bat-332 and C-20 x Bat-332 which were R x R, all F_1's were resistant. No segregation occurred in the F_2 populations of these crosses. However, with respect to the crosses Pompadour x G 05686, and C-20 x Pompadour, all F_1's were resistant and F_2's segregated into resistant and susceptible plants in a ratio of 15:1. F_3 and backcross results supported the F_2 ratio in which duplicate dominant genes at either of two loci conferred resistance in the bean cultivars C-20, Pompadour Checa and G 05686. It was inferred from these R x R crosses that C-20 (Meso-American gene pool) and G 05686 (Andean gene pool) have identical genes ($c_2 c_2 d_1 d_1$) for resistance to the Michigan 5 isolate, whereas Pompadour Checa and Bat-332 are $c_1 c_1 d_2 d_2$ and $c_2 c_2 d_2 d_2$ respectively. It can be concluded, therefore, that some of the sources of resistance share the same genes, whereas others do not. The R x R crosses also revealed that large-seeded (Andean source) beans and small-seeded (Meso-American) types can be identical in their patterns of

inheritance of resistance to particular isolates of the ALS pathogen.

The inheritance of resistance to the Colombia 10 isolate was studied in two crosses. In cross C-20 x Bat-332, which was an S x R type, all F_1's were susceptible; the F_2 population segregated into susceptible and resistant plants in a ratio of 13:3. F_3 and backcross results supported the F_2 segregation ratio, which indicated that resistance was controlled by two independent loci, with a dominant inhibitor allele for susceptibility being epistatic to the dominant allele for resistance present in Bat-332. In the cross Pompadour x Bat-332, F_1's were susceptible and F_2 segregation resulted in a ratio of 9 susceptible to 7 resistant. F_2, F_3 and backcross results indicated that two complementary recessive genes confer resistance in Bat-332 to the Colombia 10 isolate.

The inheritance of resistance to the Malawi 1 isolate was studied only in the cross C-20 x Pompadour. All F_1's were susceptible and a segregation ratio of 3 susceptible to 1 resistant was obtained in the F_2's. F_3 and backcross data supported the segregation ratio in the F_2. It was, therefore, concluded that resistance in C-20 to the Malawi 1 isolate was controlled by a single recessive gene, possibly being influenced by minor modifiers.

The only cross tested against the Puerto Rico 2

isolate was susceptible x susceptible (S x S) type. All F_1's and F_2's were susceptible, and therefore no genetic information was obtained from this cross. However, it is possible that the genes for susceptibility in both parents are identical, or genes for reaction to this isolate were so tightly linked that it was not possible to recover recombinants in the small population tested.

LITERATURE CITED

1. Alvarez-Ayala, G. 1979. Development of a method for testing resistance of Phaseolus vulgaris L. to angular leaf spot (Phaeoisariopsis griseola Sacc.). M.Sc. Thesis, McGill University, Canada. 179 pp.

2. Alvarez-Ayala, G. and H. F. Schwartz. 1979. Preliminary investigations of pathogenic variability expressed by Isariopsis griseola. Ann. Rep. bean Improv. Coop. 22:86-88.

3. Alvarez-Ayala, G. and H. F. Schwartz. 1984. Preliminary investigations of pathogenic variability expressed by Isariopsis griseola. Ann. Rep. Bean Improv. Coop. 27:22-24.

4. Barros, O., Cardona and R. L. Skiles. 1957. The severity and control of angular leaf spot of beans in Colombia. Phytopathology 47: 3.

5. Barros, O., C. Cardona and R. Cardenosa. 1958. The control of angular leaf spot of bean in Colombia. F.A.O. Plant Prot. Bull. 6:97-101.

6. Barros, O., C. Cardona, R. Cardenosa and R. L. Skiles. 1958. Angular leaf spot of bean in Colombia. Plant Dis. Reptr. 42:420-424.

7. Bose, S. K. and G. S. Sindhan. 1972. Leaf spot of French beans caused by Isariopsis griseola Sacc. and its control. Prog. Hort. 4:69-75.

8. Brock, R. D. 1951. Resistance to angular leaf spot varieties of bean . J. Aust. Inst. Agr. Sci. 17:25-30.

9. Cardona-Alvarez, C. 1956. Angular leaf spot of bean. Ph.D. Dissert., Univ. of Wisconsin. 49pp.

10. Cardona-Alvarez, C. and J. C. Walker. 1956. Angular leaf spot of bean. Phytopathology 46:610-615.

11. Cardona-Alvarez, C. 1962. Inheritance of resistance to angular leaf spot in French bean. Agric. Trop. Bogota 18:330-331.

12. Chupp, C. and A. F. Sherf. 1960. Vegetable diseases

and their control. Ronald Press, New York. 136-137.

13. Chupp, C. and A. F. Sherf. 1980. Vegetable diseases and their control. Ronald Press, New York. 64-65.

14. Cole Jr., H. 1966. Angular leaf spot associated with severe defoliation of red kidney beans (Phaseolus vulgaris) in Pennsylvania. Plant Dis. Reptr. 50:494.

15. Correa, F. J. 1984. Angular leaf spot (Isariopsis griseola Sacc.) of red kidney beans in Michigan. M. SC. thesis. Michigan State University, East Lansing. 82 pp.

16. Correa, F. J. 1987. Pathogenic variation, production of toxic metabolites, and isoenyme analysis in Phaeoisariopsis griseola (Sacc.) Ferr. Ph.D thesis. Michigan State University, East Lansing. 154 pp.

17. Correa, F. J., and Saettler, A. W. 1984. Angular leaf spot (Isariopsis griseola Sacc.) in seed fields of Michigan red kidney beans. Ann. Rept. Bean Improv. Coop. 27:28-30.

18. Correa, F. J. and A. W. Saettler. 1987. Angular leaf spot of red kidney beans in Michigan. Plant Disease. 71:915-918.

19. Ferraz, S. 1980. Angular leaf spot. In: H. F. Schwartz and G. E. Galvez (ed.), Bean Production Problems: Disease, insect, soil and climatic constraints of Phaseolus vulgaris. Centro Internacional de Agricultura Tropical, series 09EB-1, Cali, Colombia. 55-64 pp

20. Garnder, M. W. and E.B. Mains. 1929. Inddiana Plant Diseases. 1928. Ind. Acad. Sci. 39:85-99.

21. Hagedorn, D. J. and E. K. Wade. 1974. Bean rust and angular leaf spot in Wisconsin. Plant Dis. Reptr. 58:330-332.

22. Harter, L. L., and Zaumeyer, W. J. 1944. A monographic study of bean diseases and methods for their control. USDA. Agr. Tech. Bull. No. 868, 160 pp.

23. Hocking, D. 1967. A new virulent form of Phaeoisariopsis griseola causing circular leaf spot of French beans. Plant Dis. Reptr. 51: 276- 278.

24. Inglis, D. A. and D. J. Hagedorn. 1984. Temperature requirements by Isariopsis griseola for infecton and disease development on red kidney beans. Abstracts of presentations. APS-CPS Annual Meeting. August 12-16, 1984. University of Guelph, Canada.

25. Inglis, D. A., D. J. Hagedorn and R. E. Rand. 1984. Using dry inoculum in the field for testing beans for resistance to angular leaf spot. Abstracts of presentations. APS-CPS Annual Meeting. August 12-16, 1984. University of Guelph, Canada.

26. Inglis, D. A., D. J. Hagedorn and R. E. Rand. 1984. A new technique for testing beans for resistance to anthracnose and angular leaf spot. Ann. Rep. Bean Improv. Coop. 27:20-21.

27. Miles, L. E. 1917. Some diseases of economic plants in Puerto Rico. Phytopathology 7:345-348.

28. Nelson, R. R. 1973. Pathogenic variation and host resistance. In: R. R. Nelson. Breeding Plants for Disease Resistance. The Pennsylvania State University Press. p.40-48.

29. Orozco-Sarria, S. H. and C. Cardona-Alvarez. 1959. Evidence of seed transmission of angular leaf spot of bean. Phytopathology 49:159.

30. Saettler, A. W. and F. J. Correa. 1983. Angular leaf spot in seed fields of Michigan red kidney beans. Mich. Dry Bean Digest 8 (2):2-3.

31. Santos-Filho, H. P., S. Ferraz and C. Vieira. 1976. Inheritance of resistance to angular leaf spot in Phaseolus vulgaris L. Ann. Rept. Bean Improv. Coop. 19:69-70

32. Schwartz, H. F., F. Correa, P. Pineda, M. M. Otoya and M. J. Katherman. 1981. Dry bean disease losses caused by Ascochyta, angular, and white leaf spot in Colombia. Plant Disease 65:494-496.

33. Schwartz, H. F., M. A. Pastor-Corrales and S. P. Singh. 1982. New sources of resistance to anthracnose and angular leaf spot of beans (Phaseolus vulgaris L.). Euphytica 31:741-754.

34. Sharma, R. D. and H. S. Sohi. 1980. Studies on angular leaf spot of French bean Phaseolus vulgaris L.) caused by Isariopsis griseola Sacc. Indian J. Mycol. Plant Pathol. 10(2):xxvii.

35. Sindhan, G. S. and S. K. Bose. 1979. Perpetuation of Phaeoisariopsis griseola causing angular leaf spot of French beans. Indian Phtopathology 32(2):252-254.

36. Sindhan, G. S. and S. K. Bose. 1980. Effect of temperature and relative humidity on the development of angular leaf spot of French bean. Prog. Hort. 12:5-14.

37. Sindhan, G. S. and S. K. Bose. 1980. Epidemiology of angular leaf spot of French bean caused by Phaeoisariopsis griseola. Indian Phytopathology 33(1):64-68.

38. Singh, A. K. and S. S. Saini. 1980. Inheritance of resistance to angular leaf spot (Isariopsis griseola Sacc.) in French beans (Phaseolus vulgaris L.). Euphytica 29:175-176.

39. Singh, B. M. and Y. R. Sharma. 1975. Screening of bean lines for resistance to angular leaf spot caused by Isariopsis griseola Sacc. Indian Phytopath. 28:435-436.

40. Sohi, H. S. and R. D. Sharma. 1974. Mode of survival of Isariopsis griseola Sacc. the causal agent of angular leaf spot of beans. Indian J. Hortic. 31:110-113.

41. Weaver, L. O. and W. J. Zaumeyer. 1956. Angular leaf spot of bean found in Maryland. Plant Disease Reptr. 40:1092.

42. Zaumeyer, W. J. and J. P. Meiners. 1975. Disease resistance in beans. Ann. Rev. Phytopath. 13:318-320.

43. Zaumeyer, W. J. and H. R. Thomas. 1957. A monographic study of bean diseases and method for their control. USDA. Agr. Tech. Bull. No. 868:255 pp.

CHAPTER TWO

EXPRESSION OF HYBRID WEAKNESS IN PHASEOLUS VULGARIS L. AND ITS ASSOCIATION WITH SEED SIZE

7.1 INTRODUCTION AND LITERATURE REVIEW

The improvement of various characters of existing crop species conventionally involves the hybridization of cultivars from both within and between geographic origins. The hybridization within the species Phaseolus vulgaris L is achieved with relative ease and mostly results in normal hybrids. However, there have been instances where abnormal F_1s and sub-viable progenies of the F_2 and subsequent generations of crosses between normal parents have been observed.

Davis and Frazier (1964) reported abnormal progenies in the F_2 generation of crosses between true bushes and Blue Lake- derived bush snap beans. Coyne (1965), similarly observed "crippled" plants in F_2 and F_3 generations of the crosses Yellow Eye P1209806 x Great Northern (GN) Nebraska #1, and Dark Red Kidney x GN, and attributed them to two complementary recessive genes. However, in other studies, abnormal or defective plants were observed in the F_1 generation, indicating that the trait was controlled by dominant genes. F_2 segregation ratios, together with results of F_1s backcrossed to parents in various crosses

showing these defective plants indicated that the character was conferred by the interaction of two complementary dominant genes (York and Dickson, 1975, Van Rheenen, 1979 and Shii et al. 1980). Shii et al. (1980) designated these genes as DL_1 and DL_2 (for dosage dependent lethal or dwarf lethal). Muhalet (1979) observed these dwarf lethals in F_1's of some crosses and F_2's in other crosses.

The apparent incompatibility of normal bean cultivars leading to dwarf lethal plants generally arise when small-seeded parents are crossed to either medium or large seeded ones (Singh and Gutierrez, 1980 and CIAT, 1983). Electrophoretic analysis of phaseolin revealed that F_1 hyribid abnormalities resulted from crosses between "S" phaseolin (associated with small-seeded cultivars of Middle American origin) and a "T" or "C" phaseolin (found in large-seeded cultivars of Andean origin) (Gepts and Bliss,1985). The DL_1 and DL_2 genes, therefore possibily served as a genetic barrier, limiting free genetic exchange between the two cultivated common bean germplasm groups.

In the present study conducted to determine the inheritance of resistance to angular leaf spot disease in common bean (Phaseolus vulgaris L.), abnormal F_1 hybrids were observed in some crosses . In the F_2 and F_3 generations, segregation of normal to dwarf lethals was

observed (Fig.8 and 9) wihich prompted the further investigation of (1) the inheritance of the dwarf lethal character in those crosses in which they were observed, and (2) the relationship between the common bean cultivars involved in F_1 hybrid abnormalities by means of electrophoretic analysis of the phaseolin type.

Fig. 7. Segregation of dwarf lethals and normal plants in the F_2 generation of the cross C-20 x Pompadour Checa.

Fig. 8. Segregation of dwarf lethals and normal plants in the F_3 generation of the cross Pompadour Checa x BAT-332.

MATERIALS AND METHOD

8.1.1 EXPERIMENT 1: Common bean crosses and segregation of dwarf lethals.

Five common bean (P. vulgaris L.) cultivars with different seed sizes were crossed in different combinations to produce F_1's during the spring of 1985 (Table 11 and 12). Reciprocal crosses were attempted in the crosses G 05686 x BAT-332 and G 05686 x C-20, since it was difficult to achieve success in the other direction. During the winter of 1986, these F_1's and their corresponding parents were planted and reciprocal backcrosses made. All studies were conducted under greenhouse conditions.

The segregation pattern of abnormal plants, hereinafter referred to as dwarf lethals, and normal plants based on the vegetative vigour and growth of parental, F_1, F_2, F_3 and reciprocal backcross populations was studied between summer 1986 and winter 1988. The observed numbers of dwarf lethals versus normal plants in F_2's, F_3's and backcrosses were tested against theoretical ratios using a chi-square (X^2) test.

8.1.2 EXPERIMENT 2: Electrophoretic analysis of Phaseolin

The phaseolin of parental genotypes involved in the production of F_1 dwarf lethals was analysed by Horizontal

Starch Gel Electrophoresis as outlined by Weeden and Emmo (n.d.). The gel was prepared from 33 gm of starch and 330 ml of buffer. The gel buffer for Weeden"s first system consisted of 300 ml of tris-citrate at pH 8.4, and 30 ml of lithium hydroxide (LiOH)-boric acid at ph 8.1.

Table 11

Cultivars of common bean involved in F_1 dwarf lethals and their seed size.

CULTIVAR	SEED SIZE	100 SEED WEIGHT (g)
G 05686	Large	58.2
Montcalm	Large	45.6
Pompadour Checa	Medium	36.1
C-20	Small	19.2
BAT-332	Small	14.1

25g or less = small, 26 to 40g = medium, >40 = large -seed size

8.1.3 Sample Preparation

Approximately 25 mg of each imbibed seed cotyledon was crushed in single wells in a porcelain dish containing 0.2 ml of grinding buffer. The grinding buffer was made of 1.5 gm of reduced glutathione dissolved in 40 ml of deionized water. The grinding buffer was titrated to pH 7.6

with 1M tris maleate.

Table 12

Common bean crosses ,their corresponding seed sizes and growth of F_1 hybrids.

Cross	Seed size	Growth
Montcalm x G 05686	L x L	N
Pompadour x G 05686	M x L	N
C-20 x G 05686	S x L	D
C-20 x Pompadour	S x M	D
C-20 x BAT-332	S x S	N
Pompadour x BAT-332	M x S	D
G 05686 x BAT-332	L x S	D

N = Normal growth L = Large S = Small

D = Dwarf lethals M = Medium

8.1.4 Loading and running gels.

The gels were loaded at 50mA for 20 minutes and run at 45 mA for 5 hours in about 400 ml of chilled tank buffer consisting of LiOH-boric acid at pH 8.1. The voltage during these periods ranged from 270V to 300V. At the end of the run the gels were sliced and stained for phaseolin protein.

8.1.5 Staining

Stain solution consisting of 20mg of Napthol blue black in 20 ml of wash solution (5:5:1 Methanol:Water:Acetic acid) was poured over the gel and left covered at room temperature for about 45 minutes. The gel was rinsed with wash solution three times and scored as fast or slow with Sanilac and Tendergreen as controls.

RESULTS AND DISCUSSION

9.1 Experiment 1: Bean cultivars and crosses

Satisfactory success was achieved in the production of all crosses except in the cases of BAT-332 x G 05686, and G 05686 x C-20. No success was achieved when BAT-332 was used as the female parent in the cross BAT-332 x G 05686. However, in the reciprocal cross G 05686 x BAT-332, one F_1 seed was obtained. The crosses between C-20 and G 05686 were almost exclusively aborted when C-20 was used as the female parent, and resulted in only a few F_1 seeds. When G 05686 was the female parent no success was achieved in obtaining F_1 seeds.

Of the seven crosses, Montcalm x G 05686, Pompadour x G 05686, and C-20 x BAT-332 were the only ones that produced normal plants in the F_1 and subsequent generations. These crosses were of the type Large x Large, Medium x Large and Small x Small, respectively (Table 12). The other four crosses, consisting of either Large x Small, or Medium x Small produced only F_1 dwarf lethals (Table 12). From these results it was observed that the apparent incompatibility occurred only when small-seeded parents were crossed to either medium or large-seeded parents.

During the summer of 1985 when the dwarf lethals were first observed, the seedlings generated from F_1 seeds had 100% emergence and looked normal. However, after the

formation of the primary leaves, certain phenotypic abnormalities were observed which included stunted growth, chlorosis of the first two trifoliate leaves and the formation of adventitious roots on the hypocotyls. The primary leaves, which were initially green, also eventually became chlorotic. However, during the winter of 1986 when greenhouse temperature was generally lower (18-20 ^{o}C), F_1 plants looked normal except for the adventitious roots that appeared on the hypocotyls. The F_1's therefore produced many seeds as opposed to the few seed set by the F_1's during the Summer of 1985. These results suggest that the expression of the dwarf lethal condition is temperature dependent, and are in agreement with those of York and Dickson (1975) and Mok *et al*. (1980).

Though the dwarf lethal condition in the F_1s was observed in four crosses, the inheritance of the trait was studied in only two crosses, the reason for this being that only a few seeds were produced in the other two crosses.

9.2 Segregation of the dwarf lethal condition

The inheritance of the dwarf lethal trait was studied using F_2, F_3, and reciprocal backcross populations grown under greenhouse conditions. The segregation of plants was classified into two phenotypic categories as normal and dwarf lethal. According to Shii *et al*. (1980) the F_2

populations were classified as normal, F_1-like plants, sub-lethal and lethal, with F_1-like plants being temperature dependent.

The F_2 population of the cross Pompadour x BAT-332 segregated into dwarf lethals and normal plants in the ratio of 9:7 (Table 12). Ten F_2 plants consisting of three normal and seven dwarf lethals were raised to generate F_3 families. All three F_2 normal plants produced only normal F_3 families. Of the seven dwarf lethals five segregated into 9 dwarf lethal 7 normal plants. The remaining two F_2 dwarf lethals segregated in the ratio of 3 dwarf lethal to 1 normal in the F_3 generation. The results of the F_3's and F_2's were supported by the data of the backcrosses. Progenies of F_1's backcrossed to both parents segregated into dwarf lethal and normal plants in a ratio of 1:1 (Table 13).

Table 13.

Segregation for dwarf lethal and normal plants in F2 and backcross populations of the cross Pompadour x BAT-332.

Population	Number of plants			Expected	Chi-Square	P
	D	N	Total	ratio	(X^2)	
F_2	100	89	189	9:7	.726	.25-.50
$B.C_1$	2	3	5	1:1	.000	1.00
$B.C_2$	8	5	13	1:1	.308	.50-.75

$B.C_1$ = F x Pompadour D = Dwarf lethals

$B.C_2$ = F_1 x BAT-332 N = Normal plants

Similar results were obtained in the cross C-20 x Pompadour (Table 14). Ratios of 9:7 and 1:1 dwarf lethals to normal plants were observed in the F_2 and backcross populations, respectively. Ten F_2 were selected and advanced into F_3 families. Four of these F_2 plants were normal and produced only normal F_3's. Of the remaining six, two segregated into dwarf lethals and normal in the ratio of 9:7. and four into 3 dwarf lethals to 1 normal plants. The results from both crosses indicated that the dwarf lethal plants in the F_1's were inherited as two complementary dominant genes, one of each being derived from each parent. These results are consistent with those of York and Dickson (1975), Van Rheenen (1979) and Shii *et*

al (1980)

Table 14.

Segregation for dwarf lethals and normal plants in F2 and backcross populations of the cross C-20 x Pompadour.

Population	Plant Number			Expected	Chi-Square	P
	D	N	Total	ratio	(X^2)	
F_2	110	69	179	9:7	1.897	.10-.25
$B.C_1$	3	2	5	1:1	0.000	1.00
$B.C_2$	4	6	10	1:1	0.100	.50-.75

$B.C_1 = F_1 \times$ C-20

$B.C_2 = F_1 \times$ Pompadour

D = Dwarf lethals

N = Normal

9.3 Experiment 2: Electrophoretic analysis of phaseolin storage protein

The phaseolin types exhibited by the parental genotypes involved in F_1 hybrid weakness are shown in Table 15. The analysis of the seed protein revealed that the genotypes exhibited phaseolin types as first described by Gepts and Bliss (1985). C-20, Pompadour Checa, BAT-332 and G 05686 have "S", "T", "S", and "T" phaseolin, respectively. The results of this study indicate that dwarf lethals observed in the F_1's and subsequent generations arose when small-seeded parents with an "S" type phaseolin

were crossed to medium- or large-seeded parents with a "T" type phaseolin. Gepts and Bliss (1985) obtained similar results when the phaseolin types of parental genotypes known to give rise to dwarf lethals in the F_1's and F_2's were analysed.

The expression of dwarf lethal F_1 plants occuring in crosses involving Andean (medium to large-seeded) and Meso-American (small-seeded) cultivars serves as a genetic barrier between these two common bean germplasm groups. This isolating mechanism points to the fact that within cultivated common bean two distinct gene pools exist.

Table 15

Phaseolin types and seed sizes of parental genotypes involved in dwarf lethal F1 plants.

Parental genotypes	Phaseolin types	seed size	100 seed wt. (g)
C-20	"S"	Small	19.2
Pompadour Checa	"T"	Medium	36.1
Sanilac*	"S"	Small	17.9
Tendergreen*	"T"	Large	36.9
BAT-332	"S"	Small	14.1
G 05686	"T"	Large	58.2

* = controls

SUMMARY AND CONCLUSIONS

During the study conducted to determine the inheritance of resistance to angular leaf spot disease of common bean (Phaseolus vulgaris L.), certain morphological abnormalities were observed in F_1's of some crosses. In all cases, these abnormalities occurred in crosses involving small-seeded parents and medium- or large-seeded parents, and it became apparent that the abnormalities reflected a genetic disorder.

The phenotypic abnormalities consisted of stunted growth, chlorosis of trifoliate and primary leaves and the appearance of adventitious roots on hypocotyls. Dwarf lethal F_1 plants were highly influenced by temperature, with most of the plants perishing under high temperatures ($26\text{-}32^{o}C$). Results of F_2, F_3 and backcross populations of the the two crosses studied indicated that the occurrence of the dwarf lethals was inherited as two complementary dominant genes.

Electrophoretic analysis of phaseolin, the major seed protein, revealed that all the small-seeded parents (C-20, and BAT-332) had an "S" type phaseolin, whereas the medium and large-seeded parents (Pompadour and G 05686) exhibited a "T" type phaseolin. The dwarf lethal F_1 plants obtained when medium -large seeded (Andean) and small-seeded (Meso-American) cultivars are crossed supports the existence of

two distinct gene pools in cultivated common bean germplasm.

LITERATURE CITED

1. CIAT. 1983. Occurrence of F1 hybrid dwarfism in crosses between bean lines of different seed sizes. 1982 Bean Program Ann. Report, Centro Internacional de Agricultura Tropical, Cali, Colombia. 123-126p.

2. Coyne, D. P. 1965. A genetic study of "crippled" morphology resembling virus symptoms in Phaseolus vulgaris L. J. Hered. 56:162-176.

3. Davis, D. W., and W. A. Frazier. 1964. The incidence of three abnormalities in F2 progeny of crosses between true bushes and Blue Lake derived bush snap bean. Ann. Rept. Bean Impr. Coop. (New York) 7: 14-16.

4. Gepts, P. and F. A Bliss. 1985. F1 hybrid weakness in the common bean. Differential geographic origin suggests two gene pools in cultivated bean germplasm. J. Hered. 76:447-450.

5. Muhalet, C. S. 1979. Genetic system for reaction in field beans Phaseolus vulgaris L.) to three races of Colletotrichum lindemuthianum (Sacc. and Magn.) Brio. Et Cav. M. Sc. Thesis, Michigan State University, U.S.A. 100 pp.

6. Shii, C. T., M. C. Mok, and D. W. Mok. 1981. Developmental controls of morphological mutants of Phaseolus vulgaris L.: Differential expression of mutant loci in plant organs. Develop. Genet. 2:279-290.

7. Shii, C. T., M. C. Mok, S. R. Temple and D. W. S. Mok. 1980. Expression of developomental abnormalities in hybrids of Phaseolus vulgaris L.: Interaction beteween temperature and allelic dosage. J. Hered. 71:218-22.

8. Singh, S. P., and J. A. Gutierrez. 1984. Geographical Distribution of the DL1 and DL2 gene causing hybrid dwarfism in Phaseolus vulgaris L., their association with seed size, and their significance to breeding. Euphytica. 33:337-345.

9. Van Rheenen, H. A. 1979. A sub-lethal combination of two dominant factors in Phaseolus vulgaris L.

Ann. Rept. Bean Impr. Coop. (New York) 22:67-69.

10. Weeden, N. F., and Emmo, A. C. (n.d.). Horizontal Starch Gel Electrophoresis Laboratory Procedures. NYAES. Geneva, New York.

11. York, D. W., and M. H. Dickson. 1975. Segregation of a semi-lethal or crippled condition from crosses involving P. I. 165435. Ann. Rept. Bean Impr. Coop. (New York). 18:88-89

Appendix 1

Observed and expected ratios of susceptible to resistant plants in F_3 families of the cross Montcalm x G 05686 against the Michigan 5 isolate.

F_3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M501-41	3	16	6	22	3:1	0.00	1.00
85M501-1	1	0	16	16	0:1	0.00	1.00
85M501-17	1	0	23	23	0:1	0.00	1.00
85M501-12	4	19	5	24	3:1	0.06	.75-.9
85M501-50	1	0	13	13	0:1	0.00	1.00
85M501-3	3	16	6	22	3:1	0.00	1.00
85M501-23	1	0	19	19	0:1	0.00	1.00
85M501-11	4	11	4	15	3:1	0.02	.75-.9
85M501-14	2	0	13	13	0:1	0.00	1.00
85M501-19	1	0	19	19	0:1	0.00	1.00
85M501-7	6	12	5	17	3:1	0.02	.75-.9
85M501-47	6	15	0	15	1:0	0.00	1.00
85M501-38	4	11	3	14	3:1	0.00	1.00
85M501-31	1	0	20	20	0:1	0.00	1.00
85M501-20	8	16	0	16	1:0	0.00	1.00
85M501-49	8	16	0	16	1:0	0.00	1.00
85M501-76	9	13	0	13	1:0	0.00	1.00
85M501-60	1	0	18	18	0:1	0.00	1.00

Appendix 1 cont.

F3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X2	P
85M501-46	5	11	4	15	3:1	0.02	.75-.9
85M501-27	8	14	0	14	1:0	0.00	1.00
85M501-53	7	12	0	12	1:0	0.00	1.00
85M501-173	8	14	0	14	1:0	0.00	1.00
85M501-165	1	0	17	17	0:1	0.00	1.00
85M501-189	1	0	18	18	0:1	0.00	1.00
85M501-192	9	14	0	14	1:0	0.00	1.00
85M501-164	8	16	0	16	1:0	0.00	1.00
85M501-177	9	16	0	16	1:0	0.00	1.00
85M501-161	5	8	3	11	3:1	0.03	.75-.9
85M501-180	4	14	4	18	3:1	0.00	.75-.9
85M501-181	3	14	3	17	3:1	0.18	.50-.7

85M501 = Montcalm x G 05686

S = Susceptible

R = Resistant

Appendix 2

Observed and expected ratio of susceptible to resistant plants of F_3 families of the cross Pompadour Checa x G 05686 to the Michigan 5 isolate.

		Number of Plants Expected					
F_3 Plants	Rating	S	R	Total	Ratio	X^2	P
85M505-7	7	18	0	18	1:0	0.00	1.0
85M505-19	7	10	0	10	1:0	0.00	1.0
85M505-13	8	10	0	10	1:0	0.00	1.0
85M505-136	7	15	0	15	1:0	0.00	1.0
85M505-152	8	12	0	12	1:0	0.00	1.0
85M505-26	1	0	11	11	0:1	0.00	1.0
85M505-31	1	0	21	21	0:1	0.00	1.0
85M505-53	1	0	16	16	0:1	0.00	1.0
85M505-44	2	2	20	22	15:1	0.01	.75-.90
85M505-122	1	0	12	12	0:1	0.00	1.0
85M505-130	1	6	10	16	3:1	0.79	.25-.50
85M505-102	1	0	11	11	0:1	0.00	1.00
85M505-110	1	0	13	13	0:1	0.00	1.00
85M505-120	1	7	11	18	3:1	1.20	.25-.50
85M505-112	1	0	15	15	0:1	0.00	1.00
85M505-114	1	0	16	16	0:1	0.00	1.00
85M505-130	1	0	18	18	0:1	0.00	1.00
85M505-145	1	2	10	12	3:1	0.11	.50-.75

Appendix 2 cont.

F3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M505-118	1	0	13	13	0:1	0.00	1.0
85M505-136	1	0	15	15	0:1	0.00	1.0
85M505-132	1	2	14	16	15:1	0.26	.50-.7
85M505-128	1	0	15	15	0:1	0.00	1.0
85M505-129	1	0	12	12	0:1	0.00	1.0
85M505-140	1	0	13	13	0:1	0.00	1.0

85M505 = Pompadour Checa x G 05686

S = Susceptible

R = Resistant

Appendix 3

Observed and expected ratios of susceptible to resistant plants in F_3 families of the cross C-20 x Pompadour Checa against the Michigan 5 isolate.

F_3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M502-51	7	15	0	15	1:0	0.00	1.0
85M502-94	6	12	0	12	1:0	0.00	1.0
85M502-80	7	11	0	11	1:0	0.00	1.0
85M502-83	7	17	0	17	1:0	0.00	1.0
85M502-73	6	10	0	10	1:0	0.00	1.0
85M502-52	6	12	0	12	1:0	0.00	1.0
85M502-4	1	0	17	17	0:1	0.00	1.0
85M502-14	1	0	14	14	0:1	0.00	1.0
85M502-101	1	0	9	9	0:1	0.00	1.0
85M502-166	1	0	10	10	0:1	0.00	1.0
85M502-157	1	0	11	11	0:1	0.00	1.0
85M502-123	1	0	10	10	0:1	0.00	1.0
85M502-	1	2	19	21	15:1	0.03	.75-.9
85M502-17	1	0	17	17	0:1	0.00	1.0
85M502-46	2	3	11	14	3:1	0.00	1.0
85M502-150	1	0	12	12	0:1	0.00	1.0
85M502-32	2	3	11	14	3:1	0.00	1.0
85M502-38	2	2	20	22	15:1	0.01	.75-.9

Appendix 3 cont.

F3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M502-87	1	2	22	24	15:1	0.00	1.0
85M502-18	1	0	14	14	0:1	0.00	1.0
85M502-79	1	0	10	10	0:1	0.00	1.0
85M502-93	1	2	20	22	15:1	0.01	.75-.9
85M502-129	1	3	11	14	3:1	0.00	1.0
85M502-140	1	3	9	12	3:1	0.00	1.0
85M502-I69	1	0	11	11	0:1	0.00	1.0
85M502-192	1	0	12	12	0:1	0.00	1.0
85M502-182	2	2	19	21	15:1	0.03	.75-.9
85M502-207	1	0	11	11	0:1	0.00	1.0
85M502-200	1	0	12	12	0:1	0.00	1.0
85M502-107	1	0	14	14	0:1	0.00	1.0
85M502-63	1	3	24	27	15:1	0.420	.50-.7
85M502-38	1	0	15	15	0:1	0.00	1.0
85M502-21	1	0	11	11	0:1	0.00	1.0
85M502-70	1	0	10	10	0:1	0.00	1.0
85M502-18	1	0	13	13	0:1	0.00	1.0
85M502-75	1	0	11	11	0:1	0.00	1.0

85M502 = C-20 x Pompadour Checa

S = Susceptible R = Resistant

Appendix 4

Observed and expected ratio of susceptible to resistant plants in F_3 families of the cross C-20 x BAT-332 against the Colombia 10 isolate.

F_3	Plants	Rating	Numbers of Plants S	R	Total	Expected Ratio	X2	P
85M504-128	8	30	0	30	1:0	0.00	1.0
85M504-43	4	30	9	39	3:1	0.01	.90-.95
85M504-97	6	60	0	60	1:0	0.00	1.0
85M504-109	5	45	0	45	1:0	0.00	1.0
85M504-9	3	11	36	47	1:3	0.007	.90-.95
85M504-57	4	39	11	50	3:1	0.107	.50-.75
85M504-118	4	30	8	38	3:1	0.14	.50-.75
85M504-131	4	32	12	44	3:1	0.03	.75-.90
85M504-108	7	28	0	28	1:0	0.00	1.0
85M504-139	4	34	13	47	3:1	0.064	.75-.9
85M504-3	6	30	5	35	13:3	0.028	.75-.9
85M504-93	5	30	7	37	13:3	0.063	.75-.9
85M504-179	7	28	0	28	1:0	0.00	1.0
85M504-1	3	8	30	38	1:3	0.14	.50-.75
85M504-159	3	6	22	28	1:3	0.048	.75-.9
85M504-12	7	40	0	40	1:0	0.00	1.0
85M504-39	7	35	0	35	1:0	0.00	1.0
85M504-20	1	0	42	42	0:1	0.00	1.0
85M504-22	4	25	7	32	3:1	0.041	.75-.9

Appendix 4 cont.

F3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M504-95	2	0	32	32	0:1	0.00	1.0
85M504-99	6	32	0	32	1:0	0.00	1.0
85M504-124	2	0	45	45	0:1	0.00	1.0
85M504-82	6	15	31	46	1:3	0.04	.75-.9
85M504-44	3	6	20	26	1:3	0.00	1.0
85M504-96	5	18	7	25	3:1	0.01	.9-.95
85M504-149	2	13	37	50	1:3	0.00	1.0
85M504-160	2	4	15	19	1:3	0.02	.75-.9
85M504-98	2	0	39	39	0:1	0.00	1.0
85M504-53	5	37	17	54	3:1	0.87	.25-.5
85M504-18	5	26	11	37	3:1	0.25	.50-.7

85M504 = C-20 x BAT-332

S = Suceptible

R = Resistant

Appendix 5

Observed and expected ratios of susceptible to resistant plants in F_3 families of the cross Pompadour Checa x Bat-332 against the Colombia 10 isolate.

F_3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M503-17	1	0	12	12	0:1	0.00	1.0
8M5503-5	1	0	11	11	0:1	0.00	1.0
85M503-47	1	0	14	14	0:1	0.00	1.0
85M503-123	1	0	13	13	0:1	0.00	1.0
85M503-90	1	0	10	10	0:1	0.00	1.0
85M503-93	1	0	10	10	0:1	0.00	1.0
85M503-88	2	0	11	11	0:1	0.00	1.0
85M503-124	4	12	3	15	3:1	0.020	.75-.9
85M503-79	6	10	0	10	1:0	0.00	1.0
85M503-69	9	10	0	10	1:0	0.00	1.0
85M503-121	6	6	4	10	9:7	0.057	.75-.9
85M503-143	5	8	5	13	9:7	0.011	.75-.9
85M503-132	7	10	0	10	1:0	0.00	1.0
85M503-138	5	9	2	11	3:1	0.03	.75-.9
85M503-160	5	7	5	12	9:7	0.021	.75-.9
85M503-165	7	11	0	11	1:0	0.00	1.0
85M503-172	4	8	5	13	9:7	0.01	.9-.95
85M503-136	4	9	2	11	3:1	0.03	.7-.9

Appendix 5 cont.

		Number of Plants			Expected		
F$_3$ Plants	Rating	S	R	Total	Ratio	X^2	P
85M503-212	4	10	0	10	1:0	0.00	1.0
85M503-179	4	7	6	13	9:7	0.031	.75-.9

85M503 = Pompadour Checa x BAT-332

S = Susceptible

R = Resistant

Appendix 6

Observed and expected ratios of susceptible to resistant plants of F_3 families of the cross C-20 x Pompadour Checa against the Malawi 1 isolate.

		Number of Plants			Expected		
F_3 Plants	Rating	S	R	Total	Ratio	X^2	P
85M502-53	4	17	7	24	3:1	0.04	.75-.9
85M502-45	2	13	4	17	3:1	0.02	.75-.9
85M502-6	1	0	21	21	0:1	0.00	1.0
85M502-56	4	14	7	21	3:1	0.39	.50-.75
85M502-42	6	16	6	22	3:1	0.00	1.0
85M502-17	1	0	17	17	0:1	0.00	1.0
85M502-23	4	11	4	15	3:1	0.02	.75-.9
85M502-46	2	0	14	14	0:1	0.00	1.0
85M502-27	6	16	0	16	1:0	0.00	1.0
85M502-44	6	16	5	21	3:1	0.02	.75-.9
85M502-20	6	7	2	9	3:1	0.04	.75-.9
85M502-30	5	12	5	17	3:1	0.02	.75-.9
85M502-142	8	15	0	15	1:0	0.00	1.0
85M502-150	1	0	12	12	0:1	0.00	1.0
85M502-119	3	14	7	21	3:1	0.39	.50-.75
85M502-98	1	0	14	14	0:1	0.00	1.0
85M502-172	7	15	0	15	1:0	0.00	1.0
85M502-189	4	12	6	18	3:1	0.29	.50-.75

Appendix 6 cont.

F3 Plants	Rating	Number of Plants S	R	Total	Expected Ratio	X^2	P
85M502-134	7	15	0	15	1:0	0.00	1.0
85M502-80	7	11	0	11	1:0	0.00	1.0
85M502-158	7	11	0	11	1:0	0.00	1.0
85M502-179	6	14	0	14	1:0	0.00	1.0
85M502-157	1	0	11	11	0:1	0.00	1.0
85M502-101	1	0	9	9	0:1	0.00	1.0
85M502-166	1	0	10	10	0:1	0.00	1.0
85M502-180	7	10	0	10	1:0	0.00	1.0
85M502-8	7	12	0	12	1:0	0.00	1.0
85M502-123	1	0	10	10	0:1	0.00	1.0
85M502-130	7	12	0	12	1:0	0.00	1.0
85M502-29	2	0	11	11	0:1	0.00	1.0

85M502 = C-20 x Pompadour Checa

S = Susceptible

R = Resistant