"DETERMINATION OF PATHOGENIC VARIATION IN ISARIOPSIS GRISEOLA SACC. AND PSEUDOMONAS SYRINGAE pv. PHASEOLICOLA (BURK., 1926) YOUNG, DYE AND WILKIE 1978".

BY

ROBIN ARANI BURUCHARA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signed: _____ Date 24/4/84

R.A. Buruchara

This thesis has been submitted for examination with our approval as University supervisors.

Signed: _____ Date 25/4/84

Prof. D.M. Mukunya
Associate Professor
Department of Crop Science
Faculty of Agriculture
University of Nairobi.

Signed: _____ Date 30/4/84

Dr. E.M. Gathuru
Senior Lecturer in Plant Pathology
Department of Crop Science
Faculty of Agriculture
University of Nairobi.

DEDICATED TO ALL THOSE CITIZENS OF OUR NATION WHO HAVE WORKED HARD FOR THE DEVELOPMENT AND ENHANCEMENT OF PLANT PATHOLOGY AND WHO HAVE BEEN TO ME, A SOURCE OF INSPIRATION.

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ABSTRACT

Occurrence and extent of pathogenic variation and virulence was determined in Isariopsis griseola Sacc. and Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie 1978 using 21 and 30 isolates respectively, collected from naturally infected bean plants in important bean growing areas of Colombia.

I. griseola did not show any correlation between variation in conidial size and some cultural characteristics with the observed variation in pathogenicity. Conidial length of 10 isolates varied between 18-76µ with a mean of 38.5µ. The width varied between 3.8 - 8.8µ with an average of 6.4µ, whereas the number of septa varied between 0-7 with a mean of 3. These parameters varied significantly both within and between isolates.

The optimum growth temperature of the fungus in culture media was 24 C. No difference in the amount of mycelial growth at 14, 19, and 24 C was observed between the isolates. Differences in sporulation among some isolates were significant but

all except IG2 - 78 had an optimum sporulation temperature of 24 C after 10 days of incubation. IG2 - 78 produced significantly more conidia at 19 C than at 24 C. No correlation between the optimum growth and sporulation temperature of the isolates with the mean temperatures of their sources of origin was observed.

The 21 isolates of I. griseola were differentiated into seven pathotypes based on differential reactions of 6 bean lines/cultivars, demonstrating occurrence of physiological specialization in the fungus. The reactions used, were independent of environmental temperatures under which plants were kept after inoculation. The cultivar - isolate interactions obtained implied a presence in the cultivar of specific resistance which was effective against some and not all the pathotypes. Cultivar G 2575-10P-2C was susceptible to all but no cultivar was immune to all pathotypes. Two out of the seven pathotypes induced a susceptible reaction on cultivar G 1805-1P-1C but of very low (disease index scale of 2) disease severity. Some of the cultivars considered susceptible appeared to have partial resistance, expressed as

reduced, apparent infection rate (r) of angular leaf spot in the field.

The isolates of Ps. syringae pv. phaseolicola were separated into race 1 and 2 on the basis of their pathogenicity on the cultivar 'Red Mexican UI 3'. Race determination was based on both leaf and pod reactions. Standard isolates of race 1 and 2 were used for comparison. Twenty Pasto isolates were classified as race 2 whereas, 7 isolates from Popayan, 1 from Palmira and 2 from Tenerife were identified as race 1.

The isolates were also classified in their decreasing order of virulence by using the diameters of watersoaking lesions caused on pods during a period of 5 days on cultivars 'Seminole', 'G.N. Nebraska No. 1 Sel 27' and 'Wisc HBR 72'; following inoculation using a hypodermic needle. The most virulent isolates were from Pasto; some of which were more virulent than the standard race 2 used for comparison. Isolates differentiated into race 1 and 2 were not homogenous in virulence. Some isolates belonging to race 1 were more virulent than some of the race 2 isolates or vice versa apparently, due to either isolate and/or cultivar effect.

Cultivar 'Wisc. HBR 72' had a high foliar resistance but a susceptible pod reaction (mean lesion diameter 3.1 mm; dispersion 0.5 - 5.3 mm) comparable to the susceptible cultivar 'Seminole' (mean lesion diameter 2.8 mm; dispersion 0.5 - 4.8 mm). 'G.N. Nebraska No. 1 Sel 27' had a mean lesion diameter of 1.8 mm and a dispersion between 0.4 - 2.8 mm. The increasing or decreasing order of virulence was similar in all the three cultivars as was indicated by the high correlation coefficients ($r = 0.86 - 0.87$) between them.

The present studies unequivocally show that both bean pathogens exhibit pathogenic variation. A high degree of cultivar - isolate specificity was observed with I. griseola. Specificity was also shown with isolates of Ps syringae pv. phaseolicola when using 'Red Mexican UI 3'. However, virulence of the bacterial isolates varied continuously in a manner which nullify the present race categorization.

The practical importance of these findings are that they provide basic information about the pathogens, that must be considered before starting a program to either incorporate or develop resistance against angular leaf spot and halo blight in

P. vulgaris. The presence in some bean cultivars of pathotype specific resistance and in others partial resistance characterized by reduced rates of disease increase, indicate the available possibilities of recombining resistance against I. griseola. To grade pathogenicity and virulence of isolates of Ps. syringae pv phaseolicola, leaf and pod reactions of various bean cultivars, and not just one, must be based on. In turn, evaluation and improvement of resistance of bean cultivars against the bacterium should consider both the leaf and pod components of the plant.

1. INTRODUCTION

One of the major nutritional problems in many of the developing countries in the low land tropics is the lack of sufficient dietary proteins (Roberts, 1970). A substantial part of the population in these areas, mostly low and medium income families, is not able to obtain or afford the relatively expensive and/or scarce animal protein sources (Roberts, 1970; Smartt, 1976; Jalil, 1977). Hence, the cultivation of food legumes in such areas remains an important nutritional aspect, as they offer a cheaper alternative source of proteins (Jalil, 1977). Food intake trends show that in many of these countries grain legumes are consumed with various other stapple foods such as maize, rice, cassava or plantains depending on locality (Stanton, 1966; Smartt, 1976; Bressani and Elias, 1978). In Africa dry beans (Phaseolus vulgaris L.) cowpeas (Vigna unguiculata (L) Walp.) and lima beans (Vigna radiata (L) Wilczek) are readily acceptable (Stanton, 1966; Smartt, 1976). In Mexico and other parts of Latin America, dry beans constitute the main edible legume that is grown

and consumed (Bressani, 1972; Zaumeyer and Meiners, 1975; Smarrt, 1976; Graham, 1978). In Kenya, beans are the major legume crop grown followed by cowpeas, and pigeon peas (Cajanus cajan (L) Missp.) (Kenya Government Statistical Abstracts, 1969).

The protein content in P. vulgaris L. ranges between 20-25%, compared with 23.4% in cowpeas, 20.7% in lima beans, and 29-51% in soya beans (Glycine max (L) Merr.) (Leakey, 1970; Smartt, 1976; Bressani and Ellias, 1978). Cereals and beans complement each other very effectively in amino acids, the former providing methionine and the latter lysine (Bressani and Ellias, 1978).

Beans are primarily grown by small scale farmers under various cropping systems, usually, in mixed cropping (Graham, 1978; Sanders and Schwartz, 1979). Due to a combination of various factors, production lags behind consumption (Roberts, 1970; Jalil, 1977). In Latin America bean yields average 600 kg/ha in association with maize or 800 kg/ha in monoculture (Sanders and Schwartz, 1979); whereas the average yield for Africa ranges between 300-600 kg/ha (Roberts, 1970; FAO Production Year Book, 1971). However, it has been shown that

under experimental conditions yields of up to 5 tons/ha can be obtained in monoculture (Roberts, 1970; Cartree and Hanks, 1974; CIAT, 1974) and 2 tons/ha in association with maize (Francis et. al., 1977). Some of the limiting factors that contribute to low yields include agronomical constraints such as poor soil fertility, variable weather conditions and use of cultivars with low yielding potentials (Roberts, 1970). However, bean diseases and pests are considered to be the major factors responsible for the low yields (Wellman, 1968; Zaumeyer and Meiners, 1975; Sanders and Schwartz, 1979).

There are more than 200 diseases of beans (Mancia and Cortez, 1975; Schwartz and Katherman, unpublished). These include diseases caused by fungi, bacteria, viruses and mycoplasma. The importance of each disease depends on the pathogen distribution, susceptibility or resistance of the bean cultivars and the prevailing environmental conditions. Some of the bean diseases considered to be of major economic importance in Latin America include: angular leaf spot, anthracnose, bean common mosaic, bean golden mosaic, common bacterial

blight and rust (Graham 1978; Proc. Workshop on Bean Anthracnose, Angular Leaf Spot and Common Bacterial Blight, 1979; Sanders and Schwartz, 1979). In Kenya, angular leaf spot, anthracnose, bean common mosaic, halo blight and rust are considered to be economically important, causing considerable damage on beans (Ministry of Agriculture-Kenya-GLP Phase III, 1979; Njuguna et. al., 1980).

Angular leaf spot of beans caused by Isariopsis griseola Sacc., 1878. (Phaeoisariopsis griseola (Sacc.) Ferraris, 1909) has a wide distribution and is considered a major problem in many of the bean growing regions (Zaumeyer and Thomas, 1957; Zaumeyer, 1968; Gutierrez et. al., 1975; Graham, 1978; Proc. Workshop on bean Anthracnose, Angular Leaf Spot and Common Bacterial Blight, 1979). Occurrence of this disease is often sporadic and when environmental conditions are favourable, infection can reach epidemic levels. Yield losses in beans due to the disease have been reported to be as high as 50% in the U.S.A. (Cole, 1966; Hagedorn and Wade, 1974), 40-60% in Colombia (Barros et. al., 1957), and 80% in Mexico (Crispin et. al., 1976).

Halo blight of beans caused by Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie 1978 (Pseudomonas phaseolicola (Burk). Dowson, 1943) has a worldwide distribution (Zaumeyer and Thomas, 1957; Hale and Taylor, 1973; Coyne and Schuster, 1979; Schwartz, 1979). Disease development is favoured by cool temperatures and high rainfall. Yield losses in experimental fields have been estimated to range between 23-40% (Saettler and Potter, 1970).

Recommended methods for the control of these two diseases include: use of chemicals (Barros et. al., 1958; Hagedorn et. al., 1969; Taylor and Dudley, 1977; Singh and Sharma, 1975), clean seed, crop rotation and removal of previously infected crop debris (Cardona-Alvarez, 1956; Zaumeyer and Thomas, 1957; Barros et. al., 1958; Grogan and Kimble, 1967). However, the effectiveness of these methods are limited due to the high production costs of using chemical control, the ability of the pathogens to survive in plant debris for a long period of time and land availability to practice crop rotation. On the contrary, the development and introduction of multiple disease

resistant bean cultivars, combined with other pest control practices, is regarded to be an effective and relatively cheap means of reducing disease incidence and, consequently, reducing yield losses.

Centro Internacional de Agricultural Tropical (CIAT), has a world-wide responsibility to develop technology needed to increase bean yields in low land tropics in collaboration with national programs. One way of achieving this goal involves the testing of the available germplasm and development of improved cultivars with multiple-disease resistance (CIAT Highlights, 1978). However, for breeding programs to succeed, the extent of pathogenic variation existing within the pathogen populations must be determined (Schwartz, unpublished; Williams, 1978; Zaumeyer, 1968), by race surveys and virulence analyses. Disease screening, then, can be based on a wide-based pathogenic spectrum in order to obtain relatively stable resistance.

Although some workers (Villegas, 1959; Silvera, 1967; Alvarez-Ayala and Schwartz, 1979), have noted pathogenic variation between different

isolates of Isariopsis griseola Sacc., no experimental work has been done to confirm it. The aim of this investigations was thus to:-

(a) determine if there is any relationship between variation of some cultural and morphological characters of the fungus with pathogenic variation and/or geographical source of origin.

(b) Confirm the existence of pathogenic variation in I. griseola and its implication on the breeding for disease resistance; and to identify cultivars that may serve as differentials.

Physiological races (race 1 and 2) of Ps. syringae pv. phaseolicola (Burk, 1926) Young, Dye and Wilkie 1978 have been reported by various workers (Walker and Patel, 1964; Epton and Deverall, 1965; Schuster et. al., 1965; Hale and Taylor, 1973). The distinction between the races is based on the reaction of bean cultivar 'Red Mexican UI-3', which is resistant to race 1 but susceptible to race 2. On the contrary, Schroth et. al. (1972), and Szarka and Velich (1979) observed that differentiating the two races on the

basis of 'Red Mexican UI-3' was simply separating the isolates with different degrees of virulence and that isolates of neither race 1 nor race 2 were homogenous with respect to virulence. Hence, determination of the race composition and variation in virulence are essential in resistant breeding to halo blight.

Little or no work has been done on halo blight in Colombia, apart from what CIAT has initiated (Schwartz, personal communication). Despite the fact that the disease is not a serious problem in Colombia studies on pathogenic variation and virulence are necessary so that the most virulent strains can then be utilized by CIAT in the genetic improvement of bean germplasm for areas where the disease is economically important. The aim of this part of the study was to determine the pathogenic variation and virulence of isolates of Ps. syringae pv. phaseolicola collected from some of the bean growing areas of Colombia.

PART I

PATHOGENIC VARIATION IN *Isariopsis griseola* Sacc.

2.0 LITERATURE REVIEW

2:1 Nomenclature of *Isariopsis griseola* Sacc.

Isariopsis griseola Sacc on *Phaseolus vulgaris* L. was described for the first time by Saccardo (1878) in Italy. Later, the same organism was described under the names; *Graphium laxum* Ell. (Ellis, 1881), *Isariopsis laxa* (Ell.) Sacc. (Saccardo, 1886), *Cercospora columnare* Ell. and Ev. (Ellis and Everhart, 1893), *Phaeoisariopsis griseola* (Sacc.) Ferr. (Ferraris, 1909) and *Lindaumyces griseola* Gonz-Frag. (Gonzalez-Fragoso, 1927). However, after a critical review of these descriptions, Harter and Zaumeyer (1944) came to a conclusion that all the later names were synonymous with *Isariopsis griseola* Sacc.

2.2 Variation in morphology of reproductive structures

When plant parts infected with the fungus are placed under favourable environmental conditions,

the pathogen produces clusters of parallel columnal conidiophores known as synnemata. The number of conidiophores to a synnema and size of the synnema in I. griseola show considerable variation. Miles (1917) observed that, the synnemal conidiophores varied in number, ranging from 8-40. Various workers have also reported varying sizes of the synnema, ranging between 94-680µ in length and 20-70µ in width (Saccardo, 1886; Miles 1917; Srinivasan, 1953; Zaumeyer and Thomas, 1957; Hocking, 1967; Ellis, 1971).

Conidia, produced at the tips of conidiophores, are pale gray, cylindrical to spindle shaped, sometimes curved, smooth and septate (Zaumeyer and Thomas, 1957; Ellis, 1971). Their sizes have also been noted to show variation. The length and the width of the conidia are reported to range between 20-80µ and 3.4 -8.8µ respectively (Saccardo, 1886; Benlloch 1944; Zaumeyer and Thomas, 1957; Llanos, 1957; Hocking, 1967; Ellis, 1971). Zaumeyer and Thomas (1957) observed that I. griseola had conidia with one to three septa and rarely four. However, Benlloch (1944), Hocking (1967), and Ellis (1971) found

conidia which had up to six septa. Llanos (1957) observed that conidia with three and four septa were more common, but those with one and five septa were rare and it was very difficult to find a conidium without a septum.

There is no evidence to suggest that some relationship exists between these morphological variations and any physiological or pathogenic characteristics of the fungi. Hocking (1967) reported a virulent isolate which caused circular lesions on trifoliate leaves of beans, but which was morphologically indistinguishable from an isolate which caused angular leaf spot, with respect to conidial size and the number of septa per conidium, except that, the synnemata were somewhat longer with the former isolate and also occurred on the lower as well as on the upper surface.

2:3 Variation in growth and sporulation

Environmental conditions are known to greatly influence the growth, development and reproduction of many fungi (Stakman and Harrar, 1957; Hawker, 1966; Berger and Hanson, 1971).

These conditions which include such factors as light, temperature, pH, and available nutrients, exert their influences, either independently or in an interactive manner (Calpouzos and Stallknecht, 1965; Stackman and Harrar, 1957; Hawker, 1966; Alvarez-Ayala, 1979).

In the literature, there are a few conflicting reports concerning the optimum conditions for growth and sporulation of I. griseola. Brock (1951) cultured the fungus on potato dextrose agar (PDA) plus bean pod extract medium at 27° C for 21 days, but did not indicate whether the temperature used was optimal for growth and sporulation. Cardona-Alvarez (1956) observed on PDA plus bean leaf extract medium, that the fungus was capable of growing between 8 - 28° C, but optimum growth was attained at 24° C. He did not observe any growth at 32 and 36° C. Later, reports on the growth of the fungus by Llanos (1957) on PDA, Silvera (1967) on bean leaf dextrose agar (BLDA), Santos-Filho (1976) on 'baby food'-calcium carbonate-agar, and Avila (1979) on V-8 juice agar (V-8A) showed that optimum growth occurred at 24° C. Alvarez-Ayala (1979) also observed that, at 24° C, the fungus produced a highly sporulating mycellium on

the medium BLDA, potato yeast dextrose agar (PYDA) and V-8A. Silvera (1967) and Avila (1979) observed no growth of I. griseola at 30° and at 28° C, respectively.

Llanos (1957) obtained good sporulation of I. griseola at 24 C after eight days of incubation under continuous darkness. Santos-Filho (1976) also got similar results and found that sporulation of the fungus in the dark was superior, when compared to an alternate light and darkness or continuous light treatment. Silvera (1967) found no difference in growth or time to sporulation of eight isolates under five different temperature conditions. He however, noted that sporulation started much earlier (after five days) at 25° C than at any other higher or lower temperature condition tested. Using detached bean leaves, he also demonstrated that good coremial growth and sporulation of the fungus occurred at 25° C. Alvarez-Ayala (1979) reported that better growth and sporulation occurred between 14 and 19 C after 20 days of incubation on V-8A. He observed no significant difference in sporulation between 14 C and 19 C. On a separate experiment, however, he

observed that maximum conidium production occurred between 9 - 12 days of incubation in the dark at 19 C and that there was a significant decrease in the number of conidia produced after the 12th day. Avila (1979) claimed that maximum sporulation occurred at 16 C after 10 days of incubation.

The above review of previous studies show that, for most of the work, the optimum growth temperature was the same as the optimum sporulation temperature. However, Avila (1979) found that the two are not the same, and observed that the best growth temperature was 24°C whereas, the best sporulation temperature was 16°C. The differences in optimal growth and sporulation requirements reported may be due in part to the different media used, the environmental conditions or the techniques employed. The differences may also be due to the inherent characters of the isolates studied.

2:4 Pathogenicity of *Isariopsis griseola* Sacc

Studies on bean varietal reaction to inoculation with *Isariopsis griseola* were first made by Gardner and Mains (1930) in United States

of America (USA). They found that 'Kentucky Wonder', was the most resistant of 40 Phaseolus vulgaris L. cultivars tested under greenhouse conditions. In a study using a single isolate, Brock (1951) in Australia grouped 154 P. vulgaris and 2 P. multiflorus Wild. cultivars into five groups, ranging from highly susceptible to highly resistant. The latter group included P. vulgaris L. cultivars 'Alabama No. 1', 'California Small White' and 'Epicure'. He noted that resistant cultivars were either, runner beans or field beans. In 1956 the bean cultivar 'Cauca 27 A' was reported (Colombian Agric. Res. Prog., Min. Agric. and Rockefeller Foundation) to have angular leaf spot resistance. Later, Olave (1958) made similar observations and noted that cultivars 'Mexico 11' and 'Mexico 12' were also resistant. Singh and Sharma (1975) in India, made field evaluation of 40 bean lines using the powder of infected bean leaves as inoculum and found that the four lines; 'EC 38921', 'EC 44621', 'PLB 148' and 'Kentucky Wonder' were completely disease free. In similar studies resistance to angular leaf spot has been reported in P. vulgaris L. line 'O258' in Colombia

(Cardona-Alvarez, 1958), cultivars 'Boriole' and 'San Fiacre' in Spain (Puerta and Alonso, 1958), cultivars 'S-29-N' and '15-Guatemala-2524' in Costa Rica (Silvera, 1967), cultivar 'Caraota-260' in Brazil (Santos-Filho et. al., 1976), and cultivar 'P 525' (G 4421) in Mexico (Avila, 1979). Singh and Saini (1980) in India, reported that a P. coccineus L. cultivar, 'PLB 257' was resistant to angular leaf spot but they could not find any resistant P. vulgaris L. cultivar under field conditions.

Some of the cultivars previously reported to be good sources of resistance to angular leaf spot in various countries have been found to be susceptible in either the same or different country. In Brazil, cultivars 'Manteigao Preto 20' and 'Cauca-168-N' were initially reported to have high degrees of resistance (Vieira, 1964; Guazzelli, 1971, respectively, In Costa, 1972). Later, Santos-Filho et. al. (1976) found the two cultivars to be highly susceptible. They nevertheless, observed that 'Caraota 260' was highly resistant to a mixture of 10 local isolates. These later results were in agreement with earlier

observation by Vieira (1974) under field conditions. Studies conducted at Centro Internacional de Agricultura Tropical (CIAT) by Alvarez-Ayala and Schwartz (1979) showed that plants grown from seeds of 'Caraota 260' from Brazil were susceptible to three of the five isolates used in greenhouse tests. They also found that cultivars, 'Alabama No. 1' and 'Cauca-27A', both previously reported as resistant, were susceptible to some of the Colombian isolates of I. griseola tested.

Resistance of some of the bean cultivars has been shown to be conferred by either one or more independent recessive or dominant genes. Barros et. al. (1957) found that in most of their simple crosses, resistance was governed by a recessive character controlled by two or three independent factors. Santos-Filho et. al. (1976) in Brazil used a single, highly pathogenic local isolate to test resistance in crosses between 'Caraota 260' (resistant) and 'Venezuela 350' (susceptible). Resistance in 'Caraota 260' was shown to be governed by a single recessive factor. Resistance in the bean cultivars 'Decal', 'Maravilla' and 'Huila 14' has also been attributed

to three recessive factors (Colombian Agric. Res. Prog., Min. Agric. and Rockeffer Foundation, 1956). Crosses made by Singh and Saini (1980) between a P. coccineus cultivar 'PLB 257' (resistant) and a P. vulgaris cultivar 'Contender' (susceptible), showed that, 'PLB 257' carried a recessive gene imparting resistance to angular leaf spot. However, in some cases resistance has been found to be dominant (Barros et. al., 1957). Cardona-Alvarez (1958) made direct and reciprocal crosses between the resistant line '258' and a susceptible line '233'. Resistance was found to be monogenic and dominant in the resistant line when tested against a single isolate. These results indicate that there exists variation in isolate-cultivar interaction with regard to resistance or susceptibility which in these cases seem to vary depending on either cultivar or isolate or even both.

Some investigators in the past have given indications that, I. griseola exhibits pathogenic variation. Brock (1951) found that, although there were differences in pathogenecity of 13 field isolates, their order was the same in cultivars

'Brown Beauty' and 'Red Mexican' tested. Cardona-Alvarez (1956) however, did not find any difference in pathogenicity of 10 single-spore isolates on cultivar 'Idaho Refugee'. Silvera (1967) obtained similar results with seven isolates tested on eight cultivars, and did not observe any evidence of pathogenic specialization. Llanos (1957) reported that, I. griseola isolates did not change their pathogenic capacity after several successive conidial transfer and maintenance on artificial media. However, Villegas (1959) was able to group 33 single-spore isolates collected from Colombia, into 13 races on the basis of their differential reactions with 14 bean lines but doubted the genetic purity and uniformity of his lines. Using young seedlings, he found that only two of the 14 lines showed resistance to most of the isolates but none was immune to all isolates. Preliminary studies by Alvarez-Ayala and Schwartz (1979) at CIAT, using five isolates from Colombia and five cultivars, also suggested that pathogenic variation in I. griseola exist.

The conflicting reports on varietal resistance or susceptibility to the angular leaf

spot pathogen may be attributed to genotypic differences of the cultivar tested, variation in testing techniques and conditions, or pathogenic variation of the isolates used. Leach et. al. (1939) pointed out that, if pathogenic races exist in different areas, one variety resistant in one area may be found susceptible in another. The existence of ecological races with plant pathogenic organisms has also been suggested by some investigators (Line and Bugbee, 1964; Brinkerhoff, 1970) and demonstrated by Hill and Nelson (1976) with race T of Helminthosporium maydis Nisikado and Miyake. These are populations of organisms that are adapted to a particular ecological condition due to their inherent genetic characteristics. Evidence of their existance in I. griseola does not exist.

2.5 Epidemiology

Relationship between environmental conditions and pathogenicity of Isariopsis griseola in the development of angular leaf spot has been demonstrated. Exposure of plants to high relative humidity (95-100%), immediately after inoculation, has been shown to be

an essential pre-requisite for infection on susceptible cultivars (Cardona-Alvarez and Walker, 1956; Llanos, 1957; Silvera, 1967; Alvarez-Ayala, 1979; Avila, 1979). Llanos (1957) reported that the period in which inoculated plants were maintained under high relative humidity influenced disease expression. Periods of 48-72 hrs have been found adequate for infection to occur (Cardona-Alvarez and Walker, 1956; Llanos, 1957; Alvarez-Ayala, 1979; Avila, 1979).

Cardona-Alvarez and Walker (1956) observed that under high relative humidity, infection occurred and disease developed rapidly from 16 to 28 C, with an optimum at 24 C. Infection did not occur at 32 C. Working under field conditions, Sindhan and Bose (1980) however, concluded that relative humidity and precipitation were more important than temperature in disease development.

Studies with cultivar 'Idaho Refugee' by Cardona-Alvarez and Walker (1956), showed that plants ranging in age between 10 to 60 days, were equally susceptible. All of them exhibited a similar sequence of infection and disease development despite their age differences. Olave (1958) also

found that, 20-day-old plants of cultivar 'Algarrobo' showed the same disease severity as 30-day-old plants, 15 days after inoculation. Sindhan and Bose (1980) however, reported that two-week-old plants of cultivar 'Black Queen' remained healthy after inoculation whereas, three-week-old plants were infected but were less susceptible than the four-, five- and six-week-old plants. Maximum infection occurred with six-week-old plants. The same workers also implicated late infection on the same cultivar in the field condition as being due to resistance of plants at a younger age. Villegas (1959) did not observe this phenomenon in his studies with various lines using very young seedlings.

3 MATERIALS AND METHODS

3:1 Sources of Isariopsis griseola Sacc. isolates

Isolates of Isariopsis griseola Sacc. used in these studies, were obtained from some of the main bean growing regions of Colombia, representing varying ecological conditions (Table 1). Isolation was made from naturally infected bean (Phaseolus vulgaris L.) plants growing in farmers' fields and at the Centro Internacional de Agricultural Tropical (CIAT) and Instituto Colombiano Agropecuario (ICA) experimental fields. A total of 21 isolates were used; fifteen of which were collected and isolated during the period between November 1979 and March 1980, whereas, five isolates had been isolated earlier and were maintained at the CIAT Bean Pathology Section. One isolate was received from Dr. Donald Hagedorn, Department of Plant Pathology, University of Wisconsin, USA.

3:2 Isolation and storage of isolates

Isolation was made from lesions on infected leaves and/or pods, showing fungal sporulation. In case of non-sporulated lesions, the fungus was

Table 1. Areas of origin of Isariopsis griseola Sacc. isolates and their climatological conditions[a].

Area of origin	Number of isolates	Climatological conditions		
		Altitude (m)	Mean annual rainfall (mm/yr)	mean annual temperature (C)
Cajibío	2	1700-1800	-[b]	-
CIAT-Palmira	2	1000	1000	24
La Selva (Rio Negro)	2	2200	1500	18
Obonuco	1	2710	575	13
Pitalito (Huila)	1	900-1300	1000-1500	17-23
Popayán	7	1850	1600	18
Restrepo	1	1500	1000	20
Santander de Quilichao	2	1052	1845	25
Tenerife	2	2609	1356	13.5
Wisconsin (USA)	1	-	-	-

[a]Source of climatological data: CIAT Annual Report 1980; Instituto Colombiano de Hidrología, Meteorología y Adecuacion de Tierras (HIMAT), Cartago, Valle, Colombia.

[b]no data available.

induced to sporulate before isolation. To induce sporulation, small pieces of infected leaves or pods bearing lesions were surface sterilized for two minutes in 1% (W/V) sodium hypochlorite, double rinsed with sterile distilled water and dried between sterile filter papers. The tissues were then placed in sterile glass petri dishes containing moistened filter papers and were incubated in a Fries 815 low temperature incubator (Precision Scientific Co.) at 19 C and observed after 48 hours. The needle tip method (Diaz et. al., 1965; Santos-Filho et. al., 1976; Alvarez-Ayala, 1979) was used to isolate the fungus from infected tissues. Using, Stereozoom 7 model dissecting stereoscope (Bausch and Lomb Co.), synnemata bearing conidia were touched with a fine tip of a needle bearing a tiny piece of agar, without touching the leaf surface. Both the conidia and the agar piece were planted on V-8 juice agar medium (juice of 8 vegetables, Campbell Soup Co., Camden, N.Y., 200 mℓ; $CaCO_3$, 3 g; bacto-agar (Difco), 18 g; distilled water, 800 mℓ) and the petri dishes wrapped up with an aluminium foil to exclude light (Llanos, 1957; Santos-Filho, 1976; Alvarez-Ayala, 1979) and

incubated at 19 C (Alvarez-Ayala, 1979). Cultures obtained thereof were purified by two consecutive conidial transfers, after which single conidial cultures were prepared and used in all subsequent tests. The isolates were maintained and stored on V-8A medium in sealed test tubes and/or petri dishes at 4 C and were periodically transferred on to a fresh medium.

3:3 Determination of variation in conidial size, growth and sporulation of isolates on artificial medium.

3:3:1 Conidial size

A total of ten isolates from Cajibio (IG 11-80), CIAT-Palmira (IG 4 - 78), La Selva (IG 15-80), Obonuco (IG 3-78), Pitalito (IG 21-80),Popayan (IG 1-77 IG 6-79, IG 10-79) and Tenerife (IG 2-78, IG 13-80) were used to determine variation in conidial size. Single conidial cultures were grown in 9 cm diameter petri dishes containing V-8A medium for 10 days in a Freas 815 low temperature incubator (Precision Scientific Co.) set at 24 C. A completely randomized design was used with 4 replicates per treatment (isolate). On to each plate culture, 5 mℓ of distilled water was added, and using a wire loop, the culture surface was gently scrapped to make a

conidial suspension. One hundred conidia per isolate were measured to determine the length, width and the number of septa, using a Dynazoom compound microscope (Bausch and Lomb Co.) with a micrometer. Determination of the width was done by measuring the widest section of the conidia.

3:3:2 Determination of growth.

Eight isolates from different areas of Colombia with different temperature regimes were used (Table 2). The isolates had been stored for varied periods of time at 4 C but incubated at 19 C during regular transfers and increase. Conidial suspensions, were prepared as described in section 3:3:1 and their concentrations were determined using the Improved Neubauer hemacytometer (American Optical Corporation, Buffalo, New York) and adjusted to 10×10^4 conidia per milliliter. Ten drops from each suspension were plated on each petri dish containing about 20 mℓ of V-8A medium. Incubation was made at 14, 19, 24 and 29 C in the dark. Three petri dishes were used for each isolate at each temperature.

Table 2. Mean annual temperatures for areas of origin of Isariopsis griseola Sacc. isolates used in determination of growth and sporulation of the fungus.

Isolate	Area of origin	Mean annual Temp. (C)
IG1-77	Popayán	18
IG2-78	Tenerife	13.5
IG13-80	Tenerife	13.5
IG3-78	Pasto	13
IG14-80	La Selva	18
IG16-80	CIAT-Palmira	24
IG19-80	Santander de Quilichao	25
IG21-80	Pitalito-Huila	17-23

3:3:3 Determination of Sporulation

Based on results of the growth experiment, the amount of sporulation by each isolate was estimated at 19 and 24 C after 10 days of incubation in the dark. Preparation of the inocula and plating was done as described in Section 3:3:1. To estimate sporulation, spores were collected using a modification of the method described by Calpouzos and Stallknecht (1965). From each plate, three 4 cm^2 pieces of the culture with agar were cut. Each piece was introduced into a test tube containing 10 mℓ of distilled water and shaken vigorously for two minutes on a lab-line super mixer (Arthur H. Thomas Co.). From each conidial suspension, at least four counts were made by means of the Improved Neubauer hemacytometer (American Optical Corporation, Buffalo, New York). The number of conidia per milliliter multiplied by a factor of 2.5 gave the number of conidia/1 cm^2 of the culture (number of conidia/4 cm^2 = number of conidia/10 mℓ distilled water. Therefore, number of conidia/1 cm^2 = number of conidia/2.5 mℓ of distilled water = number of conidia/1 mℓ of distilled water x 2.5). Three petri dishes were used for each isolate at each temperature tested to obtain the conidial counts.

A completely randomized design was used and the data was analysed statistically. Following results obtained in this study, a second similar study was conducted to compare sporulation of four isolates at 19 and 24 C, after 7, 10 and 13 days of incubation in the dark.

3:4 Pathogenic variation

3:4:1 Sources of seeds of *Phaseolus vulgaris* L. cultivars

Selection of bean (*Phaseolus vulgaris* L.) cultivars used in this study was based on literature reports regarding their susceptibility or resistance to angular leaf spot; previous results of germplasm evaluations; and preliminary tests with *Isariopsis griseola* isolates conducted at CIAT. The cultivars were 'Alabama No. 1', 'California Small White No. 643', 'Caraota-260', 'Cauca 27A', 'G 01805-1P-1C' (Mexicano), 'G 02575-10P-2C' (Col. No. 328), 'G 02858' (Zacaticano) and 'ICA-Duva'. Seeds of 'Caraota-260' were received from Dr. C. Vieira, Federal University of Vicosa, Brazil. Seeds of the other cultivars were obtained from the Bean Breeding and the Genetic Resources Sections of the

Centro Internacional de Agricultura Tropical. Single plant selection and seed increase were made for all the cultivars. Seeds were planted in 15 cm diameter pots containing a steam sterilized mixture of soil and sand (5:1 V/V), in a greenhouse with a mean temperature of 24 C (range 19 C (night) to 30 C (day)). Plants were inoculated when they were 19-20 days old.

3:4:2 Inoculum preparation

The isolates were grown on V-8A at 24 C in the dark. Inoculum for each isolate was prepared individually, by gently scraping the surface of 10 day old cultures with a wire loop in the presence of distilled water. The conidial suspension was then passed through a double layer of cheesecloth and its concentration was determined by using an Improved Neubauer hemacytometer (American Optical Corporation, Buffalo, New York) and then adjusted to 2×10^4 conidia per milliliter. Triton AE (0.1% V/V) was added as an emulsifier and surfactant.

3:4:3 Inoculation procedure

Inoculations were made in the greenhouse using single spore cultures of the 21 isolates

Table 3. Areas of origin and identification of *Isariopsis griseola* Sacc. isolates.

Area of origin	Isolate identification[a]	
Cajibio	IG 11-80	
	IG 12-80	
CIAT- Palmira	IG 4-78	
	IG 16-80	
La Selva (Rio Negro)	IG 14-80	
	IG 15-80	
Obonuco	IG 3-78	(Obonuco)
Pitalito-Huila	IG 21-80	(Pitalito-Huila)
Popayan	IG 1-77	(IG 23)
	IG 5-78	
	IG 6-79	
	IG 7-79	
	IG 8-79	
	IG 9-79	
	IG 10-79	
Restrepo	IG 17-79	
Santander de Quilichao	IG 18-80	
	IG 19-80	
Tenerife	IG 2-78	
	IG 13-80	
Wisconsin, U.S.A.	IG 23-80	

[a]Collection number assigned to an isolate of *Isariopis griseola* and the year of isolation. Example: IG 11-80, refers to isolate number 11, isolated in 1980.

(Table 3), at late afternoon. The inoculum was sprayed on the abaxial surface of the first and second trifoliate leaves using a De Vilbiss No. 15 atomizer, attached to a compressed airline at 15 p.s.i (1.05 kg of force/cm^2) and held at a distance of about 10-15 cm from the leaf, until the runoff occurred (Plate 1). The plants were immediately placed in a humidity chamber with relative humidity of 95-100% and temperature at 22 ± 2 C for four days, after which they were moved onto a greenhouse bench. The experiment was repeated three times, using completely randomized designs with nine replicates per treatment.

3:4:4 Disease evaluation

Plants were observed for symptoms six days after inoculation and on a daily basis up to three weeks. Disease severity assessment was made 14 days after inoculation and was based on a CIAT evaluation scale of 1 to 5 (Plate 2) representing varying percentages of actual leaflet area covered with lesions where:

1 = no apparent symptoms

2 = less than 2% of actual leaflet area covered with lesions

3 = 3-10% of actual leaflet area covered with lesions

4 = 11-25% of actual leaflet area covered with lesions and sometimes with limited chlorosis

5 = more than 26% of actual leaflet area covered with lesions often accompanied with chlorosis.

Standard area diagrams (Figure 1) were made representing the above scale and were used as a guide to estimate disease severity. Also, diagrams (Figure 2) representing the disease severity scale deviced by Horsfall and Barratt (1945) were used for comparison. In addition, incubation period (days from inoculation to the time the lesions were first observed), and chlorosis due to infection were also evaluated.

Plate 1. Inoculation of the first trifoliate leaves of beans with a conidial suspension of Isariopsis griseola Sacc. using a De Vilbiss atomizer connected to an airline from an air compressor.

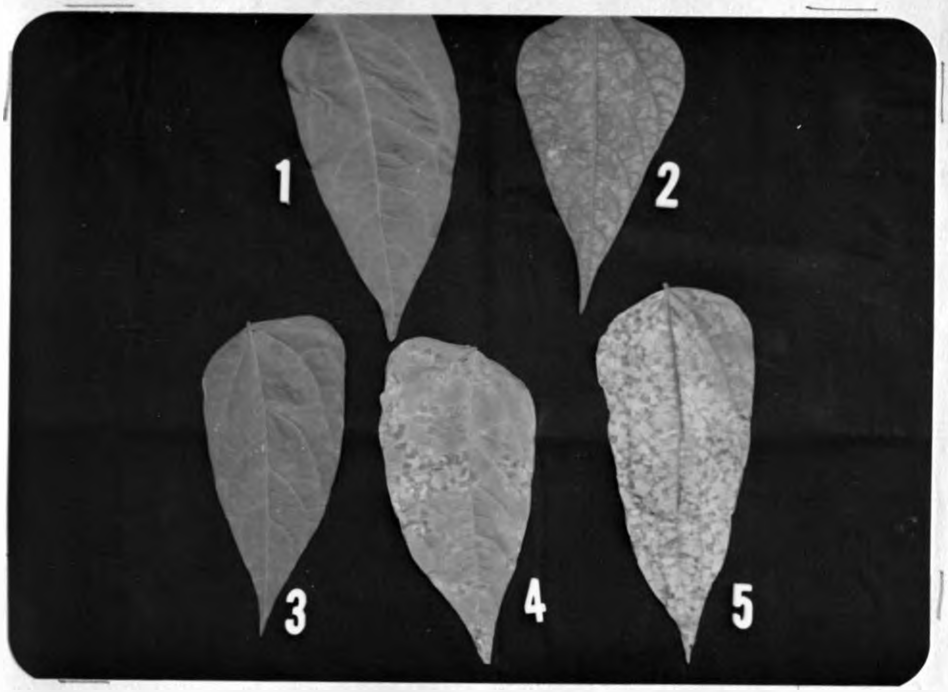


Plate 2. The mid-points of the five classes used in the assessment of severity of angular leaf spot based on the percentage of the leaf area covered with lesions;
1 = 0%, 2 = $\leq$ 2%, 3 = 3-10%, 4 = 11-25%, 5 = $\geq$ 26%.

Figure 1. Disease diagrams illustrating the angular leaf spot severity assessment scale used, and the corresponding percentage of actual leaf area covered with lesions.

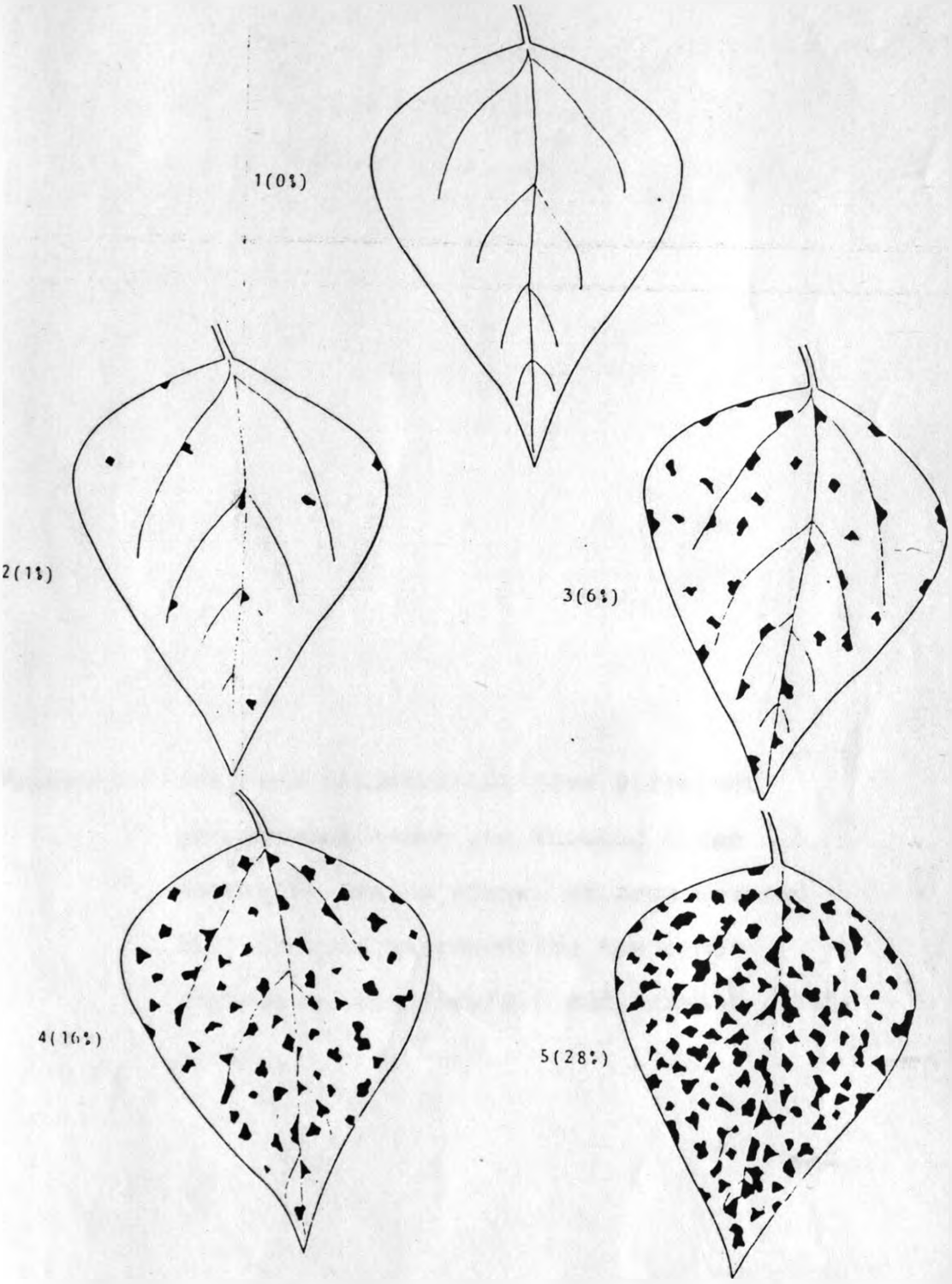
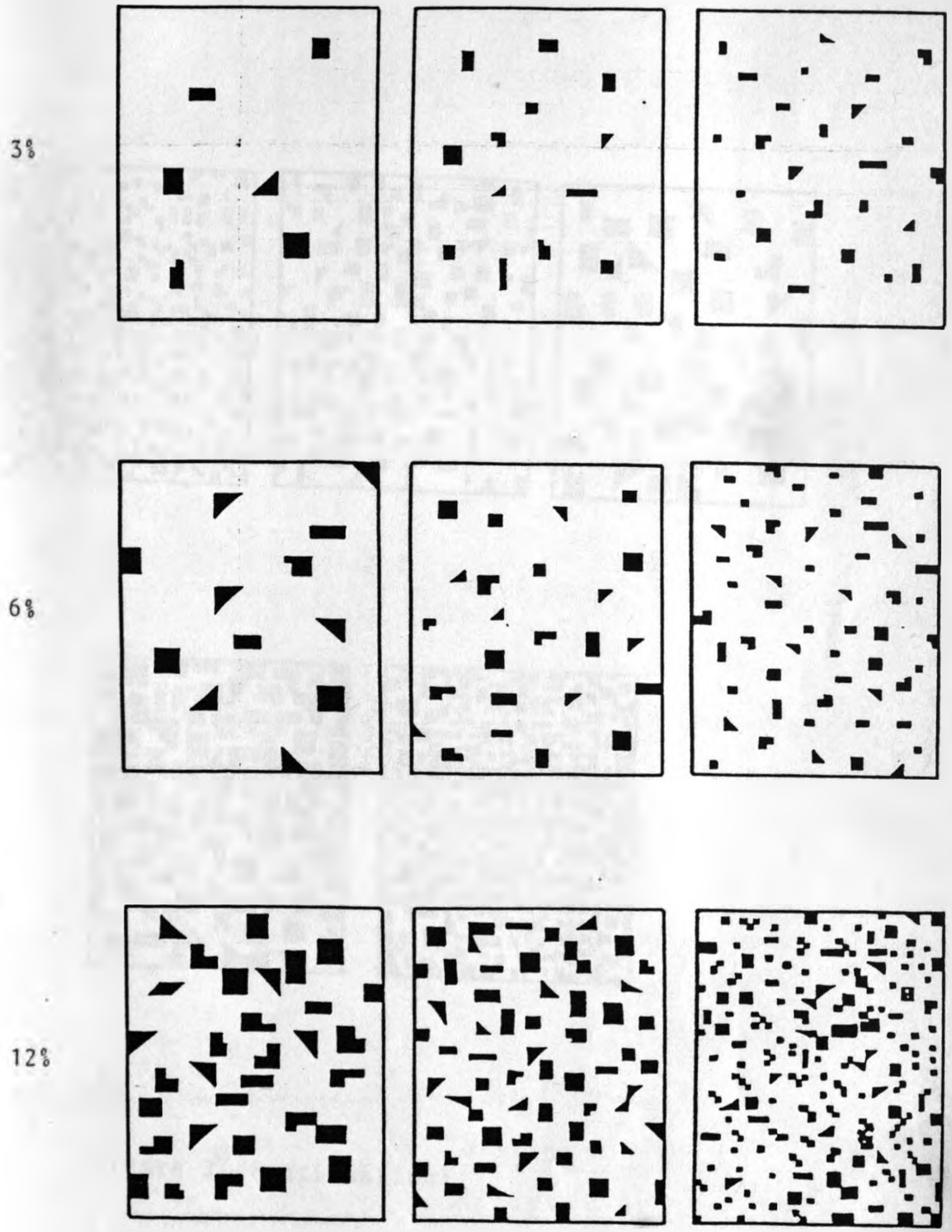
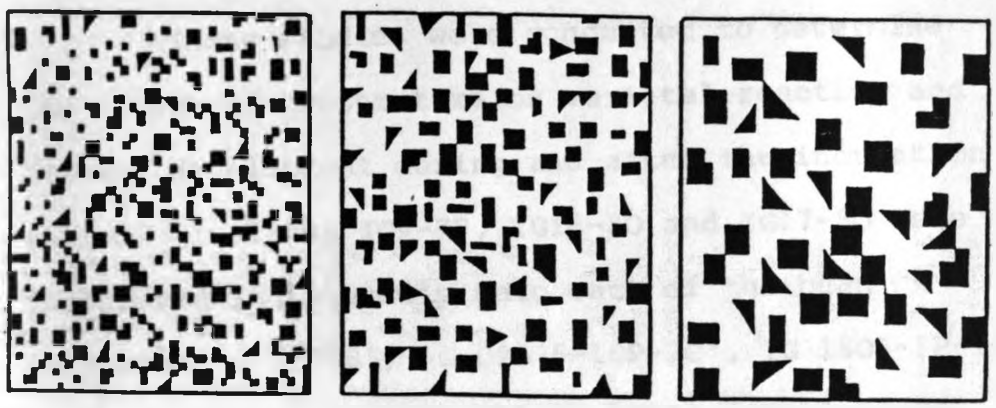

1(0%)
2(1%)
3(6%)
4(16%)
5(28%)

Figure 2. Diagrams illustrating five different percentages (each one showing three different lesion sizes) of area covered with lesions representing the upper limites of the Horsfall and Barratt (1945) scale.


3%
6%
12%

25%



50%

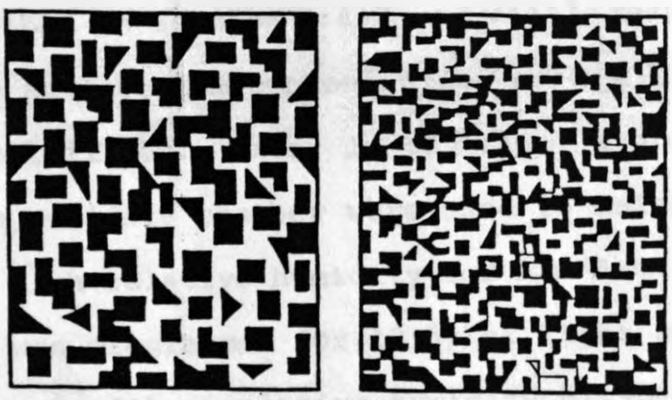


Figure 2 (Continuation)

3:5 The effect of temperature on varietal reaction and development of angular leaf spot.

These studies were conducted to determine the effect of temperature on varietal reaction and disease development during and after the incubation period. Isolates IG9-79, IG15-80 and IG17-80 were inoculated individually onto each of the bean cultivars 'G 02858', 'G 02575-10P-2C', 'IG 1805-1P-1C' and 'ICA-Duva' whose reactions after inoculations with the three isolates were known. Inoculum preparation and inoculations were conducted as described in Section 3:4:2 and 3:4:3 respectively, using an inoculum concentration of 2×10^4 conidia per milliliter. After inoculation, a growth room and a humidity chamber were used to maintain plants at a high relative humidity for four days. The growth room was programmed for 12 hr of light and 12 hr of darkness with a relative humidity of 90-100% and a temperature of 16 ± 1 C. Light was supplied by eight metal halide lamps (General Electric Co., Hendersonville, N.C.) with a polythene shade below them, and at bench level measured 1-2 klux. The average temperature in the humidity chamber was

$22^{\pm}$ 1 C with a relative humidity of 95-100% and plants received about 12 hr of natural light. At the end of four days plants were moved from each temperature condition to greenhouse benches at an average temperature of 24 and 30 C, and relative humidities of 72 and 60%, respectively. One group of plants was kept in the growth room at 16 C with a relative humidity of 70%. Fifteen days after inoculation plants were evaluated for disease reaction. On susceptible cultivars, the number of lesions on the first trifoliate leaves were counted. At least five plants per cultivar were tested for each treatment. The latter consisted of isolate x cultivar x temperature one(humidity chamber/growth room) x temperature two (growth room/greenhouse benches) combination.

3:6 The effect of individual and mixtures of isolates of *Isariopsis griseola* Sacc. on disease severity.

In the course of preliminary studies, it was observed that, on some bean cultivars, inoculations made with a mixture of isolates (concentration, 2×10^4 conidia/mℓ) resulted in lower disease severity than

when some of the isolates (components of the mixture) were inoculated individually at the same concentration (2×10^4 conidia/mℓ). An investigation was therefore conducted to verify these observations and to determine the influence of a mixture of isolates of I. griseola on disease severity.

Seven isolates (IG 1-77, IG 5-78, IG 6-79, IG 7-79, IG 8-79, IG 9-79, IG 10-79) of I. griseola from Popayan were used. Two bean cultivars, 'G 02575 - 10P-2C' and 'G 02858', observed from previous studies to be susceptible (with varying degrees of disease severity) to the seven isolates, were tested. Inoculum preparation and inoculation were conducted as described in Section 3:4:2 and 3:4:3 respectively. Plants were inoculated with each isolate singly at a concentration of 2×10^4 conidia/mℓ. Two mixtures each containing all the seven isolates, one in which the final concentration was 2×10^4 conidia/mℓ (each isolate present at a concentration of 2857 conidia/mℓ) and another in which the final concentration was 14×10^4 conidia/mℓ (each isolate present at a concentration of 2×10^4 conidia/mℓ) were similarly inoculated. Also, isolate IG 9-79 was inoculated singly at a concentration of

14 x 10^4 conidia/mℓ. A completely randomized design was used with 18 replicates (plants) per treatment. Disease severity assessment was estimated as described in Section 3:4:4.

3:7 Determination of the progress of angular leaf spot in the field.

In germplasm screening tests and preliminary field studies using isolate IG 1-77, cultivars susceptible to the latter were observed to vary in time when symptoms appeared, and in the rate and extent which disease severity increased. During the long rain season of March through June 1981, a trial was mounted to study progress of angular leaf spot disease on 14 bean cultivars ('Alabama No. 1', 'A 43-1P-(18)P', 'BAT 67-(20)P', 'BAT 452-(18)P', 'BAT 508-(25)P', 'BAT 527-(20)P', 'BAT 966-1P-(20)P', 'BAT 1057-1P-(24)P', "Caraota-260' 'Cauca-27A', 'Epicure', 'G 1805-1P-1C' 'G 2858', 'G 4421'), artificially infected under field conditions. Rainfall distribution during the experimental period is shown on Figure 3. Each cultivar was planted in 4-row plots, two meters in length and replicated three times. Isolate IG 1-77 was used

to artificially start epidemics. The inoculum was prepared as described above (Section 3:4:2) and inoculation was carried out in late afternoon using a motorized knapsack sprayer at a spore concentration of 2×10^4 conidia per milliliter of water. Two inoculations were made; one, 4 weeks after planting and the second one, a week later. Observations were made every week starting when symptoms were first seen (12 days after the first inoculation).

Disease incidence was assessed for each cultivar as defined by James (1974). Disease severity was estimated as described above (Section 3:4:4) on twenty randomly chosen plants per plot. Thus during the whole season the same plants had been observed. The disease severities estimated were adjusted such that the five-degree scale of 1, 2, 3, 4, 5 was represented by proportions of 0, 0.25, 0.5, 0.75 and 1 respectively and then subjected to logit transformation (Van der Plank, 1963; Kranz, 1974). Van der Plank's average apparent rate of disease increase r (1963) was estimated for eight cultivars ('Alabama No. 1', 'BAT 67-(20)P', 'BAT 452-(18)P', 'BAT 966-1P-(20)P',

'Caraota-26O', 'Cauca-27A', 'G 2858', 'G 4421') selected for their varying susceptibility to isolate IG 1-77, using the formula;

$$r = \frac{1}{t_2 - t_1} \left(\log_e \frac{x_2}{1 - x_2} - \log_e \frac{x_1}{1 - x_1} \right)$$

where t_1 and t_2 denotes time one and two respectively and $1 - x$, the correction factor for the decreasing amount of healthy tissues.

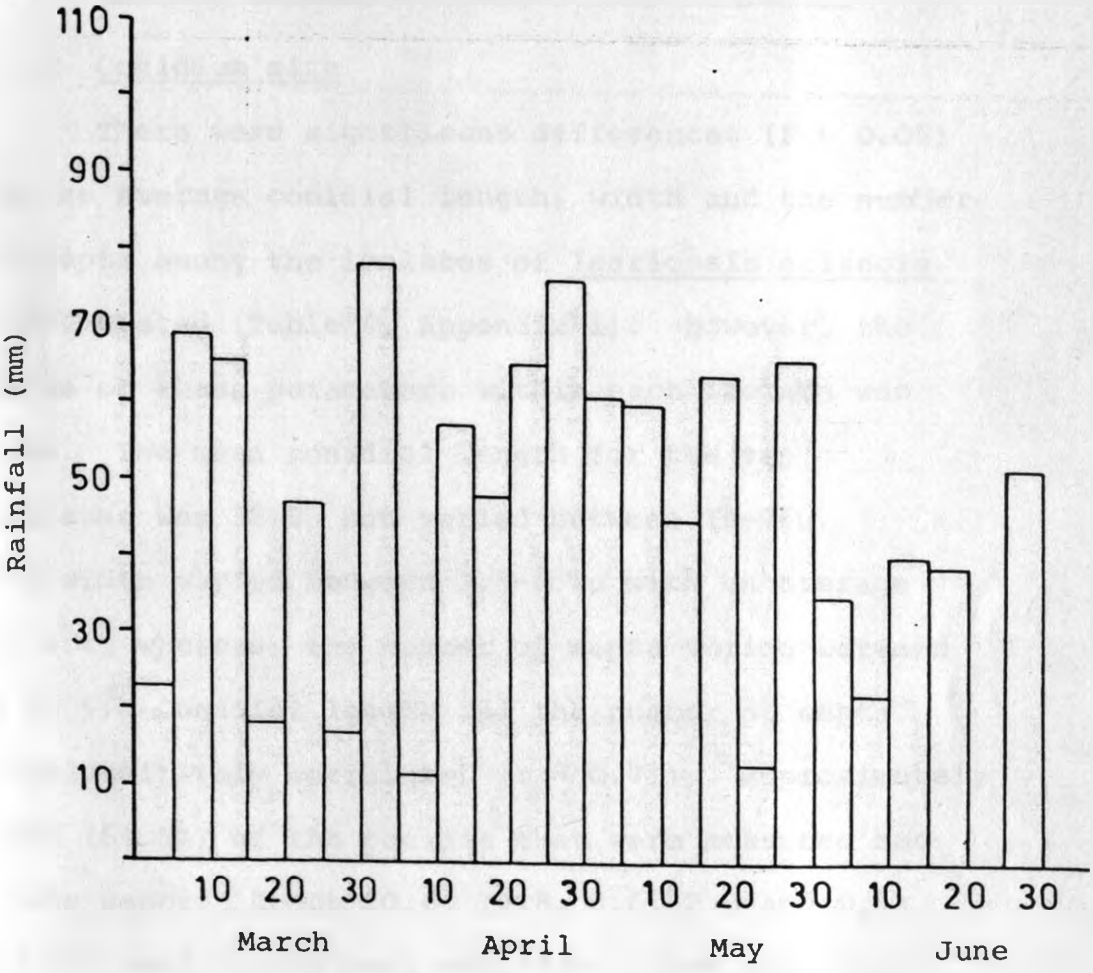


Figure 3. Rainfall (mm) distribution (at 5-day-intervals) during the long (March - June, 1981) rain season at Popayan, Cauca, Colombia.

4 RESULTS

4:1 Variation in conidial size, growth and sporulation of isolates of *Isariopsis griseola* Sacc.

4:1:1 Conidium size

There were significant differences (P = 0.05) in the average conidial length, width and the number of septa among the isolates of *Isariopsis griseola* Sacc. tested (Table 4, Appendix 1). However, the range of these parameters within each isolate was wide. The mean conidial length for the ten isolates was 38.8µ but varied between 18-76µ. The width varied between 3.8-8.8µ with an average of 6.4µ whereas, the number of septa varied between 0 to 5. Conidial length and the number of septa were positively correlated (r = 0.71). Approximately half (51.5%) of the conidia that were measured had three septa. About 20.8, 19.8, 6.6, 2.0 and 0.3% of the conidia had two, one, four, five and no septum respectively. Isolate IG 21-80 had conidia with six and seven septa (Plate 3, C, D,) but their occurrence was rare.

4:1:2 Growth on artificial medium

The growth of the eight isolates of *I.*

griseola on V-8 juice agar was compared on the basis of visual observations of the colour and amount of mycelial growth. Growth with all the isolates was very slow at 14 C but increased with temperature to an optimum at 24 C after which, there was a decline in growth such that no growth occurred at 29 C (Plate 4). When some of the petri dishes incubated at 29 C for 5 days and showing no growth were transferred to 19 C, growth occurred four days later whereas no growth occurred with those maintained at 29 C for three weeks. No visual differences were observed between the isolates in the amount of growth at 14, 19 and 24 C. There was no correlation between growth of the isolates at the different temperatures and the temperatures of the areas of origin of the isolates. Some of the isolates (IG3-78, IG 14-80) produced grey mycelia, in contrast to the dark mycelia found to be characteristic of many of the isolates (Plate 5). Appearance of the grey mycelia was enhanced when isolates were incubated at 24 C. Such cultures had fewer numbers of conidia than the dark mycelial cultures.

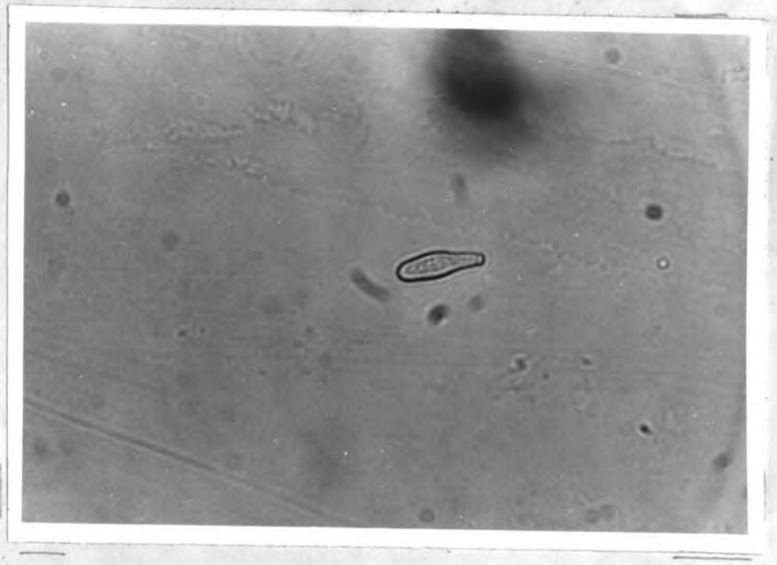
Table 4. The mean[x] and range in conidial length (μ), width (μ) and number of septa of 10 isolates of Isariopsis griseola Sacc.

Isolate	Length		Width		Number of Septa	
	Mean	Range	Mean	Range	Mean	Range
IG 13-80	47.3[a]	29 - 76	6.6[b]	5.0 - 8.8	2.9[a]	1 - 5
IG 21-80	43.4[b]	21 - 61	6.3[d]	5.0 - 8.1	2.8[a]	0 - 4
IG 15 - 80	42.8[b]	24 - 61	6.4[d]	5.0 - 7.5	2.8[a]	1 - 5
IG 1 - 77	39.4[c]	25 - 53	6.8[a]	5.0 - 8.1	2.7[a]	1 - 5
IG 10 - 79	37.2[d]	23 - 63	6.9[a]	3.8 - 8.8	2.4[b]	1 - 4
IG 4 - 78	37.0[d]	26 - 55	6.9[a]	5.0 - 8.8	2.2[bc]	1 - 4
IG 3 - 78	36.7[d]	18 - 61	5.3[f]	3.8 - 6.3	2.2[bc]	1 - 4
IG 2 - 78	35.6[d]	23 - 54	6.1[d]	5.0 - 7.5	2.1[c]	1 - 4
IG 6 - 79	35.2[d]	23 - 53	6.4[cd]	4.4 - 7.5	2.4[b]	1 - 4
IG 11 - 80	33.1[e]	20 - 48	6.5[bc]	5.0 - 8.8	2.4[b]	1 - 4

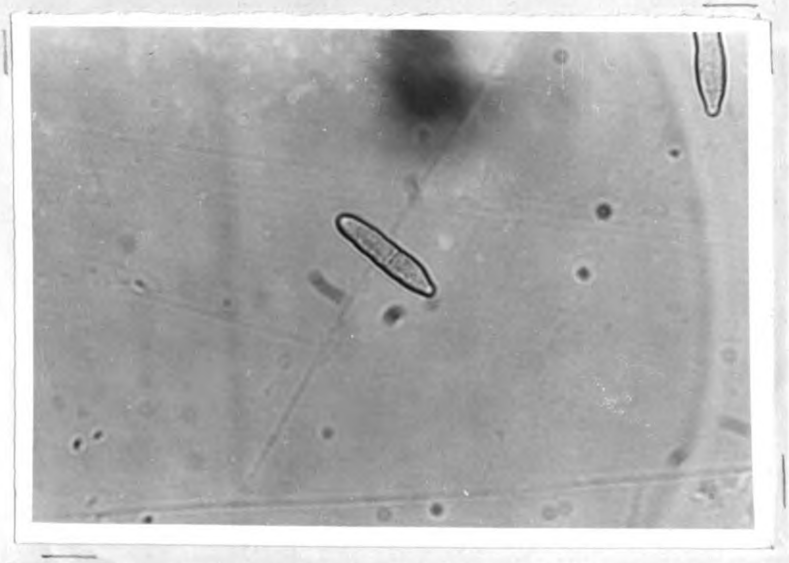
[x]Average of 100 conidia.

Means followed by the same letter within each column are not significantly different at P = 0.05 according to Duncan's new multiple range test.

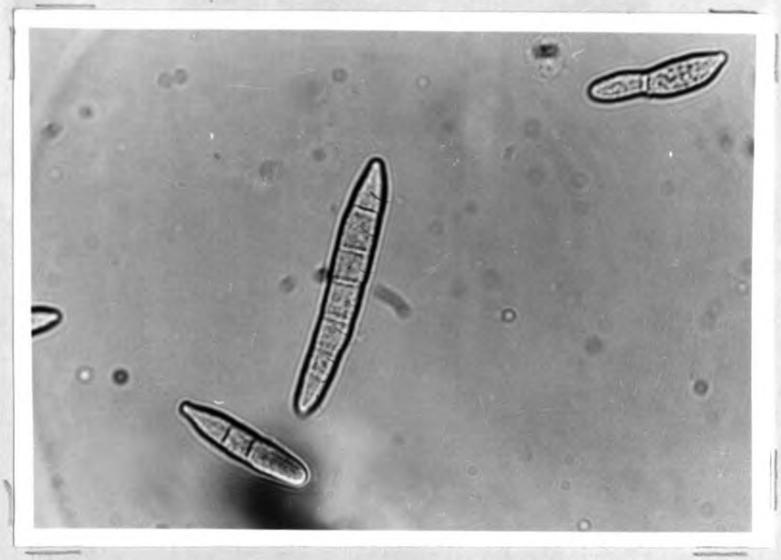
Plate 3. Conidia of Isariopsis griseola Sacc. (isolate IG 21-80) from a 10-day-old culture incubated on V-8 juice agar at 24 C in the dark, showing variation in the number of septa. A, no septum; B, 1 septum; C, 6 speta; D, 7 septa.



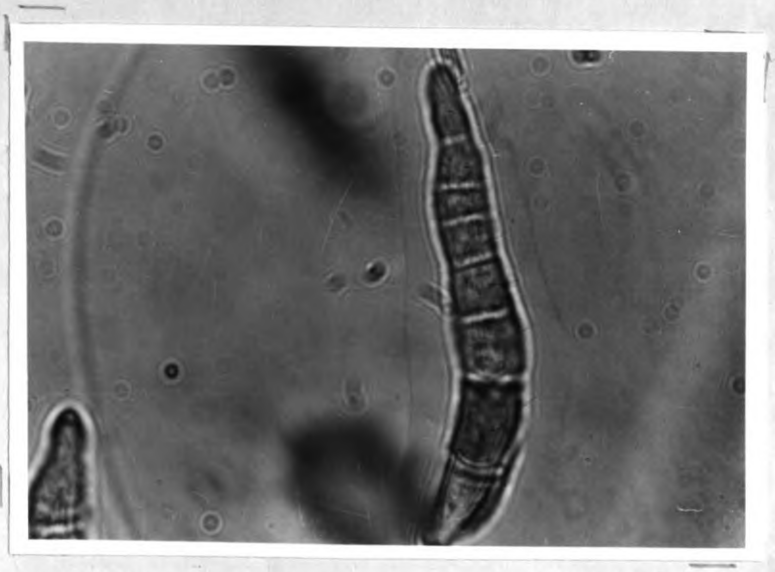
A.



B.



C.



D

Plate 3.(Contd.)

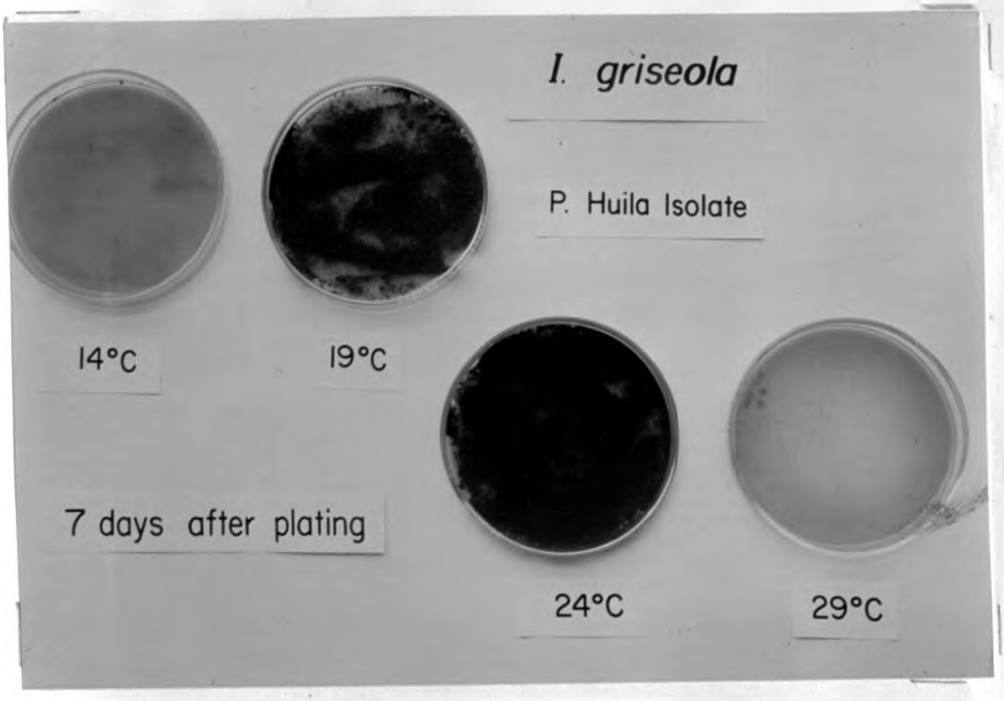


Plate 4. Mycelial cultures of Isariopsis griseola Sacc. (isolate IG 13-80) grown on V-8 juice agar, seven days after incubation at 14, 19, 24 and 29 C in the dark.

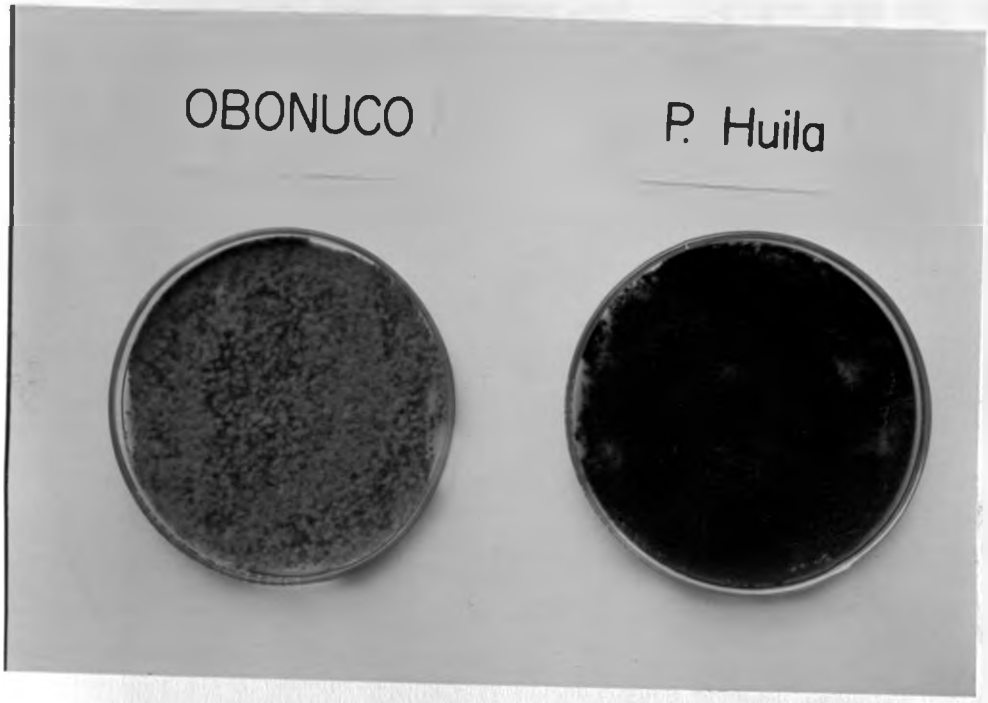


Plate 5. Cultures of Isariopsis griseola Sacc. showing the grey mycelia (isolate IG 3 - 78, Obonuco) and the dark mycelia (isolate IG 21 - 80 (Pitalito-Huila)), after seven days of growth on V-8 juice agar in the dark at 24 C.

4:1:3 Sporulation on artificial medium

In the initial experiment, determination of sporulation for seven isolates of Isariopsis griseola was made after 10 days of incubation on V-8A medium in the dark at 19 and 24 C. Sporulation was found to be affected by temperature, isolate and their interaction (Appendix 2, Table 5). Significantly ($P = 0.01$) more conidia were produced at 24 than at 19 C by six of the seven isolates tested whereas, one isolate (IG 2 - 78) produced more conidia at 19 than at 24 C (Table 5).

When the experiment was repeated using 4 isolates and determining sporulation after 7, 10 and 13 days of incubation, similar results as in the previous experiment were obtained (Table 6, Appendix 3) Isolate IG 2 - 78 consistently produced less number of conidia at 24 than at 19 C but the contrary was true with the other 3 isolates (Figure 4A). There was no significant difference in conidia production between the 10th and the 13th day of incubation, but sporulation at both periods was about twice the amount produced after seven days of incubation. There were significant differences in

Table 5. Effects of isolate and temperature on conidial production of seven isolates of *Isariopsis griseola* Sacc. grown on V-8 juice agar in the dark.

Isolate	Number of conidia x $10^4/cm^2$ of mycelia culture		
	Temperature C : 19	24	: Average
IG 19 - 80	3.52[x]	13.94	8.73a[y]
IG 2 - 78	16.80	5.98	11.38ab
IG 3 - 78	5.32	19.92	12.62ab
IG 15 - 80	7.87	20.57	14.22ab
IG 21 - 80	24.30	45.86	35.08c
IG 1 - 77	24.71	48.38	36.55c
IG 16 - 80	24.38	54.84	39.61c
Average of 7 isolates	15.27a[z]	29.93b	

X = Means of 12 values

Y = Means of 24 values

Z = Means of 84 values

Means followed by the same letter within columns are not significantly different at P = 0.05 according to Duncan's multiple range test.

Table 6. Effect of isolate, period of incubation and temperature on conidial production of four isolates of Isariopsis griseola Sacc. grown on V-8 juice agar in the dark.

	Number of conidia x $10^4/cm^2$ of mycelial culture					
	Days of incubation			Temperature :		Average
	7	10	13	19	24 :	
IG 2 - 78	7.43a[X]	14.24b	14.33b	18.25a[Y]	5.75b	12.00a[Z]
IG 21 - 80	25.70b	52.90c	65.86c	36.29b	60.02c	53.55b
IG 13 - 80	29.77b	60.09c	70.78c	32.52b	74.57c	48.15b
IG 16 - 80	30.18b	60.30c	71.60c	41.84b	66.20c	54.02b
Average of four isolates	23.27a[R]	46.88b	55.64c	32.23a[Q]	51.66b	

X Means of 24 values

Y Means of 36 values

Q Means of 144 values

R Means of 96 values

Z Means of 72 values

Means followed by the same letter within columns are not significantly different at P = 0.05 according to Duncan's new multiple range test.

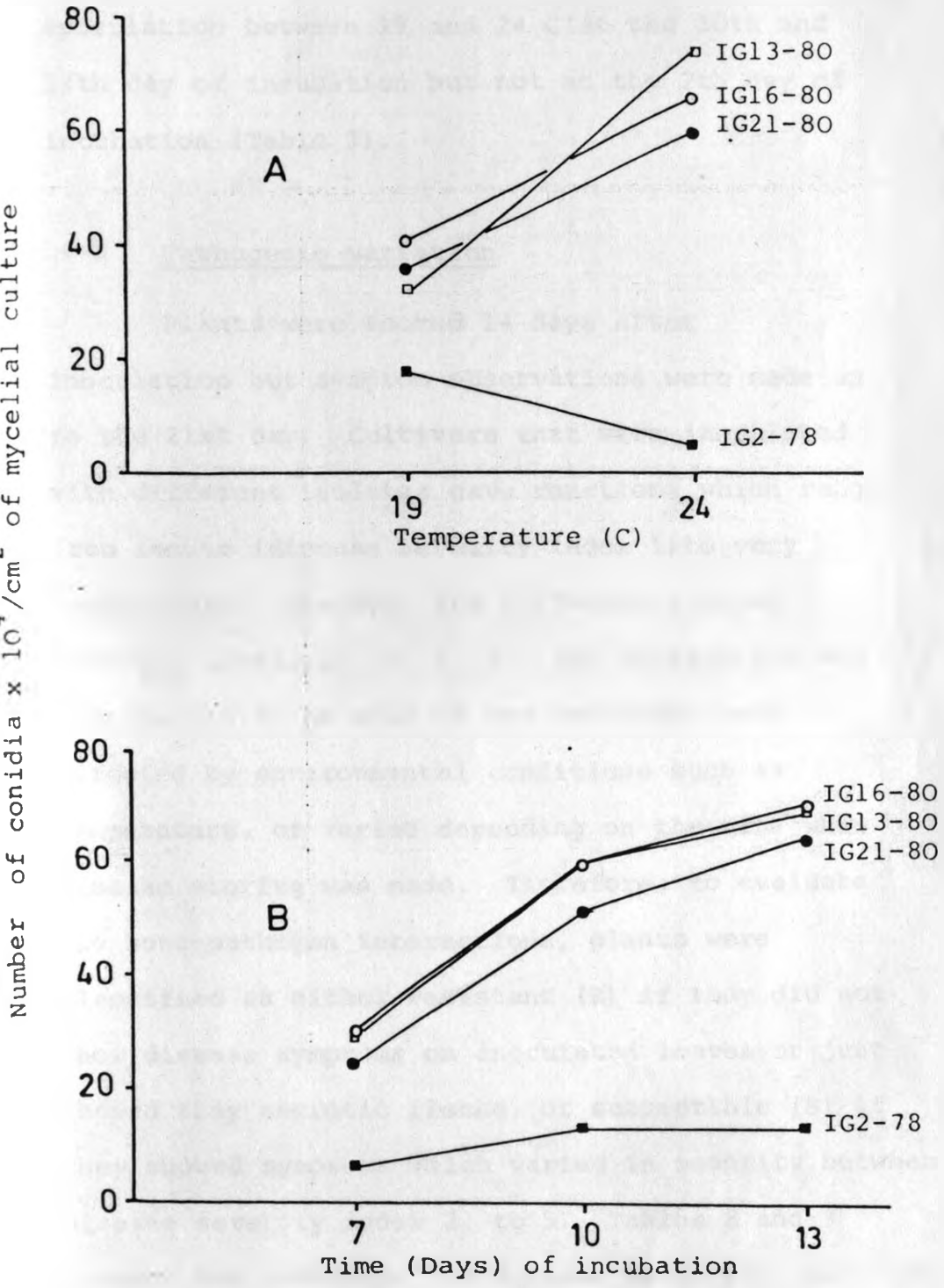


Figure 4. Effect of incubation temperature (A) and period of incubation (B) on conidia production by cultures of four isolates (IG2-78, IG13-80, IG16-80 and IG21-80) of Isariopsis griseola Sacc. incubated in the dark on V-8 juice agar.

sporulation between 19 and 24 C at the 10th and 13th day of incubation but not at the 7th day of incubation (Table 7).

4:2 Pathogenic variation

Plants were scored 14 days after inoculation but symptom observations were made up to the 21st day. Cultivars that were inoculated with different isolates gave reactions which ranged from immune (disease severity index 1) to very susceptible. However, the different disease severity levels (2, 3, 4, 5; for description see Section 3:5:5) on some of the cultivars were affected by environmental conditions such as temperature, or varied depending on the time when disease scoring was made. Therefore, to evaluate the host-pathogen interactions, plants were classified as either resistant (R) if they did not show disease symptoms on inoculated leaves or just showed tiny necrotic flecks, or susceptible (S) if they showed symptoms which varied in severity between disease severity index 2 to 5. Tables 8 and 9 present the reactions and disease severities induced by the 21 isolates of *I. griseola* on the six bean

Table 7. Effect of the interaction between isolates, period of incubation and temperature on conidial production by 4 isolates of Isariopsis griseola Sacc. after 7, 10 and 13 days of growth on V-8 juice agar medium at 19 and 24 C in the dark.

Isolate	Area of origin	Number of conidia x $10^4/cm^2$ of mycelial culture					
		7 days		10 days		13 days	
		19C	24C	19C	24C	19C	24C
IG 2 - 78	Tenerife	9.27[Z]	5.49	23.70	4.77	21.68	6.99
IG 21 - 80	Pitalito-Huila	22.33	37.20	36.09	84.08	39.15	102.42
IG 13 - 80	Tenerife	15.52	35.98	39.99	65.81	53.45	78.28
IG 16 - 80	CIAT-Palmira	24.88	35.47	48.27	72.32	52.37	90.82

Z Means of 12 replications

LSD (P = 0.05) = 19.2

cultivars which were finally used. Inoculated plants of the cultivars gave fairly uniform reactions during each trial. Similarly the cultivars showed no variation in reaction despite the fact that the trials were conducted at different times. However, the amount of disease on some of the susceptible cultivar reactions showed a tendency to vary (Table 8).

Isolates interacted differentially with host varieties. Some of the isolates induced very susceptible reactions on some bean cultivars, whereas, on other cultivars the same isolates were not infective at all. For example, isolates IG 17-80 and IG 9-79 invoked a susceptible reaction on bean cultivar 'G 2575-1OP-2C' (plate 6). However, using the same inocula only isolate IG 9-79 and not IG 17-80 induced symptoms on 'G 2858' (Plate 7) and on 'Alabama No. 1' (Plate 8 Tables 8, 9).

The 21 isolates could be grouped into seven pathotypes based on the six bean cultivars. Cultivar 'G 2575-1OP-2C' was susceptible to all isolates whereas 'G 1805-1P-1C' was infected by four isolates belonging to two pathotypes. No cultivar was immune to all isolates. Some of the isolates from the same

Table 8. Reactions induced on bean (Phaseolus vulgaris L.) cultivars and lines by isolates of Isariopsis griseola Sacc. during three different trials.

	Disease severity ratings[a]																	
	'Alabama No. 1'			'G 2858'			'G 2575-10P-2C'			'ICA-Duva'			'Caraota 260'			'G1305-1P-1C'		
	TRIALS																	
Isolates	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
IG 2 - 78	1[b]	1	1	1	1	1	3	4	3	1	1	1	1	1	1	1	1	1
IG 13 - 80	1	1	1	1	1	1	4	4	3.5	2.5	3	3.5	1	1	1	1	1	1
IG 14 - 80	1	1	1	1	1	1	4	4	2	2	2	2	1	1	1	1	1	1
IG 15 - 80	1	1	1	1	1	1	5	5	3	2	2	2	1	1	1	1	1	1
IG 17 - 80	1	1	1	1	1	1	5	5	5	5	3	4	1	1	1	1	1	1
IG 23 - 80	1	1	1	1	1	1	4	5	4	3	2	3	1	1	1	1	1	1
IG 12 - 80	1	1	1	1	1	1	5	5	5	5	4	3	1	1	1	1	1	1
IG 21 - 80	1	1	1	1	1	1	4	5	5	5	4	4	1	1	1	2	1.5	2
IG 3 - 78	4	4	3	2	3	3	2	3	3	1	1	1	1	1	1	3	2	2
IG 5 - 78	2	4	4	2	3	3	2	2	2	1	1	1	1	1	1	1	1	1
IG 6 - 79	2	4	3	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1
IG 7 - 79	3	3	3	3	2	3	2	2	2	1	1	1	1	1	1	1	1	1
IG 8 - 79	3	3	3	3	3	3	3	2	2	1	1	1	1	1	1	1	1	1
IG 1 - 77	5	5	4	5	4	4	2	3	3	3	3	3	3	4	3	1	1	1
IG 4 - 78	2	4	4	3	2	3	2	2	2	2	2	2	2	4	3	1	1	1
IG 16 - 80	4	5	4	3	3	3	4	2	3	3	2	3	2	3	3	1	1	1
IG 11 - 80	2	3.5	3	3	4	3	3	5	3	3	3	2	3	3	3	1	1	1
IG 19 - 80	5	5	5	5	5	5	4	3	3	5	3	3	3	3	3	1	1	1
IG 9 - 79	5	5	4.5	5	5	4.5	4	4	2	3	3	3	3	5	3	2	2	2
IG 10 - 79	5	5	4	5		4	3	2	2	2	2	2	3	4	2	2	1.5	2
IG 18 - 80	2	3	3	4	4	4	4	2	3	1.5	2	2	3	3	3	3	2	2

a Disease severity was rated on a scale of 1-5 in which 1 = no apparent infection; 2 $\leq$ 2%; 3 = 3-10%; 4 = 11-25%; 5 $\geq$ 26% of actual leaflet area covered with lesions.

b Values are averages of 9 replicates (plants)

geographical area varied pathogenically. For example, the seven isolates from Popáyan could be differentiated into three pathotypes on the basis of reactions of bean cultivars 'ICA-Duva', 'Caraota-260' and 'G 1805-1P-1C'. There were also some variation among isolates within each pathotype with respect to disease severity on a susceptible bean cultivar (Table 8). Some of the isolate-cultivar interactions consistently resulted in low disease severity and were not influenced by variation in temperatures. It was generally noted, however, that high disease severity levels (disease index 4 and 5) had relatively lower incubation (period from inoculation time until when lesions appeared) than lower disease severity levels (disease index 2), but this parameter was not reliable in comparing isolates as it was influenced by environmental conditions.

There was a clear distinction between chlorosis of leaves due to infection by I. griseola and natural chlorosis due to the age of the plant. The former was observed not to be always a function of disease severity. On some cultivars, infected leaves became chlorotic when inoculated with certain isolates even though the percentage leaf

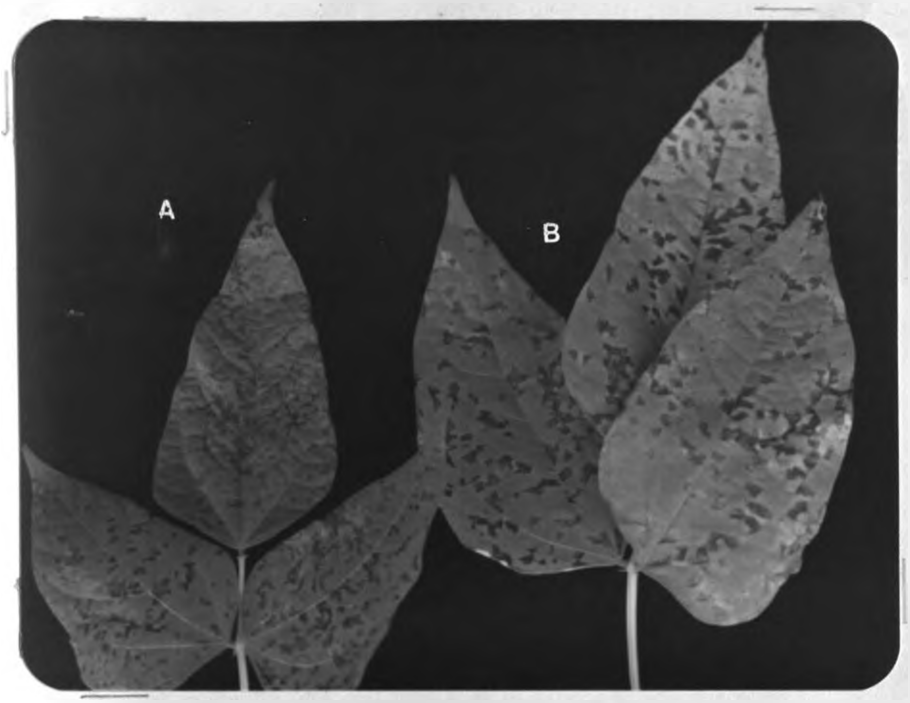


Plate 6. Trifoliate leaves A and B of bean (Phaseolus vulgaris L.) cultivar 'G 2575-10P-2C' showing necrotic lesions 10 days after being inoculated with isolates IG 9-79 and IG 17-80 respectively of Isariopsis griseola Sacc.

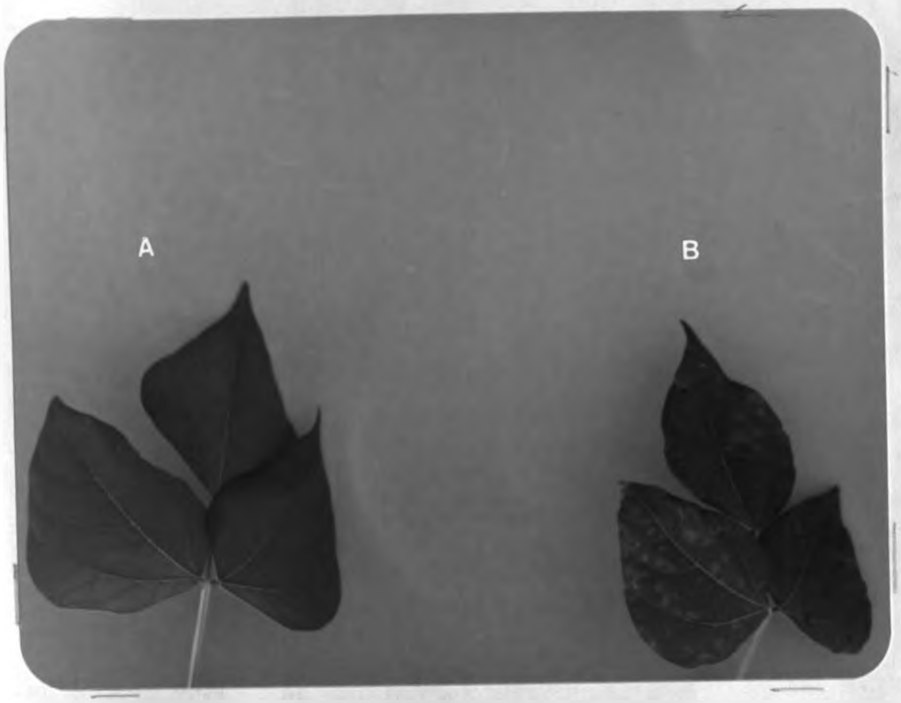


Plate 7. Trifoliate leaves of bean (Phaseolus vulgaris L.) cultivar 'G 2858'; A, showing no symptoms (resistant) compared with B, showing necrotic lesions (susceptible) 9 days after being inoculated with isolates IG 17-80 and IG 9-79 respectively of Isariopsis griseola Sacc.

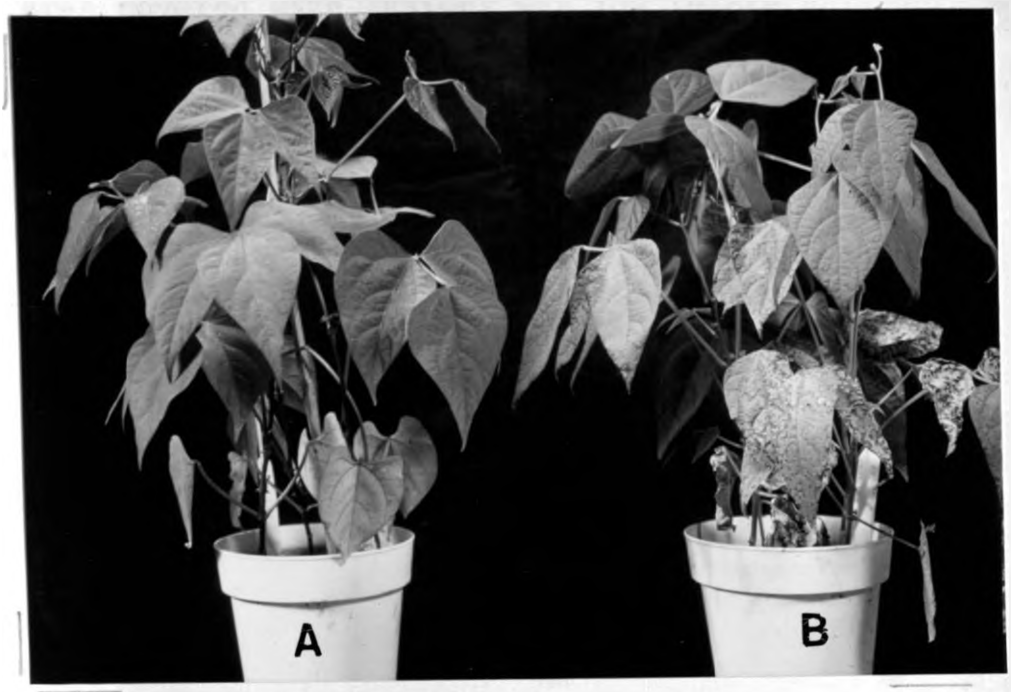


Plate 8. Bean (Phaseolus vulgaris L.) cultivar Alabama No. 1' (A) showing no symptoms (resistant) and 'G 2575-1OP-2C- (B) showing numerous necrotic lesions and start of chlorosis (susceptible) on the first trifoliate leaves, ten days after inoculation with isolate IG 17-80 of Isariopsis griseola Sacc.

area covered with lesions was low (Plate 9B), whereas, other cultivars such as 'G 2858' showed little or no chlorosis inspite of the high level of disease severity observed on them (Plate 9A). Premature defoliation of leaves of 'G 2858' occurred without necessarily being chlorotic. However, in most cultivars this defoliation occurred following chlorosis regardless of the level of disease severity.

There were differences among different susceptible bean cultivars in reaction and the size of lesions that developed on leaves inoculated with a standard amount of inoculum of the same or different isolates. For example, after being inoculated with isolate IG9 - 79, cultivar 'Alabama No. 1' exhibited intense chlorosis, medium size lesions which were clearly delimited but with a bit of coalescing whereas, "California Small White No. 643" exhibited little chlorosis, medium size lesions surrounded by a spreading 'burning' reaction which later became necrotic (Plate 10). Cultivars 'G 2575-10P-2C' and 'Caraota-260' found to be susceptible to isolates in pathotype 7 (Table 9) varied markedly from each other with regard to the

Table 9. Differential reactions of bean (Phaseolus vulagaris L.) cultivars and lines inoculated with 21 isolates of Isariopsis griseola Sacc. from Colombia and U.S.A.

Isolate	Origin	Patho-type	Host reaction[a] Cultivars/Lines 'G 2575-10P-2C'	'Alabama No. 1/ 'G 2858'	'ICA-Duva'	'Caraota-260'	'G 1805-1P-1C'
IG 2 - 78	Tenerife	1	S	R	R	R	R
IG 12 - 80	Cajibio	2					
IG 13 - 80	Tenerife	2					
IG 14 - 80	La Selva	2	S	R	S	R	R
IG 15 - 80	La Selva	2					
IG 17 - 80	Restrepo	2					
IG 23 - 80	Wisconsin (USA)	2					
IG 21 - 80	Pitalito-Huila	3	S	R	S	R	S
IG 3 - 78	Pasto	4	S	S	R	S	R
IG 5 - 78	Popayán	5					
IG 6 - 79	Popayán	5	S	S	R	R	R
IG 7 - 79	Popayán	5					
IG 8 - 79	Popayán	5					
IG 1 - 77	Popayán	6					
IG 4 - 78	CIAT-Palmira	6					
IG 16 - 80	CIAT-Palmira	6	S	S	S	S	R
IG 11 - 80	Cajibío	6					
IG 19 - 80	Santander de Quilichao	6					
IG 9 - 79	Popayán	7					
IG 10 - 79	Popayán	7	S	S	S	S	S
IG 18 - 80	Santander de Quilichao	7					

[a] foliar disease reaction R = Resistant. No apparent disease symptoms
S = Susceptible. Varying degrees of disease severity.

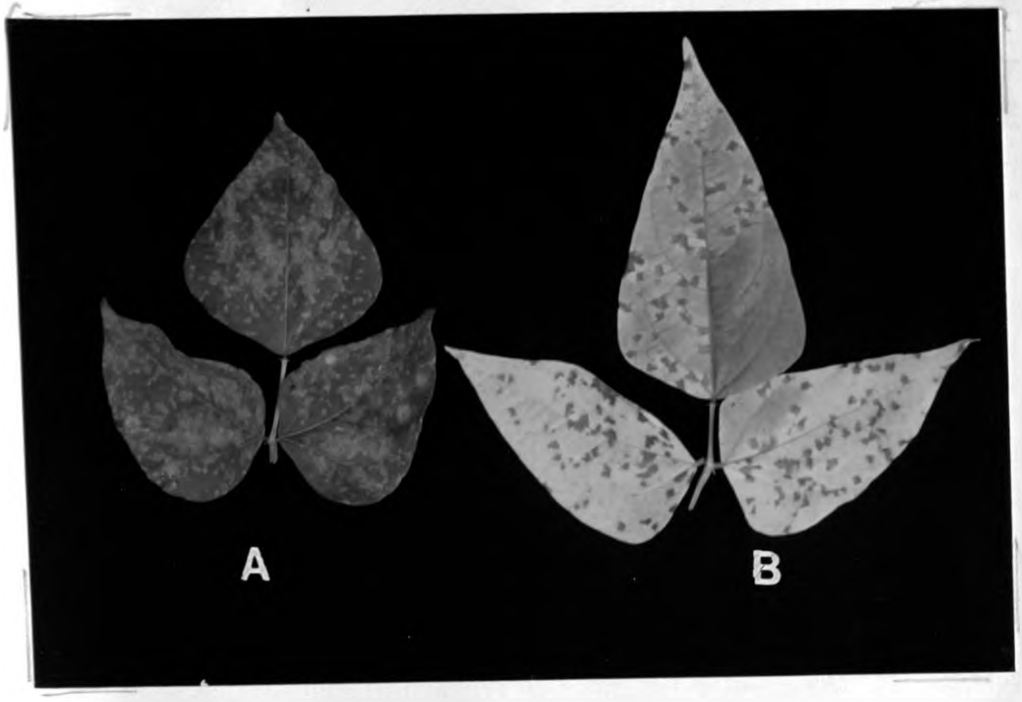


Plate 9. Trifoliate leaves of bean (Phaseolus vulgaris L.) cultivars 'G 2858' (A) showing no chlorosis and 'G 2575-10P-2C' (B) showing chlorosis, 10 days after being inoculated with isolates IG 9-79 and IG 17-80 respectively, of Isariopsis griseola Sacc. The percentage leaf area covered with necrotic lesions on 'G 2575-10P-2C' is relatively much lower than that on 'G 2858'.

type and size of lesions caused by the different isolates within the pathotype. 'Caraota-260' gave relatively small and delimited lesions following inoculation with isolate IG 18-80 whereas, 'G 2575-10P-2C' exhibited large lesions which enlarged rapidly and coalesced after inoculation with isolate IG 9-79 (Plate 11).

4:3 Effect of temperature on cultivar reactions and development of angular leaf spot

Reactions of the four bean cultivars ('ICA-Duva', 'G 1805-1P-1C', 'G 2575-10P-2C', 'G 2858') inoculated with three isolates (belonging to two pathotypes) of I. griseola and incubated at different temperature conditions are shown in Table 10. Individual cultivars inoculated with each of the isolates gave reactions which were found not to be influenced by the range of the incubation temperature tested. Under the varying conditions of the latter, each cultivar uniformly exhibited either a susceptible or a resistant reaction. Reactions of these cultivars were similar to those shown on Table 9.

Table 10. Differential reaction of bean (Phaseolus vulgaris L.) cultivars and lines inoculated with 3 isolates of Isariopsis griseola Sacc. and maintained at two successive different incubation temperatures[a]

Isolate	Cultivar/ line	Host Reaction[b] Initial incubation Temperature C. (T_1) 16 — Subsequent incubation Temperature C. (T_2) 16	24	30	Initial incubation Temperature C. (T_1) 22 — Subsequent incubation Temperature C. (T_2) 16	24	30
IG15-80 (Pathotype 2)	'G2575-10P-2C'	S	S	S	S	S	S
	'G2858'	R	R	R	R	R	R
	'ICA-Duva'	S	S	S	S	S	S
	'G1805-1P-1C'	R	R	R	R	R	R
IG17-80 (Pathotype 2)	'G2575-10P-2C'	S	S	S	S	S	S
	'G2858'	R	R	R	R	R	R
	'ICA-Duva'	S	S	S	S	S	S
	'G1805-1P-1C'	R	R	R	R	R	R
IG9-79 (Pathotype 7)	'G2575-10P-2C'	S	S	S	S	S	S
	'G2858O'	S	S	S	S	S	S
	'ICA-Duva'	S	S	S	S	S	S
	'G1805-1P-1C'	S	S	S	S	S	S

[a]Condition 1 (T_1) = Inoculated plants were incubated at 16 and 22C with relative humidity of 95-100% for 4 days.

Condition 2 (T_2). Plants from each of the 2 sets of temperature in condition 1 were maintained at 16, 24 and 30C at relatively lower and uncontrolled relative humidities.

[b]Foliar reaction = R = Resistant. No apparent disease symptoms.
S = Susceptible; with varying degrees of disease severity.

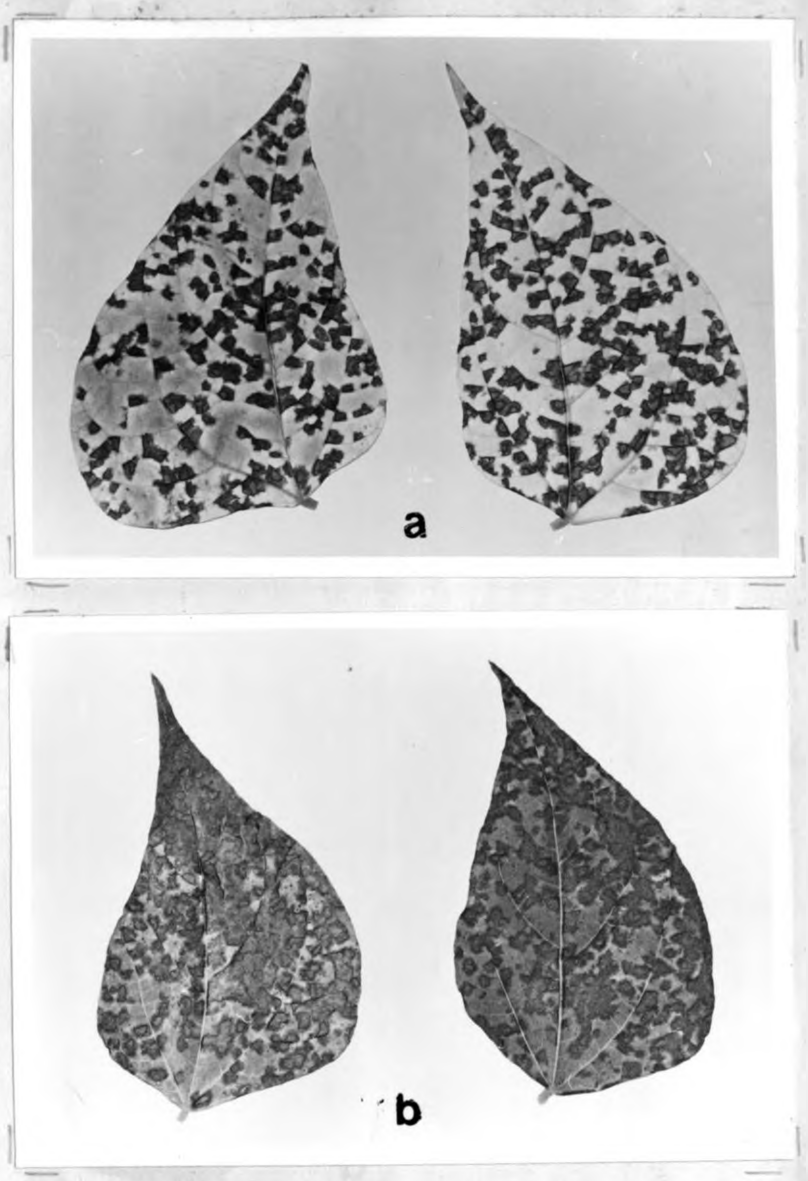


Plate 10. Leaflets of bean (Phaseolus vulgaris L.) cultivar 'Alabama No. 1' (a) showing the type and size of lesions compared with those of, and the 'burning' reaction on 'California Small White No. 643' (b), 14 days after inoculation with isolate IG 9-79 of Isariopsis griseola Sacc.

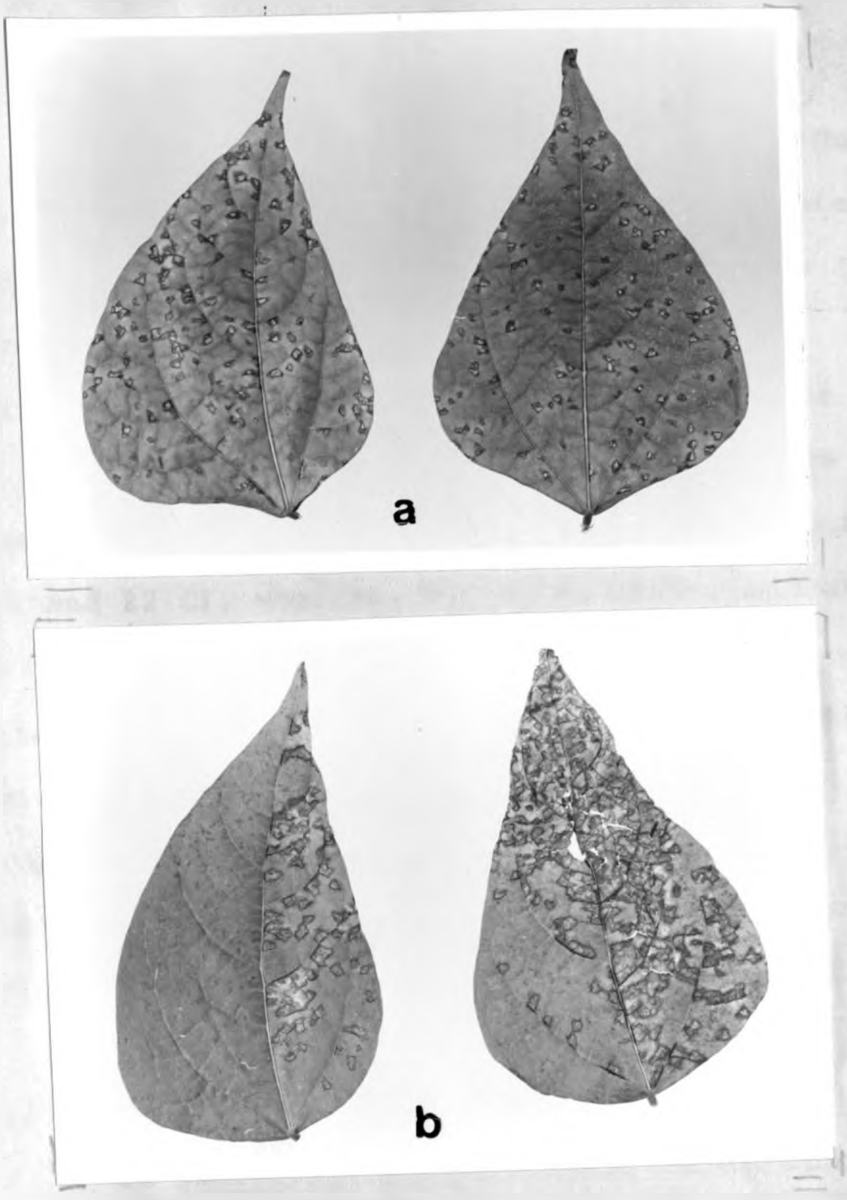


Plate 11. Leaflets of bean (Phaseolus vulgaris L.) cultivar 'Caraota-260' (a) showing small, delimited lesions compared with those of 'G 2575-1OP-2C' (b) showing large coalescing lesions, 14 days after inoculation with isolates IG 18-80 and IG 9-79 respectively of Isariopsis griseola Sacc.

On cultivars 'G 2575-1OP-2C' and 'ICA-Duva', which were susceptible to all the three isolates, temperature was however observed to influence the incubation period of angular leaf spot. Incubation period of plants finally maintained at 24 and 30 C varied between five to seven days regardless of the initial incubation temperature (16 and 22 C), whereas, for those maintained at 16 C it was 14 to 16 days. Disease severity attained at the latter condition after three to four weeks, was comparable to that of plants incubated for two weeks at 24 and 30 C. Similarly defoliation and chlorosis due to infection occurred relatively earlier at 24 and 30 C than at 16 C.

4:4 Effects of individual and mixtures of isolates of *Isariopsis griseola* Sacc. on disease severity.

Disease incited by the isolates of *I. griseola* used, was less severe on cultivar 'G 2575-1OP-2C than on cultivar 'G 2858', but the order of virulence of the isolates was similar on both cultivars (Table 11). The isolate mixture A inoculated at a concentration of 14×10^4 conidia/mℓ

Table 11. Disease severity on two bean (*Phaseolus vulgaris* L.) lines inoculated with single and mixtures of *Isariopsis griseola* Sacc. isolates at two (2×10^4 and 14×10^4 conidia/mℓ) inoculum concentration levels.

	Number of isolates	Final inoculum concentration ($\times 10^4$ conidia/mℓ)	Mean disease severity[x] values[a]		
			Bean Lines		
			'G 2858'	'G 2575-10P-2C'	Average
Mixture A	7	14	5.00	3.00	4.00[bi]
IG9-79	1	14	5.00	3.00	4.00[b]
IG9-79	1	2	5.00	3.00	4.00[b]
IG10-79	1	2	4.50	3.00	3.75[c]
IG1-77	1	2	4.00	2.50	3.25[d]
Mixture B	7	2	4.20	2.00	3.11[e]
IG5-78	1	2	4.00	2.00	3.00[f]
IG7-79	1	2	3.00	2.00	2.50[g]
IG6-79	1	2	2.00	2.00	2.00[h]
IG8-79	1	2	2.00	2.00	2.00[h]
Average			3.87	2.45	

a = All disease severity values are averages of 18 replications.

i = Means followed with the same letter in the last column are not significantly different, P = 0.05 according to Duncan's multiple range test.

x = Disease severity was rated on a scale of 1-5 in which 1 = no apparent infection; 2 = ≤2%; 3 = 3-10%; 4 = 11-25%; 5 = ≥ 26% of actual leaflet area covered with lesions.

and isolate IG 9-79 inoculated at concentrations of 2 x 10^4 and at 14 x 10^4 conidia/mℓ induced the highest degree of disease severity. Inoculating the cultivars with isolate mixture B using a concentration of 2 x 10^4 conidia/mℓ, gave disease severity significantly less than that caused by isolates IG 1 - 77, and IG 10 - 79, but more than the disease severity induced by isolates IG 5-78, IG 7-79, IG 6-79, and IG 8-79 all inoculated singly at a concentration of 2 x 10^4 conidia/mℓ.

4.5 Progress of angular leaf spot in the field

A tabular presentation of disease incidence and severity for 14 bean cultivars is given on Table 12 and shows four general patterns. First, there are those cultivars ('G 1805-1P-1C' and 'BAT 527-(20)P') which did not show any disease symptoms throughout the observation period suggesting that they were probably immune to the IG 1-77 isolate of *I. griseola*. Secondly, there are those ('BAT 966-1P-(20)P' and 'Caraota-260') which had relatively low disease incidences ($\leq$ 50%) and whose disease severity was also low (disease severity

index $\leq$ 2.5). Thirdly, there are the cultivars ('A43 - 1P - (18)P', 'BAT 67 - (20)P' and 'BAT 452-(18)P') which despite their high final incidences had low disease severities ($\leq$ 2.8). Lastly, there are those ('Alabama No. 1', 'BAT 508-(25)P', 'Cauca-27A' and 'G 2858') which showed high disease incidences and severities.

Disease progress curves for 8 of the 14 bean cultivars are shown on Figure 5. Logit transformed (Van der Plank, 1963; Kranz, 1974) values for disease severities graphically presenting disease increase are given in Figure 6. Computation of disease increase rates r by Van der Plank's formula (1963) showed that cultivars 'G 2858' and 'Alabama No 1' had the highest r-values indicating a relatively higher rate of disease increase on these cultivars than on the rest. Disease on cultivars 'Alabama No 1' 'Cauca-27A', 'G 2858' and 'G 4421' appeared at the same time but much earlier than on the other cultivars implying a longer incubation period on the latter group.

Table 12. Disease incidence and average severity (during a period of 5 weeks) on bean (Phaseolus vulgaris L.) lines and cultivars inoculated with IG1-77 isolate of Isariopsis griseola Sacc. at Popayán, Colombia, April-May, 1981.

	Disease incidence[a] and Severity[b]							
	Days after inoculation							
	12		19		26		33	
Cultivar/Line	Incidence(%)	Severity	Incidence%	Severity	Incidence%	Severity	Incidence%	Severity
A43-1P-(18)P	0	1.0[c]	68	2.5	99	2.5	100	2.6
Alabama No. 1	90	2.0	100	3.3	100	3.8	100	4.5
BAT 67-(20) P	65	2.0	82	2.5	95	2.7	100	2.8
BAT 452-(18)P	0	1.0	73	2.5	95	2.7	100	2.8
BAT 508-(25)P	75	2.0	94	3.0	100	3.1	100	4.2
BAT 527-(20)P	0	1.0	0	1.0	0	1.0	0	1.0
BAT 966-1P-(20) P	0	1.0	0	1.0	10	1.8	18	2.0
BAT 1057-1P-(24) P	0	1.0	86	2.6	100	3.6	100	3.6
Caraota-260	0	1.0	24	2.1	32	2.5	50	2.5
Cauca-27A	95	3.0	100	3.4	100	3.8	100	4.5
Epicure	80	2.0	96	3.3	100	3.4	100	3.5
G 1805-1P-1C	0	1.0	0	1.0	0	1.0	0	1.0
G 2858	95	3.0	100	4.0	100	4.6	100	4.8
G 4421	60	2.0	83	3.0	100	3.5	100	3.5

[a]Disease incidence was rated as the number of plants infected out of all plants (99-120) inoculated.

[b]Disease severity was rated on a scale of 1-5 in which 1 = no apparent infection 2 = ≤ 2%; 3 = 3-10%; 4 = 11-25%; 5 = ≥ 26% of actual leaflet area infected.

[c]Values are an average of 20 sampled plants.

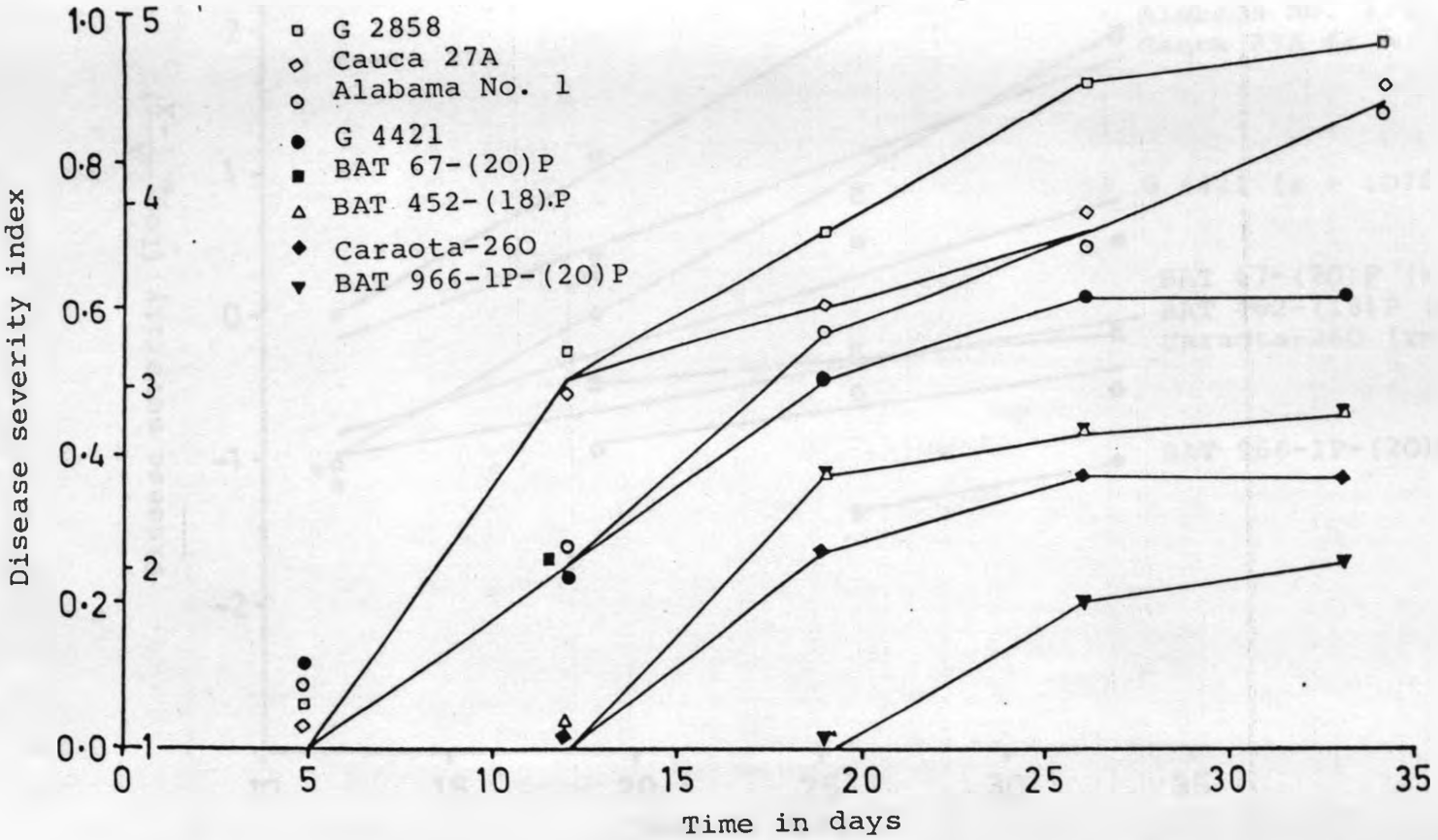


Figure 5. Disease progress curves for eight bean (Phaseolus vulgaris L.) cultivars artificially inoculated with isolate IG1-77 of Isariopsis griseola Sacc. at Popayán, Colombia.

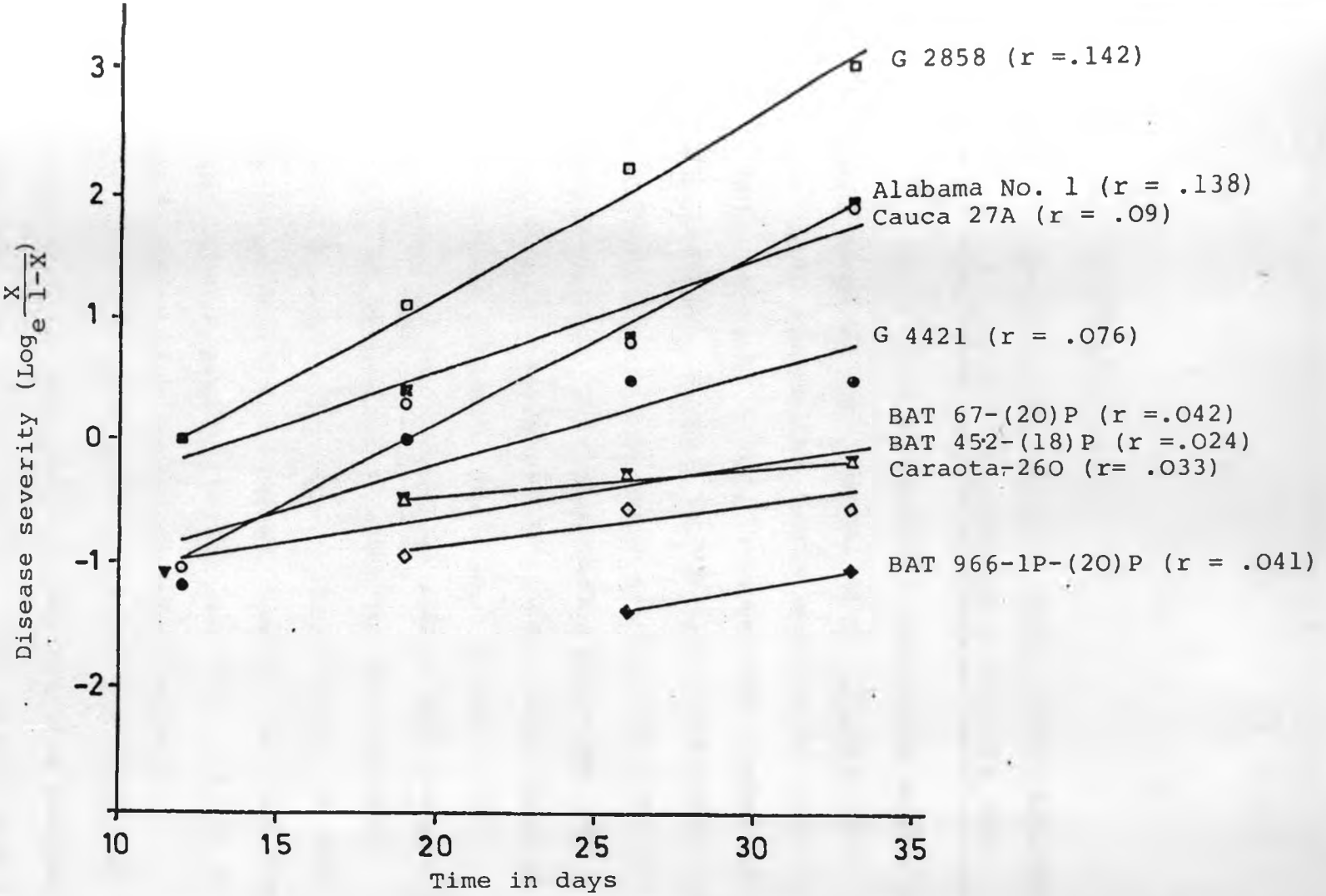


Figure 6. Apparent infection rates (r) for artificially induced infection of angular leaf spot on 8 bean (Phaseolus vulgaris L.) cultivars at Popayán, Colombia.

5. DISCUSSION

5.1 Variation in conidial size, growth and sporulation of isolates of Isariopsis griseola Sacc.

Mean conidial lengths, widths and number of septa obtained for isolates of I. griseola that were used, agreed with those reported by Saccardo (1886), Benlloch (1944), Zaumeyer and Thomas (1957), Llanos (1958), Hocking (1967) and Ellis (1971), who had found that a wide range in conidial size in the fungus exists. The parameters determined were significantly different (P = 0.05) among some of the tested isolates (Table 4). However, the variation of each parameter within each isolate was wide. Mean conidial lengths for the isolates, IG3 - 78, IG4 - 78, IG6 - 79 and IG10 - 79 were not significantly different despite the fact that they were different from isolate IG1 - 77 all of which had a same area of origin (Table 1). However, the mean conidial widths and number of septa for the same isolates did not show similar trends; and these parameters were significantly different among some of these isolates.

The most prevalent conidia were those with

three septa (51%), an observation similar to that made by Llanos (1958) who found that conidia with three and four septa to be more common. .The present study showed that conidia without septa and those with five or six septa were the least prevalent. One isolate IG21 - 80 had conidia with seven septa (Plate 3D), a feature which has not been recorded before. The occurrence of the latter was nonetheless rare. The two traits, conidial length and the number of septa showed a high correlation ($r = 0.71$), unlike those between conidial width and length ($r = 0.15$) or width and number of septa ($r = 0.18$).

From the results obtained, it is apparent that conidial size among the isolates of I. griseola vary considerably. This variation which reflects the probable genetic variation inherent in the fungus would probably enable the organism to respond differently under varying environmental conditions such as temperature, moisture, pH and nutrients (Stakman and Harrar, 1957; Hawker, 1966). In 1971, Berger and Hanson found that the length, shape, and number of septa of Cercospora zebrina Pass. were influenced by environmental factors especially by moisture and temperature. The extent of

variation of these characters observed in I. griseola limits their usefulness as means of separating isolates into groups with respect to prevailing environmental conditions or their areas of origin.

Growth of the eight isolates of I. griseola at each of the four different incubation temperatures showed no appreciable differences. However, there was clear distinction in growth of the fungus between the incubation temperatures 14, 19, 24 and 29 C after seven days of plating on V-8 juice agar medium (Plate 4). It was apparent that growth occurs at 14 C but much more slowly than at 19 C. The optimum growth temperature was 24 but no growth occurred at 29 C. Effect on growth at 29 C cannot be said to be due to the destructive effect of temperature on the isolate or medium, because when these plates were transferred into incubators set at 19 and 24 C growth occurred after three to four days. It however, appears to be inhibitive. Growth at 14 C was slow for all isolates. It has bowever been reported that growth can still occur at temperatures as low as 8 C and as high as 28 C. (Cardona-Alvarez, 1956). Silvera (1967) and Avila (1979)

also observed that the upper temperature limit for growth of I. griseola was 30 and 28 C respectively. The optimum growth temperature obtained (24 C) in the present study was in agreement with that obtained by Cardona - Alvarez (1956), Llanos (1957), Silvera (1967), Santos - Filho (1976), and Avila (1979) on potato dextrose agar (PDA) plus bean leaf extract, PDA, bean leaf dextrose agar (BLDA), 'baby food' - Calcium Carbonate - agar, and V-8 juice agar media respectively. Noting that these workers obtained similar results while working with different isolates, different media and at different times, the present study conclusively shows that the fungus does not present significant variation with regard to optimum growth temperature and little if any of its cardinal temperature points. The decline in ability to grow on either side of the optimum growth temperature is evidently unequal; being very slow before and very rapid as temperature increases above the optimum. This phenomenon is however, not surprising and is frequent in fungi (Deverall, 1965).

Growth of the different isolates of I. griseola used would have been expected to be different at the different incubation temperatures due to the varied temperature regimes (13.5 - 24 C) found in their natural environments (Table 2). This, however, was not the case implying that in the different areas of origin, the different isolates may only be having varying rates of growth and development. Growth of fungi on artificial media is probably not the same as under natural (field) conditions. But evidence obtained by Edson and Shapolov (1919) on Fusarium oxysporium Schlecht. and Verticillum albo-atrum Rke. and Berth. showed that there are good correlations between the optimum growth temperatures in media, and the temperatures at which these organisms are serious plant pathogens, in the field.

The appearance of the grey mycelia on cultures of isolates IG3 - 78 (obunuco) (Plate 5) and IG14 - 80 (La Selva) and on cultures of other isolates not included in this particular study is a feature observed and also reported previously by Alvarez - Ayala (1979) to occur in I. griseola. He explained this appearance to be probably due to growth of secondary mycelia arising from the

normally uninhibited germination of conidia produced by the primary mycelia. In the present study this phenomenon was not found to be consistent for some of the isolates during their repeated subculturing. But on cultures of isolates IG3 - 78 and IG14 - 80, grey mycelia were consistently observed, implying an isolate effect. This type of mycelia appeared earlier on cultures incubated at 24 C than those at 19 or 14 C. The extent or degree of appearance increased with the increase in length of incubation period. Given this general inconsistency among some isolates, the appearance of the mycelia is probably dependent on isolates, and/or environmental conditions particularly temperature, moisture and culture medium or an interaction of these factors. Most cultures with the grey mycelia had also low conidial harvest, but the petri dishes containing these cultures often had condensed moisture below their covers. In the presence of high relative humidity or free moisture and under favourable temperatures, conidia of I. griseola readily germinate. This may be why these cultures had lower conidial harvest a feature also noted at times on some of the dark mycelial cultures. It is, however, interesting to note that after two to three years of storage (at 4 C)

and frequent subculturing (at 19 C), isolates such as IG1-77, IG6 - 78 and IG4 - 78 produced highly sporulating dark mycelial cultures despite the fact that IG1 - 77 had been reported by Alvarez - Ayala (1979) to produce grey mycelia at some stage of its subculturing. The predominant determinants in the production of grey mycelial cultures appears to be the environmental conditions prevailing during the growth of the fungus and the period of time under which the cultures are subjected to these conditions.

It was not possible and not even correct to compare sporulation of cultures incubated at 14 C for ten days. This was because after this period of time growth of the fungus at 14 C was still minimal and required a relatively longer incubation time to attain a comparable stage, by which time sporulation at 19 and/or 24 C would have reached a maximum point and the number of conidia would have either attained a maximum level as was found (Table 7, Fig. 4B) or started to decline as reported by Alvarez - Ayala (1979).

Sporulation was found to be influenced by

the isolate, temperature and the interaction between them (Appendix 2, 3). The time at which sporulation was determined was also found to be a significant factor (Table 6). The differences observed in sporulation of different isolates may be an indication of the variation that exists among the different isolates of I. griseola with respect to this trait. The significant temperature - isolate interaction effect was partly, but mainly due to isolate IG2 - 78 (Table 5, 6, Figure 4A). The latter expressed a trend contrary to the rest of the isolates and which appeared to be inherent in the isolate. It could have been argued that more sporulation at 19 than at 24 C was probably due to the influence of low storage (4 C) and subculturing (19 C) temperatures on the isolate. This was observed not to be a general characteristic of the I. griseola fungus since isolates such as IG1 - 77 and IG3 - 78 which were also maintained under the same environmental conditions and over the same period of time did not show similar effects, implying that the character in IG2 - 78 was not adaptive. The sporulation behaviour of IG2 - 78 was probably due to either inhibition of sporulation at 24 C and

not at 19 C or because sporulation of the culture started much earlier at higher temperatures (24 C) so that on the 7th day, the number of conidia were already on the decline due to the uninhibited germination. In 1976, Silvera, obtained evidence that sporulation of isolates of I. griseola started as early as five days when incubated at 25 C on bean leaf dextrose agar medium.

The optimum sporulation temperature (24 C) observed for most isolates was in agreement with the results of Silvera (1967) and Santos-Filho (1976) but not with those of Alvarez-Ayala (1979 and Avila (1979) who found them to be 14 - 19 C and 16 C respectively. Alvarez-Ayala (1979) who did not observe significant differences in sporulation in cultures which had been incubated at 14 and 19 C for 20 days; noted on a separate experiment that, the number of conidia declined significantly after reaching a maximum on the 9th to the 12th day of incubation at 19 C. Due to the slow rate of growth and development of the fungus at 14 C, the time at which maximum conidial production occurred was likely to be more delayed than it would be at 19 C or at 24 C. But since the number of conidia in the

latter temperatures started declining after the 12th day, then comparison made after 20 days was not justified and the number of conidia at 14 and 19 C would have been expected to be more or less the same. This may also explain in part the observation made by Avila (1979) where optimum sporulation occurred at a relatively lower temperature (16 C) after a period of 10 days. The most likely influencing factors may have been the isolate and/or the environmental conditions (both ambient and nutritive) employed.

In studies where large numbers of conidia of I. griseola are required for artificial inoculation of plants (in greenhouse or field), it is imperative to understand well the conditions under which maximum conidial production occurs. From the foregoing account, it is clear that for most of the isolates, optimum sporulation occurs at 24 C after a period of 10 - 13 days of incubation on V-8 juice agar medium. It is also certain that although variation in sporulation does occur among different isolates of I. griseola the major sources of variation are the environmental conditions. Inspite of the fact that sporulation structures in culture

media and under natural (field) conditions are morphologically different, the optimum temperature for development and sporulation in both conditions are similar. During a period of continuous moisture and favourable temperature, the fungus infecting a susceptible cultivar is able to sporulate 9 to 12 days after infection (Cardona-Alvarez, 1956) a period which is comparable to that obtained in culture media.

There is a general agreement on the factors that influence growth of I. griseola. So far the only conflicting reports are those regarding sporulation particularly the best sporulating temperature - time combination. These differences and any other that may occur can be influenced by some or all of the factors tested here (isolate, temperature, time to sporulation) and also by media, light (Alvarez-Ayala, 1979) and by the interactions among some or all of these factors.

5:2 Pathogenic variation

Indications of field occurrence of physiological races of plant pathogens are generally based on two criteria;

a) The gradual 'breakdown' of disease resistance in a variety in a given area and

b) The large differences in varietal reactions at different locations or countries (Chiu et. al., 1965).

Some of the common plant pathogens such as Puccinia graminis var tritici Erik. and Hen. on wheat (Triticum Spp.), Uromyces phaseoli (Pers.) Wint. and Colletotrichum lindemuthianum (Sacc. & Magn.) Scrib. on beans (Phaseolus vulgaris L.), and Pyricularia oryzae Cav. on rice (Oryza sativa L.) are a few of many fungi which exhibit considerable degrees of pathogenic variability and have been shown to meet one or both of the above criteria (Stakman et. al., 1962; Harter and Zaumeyer, 1941; Barrus, 1911; Goto, 1965). After reviewing results of numerous tests aimed at identifying resistant rice varieties against P. oryzae, Ou (1971) found that varietal reactions varied from locality to locality as well as from season to season in the same locality.

In the past, bean cultivars that have been reported to have resistance against I. griseola include; 'Kentucky Wonder' (Gardner and Mains,

1930), 'Alabama No. 1'. 'California Small White No. 643', 'Epicure' (Brock, 1951) 'Cauca-27A' (Colombian Agric. Res. Prog., Min. Agric. and Rockefeller Foundation, 1956; Olave, 1958), 'Caraota - 260' (Santos - Filho et. al., 1976) and 'G4421' (Avila, 1979). However, Alvarez-Ayala and Schwartz (1979) observed that cultivars 'Alabama No. 1', 'Cauca 27A and 'Caraota - 260' were susceptible to 1, 1 and 3, isolates respectively out of the 5 isolates they used. In the present study all the above seven cultivars were susceptible to at least one or more of the 20 Colombian and 1 USA isolates of I. griseola tested. However, some of the cultivars were resistant (immune) to some of the isolates (Tables 8, 9, 12; Plates 10, 11, 12) implying that there exists a host - pathogen specialization relationship.

Reports by different early workers showing that the above cultivars were resistant to angular leaf spot indicates that they may have used isolates with similar pathogenicity as those used in the present study which could not overcome corresponding genes for resistance. Although it now appears that their generalizations about

resistance of these cultivars were erroneous, such a situation would be expected particularly when considering that their results were based on tests which employed one or two isolates or locality.

The methods of testing and evaluations employed by these workers may also have been responsible for the results they obtained. In identifying sources of resistance to I. griseola, Brock (1951), Santos-Filho et. al. (1976) and Avila (1979) inoculated and evaluated bean plants grown in the greenhouse scoring them on the basis of the number of lesions per plant. Alvarez-Ayala (1979) compared four methods of assessing angular leaf spot severity which consisted of determining the number of lesions per 4 cm^2 of leaf area, lesion area, and two scales representing percentages of leaf area covered with lesions, and found that all lead to the same relative rating of the host resistance and isolate virulence. On the other hand, Singh and Sharma (1975), made tests under field conditions and found 'Kentucky Wonder' to be immune. In this and similar cases, it should be appreciated that even with very compatible host - pathogen relationships (host susceptible) ideal environmental

conditions (high relative humidity and optimum temperature) are essential prerequisites to ensure successful infection by I. griseola (Cardona-Alvarez and Walker, 1956; Llanos, 1958; Silvera, 1967; Alvarez-Ayala, 1979; Avila, 1979). It is thus possible to have disease escape which may be confused for resistance, if these conditions are not met.

Other experimental factors which could have also influenced previous results are; the nature and concentration of the inoculum used and the age and media of growth of the plants. To determine the effects of any of the above factors on varietal reactions, it is imperative that the effects of all other factors be held at a more or less constant level.

Demonstration of occurrence of physiological races in fungi is usually based on various types of host reaction arising from various host-pathogen interactions. The host reactions, which can be either qualitative or quantitative are normally quantified in either specific or arbitrary units which may have physiological or epidemiological significance and which are usually categorized as

either resistant or susceptible. They include reactions such as (a) susceptible or resistant (immune, hypersensitive reaction) of host plants such as was observed on Phaseolus vulgaris L. when inoculated with races of Colletotrichum lindemuthianum (Sacc. and Magn.) Scrib. by Leakey and Simbwa-Bunya (1972), (b) infection types as on wheat cultivars inoculated with races of Puccinia graminis var tritici Erik. and Hen. (Stakman et. al., 1962), (c) percentage of infected (wilted) plants as on peas (Pisum sativum L.) inoculated with races of Fusarium oxysporium Schlecht. f. sp. pisi (Van Hall) Snyd. and Hans. (Armstrong and Armstrong, 1981), (d) percentage of leaf area covered with lesions as on wheat cultivars infected with races of Septorium nodorum Berk. (Rufty et. al., 1981) and (e) sporulation capacities of different races of a pathogen in a given host (Nelson, 1973). The host-pathogen interactions which result in host reactions a, b, c, and d measure the host resistance and are a result of a disturbed host physiology whereas e relates mainly to the epidemiology capabilities of the pathogen.

In the present study, isolate and pathotype differentiation was based on qualitative rather than on quantitative reactions of the host plants. With the methods and conditions used, results were entirely clear cut and cultivars were scored as susceptible, corresponding to the symptoms indexed two to five on the Centro Internacional de Agricultura Tropical (CIAT) angular leaf spot evaluation scale of 1 to 5 or resistant,with no visible symptoms of infection. Disease severity corresponding to disease index 2 was considered a susceptible reaction although it corresponded to a non - or little - sporulating lesion type and would be practically regarded as 'resistant'. The susceptible and resistant host reactions were manifested consistently in most of the bean cultivars used as differentials after repeated testing. They were hence considered reliable under the conditions tested. The resistant reaction was not influenced at all when incubation temperatures were varied between 16-30 C (Table 10). However, the susceptible reactions (disease index 2, 3, 4, 5) corresponding to the different levels of disease severity varied with change in incubation temperature.

The effect of temperature seemed to be in the direction of more disease severity at higher temperatures. Nevertheless, it was evident that some isolate - pathogen combinations consistently gave mild symptoms corresponding to disease, severity index 2 despite variation in ambient temperatures.

All isolates of I. griseola were pathogenic and infected all tested cultivars, but differentially (Table 8). Isolates which infected and resulted in a susceptible cultivar reaction seemed to have genes for pathogenicity which overcame the matching genes for resistance in the cultivars. However, the resistant reaction observed, appeared to be governed by genes which conferred a more or less immune reaction against some of the isolates. This implied that in the latter case, the isolates probably lacked the matching genes for pathogenicity to overcome resistance genes in the cultivars. It was thus clear that the effects of resistant genes were dependent on the isolate used, a phenomenon usually observed where race specific or vertical major genes are involved (Flor, 1971; Day, 1974) and also an indication of high degree host - pathogen

specificity between bean cultivars and isolates of I. griseola. The type of resistance conferred by the genes which gave the resistant reaction are most likely, the ones that have been referred to as vertical, differential, or race specific (Eenink, 1976; Van der Plank, 1975). Van der Plank, (1968) considers vertical resistance to be monogenically or oligogenically controlled, giving a discontinuous variation in the level of resistance or a qualitative expression.

Studies made in the past regarding inheritance of resistance to angular leaf spot, showed that resistance in the bean (P. vulgaris L.) cultivars 'Caraota 260', 'Decal', 'Maravilla' and 'line 258' as well as P. coccineaus L. cultivar PLB 257, were governed by either one or two to three independent factors (Barros et. al., 1957; Cardona-Alvarez, 1958; Santos-Filho et. al., 1976; Singh and Saini, 1980), which is characteristic of race - specific resistance (Van der Plank, 1968). This type of resistance is often complete (Eenink, 1976), but its level may be influenced by genetic dossage effect (Dunn and Namm, 1970), modifier genes (Rousele, 1974), or the physiological age of the plant (Bartos et. al.,

1969). Extraneous factors such as soil temperature, air humidity or light intensities also appear to raise or lower the level of this monogenic resistance (Hubbeling, 1966; Walker and Williams, 1973).

The presence of a 'resistant' reaction on some bean cultivars characterized by visible lesions corresponding to disease severity index 2 rather than an immune reaction, was probably due to the effect of any one or more of the above factors which influence the level of monogenic resistance. Alternatively, it may have been due to a mechanism of resistance of a different nature from the one conferring immunity.

Apart from varying qualitatively, race-specific resistance can also vary quantitatively (Ou, 1979; Johnson, 1975) a feature previously reserved for the type of resistance described as race - nonspecific or uniform resistance. Similarly the latter can also have race specific effects which are usually so small that they cannot be differentiated from the experimental error (Parlevliet, 1978). The general impression given by cultivars with race - nonspecific resistance is the absence of race -

specific effects. However, the existance of the latter does not exclude the presence of the former in the same cultivar; in fact race - nonspecific resistance is probably always present along with race - specific resistance (Van der Plank, 1968).

With a low degree of host-pathogen specificity, the two forms of resistance are epidemiologically similar in that both reduce the rate at which the epidemic proceeds. The importance of differentiating the two forms of resistance is that, race - nonspecific type is more durable because the pathogen population finds it more difficult to adapt to the several loci involved (Parlevliet, 1978). Whereas, with race - specific resistance, the pathogen population tends to adapt to the host more easily and results in a 'loss' of resistance.

Due to distinct differences in responses of the differential cultivars and the little or no effect of the environmental conditions on the reactions of the latter, these studies establish that physiological specialization exists in I. griseola. The author proposes to classify isolates of the fungus into separate pathotypes. Designation

of the tested isolates into races is deliberately avoided here since the genetic constitution of differential cultivars responsible for the differential reaction is not known. Ideally, the systematic designation of races should be based on known resistant genes or genetic compositions of differential cultivars after analysing them genetically as is, with races of bean common mosaic virus (Drijfhout, 1978) or Piricularia oryzae (Ou, 1979).

Although it remains to be determined whether the pathotypes identified in Colombia represent pathotypes that occur naturally in the field, seven pathotypes out of 20 isolates indicates high variability in the fungus. The isolate obtained from Wisconsin, USA could be grouped with Colombian isolates belonging to pathotype 2 (Table 9) suggesting that pathotypes identified in Colombia might also be occuring elsewhere. The apparent high pathogenic variation observed among the Colombian isolates was probably due to the varied number and complex gene constitution of the differential cultivars. Under such circumstances, isolates belonging to the same race (or pathotype) when tested

on a few differential cultivars may be separated when more differentials are used. Although using additional differentials gives the impression of high variation and makes the procedure or race differentiation more complicated, the latter, however, becomes more exact (Kirally, 1974). Alternatively the variation observed was possibly as a result of the capacity of the fungus to adapt to the genetically variable bean cultivars grown in Colombia. The fact that seven isolates (including some of the most virulent) from Popayan were grouped into three distinct pathotypes may be a case in point. Popayan is one of the sites where the CIAT Bean Program conducts various trials in agronomy, plant breeding and plant pathology. Tests on plant pathology involves, induction of artificial epidemics of angular leaf spot on a large number, sometimes over 10,000 accessions of bean, germplasm which most likely contain very variable and complex gene compositions. Experience with Phytophthora infestans (Mont.)de Bary (Niederhauser, 1961) and P. oryzae (Ou, 1979) has shown that the prevalence of races depends on their stability and the genotypes of the cultivars grown. Disease incidence

of a cultivar in the field, however, is determined by the population of virulent races found there but not by the number of virulent races (Kozaka, 1979). This implies that in areas where more cultivars with complex genotypes are grown widely, the pathotypes or races of a pathogen may be expected to be complex or more variable and vice versa.

Since the perfect state of the fungus has not yet been found to occur naturally, the cultural, morphological and pathogenical variations observed in I. griseola are probably due to the genetical changes brought about by either one or more of the mechanisms of mutation or heterokaryosis and parasexualism. So far no cytogenetic evidence of these genetical changes has been demonstrated and hence no exact cytogenetic explanation for the variation is available.

The differential reactions observed in cultivars 'Alabama No. 1', 'Caraota-260', 'ICA-Duva', 'G1805-1P-1C', 'G2575-10P-2C', and 'G2858', after inoculation with Colombian isolates of griseola (Table 9) prompted the author to propose them as preliminary differential cultivars in the identification of pathotypes of the pathogen. The

differential range of these cultivars may however, not be complete but forms a basis upon which improvement can be made until such a time that pure lines, with proven genomes are developed. Since the genomes of these differentials are unknown, their use in different countries may give results not comparable to those obtained in Colombia. This can be expected if the pathogen population is constituted of pathotypes different from those occuring in Colombia or if the bean lines or cultivars used elsewhere are: (1) genetically impure, (ii) have deviating resistance spectrum from those used in the present study, or (iii) if there are differences in ages of test plants, testing and evaluation methods. To avoid discrepancies in results, co-operative programs between interested countries have to be made to develop regional and/ or international set of differentials. This would entail, development, multiplication and distribution of seeds of differentials with known genomes. It would also require the standardization of testing and evaluation procedures. Because of the large bean germplasm collection (over 30,000 accessions) maintained at CIAT (CIAT, 1981) and the world-wide

mandate the latter has on research with beans, the institute would probably be in a better position to co-ordinate such a program in close collaboration with National Research Institutes of interested countries. This would greatly facilitate a standardized system of characterizing and identification of pathotypes or races of I. griseola to avoid confusion.

5:3 Effect of individual and mixtures of isolates of Isariopsis griseola Sacc. on disease severity.

In screening bean germplasm in the greenhouse and in the field, good results have been obtained at CIAT (CIAT, 1979) using a spore suspension of 2×10^4 conidia/mℓ. Alvarez-Ayala (1979) also found the conidial concentration to be optimal and that increase of spore concentration above it increased disease severity at a decreasing rate. It has been demonstrated in the present studies that, the seven Popayan isolates could be grouped into 3 distinct pathotypes (Table 9). However, all these isolates incited symptoms on cultivars 'G2575-10P-2C' and

'G2858' and showed variation in virulence in a more or less the same order on both cultivars (Table 11). A comparison between inoculation of single and mixtures of I. griseola isolates on disease severity showed that mixture 'B' inoculated at a concentration of 2 x 10^4 conidia/mℓ of water (each isolate present at a concentration of 2857 conidia/mℓ) gave a significantly lower disease severity than either isolate IG9 - 79 or IG10 - 79 inoculated singly at the same concentration. On the other hand, disease severity invoked by isolate mixture 'A' at a concentration of 14 x 10^4 conidia/mℓ (each isolate present at a concentration of 2 x 10^4 conidia/mℓ) was significantly higher than that of mixture 'B' and was comparable to that caused by isolate IG9 - 79 inoculated at 2 x 10^4 and 14 x 10^4 conidia/mℓ. The significant difference obtained in disease severity between the isolate mixture 'B' and either isolate IG9 - 79 or IG10 - 79 may have been due to:-

(i) variation in pathogenicity of the isolates in the mixture and

(ii) the fact that each isolate was present at a concentration of about one-seventh of the final mixture.

Hence the effect of each isolate in the mixture was reduced because of the low individual isolate concentration. Despite the fact that pathogenic variation in both mixtures 'A' and 'B' was the same, the former caused significantly more disease than the latter. This probably was because in mixture 'A' each isolate was present at a higher concentration, sufficient to cause more infection on a susceptible cultivar.

Results of this study have a direct bearing in the development of methods of screening bean germplasm for resistance against angular leaf spot disease. The existence of pathogenic variability in I. griseola makes it necessary for cultivars to be evaluated using isolates which are representative of the existing pathotypes or races. If the germplasm is large, it entails using mixtures of the isolates, some of which may not be pathogenic to some of the cultivars. It has presently been shown that, under such circumstances, it is imperative that the final inoculum concentration be based on the extent of the existing variation and the number of the isolates in the mixture. The common practice has been one where conidial

suspensions of isolates are mixed and then the concentration of the mixtures is adjusted to 2×10^4 conidia/mℓ (CIAT, 1977). This practice is liable to give erroneous results particularly if some of the tested cultivars have some degree of resistance against some and not all isolates in the mixture. This is because the concentration of those isolates to which the cultivar may be susceptible are reduced so that they do not exert sufficient disease pressure on the cultivar. This may result in the latter being erroneously evaluated as resistant. To ensure that each isolate is present at optimum infective concentration, the conidial concentration for each isolate should be adjusted before mixing them so that in the final mixture each isolate is present at a concentration of 2×10^4 conidia/mℓ. This will however, mean that the final concentration will be a multiple of 2×10^4 depending on the number of the isolates used.

5:4 Progress of angular leaf spot in the field

This experiment was carried out in a season favouring development of angular leaf spot and, therefore, it was possible to characterize reactions

of bean cultivars under natural conditions. Reactions of some bean cultivars to I. griseola isolate IGl - 77 in the field was comparable to that observed in the greenhouse. Cultivars Gl805-lP-lC', and 'BAT 527-(20)P' were immune to the isolate whereas others expressed varying degrees of resistance and susceptibility (Table 12).

Disease progress curves for the eight cultivars whose progress was monitored exhibited more or less sigmoid or S-shaped patterns. The logit transformation of disease proportions and determination of the apparent infection rates facilitated interpretation and comparison of disease increase between cultivars.

The apparent infection rate, r, measures the speed of the disease and provides a quantitative assessment of the effects of the hosts, weather, races of a pathogen or treatments on cultivars such as chemical control or partial resistance on epidemics (Zadoks and Schein, 1979; Van der Plank, 1963). This is because r can be affected by weather conditions, resistance or susceptibility of the hosts and the amount and nature of the inoculum

(Van der Plank, 1963). Since the testing of cultivars was carried out under the same environmental and soil conditions and were uniformly treated, the difference in r values observed probably reflected partial resistance differences inherent among cultivars tested. The individual r values of cultivars 'BAT 67 - (20)P', 'BAT 450 - (18)P', 'BAT 966 - 1P - (20)P', were one-third that of 'G2858'. This may be interpreted by Van der Plank's model (1963) to mean that the former probably had partial resistance against the 1G1 - 77 isolate expressed as r reducing resistance.

The cultivars also showed differences in the time when symptoms appeared and the extent of disease severity attained. On more susceptible cultivars, infection started earlier and at first increased rapidly but later it did so at a decreasing rate apparently due to diminishing available healthy tissues. On more resistant cultivars the symptoms appeared a week or so later, an effect wich delayed epidemics on these cultivars and which may be attributed to either long latent periods or race - specific resistance in the cultivars (Parlevliet, 1979; Van der Plank, 1975).

Van der Plank (1968) considered r - reducing resistance to be of the race nonspecific type. However, Parlevliet (1979), Toriyama (1975), and Ou (1979) reckoned that this type of resistance which is incomplete can also be race specific.

Despite the fact that the present study was carried out in one season using one isolate there are indications that r - reducing resistance against I. griseola occurs in some of the bean cultivars. Whether or not this resistance is race specific is not yet known. Reduction of r reduces the epidemic development of the disease by decreasing the reproduction rate of the pathogen (Parlevliet, 1979). The factors of resistance which lead to the latter are the reduction in infection frequency or lesion numbers, lengthening of the latent period and the decrease in sporulation capacity. These aspects need to be considered and incorporated in future studies when attempting to develop stable resistance against I. griseola in beans.

PART II: VARIATION IN PATHOGENICITY AND VIRULENCE IN *Pseudomonas syringae* pv. *phaseolicola* (BURK., 1926) YOUNG AND DYE AND WILKIE, 1978.

6. LITERATURE REVIEW

Pseudomonas syringae pv. *phaseolicola* (Burk., 1926) Young, Dye and Wilkie, 1978 is a causative organism of halo blight on *Phaseolus vulgaris* L. The organism is also referred to in the literature as *Pseudomonas phaseolicola* (Burk., 1926) Dowson, 1943. The latter name was substituted with the former following a review and re-organization of taxonomy and nomenclature of phytopathogenic bacteria (Dye *et. al.*, 1980).

Halo blight of beans has a worldwide distribution and is a serious pathogen in areas with cool to moderate (12 to 24 C) temperatures (Schwartz, 1979). In Colombia, the disease is more prevalent in the cool bean growing regions of Pasto La Selva and Popayan (Schwartz, Personal Communication). However,in Kenya, it is widely distributed, occuring more frequently in cool,bean growing areas with high

rainfall. (Kinyua and Mukunya, 1981).

During periods of unfavourable conditions, the pathogen survives in infected seed or plant debris on the soil surface. The pathogen enters plants through wounds or stomata under conditions of high relative humidity or free moisture (Saettler and Potter, 1970; Schuster and Coyne, 1975; Walker and Patel, 1964; Zaumeyer and Thomas, 1957). Initial symptoms appear three to five days after infection as small watersoaked spots (Omer and Wood, 1969; Weber, 1973; Zaumeyer and Thomas, 1957). On leaves, chlorotic halos develop around the infection points. During severe infections, the stem and pods may also be infected. Necrotic spots on any of the plant parts infected, produce a light cream coloured exudates which when dry forms a shiny crust. Lesions on pods eventually become depressed and tan in colour (Vock, 1978). A systemic chlorosis of the plant may occur (Coyne and Schuster, 1974; Zaumeyer, 1932), due to a non-host specific toxin called phaseolotoxin produced by the bacteria during infection (Coyne et. al., 1971; Hoitink et. al., 1966; Walker, 1969).

The bacteria invades the developing seed through the upper pod suture and may be carried by mature seed internally or externally (Fahy and Lloyd, 1983; Grogan and Kimble, 1967; Schuster and Coyne, 1975; Zaumeyer, 1932). Infected seeds are often wrinkled and irregular in shape and are a principle means of dissemination (Taylor et. al., 1978; Weber, 1973; Zaumeyer and Thomas, 1957).

The organism is a single celled straight rod which is motile due to multitrichous flagella. It is gram negative, strictly aerobic and does not require growth factors (Fahy and Lloyd, 1983; Sands et. al., 1980; Weber, 1973). Its optimum growth temperature is between 20 - 30 C and colony growths are usually white to creamy in colour with bluish hues (Sands et. al., 1980; Weber, 1973).

In 1943, Adam and Pugsley observed that Ps. syringae pv. phaseolicola could form both rough and smooth colonies. According to the authors these colony forms were different from one another serologically and in their reaction to bacteriophages, but they did not carry out pathogenicity tests. The rough forms were later shown by Corey and Starr (1957)

to produce less extracellular polysaccharides and to be weaker in virulence than the smooth forms. Extracellular polysaccharides produced by Ps. syringae pv. phaseolicola have been associated with pathogenicity of the bacteria on susceptible bean leaves (Epton et. al., 1977; El Banoby and Rudolf, 1978).

Using 13 isolates, Jensen and Livingstone (1944) were the first to demonstrate pathogenic variation in Ps. syringae pv. phaseolicola. However, no differences were observed among the isolates in physiological tests except for slight differences in growth rates which were not correlated with variation in pathogenicity. Patel and Walker (1965), reported the occurrence of races 1 and 2 in the U.S.A. These races were characterized based on the reaction of inoculated bean cultivar 'Red Mexican UI 3' which is resistant to race 1 but susceptible to race 2. On the same basis occurrence of the two races has been reported again in U.S.A. (Schuster et. al., 1965), Great Britain (Epton and Deverall, 1965; Wharton, 1967), New Zealand (Hale and Taylor, 1973), Bulgaria (Poryazov, 1975b) and Kenya (Kinyua and Mukunya, 1981). Characterization of the

isolates into the two race groups has been based on leaf and/or pod reaction (Patel and Walker, 1965; Hale and Taylor, 1973) following inoculation with the bacteria and on bacteriophage tests (Taylor, 1970; Hale and Taylor, 1973).

Great Northern (G.N.) bean lines such as 'G.N. Nebraska No. 1 Sel 27' and 'G.N. UI 59' were initially found to be resistant to both race 1 and 2 (Coyne et. al., 1971; Coyne and Schuster, 1974a) but later they were observed to be susceptible to some of the isolates present in U.S.A. (Coyne et. al., 1979) and Bulgaria (Poryazov, 1975a). These isolates were considered to be more virulent than race 2 of Patel and Walker (1965). However, studies made by Schroth et. al. (1971) showed that there are possibly many strains of Ps. syringae pv. phaseolicola which vary in virulence. Neither race 1 nor race 2 isolates were homogeneous with respect to virulence when tested on leaves of certain bean cultivars. They considered separating isolates of Ps. syringae pv. phaseolicola into race 1 and 2 on the basis of their reaction on 'Red Mexican UI 3' as only separating strains of the bacteria with different

degrees of virulence. Later, Szarka and Velich (1979) made similar observations and concluded that isolate classification into race 1 and 2 only amounts to classifying isolates with increasing pathogenicity.

Improvement and development of bean cultivars with stable resistance is one of the strategies used by the Centro Internacional de Agricultura Tropical (CIAT) to control bacterial diseases and increase bean yields. The occurrence of strains of a bacterial pathogen is a major consideration in breeding programs seeking to obtain stable disease resistance in their cultivars. This is because new strains are continually being produced by mutation and makes it essential to routinely characterize them. Besides, the interaction of strains of Ps. syringae pv. phaseolicola as showed by Omer and Wood (1969) indicate that, the use of indiscriminate mixture of weak and virulent strains may affect disease expression and result in erroneous conclusions.

The present study was conducted to investigate the extent of variation in pathogenicity

and virulence among isolates of Ps. syringae pv. phaseolicola collected from Colombia, so that the information can be used in evaluation of bean germplasm and breeding for disease resistance at CIAT; and to compare variation in virulence of the isolates classified into the two race groups, with respect to different bean cultivars.

7. MATERIALS AND METHODS.

7:1 Areas of origin of Isolates of Pseudomonas syringae pv. phaseolicola (Burk. 1926)Young, Dye and Wilkie, 1978.

Thirty isolates of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie, 1978 collected from Palmira, Pasto, Popayan and Tenerife, bean growing regions of Colombia were used in this study. In addition, race 1 and 2 received from Dr. D.J. Hagedorn, Department of Plant Pathology, University of Wisconsin, U.S.A; isolate HB 16 reported to be a race 2 (Coyne et. al., 1979) from Dr. M.L. Schuster, Department of Horticulture, University of Nebraska, and isolate OHB from Dr. S.V. Beer, Department of Plant Pathology, Cornell University, U.S.A., were included for comparison (Table 13).

7:2 Isolation and identification of Isolates of Pseudomonas syringae pv. phaseolicola

To isolate bacteria from the host, lesions and parts bordering them were cut from leaves of naturally infected bean plants. They were surface

sterilized by submerging them in 0.5% (w/v) sodium hypochlorite for 2 minutes, rinsed twice with sterile distilled water and then macerated in a test tube containing sterile distilled water. The bacterial suspension was then streaked on nutrient agar (NA) medium (beef extract, 2g; peptone (Difco), 3g; bacto-agar (Difco), 15g; distilled water, 1000 mℓ), and incubated at 27 C in a precision gravity convection incubator (Precision Scientific Co.).

The bacterial cultures were purified by a series of single colony transfers and maintained in NA slants at 4 C and in lyophilized forms. They were identified as Ps. syringae pv phaseolicola by biochemical and pathogenicity tests (Lelliot et. al., 1966).

7:2:1 Biochemical tests

7:2:1:1 Flourescein production

Bacterial cultures, two days old, were streaked on King's B medium (proteose peptone (Difco), 20g; K_2HPO_4 (anhydrous), 15g; Mg $SO_4.7H_2O$, 15g; Difco agar, 15g; glycerol, 15 mℓ; distilled water, 1000 mℓ)

(King et. al., 1954) contained in petri dish. After three days of incubation at 27 C in a Precision gravity convection incubator (Precision Scientific Co.) the plates were examined in a dark room under ultra-violet light (wavelength 375 nm) for a blue-green flourescence. Three plates per isolate were used.

7:2:1:2 Levan production

Levan production was determined on nutrient agar medium containing 5% (W/V) sucrose. Two days old bacterial cultures were streaked on the medium and incubated at 27 C in a Precision gravity convection incubator (Precision Scientific Co.).

7:2:1:3 Oxidase test

Oxidase test was performed using Kovac's method (1965). A piece of Whatman's No. 1 filter paper was soaked in 1% acqueous solution of tetramethyl-P-phenylenediamine dihydrochloride. With the use of a glass rod, 24 hr old bacterial cultures grown on nutrient agar medium, were

thoroughly smeared on to the reagent-impregnated filter papers and observed for any colour change. A minimum of three trials per isolate were performed.

7:2:1:4 Catalase Reaction

Three to four drops of 3% hydrogen peroxide were added on to 24 hr old bacterial cultures. Observations were made for any effervescence or lack of it.

7:2:1:5 Production of Hydrogen Sulphide

Twenty-four hours old bacterial cultures of the isolates were inoculated into nutrient broth (beef extract, 3g; peptone, 5g; distilled water, 1000 mℓ) placed in screw cap test tubes. Strips of lead acetate paper (50 mm by 5 mm) were introduced in the test tubes such that they were held between the caps and test tubes and suspended over the broth without touching it. The tubes were incubated at 27 C in a Precision gravity convection incubator (Precision Scientific Co.) and examined after 3, 7, and 14 days. Three tubes were used for each isolate.

7:2:1:6 Arginine Dihydrolase Test

About five millilitres of arginine medium (Difco bacto-agar, 1.0g; Nacl, 5.0g; K_2HPO_4, 0.3g; phenol red, 0.01g; L(+)-arginine monohydrochloride 10g; agar, 3g; distilled water, 1000 mℓ) were dispensed in 10 mℓ tubes. The medium was inoculated by making a stab using 24 hr old bacterial cultures. After inoculation, the medium was covered with a layer of sterile mineral oil to a depth of 60 mm and the tubes were incubated at 27 C in a Precision gravity convection incubator (Precision Scientific Co.). Observations for change of colour were made after 3, and 6 days.

7:2:2 Pathogenicity Test

7:2:2:1 Inoculum preparation

Inoculum suspensions were prepared in distilled water from 48 hr old cultures grown on yeast dextrose calcium carbonate agar (YDCA) medium (yeast extract (Difco), 10g; dextrose (Difco), 20g; $CaCO_3$, 3.5g; bacto-agar (Difco), 20g; distilled water, 1000 mℓ). The suspensions were then adjusted turbidimetrically using a Spectronic 20

spectrophotometer (Bausch and Lomb Co.) at 600 nm to a concentration of about 5×10^7 colony forming units (CFU) per millilitre of water.

7:2:2:2 Leaf Inoculation

Pathogenicity tests were conducted using the leaf reaction of bean cultivar 'Red kidney' which is susceptible to Pseudomonas syringae pv. phaseolicola (Patel and Walker, 1965). Half-expanded first trifoliate leaves of 14 days old plants were inoculated using the watersoaking method as desribed by Schuster (1953). The abaxial surface of the leaf was sprayed with the bacterial suspension using a De Vilbiss No. 15 atomizer attached to a compressed airline at 15 p.s.i (1.05 kg of force/cm^2) until water soaking appeared. Distilled water was also infiltrated on some plants used as controls. Inoculated plants were kept in the cooler part of the greenhouse where temperatures averaged 22 C.

7:3 Determination of Races of Pseudomanas syringae pv. phaseolicola.

7:3:1 Bean cultivars and Seed Source.

Bean cultivars, 'Seminole', 'Red Mexican UI 3', 'G.N. Nebraska No. 1 Sel 27' and Wisc HBR 72' were used in this study. Cultivar 'Seminole' is susceptible to both race 1 and 2 of Ps. syringae pv. phaseolicola (Hale and Taylor, 1973) whereas, 'Red Mexican UI 3' is resistant to race 1, but susceptible to race 2 (Patel and Walker, 1965). 'G.N. Nebraska No. 1 Sel 27' is reported as resistant (Coyne and Schuster, 1974a) and 'Wisc HBR 72' as highly resistant (Poryazov, 1975a; Coyne et. al., 1979) to both races.

Seeds of these bean cultivars were obtained from the Bean Pathology and the Genetic Resources Section of CIAT. For each cultivar, single plant selection and seed increase were made. Plants (2-3 seedlings/pot) were grown in the greenhouse with an average temperature of 24 C (range: 19 C (night) to 30 C (day) in 15 cm diameter pots containing steam sterilized mixture of soil and sand (5:1; V/V).

7:3:2 Inoculum Preparation

Inoculum for each isolate used in all inoculations was prepared as described in Section 7:2:2:1 above.

7:3:3 Inoculation Procedures

7:3:3:1 Leaf Inoculation

The leaf reaction of the four bean cultivars was determined by using either, young unifoliate leaves of 10 days old plants or half-expanded first trifoliate leaves of 14 days old plants. Inoculation of each isolate was carried out as described in Section 7:2:2:2. above

7:3:3:2 Pod Inoculation

Pod reaction was determined by using the needle inoculation method (Taylor, 1970; Hill et al., 1972; Russel, 1976; Szarka and Velich, 1976). Young, green pods, 10 to 15 cm long, from 50 days old plant were used. A sterile needle was dipped into a bacterial suspension and then inserted at different points a long the pod's length. The latter

were placed in 250 mℓ Erlenmeyer flasks containing a small amount of distilled water and loosely plugged at the mouth with cotton wool to create humid conditions. The contents were left at room temperature (22 - 25 C) for 5 days. Resistance was defined as the appearance of necrotic spot at the point of inoculation.

7:3:3:3 Seed Inoculation

Seed inoculation was made by infiltration under partial vacuum using a modification of Goth's method (1966). Seeds in small muslin bags were submerged in a bacterial suspension in a vacuum type glass dessicator, connected to a vacuum suction pump. They were then exposed to a partial vacuum of 415 mm of mercury for five minutes, after which the negative pressure was released suddenly. The seeds were then air dried at room temperature for three days and then planted in potted soil in the growth chamber where temperatures were maintained at 20 C.

7:4 Determination of Variation in Virulence of isolates of *Pseudomonas syringae* pv. *phaseolicola*.

Pods of cultivars 'seminole', 'G.N. Nebraska No. 1 Sel 27' and 'Wisc HBR 72' were inoculated and incubated in similar conditions as described in Section 7:3:3:2. The diameter of the water soaked spot around the point of inoculation was measured with the use of the Stereozoom-7 dissecting microscope (Bausch and Lomb Co.) and was considered to express the virulence of the isolates. A completely randomized design with 5 replicates per treatment (pods x isolate) was used. Four to six readings per pod were made.

8 RESULTS

8:1 Isolate Identification

8:1:1 Biochemical Tests

A summary of the results of biochemical tests obtained, using the 30 bacterial isolates is presented on Table 13.

8:1:1:1 Flourescein Production

All isolates tested produced an ultra-violet blue-green flourescent pigment after three days of incubation.

8:1:1:2 Levan Formation

Colony growth on nutrient agar with 5% sucrose was heavy, mucoid and convex, which was an indication of levan formation. Although all isolates exhibited this character, there was variation in the amount of growth among the isolates.

8:1:1:3 Oxidase Test

There was no colour change on the filter papers impregnated with 1% tetramethyl-p-

phenylenediamine dihydrochloride after smearing them with cultures of all bacterial isolates, indicating negative tests.

8:1:1:4 Catalase Reaction

Addition of hydrogen peroxide on the 24 hr old bacterial cultures of all isolates was accompanied with an effervescence which lasted for two to three minutes. The appearance of a gas was considered a positive catalase test.

8:1:1:5 Production of Hydrogen Sulphide

There was no colour change of the lead acetate paper suspended over the inoculated nutrient both in the test tubes, for all the isolates. This indicated that, there was no production of hydrogen sulphide, which if present would have blackened the lead acetate paper.

8:1:1:6 Arginine Dihydrolase Test

There was no colour change with any of the isolates, stab inoculated in the arginine medium and incubated under anaerobic condition, for six

days. This implied a negative test for arginine dihydrolase.

8:1:2 Pathogenicity Test

All the isolates were pathogenic to the bean cultivar 'Red Kidney'. A characteristic water-soaking spot at the site of inoculation appeared five to seven days after inoculation, and some plants exhibited systemic chlorosis.

8:2 Determination of Races

Race determination was performed by inoculation of individual isolates on leaves and excised pods of the bean cultivar 'Red Mexican UI 3', which is resistant to race 1 but susceptible to race 2 (Patel and Walker, 1965). Cultivars, 'Seminole', 'G.N. Nebraska No. 1 Sel 27' and 'Wisc HBR 72' were also included. A large amount of watersoaking at the site of inoculation indicated a susceptible reaction. Systemic chlorosis caused by toxin translocation was noted. Plants were considered resistant if they showed brown necrotic lesions with some traces of watersoaking at the site of inoculation. This is

Table 13: Reaction of 30 bacterial isolates and expected reactions of *Pseudomonas syringae* pv. *phaseolicola* (Burk. 1926) Young, Dye and Wilkie, 1978 to some biochemical tests.

Test	Reaction of the 30 isolates	Expected reaction of *Ps. syringae* pv. *phaseolicola*
Fluorescent pigment on King's Medium B.	+	+
Levan production	+	+
Arginine dihydrolase	-	-
Production of H_2S from nutrient broth	-	-
Oxidase reaction	-	-
Catalase reaction	+	+

+ = Positive reaction

- = Negative reaction

the type of resistance referred to as 'tolerance' by Patel and Walker (1965). A highly resistant reaction was one where plants showed a brown necrotic (hypersensitive) reaction on the point of inoculation without any watersoaking. Seed inoculation by partial vacuum method was also used for comparison.

All isolates except CBP 176 from Palmira were pathogenic to cultivar 'Seminole'. Large amount of watersoaking appeared on inoculated leaves 5-7 days after inoculation and some of the plants developed systemic chlorosis. The isolates that induced brown necrotic lesions on 'Red Mexican UI 3' were classified as race 1, but those that incited watersoaking (Plate 12), were regarded as race 2. Twenty Pasto isolates were classified as race 2, whereas, 7 isolates from Popayán, 1 from Palmira and 2 from Tenerife as race 1 (Table 15). Inoculations of unifoliate and first trifoliate leaves gave similar results. However, less amount of water-soaking was observed with the trifoliate leaves than the corresponding unifoliate leaves. Corresponding pod inoculation gave similar results of race characterization (Plate 13). However, isolate CBP 172,

showed to be race 1 by plant inoculation but race 2 by pod inoculation.

The line 'G.N. Nebraska No. 1 Sel 27' was resistant to all race 1 isolates. However, some isolates (PPP 7, PPP 18, PPP 20) from Pasto incited a susceptible watersoaking reaction without any systemic chlorosis. The line "Wisc HBR 72' showed the highest degree of resistance for leaf reaction to all isolates tested. However, the line gave a susceptible pod reaction to all isolates, suggesting that genetic factors governing its leaf and pod resistance are different and independent.

Most of the seeds of the cultivar 'Seminole' inoculated by infiltration under partial vacuum did not germinate whereas, the control inoculated with distilled water germinated and plants grew normally. Cultivar 'Red Mexican UI 3' was resistant to race 1 isolates. Most of the race 2 isolates incited watersoaked lesions on cotyledons, stems and leaves. Infected plants were stunted and some of them showed systemic chlorosis.

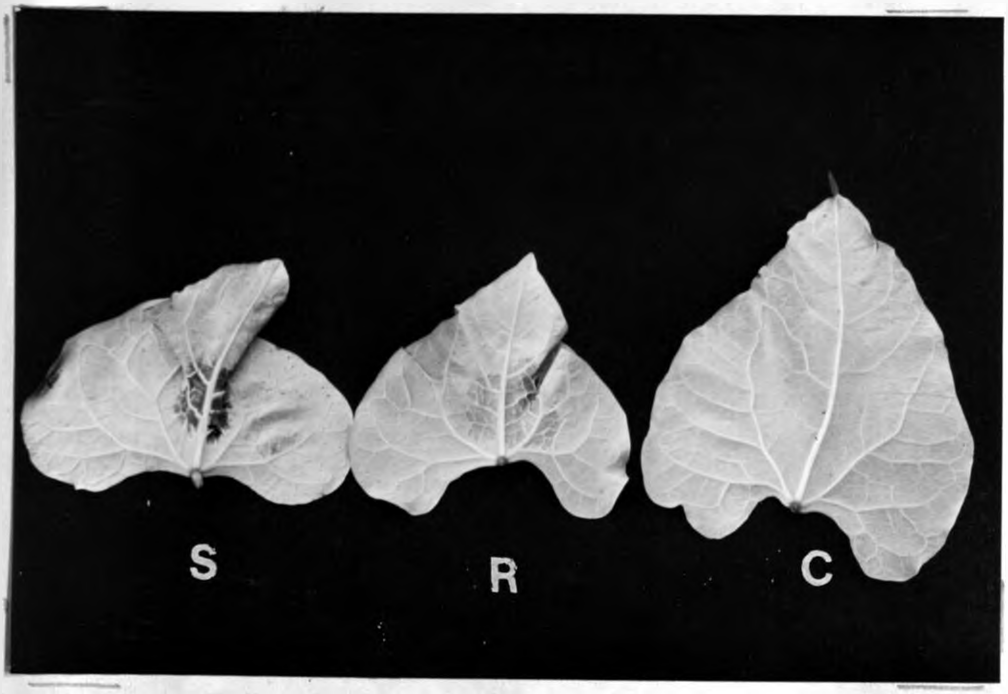


Plate 12: Primary leaves of bean (Phaseolus vulgaris L.) cultivar 'Red Mexican UI 3' showing susceptible (S) and resistant (R) reactions, and the control (C), ten days after inoculation with isolates PPP 18 (race 2), PC-4 (race 1) of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye, and Wilkie, 1978 and distilled water respectively.

Plate 13: Resistant (cv. Red Mexican UI 3') and susceptible (cv. Seminole) pod reactions, five days after inoculation with isolates PC-4 (race 1) and PPP 18 (race 2) respectively of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie, 1978.

Table 14: Race determination of 30 isolates of *Pseudomonas syringae* pv. *phaseolicola* (Burk. 1926) Young, Dye and Wilkie, 1978 from Colombia based on their pathogenicity to bean (*Phaseolus vulgaris* L.) cultivar 'Red Mexican UI-3'[a].

Isolate	Area of origin	Host	Race determination	
			Leaf	Pod
CBP-177, PC-2	Popayan	*P. coccineus*	1	1
PC-3, PC-4, PC-5, PC-6	Popayan	*P. coccineus*	1	-[b]
CBP-178	Popayan	*P. vulgaris*	1	1
CBP-172	Tenerife	*P. vulgaris*	1	2
CBP-173	Tenerife	*P. vulgaris*	1	1
CBP-176	Palmira	*P. vulgaris*	1	1
PPP-1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22	Pasto	*P. vulgaris*	2	2

a = Cultivar 'Red Mexican UI 3' is resistant to race 1 but susceptible to race 2 (Patel and Walker, 1965).

b Not tested.

8:3 Determination of Variation in Virulence

To determine the variation in virulence among the Colombian isolates, pods of cultivar 'Seminole' were used. Pods of the lines 'G.N. Nebraska No. 1 Sel 27' and 'Wisc HBR 72' were only included for comparison. The mean diameter and range of the watersoaked spots observed for each cultivar are presented on Table 15. A number of the Colombian isolates were found to induce bigger watersoaked spots and were therefore considered more virulent than the standard isolates included for comparison (Table 15 and 16). The line 'Wisc HBR 72' (with high foliar resistance) showed a susceptible pod reaction (Plate 14) similar to that of 'Seminole' (with susceptible foliar reaction). The frequency distribution of the isolates on the basis of the mean diameter of watersoaked spots of the 4 lines is shown on Figure 7. Isolate PPP 20 had the highest mean diameter value, followed by PPP 18, PPP 16 and PPP 22. The order (high to low) for most of the isolates, in diameter of the watersoaked spots was similar in the lines 'G.N. Nebraska No. 1 Sel 27', 'Wisc HBR 72' and 'Seminole'. This was

reflected in the high correlation coefficients (r = 0.87) obtained between these lines with respect to the means of the diameter of watersoaked spots (Table 17). A lower (r = 0.53, 0.57, 0.53 respectively) value was observed between the 3 lines and the cultivar 'Red Mexican UI 3' (Table 17).

On the basis of the diameter of the water-soaked spots, the virulence of the isolates varied in two ways. Some of the isolates were more (or less) virulent than others on the 3 lines; isolate PPP 20 was consistently more virulent than isolate PPP 12 (Figure 8). This type of variation was observed with most of the isolates. But virulence of other isolates depended on the cultivar; isolate PPP 22 was less virulent than isolate PPP 18 on the line 'G.N. Nebraska No. 1 Sel 27' but was more virulent on 'Wisc HBR 72'. The two types of variation were observed both within and between the isolates of the race 2 groups identified.

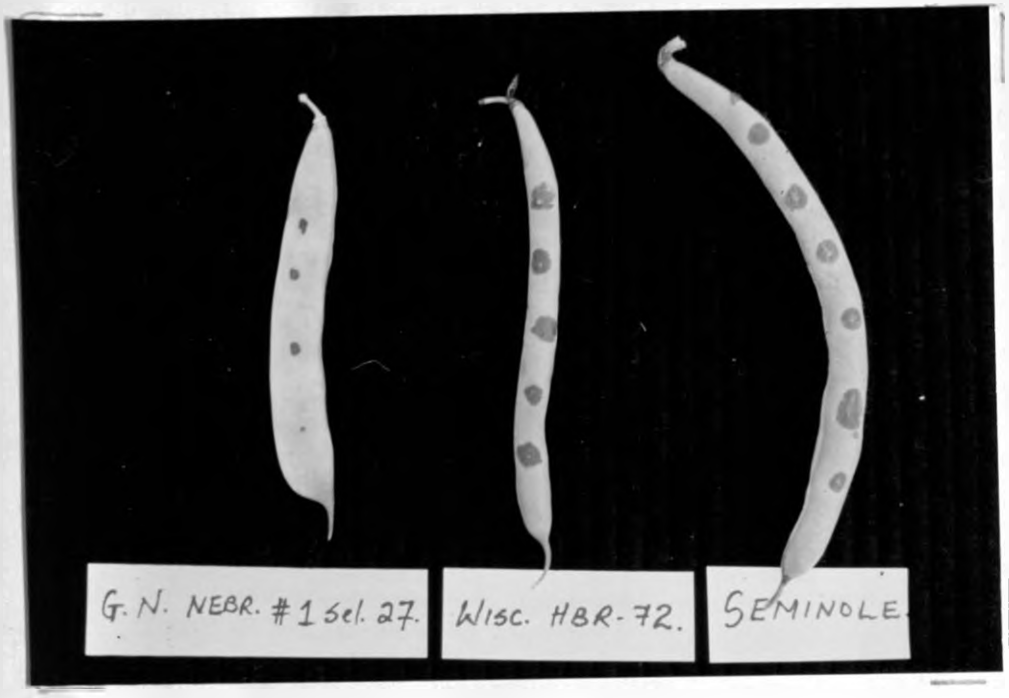


Plate 14: Watersoaked spots induced on the pods of bean (Phaseolus vulgaris L.) lines 'G.N. Nebraska No. 1 Sel 27' and 'Wisc HBR 72', and cultivar 'Seminole', five days after inoculation with isolate PPP-18 (race 2) of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young,Dye and Wilkie, 1978.

Table 15: Average diameter and range (mm) of water-soaked spots induced by thirty isolates of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie, 1978 on pods of 2 bean (Phaseolus vulgaris L.) lines and 1 cultivar tested.

Line/Cultivar	Mean diameter of watersoaked spots (mm)	Range of the diameter of water-soaked spots (mm)
'G.N. Nebraska No. 1 Sel. 27'	1.9	0.4 - 2.8
'Seminole'	2.8	0.5 - 4.8
'Wisc HBR 72'	3.1	0.5 - 5.3

Table 16: Average diameter (mm) of the watersoaked spots induced by standard isolates of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie, 1978 on pods of 2 bean (Phaseolus vulgaris L. lines and 1 cultivar.

	Average diameter (mm) of watersoaked spots			
	Standard isolates			
Line/Cultivar	Race 1[b]	Race 2[b]	HB-16(Race 2)	OHB
'G.N. Nebraska No. 1 Sel 27'	0.6	1.9	1.6	2.0
'Seminole'	1.0	1.9	2.0	1.9
'Wisc HBR 72'	0.9	3.0	2.0	2.7

[a]Average of no less than 20 values.

[b]Known isolates of race 1 and 2 supplied by Dr. J.D. Hagedorn, University of Wisconsin, USA.

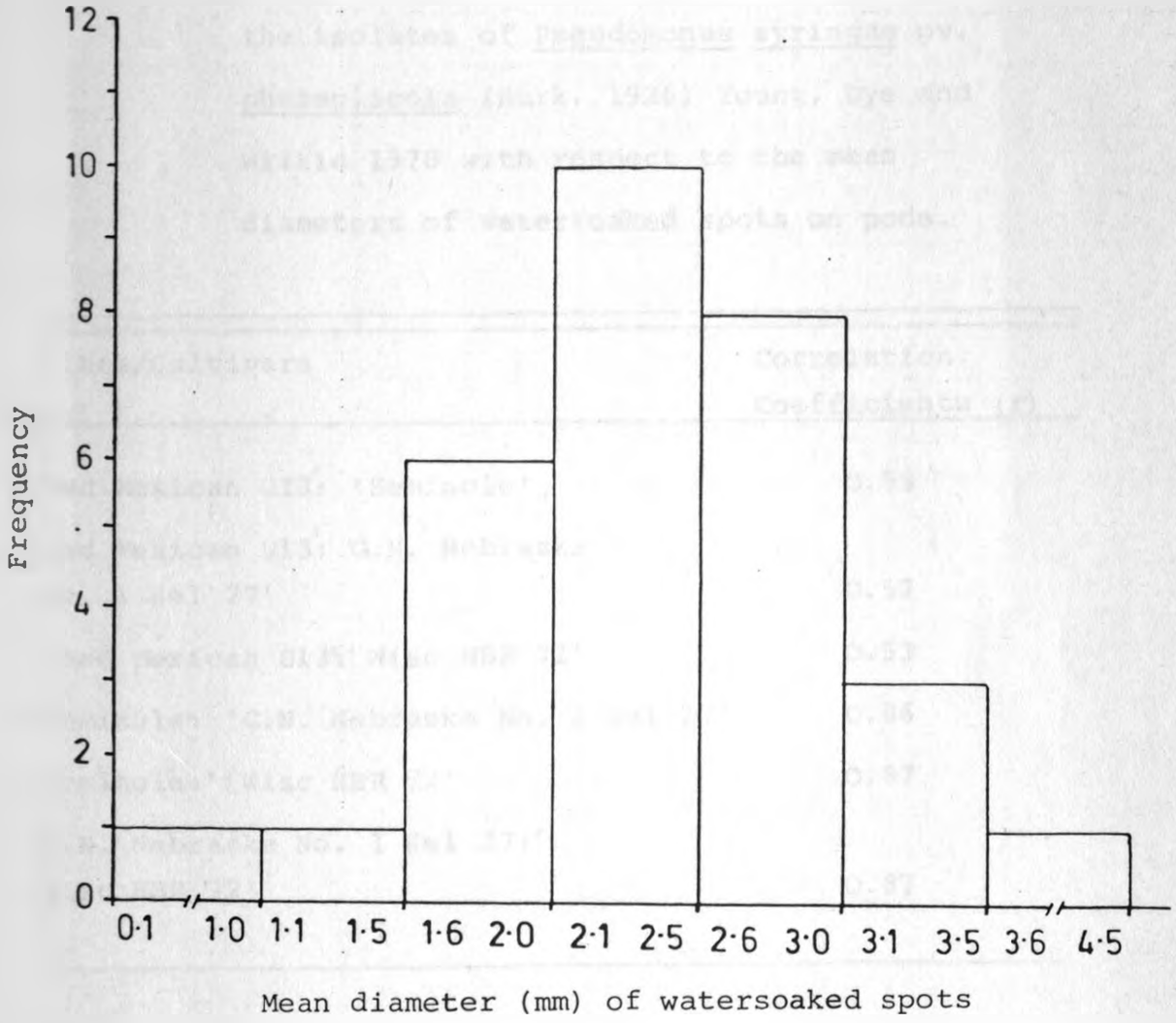


Figure 7. Histogram showing frequency distribution of 30 isolates of Pseudomonas syringae pv. phaseolicola (Burk.,1926), Young, Dye and Wilkie 1978 in relation to diameter of watersoaked spots caused on 4 bean (Phaseolus vulgaris L.) cultivars/lines.

Table 17: The correlation coefficients (r) between 4 bean (Phaseolus vulgaris L.) lines/cultivars on the decreasing order of virulence of the isolates of Pseudomonas syringae pv. phaseolicola (Burk. 1926) Young, Dye and Wilkie 1978 with respect to the mean diameters of watersoaked spots on pods.

Lines/Cultivars	Correlation Coefficients (r)
'Red Mexican UI3': 'Seminole'	0.53
'Red Mexican Ul3': 'G.N. Nebraska No. 1 Sel 27'	0.57
'Red Mexican Ul3':'Wisc HBR 72'	0.53
'Seminole': 'G.N. Nebraska No. 1 Sel 27'	0.86
'Seminole:''Wisc HBR 72'	0.87
'G.N. Nebraska No. 1 Sel 27:' 'Wisc HBR 72'	0.87

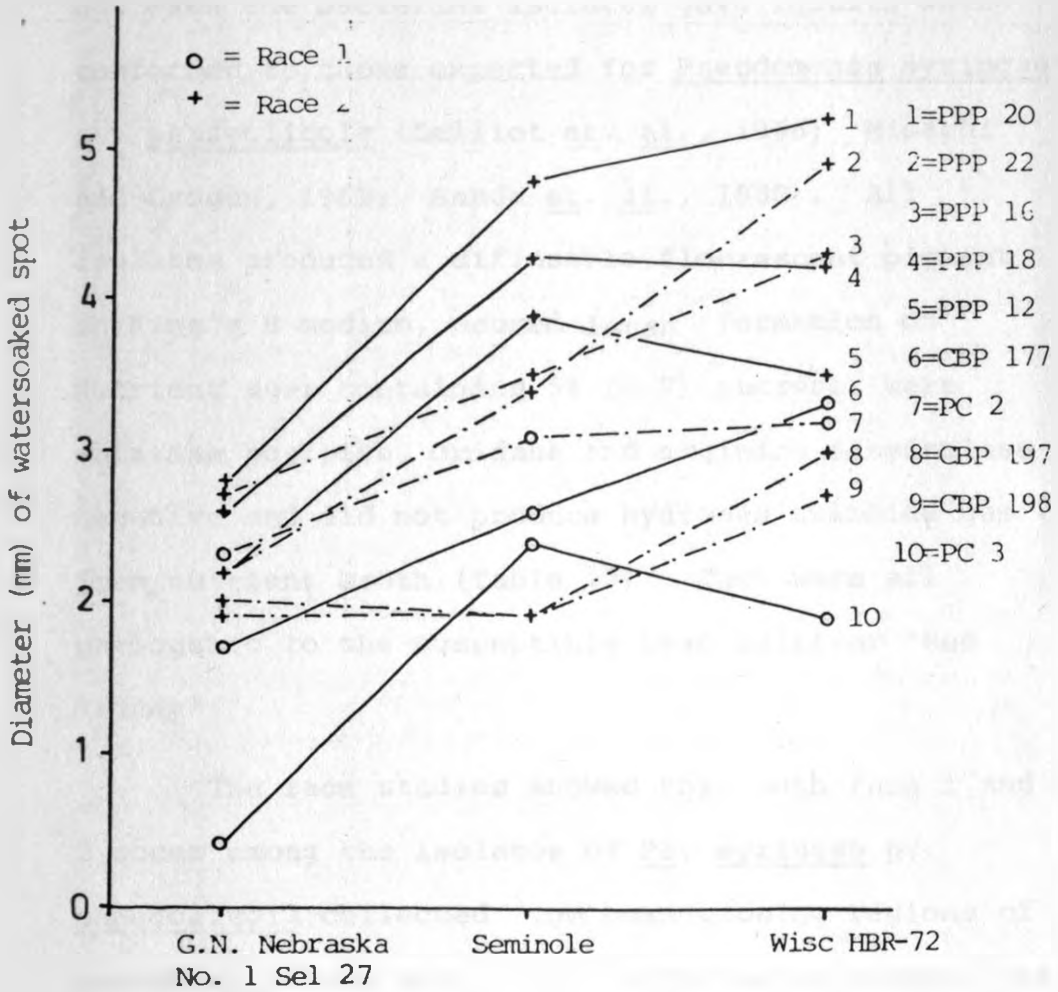


Figure 8. Variation in diameter of watersoaked spots on pods of 3 bean (Phaseolus vulgaris L.) lines inoculated with isolates of P. syringae pv phaseolicola (Burk. 1926) Young, Dye and Wilkie 1973.

9. DISCUSSION

The biochemical and pathogenicity tests carried out with the bacterial isolates gave results which conformed to those expected for Pseudomonas syringae pv. phaseolicola (Lelliot et. al., 1966; Misaghi and Grogan, 1969; Sands et. al., 1980). All isolates produced a diffusable flourescent pigment on King's B medium, caused levan formation on nutrient agar containing 5% (W/V) sucrose, were catalase positive, oxidase and arginine dihydrolase negative and did not produce hydrogen sulphide gas from nutrient broth (Table 13). They were all pathogenic to the susceptible bean cultivar 'Red Kidney'.

The race studies showed that both race 1 and 2 occur among the isolates of Ps. syringae pv. phaseolicola collected from bean growing regions of Colombia. There was a close correlation between the leaf and pod inoculation methods used to determine races on cultivar 'Red Mexican UI 3'. This was in agreement with results obtained by Hale and Taylor (1973), suggesting that leaves and pods of 'Red Mexican UI 3' react in similar way to isolates of race 1 and 2. However, the designation of isolate

CBP 172 as race 1 by leaf reaction, but race 2 by pod reaction showed an exception which was probably due to the intermediary character of the isolate's virulence, a condition not uncommon with bacterial pathogens (Dye and Starr, 1965; Hale and Taylor, 1973).

The bean line 'G.N. Nebraska No. 1 Sel. 27' was known from previous studies to show a resistant leaf reaction to both race 1 and race 2 (Coyne et. al., 1971; Coyne and Schuster, 1979). However, the watersoaking reaction incited by some of the strains from Pasto used in the present study suggested that they were able to overcome some of the genes controlling resistance to race 2. Similar observations have also been reported by Poryazov (1975a) and Coyne et. al. (1979). The 'breakdown' of resistance of 'G.N. Nebraska No. 1 Sel 27' suggests that either more virulent strains than race 2 did exist but remained undetected or mutation occurred which gave rise to more virulent strains. Emergence of new virulent strains can be attributed to: (a) genetic changes in the pathogen that has long occupied a given area, (b) introduction of an old organism to a new area or (c)changes in the cropping systems

which affect the ecological niche of the pathogen (Schuster and Coyne, 1975). Willhausen (1957) and Lincoln (1940) found that the virulence of a bacterial population increased through mutation and selection during passage through a resistant host. Anyone, or a combination of the above factors may probably have been responsible for the occurrence of the virulent strains in Colombia.

The bean line 'Wisc HBR 72', was highly resistant as expected to both race 1 and 2 and reacted to give a hypersensitive leaf reaction. This line, which carries genes for resistance from 'Red Mexican UI 3' and 'P.I. 150414' (Georgieva and Poryazov, 1980) had a susceptible pod reaction. In crosses between 'G.N. Nebraska No. 1 Sel 27', 'P.I. 150414' (resistant to both race 1 and 2) and 'Gallatin 50' (susceptible to both races), Coyne et. al., (1971), showed that systemic chlorosis and watersoaked leaf reaction were controlled by different major genes with coupling linkages involved. Later, the pods (Hill et. al., 1972) and the wilting reaction of primary leaves (Coyne and Schuster, 1974b) were also found to be controlled by major genes independent of each other and of the genes

controlling the other reactions. This probably explains the differences observed in the leaf and pod reactions of the line 'Wisc HBR 72' and suggests that only its leaf reaction has received attention in the improvement of the line's resistance. The independent reactions of the bean plant components stresses the importance of evaluating and selecting plants with both leaf and pod resistance. Seed infection or contamination with Ps. syringae pv. phaseolicola may occur through infected pods and play a significant role in the transmission of the bacteria through seed (Neergaard, 1977; Taylor et. al., 1978; Weber, 1973, Zaumeyer and Thomas, 1957).

According to Goth (1966) inoculation of seeds by partial vacuum was superior than soaking them in a bacterial suspension. He however, found that heavily infected seed disintegrated in the soil. Lack of seed germination observed with 'Seminole' and the poor germination caused by some race 2 strains on seeds of the cultivar 'Red Mexican UI 3' may have been due to similar effects. The tendency for young bean plants to be more

susceptible to bacterial pathogens (Walker and Patel, 1965) may have also played a role.

Excised plants parts have been used in plant bacteriology to study pathogenicity (Perlasca, 1960; Ark and Thompson, 1961; Klement and Lovrekovitch, 1961). Starr and Dye (1965), Russel (1976) and Szarka and Velich (1976), were able to characterize the virulence of Ps. syringae pv. phaseolicola isolates with the use of excised bean pods under specified environmental conditions. The use of pods has an advantage over the whole plants because the latter are tedious and inconvenient to use in large numbers and have variable infectivity such that large numbers would be needed to ensure statistical reliability.

Virulence determination in the present study showed that a number of the Colombian isolates were more virulent than the standard isolates used for comparison (Table 15 and 16). On the basis of the diameters of watersoaked spots, the lines 'G.N. Nebraska No. 1 Sel 27' and 'Wisc HBR 72' and cultivar 'Seminole' gave a close correlation in rating isolates in their order of virulence (Table 17).

However, reaction of some of the isolates appeared to be cultivar dependent (Figure 8) as was expressed by the statistically significant (P = 0.05) interaction obtained between isolates and cultivars (Appendix 4). The relatively lower correlation coefficients between 'Red Mexican UI 3' and the rest of the lines is due to the fact that, the former was resistant to race 1 isolates. It may not be true to assume that virulence as expressed in excised pods is the same as would be expressed in a growing plant. Rudolf (personal communication) did not observe any correlation between resistance of pods and leaves. Nevertheless, the isolates PPP 20 and PPP 18 found to incite a susceptible leaf reaction on 'G.N. Nebraska No. 1 Sel 27' were also found to be among the most virulent isolates on all the 4 bean lines based on pod reaction.

Results obtained in this study establish the occurrence of the two race groups in Colombia. However, separation of the isolates into the two races based on the leaf and/or pod reaction of 'Red Mexican UI 3' did not demonstrate the whole range of pathogenic variation of the isolates. Pathogenic variation was expressed better by the use of

excised bean pods. It was possible to show that, isolates that belonged to race 2 varied substantially in virulence. For example, the mean diameter of watersoaked spots caused on pods of cultivar 'Seminole' by race 2 (standard) was 1.9 mm, whereas, the highest value obtained on the same cultivar by isolate PPP 20 (race 2 by identification) was 4.8 mm (Table 15 and 16). It is thus apparent that development and standardization of the excised pod method complemented with the use of the leaf reaction, should adequately characterize the pathogenic variability of Ps. syringae pv. phaseolicola. This information is essential in breeding for resistance for halo blight.

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Appendix 1. Analysis of variance for conidial length, width and number of septa for ten isolates of Isariopsis griseola Sacc.

Source	df	Ms			F-Value		
		Length	Width	Number of septa	Length	Width	Number of septa
Isolate	9	1956.89	22.54	9.42	36.82**	58.65**	12.39**
Error	990	53.14	0.34	0.75			
Total	999						

** Significant at 1% level.

Appendix 2. Analysis of variance for sporulation (number of conidia x $10^4/cm^2$) of 7 isolates of Isariopsis griseola Sacc. grown at 19 and 24 C on V-8 juice agar in the dark.

Source	df	Ms	F - Value
Temperature	1	9024.40	143.02**
Isolate	6	4510.22	71.48**
Temperature x Isolate	6	1049.68	16.63**
Error	154	63.10	
Total	167		

** Significant at 1% level.

Appendix 3: Analysis of variance for sporulation (number of conidia/cm^2) of four isolates of Isariopsis griseola Sacc. after 7, 10 and 13 days of incubation on V-8 juice agar in the dark at 19 and 24 C.

Source	df	Ms	F value
Days (D)	2	26914.83	41.31**
Temperature (T)	1	27123.15	41.63**
Isolate (I)	3	29174.67	44.78**
D x T	2	1823.66	2.80^{ns}
D x I	6	1801.56	2.77**
T x I	3	9442.46	14.49**
D x T x I	6	1139.95	1.75^{ns}
Error	264	651.55	
Total	287		

ns not significant
* significant at 5% level
** significant at 1% level

Appendix 4: Analysis of variance for the diameters of watersoaked lesions on green pods of bean (Phaseolus vulgaris L.) cultivars induced by isolates of Pseudomonas syringae pv. phaseolicola.

Source	df	Ms	F-value
Variety	3	433.63	985.52**
Isolate	30	52.51	119.34**
Variety x Isolate	90	5.41	12.30**
Error	2330		
Total	2453		

** Signififant at 1% level.