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ANGULAR LEAF SPOT (Isariopsis griseola Sacc.)

OF RED KIDNEY BEANS IN MICHIGAN

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# ANGULAR LEAF SPOT (*Isariopsis griseola* Sacc.) OF RED KIDNEY BEANS IN MICHIGAN

By

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ABSTRACT

ANGULAR LEAF SPOT (ISARIOPSIS GRISEOLA SACC.)
OF RED KIDNEY BEANS IN MICHIGAN

BY

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Angular leaf spot (ALS), caused by Isariopsis griseola, severely attacked seed fields of dark red kidney bean cultivars during the 1982 and 1983 growing seasons in Michigan. Studies indicate that the pathogen is seed-transmitted. Chemical seed treatments with benomyl or captan-zineb were effective in controlling the pathogen. The pathogen overwinters at least one season under Michigan conditions, however, survivability is greater above than below soil surface. Pathogenic variation was not observed among Michigan isolates of I. griseola but was observed among isolates from different countries. Resistance to the disease was associated mainly with navy, black and pinto bean types, while susceptibility was seen with red kidney and cranberry bean types. Strategies for control of ALS in Michigan include the planting of pathogen-free seed and/or chemical seed treatment, plowing down plant debris after harvest, crop rotation, and incorporation of disease resistance into commercial red kidney bean cultivars.

To my wife

Liliana

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

# I. INTRODUCTION

Angular leaf spot (ALS), caused by the fungus Isariopsis griseola Sacc., is a major disease of dry edible beans (Phaseolus vulgaris L.) in tropical and subtropical regions of the world. The disease has been reported only sporadically in the United States and is generally not considered a disease problem. However, severe outbreaks of ALS were experienced by several Michigan seed growers of the Montcalm dark red kidney cultivar during the 1982 and 1983 growing seasons (37). The infected fields were located mainly in the North Eastern lower peninsula of Michigan, near Alpena and Rogers City.

Typical disease symptoms were observed on the leaves, pods, stems and branches of infected plants. Leaf lesions exhibited a typical angular shape and were limited by the veins; lesions were often surrounded by a chlorotic halo. Dark gray to black synnemata bearing spores were present on lesions on the lower leaf surface (Figure 1). Pod lesions consisted of large, circular, red-purple spots surrounded by a dark border. Synnemata and spores were also present on the surface of pod lesions. Severe premature defoliation was frequently noted in heavily infected plants.

The ALS disease had not been previously reported in Michigan, the largest dry bean growing state in the U.S. Thus, essentially nothing was known about the pathogen and its behavior under Michigan conditions. The present study was conducted in order to obtain information relative to:

a) Determine the reaction of commercially-grown dry bean varieties to ALS.

b) Determine the ability of the ALS pathogen to overwinter in infected plant debris.

c) Determine whether ALS is seed transmitted.

d) Study possible pathogenic variation in Michigan isolates of ALS.

# II. LITERATURE REVIEW

## 2.1 The pathogen

*Isariopsis griseola* Sacc. is an imperfect fungus which belongs to the order Moniliales, family Stilbaceae (3). The reproductive structures of the fungus are formed in lesions under favorable conditions of high humidity. Miles in 1917 (32) described such structures as coremia indicating that they are columnar, formed of rather dark brownish hyphae or conidiophores in number of 8 to 40. He also indicated that the average thickness of the coremium is 20 to 30 µ, and the length of the coremial hyphae about 200 µ. Golato and Meossi (25) reported the length to be 80 to 220 µ, while Hocking (27) indicated a range between 94 to 163 µ and Ferraz, in Schwartz and Galvez, indicated the length as 500 µ (23). Ellis (21) provided a description of the grouping of conidiophores to form a synnemata. Conidia are borne on the smooth tips of columnar hyphae, are light gray in color, cylindrical to spindleform, and slightly curved. Conidial dimensions have been reported as 43-68 µ by 3-8 µ (25,27,32).

The causal agent of angular leaf spot was first described by Saccardo in Italy in 1878 (Saccardo, 1878 in Zaumeyer and Thomas, 1957) who named it as *Isariopsis griseola* Sacc. The fungus is also synonymous with *Phaeoisariopsis griseola* (Sacc.) Ferr., *Graphium laxum* Ell., *Isariopsis laxa* (Ell.) Sacc., *Cercospora columnare* Ell. and Ev. (57), *Lindaumyces griseola* Gonz. Frag. (10), *Arthrobotryum puttemansii* Henn., and *Cercospora sthulmanni* Henn (Ferraz, in Schwartz and Galvez, 1980).

## 2.2 Isolation, Growth and Inoculation Techniques

The general method to isolate fungi has consisted of washing the infected tissue and then small portions of the tissue are disinfected with alcohol,

mercuric chloride, sodium hypochlorite, or other disinfestant, then transferred on agar slants or plates. This method does not work well for isolation of Isariopsis griseola since this pathogen grows so slowly that other contaminants suppress its growth (12,51).

Different alternate methods have been tried for isolating I. griseola which include: inducing sporulation by incubating infected, surface-disinfested stems for five days at 27 C and high humidity conditions. The stem pieces are then rolled over the surface of agar slants and the slants incubated at 27 C for 21 days (Brock, 1951). Llanos in 1956 (30) and Diaz et al in 1965 (20) reported simple isolation of the pathogen by removing the spores from infected tissue with a fine needle-tip and then transferring to a plate.

Santos-Filho in 1976 (38) obtained excellent results by removing spores from lesions with a small piece of water-agar on the tip of a needle and then transferring that piece onto the surface of a PDA plate. He also obtained good results by inducing sporulation on artificially inoculated leaves, and then preparing a suspension of spores from the lesions, which then was poured on agar plates.

Cardona-Alvarez in 1956 (10) tested 19 solid and liquid media for fungal growth and reported poor growth on most. The colonies were about 15-20 mm in diameter after 25 days of incubation in most media. Growth on liquid media was very sparse. Maximum fungal growth occurred on honey-peptone-agar at pH 5 and on PDA plus bean leaf juice, pH 6.3. Llanos (30) indicated that sporulation was abundant on PDA.

Diaz and De Armas in 1964 (19) found that the Isariopsis griseola colonies reached almost 68 mm in diameter after six days when the fungus was grown on PDA containing an exudate of black bean seeds. Silvera in 1967 (46)

obtained very good sporulation and growth in eight days when he used a medium containing water extract of bean leaves, glucose and agar.

Alvarez-Ayala in 1979 (1) obtained the highest yield of conidia from cultures grown 9-12 days on V-8 agar at 14-19 C in darkness. Spore germination was 93.5% when Tween 80 1% (w/v) was added, compared to 81.5% in water alone. Higher and lower concentrations of Tween 80 reduced spore germinability. Campos and Fucikousky in 1980 (9) reported excellent growth of I. griseola on V-8 juice agar, with optimum growth at 24 C and maximum sporulation at 16 C.

Cardona-Alvarez in 1956 (10) indicated that the fungus was capable of growing at a range of temperatures between 8 to 28 C. The optimum temperature was 24 C and no growth was observed at 32 C and 36 C. Silvera in 1967 (46) found that coremial development and production of conidia on infected leaves occurred within 24 hours at 25 C.

The most common method of inoculation for I. griseola has been the spraying of a conidial suspension or a combination of spores and mycelial fragments (7). The author atomized spore plus fragment concentrations between 3 and 4 $(10^6)$/ml to upper and lower leaf surfaces of 21 day old plants.

Alvarez-Ayala (1) and Santos-Filho (38) pointed out that a concentration of 2 $(10^4)$ spores/ml appeared to be the most practical for use in greenhouse screening tests.

Inglis et al (29) in 1984 obtained inoculum of I. griseola by drying infected bean leaves at 26-28 C and then pulverizing. Dry inoculum containing 3.0 $(10^6)$ to 4.0 $(10^7)$ conidia/g was dusted onto plants in the field following irrigation and application of a fungicide sticker. Treatments with dry inoculum had significantly higher disease leaf ratings, greater defoliation, and greater yield reduction than treatment inoculated with a suspension of conidia.

## 2.3 The host

Cardona-Alvarez in 1956 (10) reported that Phaseolus lunatus was the only species, other than Phaseolus vulgaris, susceptible to the fungus. P. multiflorus (Brock, 1951), Pisum sativum (Chupp, 1925), Vigna sinensis (Diaz et al, 1965) and P. mungo (Golato and Meossi, 1973) have also been reported as hosts. Abramanof, cited by Cardona-Alvarez, 1956 (10), found soybeans (Glycine max) as a host of Russia in 1931.

Campos in 1979 (8) reported P. coccineus, P. angularis, P. lunatus, P. acutifolius, P. calcaratus as susceptibles but Vigna sinensis, Cajanus cajan, Glycine max, Vicia faba, Medicago sativa, Pisum sativum and Lupinus sp. as resistant.

## 2.4 The disease

### 2.4.1 Host-parasite relations

A complete study of host-parasite relations was done by Cardona-Alvarez in 1956 (10). He determined that penetration occurs through stromata and after three days, the guard and adjoining mesophyll cells were killed. At nine days, the intercellular mycelium became intracellular and fungus stromata developed in the substomatal cavity. At 12 days necrosis was limited by the vascular tissue of the leaf, there was a general collapse of invaded tissue, and stromata were fully developed. Macroscopic symptoms were evident at this time and a heavy sporulation occurred when the infected tissue was exposed to continuous moisture for 48 hours.

### 2.4.2 Symptomatology

Lesions may occur on all plant parts. On leaves, lesions are initially gray or brown and become surrounded by a chlorotic halo. Lesions become necrotic and assume the typical angular shape. Lesions may increase in size,

coalesce and cause partial necrosis and yellowing of leaves followed by premature defoliation (Figure 1). Infected pods exhibit oval to circular spots with reddish brown centers surrounded by darker colored borders (Figure 2). Lesions on stems and branches appear as elongated brown areas and synnemata and spores are also produced under favorable conditions of high humidity (10,23).

Several environmental factors affect disease development and spread.

2.4.3 Relative humidity

Cardona-Alvarez (10) concluded that a minimum (3 hr) exposure to moist conditions was required for normal infection; longer periods of moisture increased disease intensity, however. A separate report in 1980 (48) found that a minimum 24 hr post inoculation period of 100% relative humidity was necessary for disease initiation, with maximum infection following a 96 hr period. Infection did not occur with 0, 6, and 12 hr post inoculation moist periods. The authors obtained maximum infection with 24 hours pre- and 96 hours post-inoculation moist periods. In contrast, Cardona-Alvarez and Walker (12) found no difference in disease development between plants with and without preinoculation treatment for 1 to 6 days.

Sindhan and Bose (48) in 1980 reported an optimum range of 90.2 and 100% RH for disease initiation and development; infection did not occur below 85.7% RH. Also, Campos (8) obtained highest infection rates when inoculated plants were incubated 56 hr at 95% relative humidity.

2.4.4 Temperature

The effect of temperature on infection and disease development has been studied by Cardona-Alvarez (10) who noted that infection and disease development occur over a wide range of temperature (16 to 28 C) with an optimum at

Figure 1. Severe symptoms of angular leaf spot on Montcalm kidney bean plants in the field.

Figure 2. Lesions caused by angular leaf spot on pods of the Montcalm cultivar.

24 C; no infection occurred at 32 C. An optimum of 24 C for initiation and development of disease was also found by Sindhan and Bose in 1980 (48) but they indicated that infection decreased dramatically below and above these temperatures. In 1984 Inglis and Hagedorn found that infection occurred most rapidly at 24 C followed by 20 C, and was lowest at 16 and 28 C. Lesion expansion was most rapid at 24 C and lowest at 16 C. Leaf chlorosis was greater and days to defoliation fewer when infection occurred at 16, 20, 24 C and the disease was allowed to develop at 20, 24 and 28 C than when the same temperatures were maintained throughout infection and disease development. Cardona-Alvarez had earlier reported that incubation period was temperature-dependent and that the period of defoliation was longer at 16 C than at 20, 24, and 28 C.

Thus it appears that many workers agree that moisture is the most critical factor in production of epidemics. Cardona-Alvarez (10) indicated that once penetration has occurred, disease development and stromata formation proceed in relatively dry atmospheres. However, he indicated that high humidity is required for coremial formation and abundant sporulation.

Sindhan and Bose in 1980 (49) also indicated that precipitation and relative humidity are more important than temperature for development of the disease in the field.

On the other hand, once spores are formed on infected parts, low humidity favors the release and dissemination of spores (10). The author concluded that the environment most favorable for epidemic development of the disease included moderate temperatures, continuous water on infected foliage and stems or high humidity for 48 hours or more, alternating with periods of low humidity. The fungus was disseminated by splashing water or wind-blown soil particles from sporulating lesions by wind currents.

2.4.5 Plant age

Age of plant is known to affect the angular leaf spot disease. For example, Cardona-Alvarez and Walker (12) indicated that plants 10 to 60 days old passed through the usual sequence of spotting, necrosis, chlorosis, and defoliation, regardless of age of the plants at the time of inoculation.

There are few reports on the occurrence of angular leaf spot in the field. In 1966, Cole (14) reported the presence of angular leaf spot on Red Kidney beans in Pennsylvania. Over a two-year period, the disease was first observed late July on the lower leaves. The disease gradually increased until early September, when numerous infected plants were dead. Although amounts of rainfall were less than normal in both years, periods of high humidity did occur during the growing season; fields exposed to the highest humidity conditions were the ones most severely affected. Weaver and Zaumeyer (56) observed two stages of disease development in Maryland, a) severe symptoms were seen on the leaves but not pods in early July, and b) severe infection of leaves and pods as well as severe defoliation when planting was delayed. Costa (15) in Brazil observed angular leaf spot disease symptoms mainly at the end of the vegetative period; however, he recognized that earlier infections could cause severe damage to the foliage.

The effect of plant age and genotype of I. griseola infection was studied by Santos-Filho et al (39). The authors inoculated plants of 30 to 75 days old; all plants were then incubated 48 hr at 20-24 C and 100% relative humidity. Disease was most intense on 30 and 45 day old plants, and yield was similarly decreased dramatically. Seed harvested from plants inoculated when 60 days old weighed less than the plants inoculated at other times, primarily as a consequence of leaf drop during seed maturation. Resistant plants were not affected by inoculation with I. griseola.

Barros *et al* (6) experienced an epidemic of ALS in Colombia in 1955. The high-yielding variety Algarrobo was planted by many farmers, as many as four crops a year without rotation, and row or overhead irrigation was used in the planting during the dry periods. They reported that yields were reduced 40 to 60 percent and concluded that the most critical conditions for the epiphytotic appeared to be a) poor rotation, b) close cropping, c) sufficient humidity from irrigation for infection to take place.

2.4.6 Yield losses

Cardona and Walker (12) indicated that while angular leaf spot is a minor disease in the U.S., the disease occasionally resulted in losses of 50 percent or more in some fields in central Wisconsin in 1954. Hagedorn and Wade (26) reported that the disease was next observed in Wisconsin in 1973, when some fields of Red Kidney beans were found infected. The authors suggested the disease was favored by days of warm, humid weather followed by relatively dry and breezy conditions. Another factor in the appearance of the disease may have been the presence of infected plant debris from previous crops.

Barros *et al* (6) reported a yield reduction of 67% due to *I. griseola* compared with a control which had been treated with a mixture of fungicides (Zineb + Thiram + Copper). Cole (14) reported that yields were 10 to 50% less than expected in Red Kidney beans according to grower reports in Pennsylvania. Yield reductions up to 80% were reported by Schwartz *et al* (43) when the susceptible black variety BAT 394 was inoculated in the field with *I. griseola*. A yield reduction of the same magnitude had been reported in Mexico by Crispin *et al* (17).

Pastor-Corrales *et al* (36) compared yields of susceptible G 2858 beans sprayed and not sprayed with the fungicide Biloxaol (Baycor). All beans

were uniformly exposed to I. griseola infection. Yields were increased 41%, 37%, and 33% when plots were sprayed 26, 40, 54 and 69; 40, 54 and 69; and 26, 40 and 54 days, respectively, after planting.

## 2.5 Resistance

Gardner and Mains (24) tested forty common bean varieties for resistance to angular leaf spot and found that Kentucky Wonder was the most resistant. Brock (7) inoculated 164 bean lines with a suspension of I. griseola spores and mycelial fragments. While no line was immune, a few highly resistant ones were free of defoliation and spore bearing lesions. The highly resistant varieties were Alabama No. 1, Cafe, California Small White, Epicure, Mexico Black, McCaslan, Navy bean, Negro Costa Rica, Scotia, and Rojo Chico. Varieties listed as resistant included Blue Lake, Blue Podded Pole, Doppette, Feijao, Golden Harvest, G 150, Grigaijy, Ideal Market, Long White Marrow, Kentucky Wonder (Brown and White seed), Mulitanho, Ousara, Poroto Arrozchileno, and Preto Brilante. Olave (34) classified as highly resistant the varieties Mexico 11, Mexico 12 and Cauca 27 A. The variety Sanchez was listed as resistant and Sangretoro, Liborino, Lines 133 and 138 as moderately resistant. He pointed out that the last two groups of varieties showed necrotic lesions only. Chupp (13) indicated that no cultivar of bush snap bean is resistant to angular leaf spot in variety test plots.

Singh and Sharma (51) inoculated 40 bean lines during 1972 and 1973 by uniformly spreading dust prepared from infected leaves onto the field at planting time. Lines EC 38921, EC 44621, PLB 148 and Kentucky Wonder were completely free of disease during both years. Silvera (46) evaluated the resistance of 527 varieties of bean and found that 2.6% were highly resistant; 46.6% moderately resistant; 28.1% susceptible; 14.8% highly susceptible

and 7.7% variable. He described the highly resistant varieties as 10 with black seed, 2 reddish-brown and 2 red.

In another study, Santos-Filho (40) reported the variety Caraota 260 as highly resistant; Col. vul. 1470 and V.I. 61 as resistant; and, as moderately resistant, varieties Diacol Nutibara, Red Kidney, Dark Red Kidney, California Small White, Michelite, Costa Rica 1031, Col. vul. 1451, 1453, 1482, 1486, 1497, and Pinto 111. Campos (8) reported the cultivar P-524 from Colombia as highly resistant.

Schwartz et al evaluated more than 13,000 accessions from the CIAT (Centro Internacional de Agricultura Tropical) germplasm bank during 1978-1981 for reactions to I. griseola. Field inoculations with a mixture of seven ALS isolates were carried out during those years. Plants showing field resistance were then inoculated with a mixture of 15 isolates from eight different regions from Colombia at a concentration of 2-4 ($10^4$) spores/ml. Resistant (R) varieties were those with no apparent infection or a few small lesions generally less than 2 mm in diameter; Intermediate (I), those with few to many lesions 2-5 mm in diameter and no appreciable local chlorosis; and Susceptible (S), those with few to many lesions greater than 5 mm in diameter, often surrounded by local chlorosis and/or premature defoliation. Only 56 accessions exhibited R or I reactions for angular leaf spot. In their publication they gave a good description of those introductions including number of accession, country of origin, growth habit, grain size and color, and reaction to the disease. The authors also observed that pathogenic variation inherent within populations of I. griseola complicates efforts to obtain sources of stable resistance by traditional screening and breeding methods. They further recommend studies to: a) investigate pathogenic variation in I. griseola, b)

develop more effective and practical systems to monitor pathogenic variability, and c) determine the nature and inheritance of new resistance sources.

Other reported sources of resistance include: Fin de Lima by Diaz et al (20); Cuva 168-N and Maintengao Preto 20 (15); Guatemalan accessions identified as 2465, 2503-12, 2504 and 2809 (42); GLP-24, a Canadian Wonder type reported to be tolerant to angular leaf spot (54) in Kenya; GLP-X.806 reported as tolerant (52); and GLP-X.92 considered to exhibit considerable resistance in the semi-arid areas of Kenya by Stoetzer et al in 1984 (55).

There are just a few studies on the inheritance of the resistance to angular leaf spot. Santos-Filho et al (41) crossed resistant variety Caraota 260 with the susceptible black bean variety Venezuela 350. All F1 plants were susceptible, and in the F2, a 3:1 segregation ratio of susceptible to resistant plants was found indicating that resistance was controlled by a single recessive gene. Singh and Saini (50) studies inheritance of resistance in crosses between resistant cultivar PLB 257 (Phaseolus coccineus) and the susceptible variety Contender (P. vulgaris). When Contender was the female parent, all 61 plants in the F1 were susceptible. In the F2 generation, eight resistant plants and 20 susceptible plants were selfed and increased. All F3 progeny raised from the eight resistant plants produced only resistant plants indicating homozygosity for resistance in the resistant F2 plants. Eight of the 20 susceptible F2 plants yielded 834 susceptible and zero resistant plants indicating homozygosity for susceptibility of the mother F2 plants. The remaining 12 progenies segregated and gave a 3:1 ratio of susceptible to resistant plants. Collectively 1098 susceptible and 355 resistant plants were produced. They concluded that PLB 257 carries a single recessive gene for resistance.

In another study Cardona-Alvarez (11) indicated that resistance was monogenic and dominant when resistant line 258 was crossed with susceptible Algarrobo and line 223. Zaumeyer and Meiners (57) reported that resistance in cultivars Decal, Maravilla, and Huila 14 was due to three recessive genes. In addition, Barros et al (5) in a review article, reported that in most published reports resistance was a recessive character and controlled by two to three independent factors.

## 2.6 Modes of survival of the fungus

Studies on the ability of the fungus to survive or overwinter have been done mainly on debris from infected plants and with contaminated seed. Cardona-Alvarez (10) harvested infected plant material from field plots near Babcock, Wisconsin, in 1954. Tissue samples were allowed to overwinter in cheesecloth bags at Madison, Wisconsin, while other samples were kept at room temperature in the laboratory. Samples were bioassayed for viable pathogen spores by suspending samples in water and inoculating susceptible plants. Such tests revealed that the fungus may survive two successive Wisconsin winters (19 months) in the debris of previously infected crops.

Cardona-Alvarez (10) reported that a very low percentage (about 1%) of germinated spores on infected material survived from November to April in Wisconsin. In another experiment Sohi and Sharma (53) reported that about 15.8% of conidia were viable after 224 days of storage under laboratory conditions, whereas only 50% of the conidia were viable after 142 days when stored in soil. They indicated that the fungus could overwinter as stromatic tissue on diseased debris. Sindhan and Bose (47) reported that conidia survived up to 6 months in plant debris under laboratory conditions and 8 months under field conditions.

Cardona-Alvarez seems to be the only one who has studied the overwintering of the fungus in the soil. In a detailed series of experiments, he showed conclusively that the fungus apparently does not overwinter in soil obtained from fields of beans infected with I. griseola.

## 2.7 Seed transmission

Reports on the seed transmissibility of I. griseola in bean are contradictory. Cardona-Alvarez (10) was unable to observe symptoms on plants grown from seed which were harvested from a field of infected plants. The germinated seeds were placed in a moist chamber near saturation at 24 C for 12 days and then removed to a greenhouse bench at 24 C. On the other hand, Orozco-Sarria and Cardona-Alvarez (35) found that approximately 50% of seed harvested from infected plants of bean line 143 yielded I. griseola when cultured on agar plates; discolored seed showed fungal growth around the hilum area after five days. Similar tests with seed of several other lines and varieties were negative. The authors reported a decline in percentage of survival of the fungus in the seed during storage from 50% at one month to 10% at nine months; no survival was observed at 12 months. To determine possible seed transmission of ALS, seeds of line 143 were planted in sterilized soil and pots and incubated in a mist chamber at 20-24 C. Initial symptoms of angular leaf spot were observed 13 days after planting and suggesed that the fungus in or on seed could be a source of primary infection. Sindhan and Bose (47) reported that the fungus remained viable in seeds for more than one year as dormant mycelium. This emphasized the importance of contaminated seed for the introduction and spread of I. griseola into new bean production areas. Sohi and Sharma (53) obtained seeds from infected pods of the Kentucky Wonder bean variety and showed that contamination by the pathogen was both external and internal.

Diaz et al (20) reported that the percentage of transmission of I. griseola in seed was low (24%) and they indicated however, that under field conditions where relative humidity is lower than optimum, little possibility of seed transmission exists.

Dhingra and Kushalappa (18) were unable to correlate seed infection with amount of infection on the plants from which the seed was harvested. The authors also found that the fungus was always associated with the hilum and that seeds were only infected when they were located under the lesion present on the pod suture. They concluded that seed transmission of I. griseola as a source of primary inoculum appears insignificant.

Sharma and Sohi (45) reported that I. griseola is restricted to the soft tissues of the hilum and the seed coat, where profuse fungal growth of conidiophores and conidia can be seen. They also observed varietal differences in amount of seed infection.

## 2.8 Pathogenic variability

Little has been done on pathogenic variability of Isariopsis griseola. Brock (7) compared the reaction of 13 isolates of the pathogen on bean varieties Brown Beauty and Red Mexican and he suggested the existance of pathogenic differences between the isolates.

Marin-Villegas (31) identified 13 physiological races among a series of isolates from three bean growing areas in Colombia. The isolates were purified using single spore transfer technique; 14 differential bean varieties were used for inoculations. The author had reason to question the homogeniety of the differential varieties, however, and indicated that additional studies were needed. Alvarez-Ayala and Schwartz (2) were able to differentiate among five I. griseola isolates from Colombia and Ecuador by inoculating the

varieties Brasil 260 (Caraota 260), Alabama 1, Red Kidney, Ica Duva, and Cauca-27. They also indicated that the isolates appeared to differ in virulence on the same variety.

A highly virulent form of Isariopsis griseola was isolated in Tanzania by Hocking (27); symptoms consisted of circular rather than angular lesions. The new form produced abundant robust synnemata on the leaf under surfaces and only a few on the upper surfaces. The synnemata were somewhat longer than those reported previously for Isariopsis griseola. Hocking was able to reproduce circular lesions when detached, unwounded bean leaves were inoculated and maintained at 100% relative humidity. He obtained infection with the new form at spore concentrations as low as $10^2$ spores/ml, compared to the $10^5$ spores/ml necessary for infection with the common forms of I. griseola.

# III. MATERIALS AND METHODS

## 3.1 Collection and pathogenicity tests of Michigan isolates of Isariopsis griseola

### 3.1.1 Collection of isolates

Isolates were obtained from various types of diseased plant tissue: a) infected leaves or pods brought to the laboratory by county agents, b) infected plant samples collected personally from fields in the Alpena-Rogers City area during the growing seasons of 1982 and 1983, and c) seeds which were found to be contaminated during the course of the present study.

Cultures of I. griseola were also obtained from the following sources: a) isolates from Colombia were kindly provided by Dr. Marcial Pastor Corrales from the Bean Pathology program, CIAT, Colombia. These isolates are identified as Col 5, Col 6, and Col 10. They had been received as Ig pop #5, Ig pop #6, and Ig pop #10 respectively; b) cultures identified as Wisconsin 1 as well as an isolate from Brazil (South America) were kindly supplied by Dr. D. J. Hagedorn, University of Wisconsin. The isolate identified as Wisconsin 3 was obtained from an infected sample sent to our laboratory by a bean grower from that state in August of 1984; c) cultures from Puerto Rico (PR - 2 and PR -3), Dominican Republic and Malawi were obtained from infected samples provided by cooperating scientists. In all cases with other than Michigan isolates, cultures, inoculum, and infected plants, were autoclaved before being discarded.

### 3.1.2 Methods of isolating the pathogen

Some but not all of the infected samples contained sporulating lesions. When lesions were present that contained synnemata with spores, a fine needle was used to remove the spores. Isolations were performed in a transfer

chamber, using a stereomicroscope to observe the mass of spores of the fungus. The fine needle, previously flamed, was dipped in sterile V-8 juice agar, and then the spores were picked up with the tip of the needle avoiding any contact with the surface of the infected tissue. The spores were then transferred to the surface of V-8 juice agar (200 ml V-8 juice, 3 g CaCO3, 18 g agar, 800 ml distilled water). Six transferences per petri dish were performed. Ioculated plates were then transferred into an incubator at 19-21 C, until well sporulated colonies formed after six days.

Single spore isolates of I. griseola were then obtained from sporulating colonies in the following way: a conidial suspension was prepared in sterile-distilled water, diluted, and poured onto the surface of petri dishes containing PDA (39 g of Difco PDA, 1000 ml distilled water). Inoculated plates were incubated at 19-21 C for 24 hours after which time several germinated spores were located under the stereomicroscope. A total of 12 of these spores plus a small amount of media were transferred with a fine needle to the surface of V-8 juice agar. The colony showing maximum sporulation under the stereomicroscope was selected, transferred, and maintained on V-8.

For samples that did not contain sporulating lesions, the infected tissue was incubated under conditions of high humidity for 48 to 96 hours. Petri dishes containing the samples on top of wet filter paper were wrapped with parafilm to maintain humidity close to 100%. During incubation, synnemata bearing spores were produced and the same procedure as above was followed to obtain single spore isolates.

For isolatin of I. griseola from infected seeds, the same technique as for sporulating lesions was followed. It should be noted that the criteria for infected seed requires the presence of synnemata and spores on the seed.

### 3.1.3 Maintenance of isolates, inoculum preparation and inoculation techniques

All isolates of I. griseola were maintained on V-8 juice agar by periodic transfer of 8 to 10 highly concentrated drops of spore suspensions to fresh plates. Spores for inoculations were obtained by scraping them from plates that had been incubated 10 to 12 days at 20 C. Spores were adjusted to a density of $10^4$ to $10^5$/ml distilled water containing 0.05% (v/v) tween 80 (Polyoxyethylene sorbitan monooleate). In preliminary greenhouse studies involving different isolates, spore concentrations and bean varieties, it was found that an inoculum containing between $10^4$ to $10^5$ spores/ml was the best to differentiate between resistant, intermediate, and susceptible reactions.

Inoculum was sprayed as a fine mist onto upper and lower leaf surfaces of leaves on test plants possessing two trifoliolate leaves. An aerosol containing can (Spra-tool No. 8011 power pack, Crown Industrial Products Co.) was used to spray the spore suspensions. Plants were maintained in a saturated mist chamber for 4 days at 22 to 28 C. During summer months of 1983 and 1984, the maximum day temperature inside the mist chamber raised up to 30-32 C. A 100% relative humidity in the mist chamber was maintained by using several humidifiers (Model 500 HERRMIDIFIER, Herrmidifier Co. Inc.) inside the chamber. The humidifiers were run continuously and were supplied with standard tap water. Plants were then removed to greenhouse benches for an additional 6 to 10 days prior to rating disease reactions. During the course of the experiments the greenhouse temperature fluctuated between 24 and 30 C. To examine pod reactions to I. griseola isolates, plants in the mid-pod filling stage of development (about 40 days after planting) were inoculated by spraying spore suspensions of the same density directly onto pods and incubating as described previously.

3.1.4 Host cultivars

Initially, 23 cultivars, representing navy, black, kidney, pinto, and cranberry types of commercial dry bean varieties were inoculated with different isolates to identify their disease reactions (Table 1). Seeds were planted using a mixture 2:1 of soil and vermiculite No. 2. Isolates used in these preliminary inoculations were Michigan 1-5 and 8, Wisconsin 1, Col 5, Col 6, and Brazil (Tables 1 and 3). Each isolate was inoculated individually to a set of cultivars because space in the mist chamber was limited. For each isolate there were two 12 cm diameter clay pots per cultivar with three plants per pot. Plants were inoculated as described previously and placed randomly in the mist chamber.

The following evaluation scales used to establish infection grades were based on percentages of leaf or pod area affected: For leaves (Figure 3):

1 - No infection (immune reaction).

2 - 1 to 15% leaf area infected (resistant).

3 - 16 to 30% leaf area infected, lesions rarely surrounded by chlorosis (intermediate reaction).

4 - 31 to 50% leaf area infected, lesions surrounded with chlorosis (susceptible).

5 - More than 50% of leaf area infected. Lesions always surrounded by chlorosis and defoliation was common (highly susceptible).

For pods:

1 - No infection.

2 - 1 to 25% pod area infected with round lesions.

3 - 26 to 50% pod area infected with round lesions.

4 - 51 to 75% pod area infected with round lesions.

5 - More than 75% pod area infected with round lesions.


Figure 3. Scale used for evaluating disease reactions caused by I. griseola

1 - No infection (immune reaction)
2 - 1 to 15% leaf area infected (resistant)
3 - 16 to 30% leaf area infected, lesions rarely surrounded by chlorosis (intermediate reaction)
4 - 31 to 50% leaf area infected, lesions surrounded with chlorosis (susceptible)
5 - More than 50% of leaf area infected. Lesions always surrounded by chlorosis, defoliation common (highly susceptible)

## 3.2 Screening for sources of resistance to Michigan isolates of I. griseola

A group of 115 cultivars and breeding lines (Table 5) were screened against one typical Michigan isolate of I. griseola selected from the preliminary studies described in 3.1.4. BAT 332 reported as resistant to Colombian isolates of the pathogen (M. P. Corrales, personal communication); G-04721, G-05686, G-04534 reported as resistant in Colombia (44); 27-R and 3M-152 observed to be resistant to Puerto Rican isolates (G. F. Freytag, personal communication), were included in the inoculations. Also included were several cultivars and lines from Malawi, which were kindly provided by Wilson Msuku.

Techniques for inoculation, incubation and evaluation were as described previously in section 3.1.3. With such a large number of host lines, it was necessary to divide this study into five groups of 23 cultivars. At each inoculation, susceptible (Montcalm) and resistant controls (C-20 and Midnight) were included.

## 3.3 Studies on seed-transmission of Angular Leaf Spot

### 3.3.1 Preliminary trials in the laboratory

In order to detect the presence of the pathogen in seed, infected pods were collected from a severely-infected field. The seed was removed from the pods and kept under laboratory conditions (23-24 C). Plastic shoe boxes (30 cm in long x 16 cm in wide x 8 cm in high) were packed with 30 cm x 16 cm sterile pieces of cotton, filter paper, and cheesecloth. The boxes were previously disintected with bleach (2% $Cl^-$) for 5 minutes. Distilled-sterile water was added in sufficient amounts to wet the filter paper and cotton. In some cases, seeds were disinfected by soaking for 2 min in bleach (2% of $Cl^-$) and blotting dry. One hundred seeds per sample were placed in the box, making sure that the hilum surface was in contact with the wet packing (Figure 4). Boxes were covered and wrapped with parafilm or tape to maintain high humidity

inside. After one week germlings were examined under the stereomicroscope for the presence of synnemata and spores of the pathogen.

Seed samples were obtained from twenty separate production fields of red kidney beans showing evidence of angular leaf spot infection. The samples were examined for incidence of seed infection by I. griseola as described previously; 100 seeds per sample were tested.

3.3.2 Detection of pathogen-infected seeds in the greenhouse

A more practical and accurate method to test for the presence of the pathogen in the seed was developed in the greenhouse. A rectangular piece of metal screen surrounded by a wood frame was used to support the seed (Figure 5). The screen was placed inside a mist chamber and elevated to a distance 12 to 15 cm above surface. Areas were delineated on the screen with wooden lables (sufficient to place 15 samples of 100 seeds each). High humidity was maintained by use of humidifiers as described previously in Section 3.1.3. After two to four days the seedlings were allowed to dry briefly and then examined under the stereomicroscope for the presence of synnemata and spores.

At the end of the 1983 growing season, 59 seed lots representing 25 seed growers were obtained and tested for seed infection. Tests were carried out during 3/7/84 and 4/17/84. Control samples consisted of: 1) a sample carrying a known level of contamination, and 2) a sample shown previously to be pathogen-free.

3.3.3 Angular leaf spot produced in plants from infected seeds

Tests were conducted to determine whether symptoms of angular leaf spot would develop from seed lots with different levels of seed infection. Several seed lots which had been tested by the seed assay method previously described at 3.3.2 and found to contain a known level of infection were selected for

Figure 4. Detection of infected seed in the laboratory by incubating seeds in a plastic shoe box

Figure 5. Detection of infected seed in the greehouse using a rectangular metal screen support

more detailed studies. Depending on seed availability, between 30 and 80 pots were used per sample. Four seeds of the test sample were inserted in the four corners of each pot. Two healthy seeds of the susceptible Montcalm variety were also planted in the center of the same pot. Controls were planted using just four healthy seeds in separate pots. Pots were placed on greenhouse benches and watered daily.

Eight to ten days after planting, the pots were moved for three to five days into a mist chamber containing two humidifiers. After this time humidifiers were turned off and the plants allowed to dry for 24 hours. A large fan was then used to blow air for 30 minutes against the plants without visible injury. The plants were immediately watered with a sprinkler head at a distance of about one meter above leaf surface so that the water splashed on the plants. Plants were incubated in the mist chamber for four additional days and then moved to a greenhouse bench.

3.3.4 Chemical treatment to control seed-borne I. griseola

The primary inoculum for many serious bean diseases in the field comes by planting infected seed. Seed-treatment with chemicals has been used to reduce or prevent the early development of many dry bean diseases; however nothing has been reported for angular leaf spot. Four fungicides were compared for their ability to control I. griseola on infected seed. These fungicides were: benomyl, which is a benzimidazole manufactured by duPont, was used at a rate of 6 g/Kg seed; multipurpose fungicide, composed of a mixture of captan (22%) and zineb (21%) and manufactured by Patterson Chemical Co., Inc., was used at the rate of 3.7 g/Kg seed; metalaxy manufactured by Ciba-Geigy was used at a rate of 1.8 g/Kg seed. The fourth fungicide used was iprodione manufactured by the Rhone-Poulenc, Inc. A rate of 2.0 g/Kg seed was used for the last fungicide.

Samples of 100 seeds with a known level of infection were treated with each fungicide; there were three replications per treatment. The fungicides were applied in water-based slurry preparations. Treated samples, plus a non-treated control sample, were tested for the presence of I. griseola as described previously in Section 3.3.2.

## 3.4 Overwintering of I. griseola

### 3.4.1 Overwintering under field conditions

Two sources of plants infected with ALS were allowed to stand in the field after the 1983 growing season: 1) plants of Montcalm that had been artificially inoculated at the Botany Farm field at Michigan State University and, 2) plants naturally infected in a seed field of Montcalm located near Rogers City, Michigan. Beginning in December of 1983, plants were removed from the field plots at five monthly intervals. Only pod and stem samples could be tested since leaves haad dropped during maturity. Plants from the Rogers City field were kindly sent every month to the laboratory by R. A. Long, County Extension Director.

Several infected pod and stem pieces from each plant were placed on a screen and incubated for five to seven days in a mist chamber at 100% RH. The samples were then removed, allowed to dry, and examined under the stereomicroscope for the presence of synnemata and spores. If spores were present, attempts were made to isolate I. griseola in pure culture; isolates obtained were tested for pathogenicity on plants of susceptible cultivar Montcalm.

### 3.4.2 Overwhelming of the fungus in infected tissue placed two to five cm and 30 cm below soil surface

During August and September of 1983, samples of stems, leaves, and pods infected with ALS were collected from plants growing at the Botany Farm,

East Lansing and at Rogers City. Pieces of 2.0 cm diamater were cut from infected leaves, while stems and pods were cut into pieces bearing typical lesions. The samples were stored at 4 C prior to use. On 12/14/83 nylon bags were filled with the infected tissue pieces. Four sites were included in this study, one at Michigan State University and three in the Rogers City area. On December 15 and 16, 1983, the nylon bags containing the infected material were buried two to five cm and 30 cm below ground, using three replications per site.

The buried samples were retrieved on June 21, 1984, at Michigan State University and on June 25 at Rogers City. Two methods were used to test for viability of I. griseola in the samples. The first method consisted of placing the stem and pod pieces on a screen and incubating for seven days in a mist chamber at 100% RH. Leaf samples were not recovered from the nylon bags.

The second method involved inoculating plants with water suspensions of macerated tissue. The suspensions were prepared by macerating the tissues from each nylon bag in a blender containing 100 ml of sterile distilled water. In this way suspensions of leaves, pods, and stems as well as soil particles were prepared for inoculations. Ten to 20 Montcalm plants possessing two trifoliolate leaves were inoculated by briefly immersing each leaflet as well as the primary leaves in the suspension. Inoculated plants were incubated in a mist chamber at 100% RH until symptoms were observed. Control plants were inoculated by immersing the leaves in sterile distilled water.

### 3.5 Variability of the pathogen

Pathogenic variation in the Michigan isolates of I. griseola was determined by inoculation tests of 12 bean cultivars (Table 15). The variety list

included BAT 332, G-05686, and Pompadour Checa which exhibited primarily resistant reactions in preliminary tests.

Isolates were inoculated five at a time for which it was necessary to erect physical barriers in the mist chamber to avoid cross-contamination between isolated (Figure 6). An inoculum concentration of $2x10^4$ spores/ml was used for all the isolates tested. Five plants per variety were planted in 10 cm diameter pots. Inoculation, incubation, and evaluation methods were the same as those described previously at Sections 3.1.3 and 3.1.4.

Figure 6. Mist chamber used to infect bean plants with *I. griseola*. Note the physical barriers used to avoid cross-contamination between isolates.

# IV. RESULTS

## 4.1 Collection and pathogenicity tests of Michigan isolates of Isariopsis griseola

An initial series of pathogenicity tests were performed by inoculating six Michigan isolates onto 23 dry bean cultivars. The results shown in Table 1 indicate that the isolates induce four types of reactions: 1) an immune reaction where inoculated plants exhibited no symptoms. This reaction is noted as (-) and corresponds to 1 in the evaluation scale described in Section 3.1.4. This reaction type was observed mainly on the navy and black bean cultivars; 2) a very susceptible reaction, which was observed mainly on the kidney and cranberry bean types. Infected leaves exhibited large numbers of angular-shaped lesions surrounded by chlorotic halos; lesions increased in size, coalesced, and defoliation was common (Figure 7). This reaction appears as (+++) and corresponds to 4 or 5 in the evaluation scale described in 3.1.4; 3) a third, or intermediate reaction was observed only on cultivar C-15 in which lesions were typically angular-shaped, but chlorosis was not always present. Lesions covered about 16 to 30% of the leaf area but did not increase in size. No defoliation was noted in infected plants. This reaction appears as (++) in Table 1 and indicates a reaction 3 in the evaluation scale described in Section 3.1.4.; a fourth reaction involved the occasional presence of small (1-3 mm diam) angular lesions on Pinto cultivars UI-111 and Olathe. This reaction is noted as (+) in Table 1 and corresponds to 2 in the evaluation scale described in Section 3.1.4.

It can be observed in Table 1 that cultivar C-15 exhibited a susceptible reaction when inoculated with isolate Michigan 4. However, a preliminary experiment where the same cultivar and isolate were involved (Table 2), indicated that the reaction of a cultivar may be affected by the inoculum

Table 1. Reactions of 23 bean cultivars to inoculation with Michigan isolates of *I. griseola*[a]

| Cultivar | Reaction Induced by Isolate[bc] | | | | | |
|---|---|---|---|---|---|---|
| | Mich 1 | Mich 2 | Mich 3 | Mich 4 | Mich 5 | Mich 8 |
| **Navy** | | | | | | |
| C-15 | ++ | ++ | ++ | +++ | ++ | ++ |
| C-20 | - | - | - | - | - | - |
| Fleetwood | - | - | - | - | - | - |
| Nep-2 | - | - | - | - | - | - |
| Neptune | - | - | - | - | - | - |
| Seafarer | - | - | - | - | - | - |
| Swan Valley | - | - | - | - | - | - |
| Tuscola | - | - | - | - | - | - |
| **Black** | | | | | | |
| B-190 | - | - | - | - | - | - |
| Black Beauty | - | - | - | - | - | - |
| Black Magic | - | - | - | - | - | - |
| Black Turtle Soup | - | - | - | - | - | - |
| Cornell 49242 | - | - | - | - | - | - |
| Domino | - | - | - | - | - | - |
| Midnight | - | - | - | - | - | - |
| **Kidney** | | | | | | |
| Charlevoix | +++ | +++ | +++ | +++ | +++ | +++ |
| Isabella | +++ | +++ | +++ | +++ | +++ | +++ |
| Montcalm | +++ | +++ | +++ | +++ | +++ | +++ |
| Redkloud | +++ | +++ | +++ | +++ | +++ | +++ |
| Sacramento | +++ | +++ | +++ | +++ | +++ | +++ |
| **Pinto** | | | | | | |
| Olathe | -/+ | - | - | -/+ | - | -/+ |
| UI-111 | +++ | - | - | -/+ | - | +++ |
| **Cranberry** | | | | | | |
| Mich. Improved Cran. | +++ | +++ | +++ | +++ | +++ | +++ |

[a]Inoculations were performed by spraying plants with spore suspensions containing $10^4$-$10^5$/ml.

[b]*I. griseola* isolates from the Rogers City-Alpena area of Michigan.

[c]Disease reactions: - = Immune, no infection; + = angular leaf spot (ALS) lesions covering 1 to 15% of leaf area; ++ = ALS lesions covering 16 to 30% of leaf area; +++ = ALS lesions covering more than 30% of leaf area, lesions increased in size, presence of chlorosis, and defoliation was common; -/+ = combination of reactions. Some plants exhibited a (-) reaction and others a (+) reaction.

Figure 7. Angular leaf spot symptoms on artificially inoculated plants of Montcalm. Note the large number of lesions formed, presence of chlorosis, coalesence and defoliation.

Table 2. Effect of spore concentration and pathogen isolate on reactions of 4 bean cultivars to *I. griseola*[a]

| Pathogen Isolate | Spore Concentration | Reaction of Cultivar[b] | | | |
|---|---|---|---|---|---|
| | | Montcalm | Olathe | C-15 | C-20 |
| Michigan 1 | $10^6$ | +++ | - | +++ | - |
| | $10^5$ | +++ | - | ++ | - |
| | $10^4$ | +++ | -/+ | + | - |
| | $10^3$ | ++ | -/+ | -/+ | - |
| Michigan 4 | $10^6$ | +++ | -/+ | +++ | - |
| | $10^5$ | +++ | - | ++ | - |
| | $10^4$ | +++ | - | + | - |
| | $10^3$ | +++ | - | - | - |

[a]Inoculations were performed by spraying plants with spore suspensions containing $10^3$ to $10^6$/ml.

[b]Disease reactions: - = inmune, no infection; + = angular leaf spot (ALS) lesions covering 1 to 15% of leaf area; ++ = ALS lesions covering 16 to 30% of leaf area; +++ = ALS lesions covering more than 30% of leaf area, lesions increased in size, presence of chlorosis, and defoliation was common; -/+ = combination of reactions. Some plants exhibited a (-) reaction and others a (+) reaction.

concentration used in the test. The results also suggested that other factors as temperature might affect the disease reactions. In the same Table 2, a very susceptible cultivar as Montcalm or very resistant as C-20 were not affected, as was the intermediate cultivar C-15, by increasing inoculum concentration.

A similar situation was observed with cultivar Pinto UI-111 which exhibited variable reactions when inoculated with a single isolate. It was also observed during the course of the present study that different factors as inoculum concentration or temperature may affect the disease reaction of this particular cultivar when exposed to infection by Michigan isolates of I. griseola.

In spite of the different types of reaction observed, the kidney and cranberry bean types are very susceptible to infection by Michigan isolates of the pathogen; the navies and blacks are very resistant except for cultivar C-15, and the pintos mostly resistant.

Different patterns of pathogenicity were noted when the same 23 cultivars were inoculated with I. griseola isolates from Colombia, Brazil, and Wisconsin (Table 3).

Isolate Col 5 induced symptoms in all of the 23 cultivars tested. However, this isolate induced slight symptoms on cultivars Montcalm, Red Kloud, Sacramento, and Charlevoix which are severely infected by the Michigan isolates. Most navy and black bean cultivars were severely infected by isolate Col 5.

Isolate Col 6 induced severe symptoms in all of the red kidney and cranberry cultivars as the Michigan isolates, but the same isolate induced slight symptoms in most of the navy and black bean cultivars.

Table 3. Reactions of 23 bean cultivars to different isolates of *I. griseola*

| Cultivar | Reaction to Isolates[ab] | | | | |
|---|---|---|---|---|---|
| | Mich | Wis 1 | Brazil | Col 5 | Col 6 |
| **Navy** | | | | | |
| C-15 | ++ | + | + | +++ | + |
| C-20 | - | - | +++ | +++ | - |
| Fleetwood | - | - | +++ | +++ | -/+ |
| Nep-2 | - | - | +++ | ++ | -/+ |
| Neptune | - | - | +++ | +++ | + |
| Seafarer | - | - | +++ | ++ | -/+ |
| Swan Valley | - | - | +++ | ++ | + |
| Tuscola | - | - | - | +++ | + |
| **Black** | | | | | |
| B-190 | - | - | +++ | +++ | + |
| Black Beauty | - | - | +++ | +++ | -/+ |
| Black Magic | - | - | +++ | +++ | -/+ |
| Black Turtle Soup | - | - | +++ | +++ | -/+ |
| Cornell 49242 | - | - | - | ++ | + |
| Domino | - | - | +++ | +++ | -/+ |
| Midnight | - | - | +++ | +++ | - |
| **Kidney** | | | | | |
| Charlevoix | +++ | +++ | ++ | + | +++ |
| Isabella | +++ | +++ | +++ | +++ | +++ |
| Montcalm | +++ | +++ | ++ | + | +++ |
| Redkloud | +++ | +++ | +++ | ++ | +++ |
| Sacramento | +++ | +++ | ++ | + | +++ |
| **Pinto** | | | | | |
| Olathe | -/+ | - | ++ | ++ | + |
| UI-111 | -/+ | - | +++ | +++ | -/+ |
| **Cranberry** | | | | | |
| Mich. Improved Cran. | +++ | +++ | +++ | +++ | +++ |

[a]Disease reactions: - = inmune, no infection; + = angular leaf spot (ALS) lesions covering 1 to 15% of leaf area; ++ = ALS lesions covering 16 to 30% of leaf area; +++ = ALS lesions covering more than 30% of leaf area, lesions increased in size, presence of chlorosis, and defoliation was common; -/+ = combination of reactions. Some plants exhibited a (-) reaction and others a (+) reaction.

[b]Mich. represents the typical reaction induced by Michigan isolates of *I. griseola*. The other isolates are from Wisconsin (Wis 1), Brazil, and Colombia (Col 5 and Col 6).

The Brazilian isolate also had a wide host range of infection, however, it induced no visible symptoms on cultivars Tuscola and Cornell 49242 (Table 3).

On the other hand, disease reactions induced on the 23 bean cultivars by Wisconsin isolate 1 (Table 3) indicated that this isolate induced a similar pattern of infection as the pattern observed for Michigan isolates. Severe symptoms were observed on the red kidney and cranberry bean types. Slight symptoms were observed on cultivar C-15 and no symptoms occurred on the other navy or black bean cultivars.

In a preliminary experiment where Brazilian and Colombian isolates were involved, it was shown that inoculum concentration also affects the disease reactions induced by these isolates as it had been observed previously for Michigan isolates of I. griseola (Table 4).

All the observations on disease reactions induced by the different isolates involved in this part of this study suggest that the isolates can be arranged into different pathogenicity groups according to the patterns of infection. However it will be a matter discussed further in the section concerning variability of the pathogen.

Isolate Michigan 5 was used for purposes of screening large numbers of genotypes.

## 4.2 Screening for sources of resistance to Michigan isolates of I. griseola

A group of 115 bean cultivars and breeding lines was tested for reaction to I. griseola to find sources of resistance in addition to those navy and black beans identified in Section 4.1 and Table 1. Isolate Michigan 5 was used for the inoculations following the techniques described in Section 3.1.3.

Table 4. Effect of spore concentration on reactions of bean cultivars to *I. griseola* isolates from Colombia and Brazil.[a]

| *I. griseola* Isolate[b] | Spore Concentration | Reaction of Cultivar[c] | | | |
|---|---|---|---|---|---|
| | | Montcalm | Olathe | C-15 | C-20 |
| Col 5 | $10^6$ | ++ | +++ | +++ | +++ |
| | $10^5$ | ++ | +++ | +++ | +++ |
| | $10^4$ | + | ++ | ++ | ++ |
| | $10^3$ | -/+ | + | + | + |
| Brazil | $10^6$ | +++ | ++ | ++ | |
| | $10^5$ | +++ | + | + | |
| | $10^4$ | ++ | - | -/+ | |
| | $10^3$ | + | - | - | |

[a]Inoculations were performed by spraying plants with spore suspensions containing $10^3$ to $10^6$/ml.

[b]Isolates from Colombia (Col 5), and Brazil.

[c]Disease reactions: - = inmune, no infection; + = angular leaf spot (ALS) lesions covering 1 to 15% of leaf area; ++ = ALS lesions covering 16 to 30% of leaf area; +++ = ALS lesions covering more than 30% of leaf area, lesions increased in size, presence of chlorosis, and defoliation was common; -/+ = combination of reactions. Some plants exhibited a (-) reaction and others a (+) reaction.

The foliage reactions of these cultivars to I. griseola, shown in Table 5, indicate that 54 (47%) of the cultivars tested showed a Susceptible (S) reaction; 49 (42.6%) a Resistant (R) reaction; 8 (7%) an Intermediate (I) reaction and 4 (3.4%) a Variable (V) reaction.

Resistant cultivars included 22 navy (small-white), 9 black, 6 pinto, 4 medium-red, 2 medium-white, 1 large-cream mottled, and 1 small-cream bean types. In addition, the following cultivars/genotypes were all resistant: Purple Hull Southern Pea and Mississippi Silver Pea cow pea (Vigna unguiculata), Evans 82 soybean (Glycine max), and the F1 generation plants from the cross Montcalm x 1212-D.

The intermediate cultivars included 2 navy (small-white), 1 medium-yellow, 1 large-purple, 1 medium-red, 1 small-brown, and 2 Phaseolus acutifolius cultivars (P 597 and Arizona Buff Tepary A).

Susceptibility was associated primarily with large and medium size seed types. None of the large red seed-coated cultivars tested was resistant. Only cultivar G 05686 (received from Colombia), a large-brown seeded type, was resistant to the Michigan isolate of I. griseola.

Within the Malawian accessions, Mulanje IB and Mulanje II, both large and red-seeded types, showed variable reactions to I. griseola. Unfortunately nothing is known about the pedigree of these accessions and it is possible that they are still segregating for reaction to the pathogen as well as for seed-coat color.

Pod reactions of 72 cultivars to the same Michigan isolate of I. griseola are seen in Table 5. Interestingly, for 21 of the cultivars, foliage and pod reactions both were resistant. In 26 of the cultivars, similarly, foliage and pod reactions were both susceptible. In 14 cases the foliage reaction was resistant while the pod reaction was either Intermediate of Susceptible. In

Table 5. Reaction of 115 bean cultivars to *I. griseola* under greenhouse conditions.[a]

| Cultivar | | Seed Characteristics | | Reaction to *I. griseola* | |
|---|---|---|---|---|---|
| | | Size | Color | Foliage[b] | Pod[c] |
| Navy | | | | | |
| 1- | Admiral | Small | White | R | R |
| 2- | Artic | Small | White | R | R |
| 3- | Aurora | Small | White | R | R |
| 4- | Bunsi | Small | White | R | R |
| 5- | C-15 | Small | White | I | S |
| 6- | C-20 | Small | White | R | I |
| 7- | Cumulus | Small | White | R | R |
| 8- | D-77196 | Small | White | R | R |
| 9- | D-79054 | Small | White | R | R |
| 10- | Fleetwood | Small | White | R | S |
| 11- | Kentwood 83 | Small | White | R | R |
| 12- | Michelite | Small | White | R | - |
| 13- | Mich. Tall Bunyan | Small | White | S | S |
| 14- | Midland | Small | White | R | R |
| 15- | Nep-2 | Small | White | R | I |
| 16- | Neptune | Small | White | R | I |
| 17- | Northland | Small | White | R | R |

[a]Reaction to *I. griseola* isolate Michigan 5. Six plants per cultivar were inoculated with a spore suspension containing $2(10^4)$/ml. Plants were incubated 4 days in a mist chamber at 100% RH and then removed to the greenhouse.

[b]Foliage reactions were evaluated according to the following scale: 1 - immune, no infection; 2 - 1 to 15% leaflet area infected with angular leaf spot (ALS); 3 - 16 to 30% leaflet area infected with ALS, lesions rarely surrounded by chlorosis; 4 - 31 to 50% leaflet area infected with ALS, lesions surrounded with chlorosis; 5 - more than 50% leaflet area infected with ALS. Lesions always surrounded with chlorosis. Defoliation was common. Plants with foliage reaction 1 or 2 were classified as Resistant (R), plants with 3 as Intermediate (I), and plants with 4 or 5 as Susceptible (S).

[c]Pod evaluations were according to the following scale: 1 - no infection; 2 - 1 to 25% pod area infected with round lesions; 3 - 26 to 50% pod area infected with round lesions; 4 - 51 to 75% pod area infected; 5 - more than 75% pod area infected with round lesions. Plants with pod reaction 1 or 2 were classified as Resistant (R), plants with 3 as Intermediate (I), and plants with 4 or 5 as Susceptible (S).

Plants were classified as Variable (V) when different reactions were observed among the inoculated plants.

Table 5. Continuation

| Cultivar | Seed Characteristics: Size | Seed Characteristics: Color | Reaction to I. griseola: Foliage[b] | Reaction to I. griseola: Pod[c] |
|---|---|---|---|---|
| Navy (cont.) | | | | |
| 18- Sanilac | Small | White | R | - |
| 19- Seafarer | Small | White | R | I |
| 20- Swan Valley | Small | White | R | S |
| 21- Tall Bunyan | Small | White | I | S |
| 22- Tuscola | Small | White | R | I |
| 23- Zircon | Small | White | R | R |
| 24- Wesland | Small | White | R | R |
| Blacks | | | | |
| 25- B-190 | Small | Black | R | I |
| 26- Black Beauty | Small | Black | R | I |
| 27- Black Magic | Small | Black | R | S |
| 28- Black Turtle Soup | Small | Black | R | S |
| 29- Cornell 49242 | Small | Black | R | S |
| 30- Domino | Small | Black | R | S |
| 31- Ebony | Small | Black | R | R |
| 32- Midnight | Small | Black | R | R |
| 33- T-39 | Small | Black | R | R |
| Pinto | | | | |
| 34- Agate | Medium | Pinto | R | R |
| 35- Olathe | Medium | Pinto | R | - |
| 36- Ouray | Medium | Pinto | R | R |
| 37- Pindak | Medium | Pinto | R | R |
| 38- Pinto 111 | Medium | Pinto | R | - |
| Dark Red Kidney | | | | |
| 39- California | Large | Dark red | S | S |
| 40- Carmine | Large | Dark red | S | S |
| 41- Charlevoix | Large | Dark red | S | S |
| 42- Montcalm | Large | Dark red | S | S |
| 43- Royal Red | Large | Dark red | S | S |
| 44- F1 (Montcalm x 1212-D) | Large | Dark red | R | - |
| Light Red Kidney | | | | |
| 45- 9482 A. A. | Large | Light red | S | S |
| 46- Cal. L. R. K. | Large | Light red | S | S |
| 47- Isabella | Large | Light red | S | S |
| 48- LR. 006 | Large | Light red | S | S |
| 49- Manitou | Large | Light red | S | S |
| 50- Red Kloud | Large | Light red | S | S |
| 51- Red Kote | Large | Light red | S | S |
| 52- Ruddy | Large | Light red | S | S |
| 53- Sacramento | Large | Light red | S | - |

Table 5. Continuation

| Cultivar | | Seed Characteristics: Size | Seed Characteristics: Color | Reaction to I. griseola: Foliage[b] | Reaction to I. griseola: Pod[c] |
|---|---|---|---|---|---|
| **Cranberry** | | | | | |
| 54- | 0 28 | Medium | Cranberry | S | S |
| 55- | MI Imp. | Medium | Cranberry | S | - |
| 56- | MI Imp. Cranberry | Medium | Cranberry | S | S |
| 57- | Taylor | Medium | Cranberry | S | S |
| 58- | UI 51 | Medium | Cranberry | S | S |
| **White Kidney** | | | | | |
| 59- | Cal. W. K. | Large | White | S | S |
| 60- | Kaboon | Large | White | S | - |
| 61- | W. K. 5408 | Large | White | S | I |
| 62- | W. K. 61144 | Large | White | S | S |
| 63- | White Kidney (ISB) | Large | White | S | S |
| **Great Northern** | | | | | |
| 64- | GH 482 | Medium | White | R | I |
| 65- | Great Northern (Rogers) | Medium | White | R | R |
| 66- | Jules | Medium | White | S | - |
| 67- | Tara | Medium | White | S | - |
| 68- | UNS 141 | Medium | White | S | R |
| **Colombian cultivars** | | | | | |
| 69- | Bat 332 | Small | Cream | R | - |
| 70- | G-02858 | Medium | Pinto | R | - |
| 71- | G-04534 | Large | Maroon-mottled | S | S |
| 72- | G-04721 | Medium | Cream-mottled | V | V |
| 73- | G-05686 | Large | Brown-mottled | R | R |
| 74- | ICA L-22 | Large | Red-mottled | S | - |
| 75- | ICA L-23 | Large | Red-mottled | S | - |
| 76- | ICA L-24 | Large | Red mottled | S | - |
| **Malawian cultivars** | | | | | |
| 77- | 336 | Medium | Dark red | R | - |
| 78- | 600-D | Large | Purple | S | I |
| 79- | 1039 | Large | Brown | S | - |
| 80- | 1212-D | Small | White | R | R |
| 81- | 1251-D | Large | Marron | S | - |
| 82- | 1768-D | Large | Dark red | S | - |
| 83- | 2482-D | Large | Brown | S | - |
| 84- | 2539-D | Large | Brown | S | - |
| 85- | 2586-D | Medium | Yellow | S | - |
| 86- | 2589-D | Medium | Yellow | S | S |
| 87- | Dova Hills | Medium | Red | S | - |
| 88- | Malawian line | Large | Maroon-mottled | S | - |
| 89- | Mulanje IA | Large | Purple | S | - |
| 90- | Mulanje IB | Large | Dark red | V | - |

Table 5. Continuation

| Cultivar | | Seed Characteristics: Size | Seed Characteristics: Color | Reaction to *I. griseola*: Foliage[b] | Reaction to *I. griseola*: Pod[c] |
|---|---|---|---|---|---|
| **Malawian cultivars (cont.)** | | | | | |
| 91- | Mulanje II | Large | Red-purple | V | - |
| 92- | Mulanje III | Large | Light red | S | - |
| 93- | Mulanje IV | Large | Purple | I | - |
| 94- | Mulanje V | Medium | Maroon-mottled | S | - |
| 95- | Mulanje VI | Large | Dark red | S | - |
| 96- | Thyolo IV | Large | Red-mottled | S | - |
| **Puerto Rican cultivars** | | | | | |
| 97- | 27-R | Large | Light red | S | S |
| 98- | 3M-152 | Large | Light red | S | S |
| **Miscellaneous** | | | | | |
| 99- | AB 136 | Medium | Red | R | - |
| 100- | Kenearly | Medium | Yellow eye | S | R |
| 101- | Maine | Medium | Yellow eye | I | I |
| 102- | Mexico 222 | Medium | Cream | S | - |
| 103- | PI 165426 | Small | Brown | V | - |
| 104- | Pompadour (Checa) | Medium | Red-mottled | R | - |
| 105- | Pompadour (Jose Beta) | Medium | Red-mottled | S | - |
| 106- | Red Mexican UI 37 | Medium | Red | R | - |
| 107- | Rufus | Medium | Red | I | R |
| 108- | Steuben | Medium | Yellow eye | S | I |
| 109- | Topcrop | Medium | Maroon-mottled | S | - |
| 110- | Viva | Medium | Cream | S | S |
| 111- | P597 (*P. acutifolious*) | | | I | - |
| 112- | Arizona Buff Tepary A (*P. acutifolious*) | | | I | - |
| 113- | 18-Purple Hull. Southern pea (*Vigna unguiculata*) | | | R | - |
| 114- | 15-Mississippi Silver Pea (*Vigna unguiculata*) | | | R | - |
| 115- | Evans 82 (*Glycine max*) | | | R | - |

five cases where the foliage reaction was susceptible, the pod reaction was either resistant or intermediate. In five cases where the foliage reaction was intermediate, the pod reaction was resistant, intermediate or susceptible.

Although no replications were done to reevaluate the foliage and pod reactions of all entries, (foliage and pod susceptible) Montcalm, (foliage resistant) C-20, and Midnight (pod resistant) were consistently used as controls in every subsequent inoculation with identical results.

## 4.3 Studies on seed-transmission of Angular Leaf Spot

### 4.3.1 Preliminary trials in the laboratory

The presence of *I. griseola* in the seed of three different sources was tested by using plastic shoe boxes as described in Section 3.3.1. The first source of seed had been harvested from visually infected pods from a severely infected field of Montcalm cultivar near Alpena, Michigan. The seed was divided into three groups: a) apparently infected, which contained seed showing discoloration, irregular shape, and/or the presence of a dark color around the hilum area; b) possibly infected, which contained small and wrinkled seeds, and c) seed apparently healthy, which contained all seeds which appeared normal in size and color. A second source of seed consisted of seeds harvested from infected Montcalm plants at the Botany Farm at M.S.U., East Lansing. Seeds were separated into groups representing those beneath pod lesions and those that were not. The third source was obtained from a severely infected seed field of Montcalm cultivar near Rogers City, Michigan.

The results shown in Table 6 indicate that the pathogen is indeed associated with the seed. A direct correlation existed between the physical condition of the seed harvested from infected pods and contamination with

I. griseola. For example, about 84% of seed showing discoloration, malformation and/or presence of a dark color around the hilum area yielded I. griseola. After surface-sterilization for 2 minutes with bleach (2% $Cl^-$), 70% of the seeds still yielded the pathogen suggesting that seed contamination may be both external and internal.

Approximately 14% of small and wrinkled seeds were contaminated by I. griseola while only 2% of apparently healthy seed were contaminated.

Within an infected pod, seeds yielded the pathogen only when they were in direct contact with the lesion; adjacent seeds were pathogen free. Thus, 10% infection was detected in seed in contact with the lesion while no infection was found in seed without contact with lesions in the same infected pod.

A bulked lot of seed harvested from a naturally-infected field near Rogers City, Michigan, carried 4.6% seed infection by I. griseola.

Fungal synnemata and spores were located at the hilum area (Figure 8) in all instances where Montcalm seeds were shown to be infected.

It should be mentioned, however, that contamination with bacteria and saprophytic fungi was common using the method for testing seed as described. Thus, it is possible that some of the seed classified as non-infected could have carried the pathogen

Nine of 20 seed samples, which were obtained from individual fields of red kidney beans grown in 1982 contained 1% or more infection by I. griseola (Table 7). Results on surface-sterilized samples indicated again that fungal contamination may be both external and internal.

Seed lot #11 exhibited 2% infection after, but not before, surface-sterilization. This suggests that seed infection may have been at lower levels than 1% and therefore not detectable in these tests which utilized only 100 seeds. Also, seed contamination with saprophytic fungi and bacteria did

Table 6. Detection of I. griseola in different seed lots incubated seven days in closed plastic boxes.

| Seed Lots[a] | Infected Seeds | Non Infected | % Infection |
|---|---|---|---|
| Alpena (diseased pods) | | | |
| 1- apparently infected | | | |
| Non-surface sterilized | 80 | 15 | 84.2 |
| Surface sterilized[b] | 70 | 30 | 70.0 |
| 2- Small-wrinkle | | | |
| Non-surface sterilized | 12 | 76 | 13.6 |
| 3- Apparently healthy | 2 | 98 | 2.0 |
| East Lansing (diseased pods) | | | |
| 4- Beneath the lesions | 10 | 88 | 10.2 |
| 5- Not beneath lesions | 0 | 96 | 0.0 |
| Rogers City | | | |
| 6- Seed field | 4 | 82 | 4.6 |

[a]Seed lots: 1 - apparently infected, seed showing discoloration, malformation, and/or presence of a dark color around the hilum.
2 - small and wrinkled seed, were not discolored and did not exhibit dark color around the hilum area.
3 - Apparently healthy, seed looking normal in size, color and any other physical aspect.
4 - beneath the lesions, seed located under a lesion in a diseased pod.
5 - no beneath the lesions, seeds not located under lesion in a deseased pod.
6 - Seed field from a severely infected field near Rogers City.

[b]Surface sterilized with bleach (2% $Cl^-$) for 2 minutes.

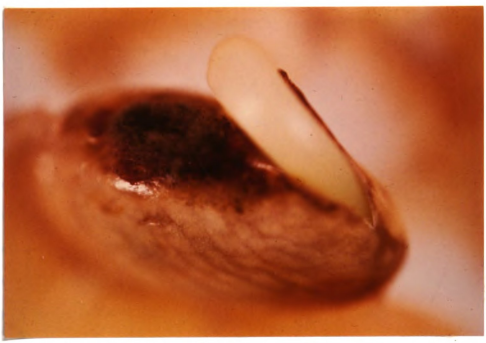

Figure 8. Infected seed showing fungal synnemata and spores located at the hilum area.

occur in many samples, making accurate evaluations under the stereomicroscope difficult.

4.3.2 Detection of pathogen-infected seeds in the greenhouse

A more practical and accurate method to test for the presence of the pathogen in seed was developed in the greenhouse using a rectangular metal screen as described in Section 3.3.2.

Several of the seed samples previously tested (4.3.1) were included in these studies. The results shown in Table 8 indicate that the Rogers City seed source had a mean infection level of 8% for non-surface sterilized seed, which is larger than the 4.6% level found previously in Section 4.3.1. For apparently healthy group of seeds from Alpena (See Section 4.3.1), the same percentage of infection (2%) was found using the metal screen method as for the plastic shoe boxes. This suggests that the pathogen can be found associated even with symptom-free or apparently healthy seed.

Two sources of seeds were sorted under the stereomicroscope as infected and non-infected, based on the presence or absence, respectively of fungal stromata. The sources were: a) 100 Montcalm seeds from Rogers City which contained an infection level about 8%, and b) a sample including seeds of different bean types and different seed coat colors harvested from pods artificially inoculated with I. griseola. The seeds were tested for the presence of the pathogen as described in Section 3.3.2. The results for the 100 Montcalm seeds indicated that of the nine seeds showing I. griseola in the mist chamber, four had previously been selected on the basis of stereomicroscope examination.

For the second group of seeds, which included 548 seeds, the results indicated that almost all of the infected seed (25.2% out of 26.8%) could have been identified by examining the seeds with the stereomicroscope.

Table 7. Detection of I. griseola in seed obtained from 1982 seed production fields of the Montcalm cultivar.[a]

| | Seed Lot[b] | Infected Seeds[c] | Non-Infected Seeds | % Infection |
|---|---|---|---|---|
| 1 | Non-surface sterilized | 1 | 99 | 1.0 |
| | Surface sterilized[d] | 1 | 99 | 1.0 |
| 2 | Non-surface sterilized | 1 | 99 | 1.0 |
| | Surface sterilized | 2 | 98 | 2.0 |
| 3 | Non-surface sterilized | 3 | 97 | 3.0 |
| | Surface sterilized | 1 | 74 | 1.3 |
| 4 | Non-surface sterilized | 5 | 95 | 5.0 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 5 | Non-surface sterilized | 1 | 67 | 1.5 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 6 | Non-surface sterilized | 0 | 100 | 0.0 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 7 | Non-surface sterilized | 2 | 98 | 2.0 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 8 | Non-surface sterilized | 0 | 100 | 0.0 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 9 | Non-surface sterilized | 0 | 100 | 0.0 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 10 | Non-surface sterilized | 2 | 98 | 2.0 |
| | Surface sterilized | 0 | 100 | 0.0 |
| 11 | Non-surface sterilized | 0 | 100 | 0.0 |
| | Surface sterilized | 2 | 98 | 2.0 |
| 12 | Non-surface sterilized | 0 | 100 | 0.0 |
| 13 | Non-surface sterilized | 3 | 93 | 3.1 |
| 14 | Non-surface sterilized | 0 | 100 | 0.0 |
| 15 | Non-surface sterilized | 0 | 100 | 0.0 |
| 16 | Non-surface sterilized | 0 | 83 | 0.0 |
| 17 | Non-surface sterilized | 0 | 90 | 0.0 |
| 18 | Non-surface sterilized | 0 | 90 | 0.0 |
| 19 | Non-surface sterilized | 0 | 44 | 0.0 |
| 20 | Non-surface sterilized | 0 | 94 | 0.0 |

Seed lots of less than 100 seeds indicate losses due to contamination with saprophytic bacteria and fungi.

[a]Seed tested by incubating in a close plastic box for 7 days and then observing the seedings under the stereomicroscope for the presence of the pathogen

[b]Twenty seed lots obtained from individual fields grown in 1982

[c]Infected seed contained synnemata and spores of I. griseola

[d]Surface sterilized seed with bleach (2% $Cl^-$) for 2 min.

Table 8. Detection of I. griseola infection in Montcalm seed lots by using a rectangular metal-screen support in the greenhouse[a]

| Seed Source | Infected Seeds[b] | Non-Infected Seed | % Infection |
|---|---|---|---|
| Rogers City[c] | | | |
| 1st replication | | | |
| Non-surface sterilized | 7 | 80 | 8.0 |
| 2nd replication | | | |
| Non-surface sterilized | 7 | 87 | 7.4 |
| Surface sterilized[d] | 3 | 97 | 3.0 |
| 3rd replication | | | |
| Non-surface sterilized | 9 | 91 | 9.0 |
| Alpena[e] | | | |
| Seed apparently healthy | 2 | 98 | 2.0 |

[a]Seed was incubated in a mist chamber for 2 to 4 days using a rectangular piece of metal screen to support the seed.

[b]Infected seed contained synnemata and spores of I. griseola.

[c]Seed field from a severely infected field near Rogers City.

[d]Surface sterilized with bleach (2% $Cl^-$) for 2 minutes.

[e]Apparently healthy seed from a field near Alpena. Seed looking normal in color, size, and any other physical aspect.

Figure 9. Angular leaf spot symptoms developing on plants grown from seed infected with I. griseola. Note the formation of fungal structures on the lesions.

These results strongly suggest that most seed infection is of an external nature and that a good estimate of seed contamination can be obtained by microscopic observation. The results also showed that seed infection occurs not only on Montcalm red kidney beans, but also on beans of different size and colors. No infection, however, was detected on the 69 black seeds present in this group.

Seed infection on bean types other than Montcalm was seen both in the hilum area and other sites of the seed coat. In Montcalm, however, the pathogen was always located in the hilum area and was never observed on the seed cotyledons or the hypocotyl of the germinating seeds.

Fifty-nine (59) seed lots from the 1983 growing season obtained from 25 different seed growers were tested together with six seed lots from the 1982 growing season previously tested in Section 4.3.1. The results (Table 9) indicate that only six out of 59 (10.2%) seed lots contained levels of infection of 1% or more. Tests for the six samples from 1982 showed that only one sample was infected after 17 months. All six samples had previously been found to carry I. griseola.

The above results suggest that seed infection may decrease with time. On the other hand, I. griseola did remain viable for at least 17 months on the infected seed sample 3-82.

### 4.3.3 Seed transmission studies with I. griseola

The following three groups of seed were examined for seed transmission using the methodology described in Section 3.3.3: a) a seed sample from Alpena, Michigan previously classified as apparently infected and found to contain 84.2% seed infection (see Table 6). Two replications of the sample were tested at different times; b) a sample from Alpena containing small and

Table 9. Percentage of seed infection by *I. griseola* in Montcalm seed fields

| Seed Lot Identity | No. Seed Tested | % Infection 1984 Tests | % Infection 1982 Tests |
|---|---|---|---|
| 1983 growing season[a] | | | |
| K1-14; 17-18;20-34; 36-40; 42-54;56-59 | 5,300 | 0.0 | - [c] |
| K15 | 100 | 1.0 | - |
| K16 | 100 | 1.0 | - |
| K19 | 100 | 1.0 | - |
| K35 | 100 | 1.0 | - |
| K41 | 100 | 2.0 | - |
| K55 | 100 | 3.0 | - |
| 1982 growing season[b] | | | |
| 3-82 (K5) | 100 | 1.0 | 3.0 |
| 4-82 (K37) | 100 | 0.0 | 5.0 |
| 5-82 (K6) | 100 | 0.0 | 1.0 |
| 7-82 (K9) | 100 | 0.0 | 2.0 |
| 10-82 (K10) | 100 | 0.0 | 2.0 |
| 13-82 (K61) | 100 | 0.0 | 3.0 |
| Infected control | 479 | 8.8 | - |
| Healthy control | 500 | 0.0 | - |

[a]Seed samples from grower fields of 1983; 100 seeds from each lot were tested 3/7/84 to 4/17/84

[b]Seed samples from grower fields of the 1982 growing season first tested on 12/28/82 and later on 4/17/84

[c]Not tested

wrinkled seeds previously found to contain 13.6% infection (see Table 6); and c) a seed sample from Rogers City previously found to contain about 8% infection (see Table 8).

The results shown in Table 10 indicate that under greenhouse conditions, symptoms of angular leaf spot developed on plants from seed lots with different levels of infection with I. griseola as well as on the healthy controls used.

For the seed sample from Alpena, the percentage of infected plants (48.9-50.3%) was lower than the actual level of seed infection (84.2%). A similar observation was noted for the Alpena sample with a seed infection level of 13.6%, in which only 5.6% of infected plants were observed. In the case of the seed lot from Rogers City with 8% of seed infection, 9% of infected plants were observed.

Symptoms were observed between 13 to 16 days after the plants were exposed to blowing air, watered with a sprinkler head, and returned to high humidity conditions.

Initial symptoms on the primary leaves were angular lesions, but with time lesions enlarged, assuming circular shape. Lesions turned necrotic and often were surrounded by a chlorotic halo. Generally one lesion was observed on the primary leaf; occasionally several were found (Figure 9). When plants bearing lesions were incubated under high humidity conditions, synnemata and spores were formed in the lesions on the undersurfaces of the leaves.

Infection observed on the controls indicated that spore dissemination occurred within the chamber. The fact that symptoms were formed in all cases between 13 to 16 days after exposing the plants to conditions favorable for infection suggests that indeed the same source of spores, spread from the infected seeds, were involved.

Table 10. Evidence of seed transmission of angular leaf spot (ALS) under greenhouse conditions in seed lots with different levels of infection by *I. griseola*

| Seed Source | No. Plants Tested | No. Infected Plants | % Infection |
|---|---|---|---|
| [a]Alpena (84.2% infection) | | | |
| 1st replication | 90 | 44 | 48.9 |
| [b]Control (center of pot) | 41 | 10 | 24.4 |
| [c]Control (separate pot) | 12 | 1 | 8.3 |
| 2nd replication | 286 | 144 | 50.3 |
| Control (center of pot) | 125 | 44 | 35.2 |
| Control (separate pot) | 24 | 6 | 25.0 |
| [d]Alpena (13.6% infection) | 267 | 15 | 5.6 |
| Control (center of pot) | 121 | 0 | 0 |
| Control (separate pot) | 50 | 0 | 0 |
| [e]Rogers City (8.0% infection) | 233 | 21 | 9.0 |
| Control (center of pot) | 105 | 9 | 8.6 |
| Control (separate pot) | 20 | 0 | 0 |

[a]Seed lot from Alpena, MI., previously shown to carry 84.2% infection with *I. griseola*

[b]Two healthy Montcalm seeds were planted in the center of each pot containing four seeds of the infected sample

[c]Three healthy Montcalm seeds per pot were planted in separate pots and placed at random in the mist chamber

[d]Seed lot from Alpena with a level of infection of 13.6%

[e]Seed lot from Rogers City, MI., with a level of infection of 8%

In many cases the healthy controls in the center of a pot were infected while the surrounding plants in the same pot were not infected. This suggests that infection in the controls occurred by spores carried during the water-splashing or air blown against the plants.

Plants grown from the same infected seed, but not exposed to the wind and splashing, remained symptomless.

4.3.4 Chemical treatment for control of seed-borne I. griseola

Samples of 100 Montcalm seeds from a lot carrying 8% infection by I. griseola were treated with the fungicides Benomyl, Multi-purpose Fungicide (mixture of Captan and Zineb), Metalaxyl and Iprodione. Treated samples plus non-treated controls were tested in the greenhouse for the presence of I. griseola. The results indicate that the pathogen can be controlled by chemical seed treatment. Results are shown in Table 11.

No fungal growth was observed on seed treated with the fungicides Benomyl or Captan-Zineb. On the other hand, synnemata and spores of I. griseola were seen on seeds treated with Rovral, Apron 25W, and the untreated control. These results indicate that seed treatment can reduce seed-borne inoculum.

## 4.4 Overwintering of I. griseola

### 4.4.1 Overwintering under field conditions

I. griseola was found to overwinter when infected Montcalm plants were allowed to stand in the field during the 1983-1984 winter. The pathogen was recovered from stem and pod tissue collected in the field at Michigan State University, East Lansing, and Rogers City. The results shown in Table 12 indicate that the pathogen was easily recovered from the tissue samples tested

Table 11. Chemical treatments to control I. griseola in infected seed of the Montcalm cultivar[a]

| Seed Treatment[b] | Total No. Seed Tested[c] | No. Seed Infected[d] | % Infection |
|---|---|---|---|
| Benomyl | 300 | 0 | 0.0 |
| Captan-Zineb | 300 | 0 | 0.0 |
| Iprodione | 300 | 9 | 3.0 |
| Metalaxyl | 300 | 10 | 3.3 |
| Untreated control[d] | 300 | 13 | 4.3 |

[a]Montcalm seed with a level of I. griseola infection about 8%

[b]Seed treatments were applied in a water-based slurry using the following rates: Benomyl, 6 g/kg seed; Captan-Zineb, 3.7 g/kg seed; Iprodione, 2.0 g/kg seed; Metalaxyl, 1.8 g/kg seed. Slurry preparations were prepared with the following concentrations in g/ml respectively: 0.11, 0.11, 0.07, and 0.07.

[c]Treatments were performed on three replications of one hundred seeds each.

[d]Number of seeds showing synnemata and spore formation on the hilum area.

during five consecutive months starting in December, 1983. Isolation and pathogenicity tests confirmed the identity of the isolates as I. griseola.

The last sample was collected at about the same time (May) when growers begin to plant beans. Thus, the pathogen overwinters at least one season under Michigan field conditions. This indicates that debris left on the field from a previous season constitutes a source of primary inoculum for the development of angular leaf spot.

4.4.2 Overwintering of I. griseola in infected tissue placed 2-5 cm and 30 cm below soil surface

Overwintering of I. griseola was tested using infected leaf, pod, and stem pieces that had been buried below soil surface prior to the 1983-1984 winter at East Lansing and Rogers City, Michigan. All infected pieces yielded fungal structures when incubated at 100% RH for seven days prior to placement in the field.

The buried samples were retrieved in June, 1984 and assayed for I. griseola in two ways: a) presence of synnemata and spores on the retrieved samples after incubation at 100% RH and, b) suspensions prepared by macerating the retrieved samples were inoculated to susceptible Montcalm plants.

Results relative to the formation of synnemata and spores of the pathogen on the retrieved samples are shown in Table 13. Few pieces (6 out of 30) of infected tissue placed 2 to 5 cm deep at East Lansing, yielded fungal structures; even fewer pieces (1 out of 30) of infected tissue buried 30 cm at the same location yielded I. griseola. No synnemata and spores were formed on samples retrieved from the three different locations at Rogers City.

The Rogers City and East Lansing samples were further tested for I. griseola by immersing each leaflet as well as the primary leaves of Montcalm plants in macerated suspensions prepared from the retrieved samples.

Table 12. Overwintering of *I. griseola* under field conditions

| Date and Location[a] | No. of Tissue Samples Tested[b] | No. of Tissue Samples with *I. griseola*[c] |
|---|---|---|
| December-January | | |
| East Lansing | 45 | 45 |
| Rogers City | 38 | 38 |
| January-February | | |
| East Lansing | 24 | 21 |
| February-March | | |
| East Lansing | 85 | 65 |
| Rogers City | 74 | 42 |
| March-April | | |
| East Lansing | 22 | 19 |
| Rogers City | 39 | 29 |
| April-May | | |
| East Lansing | 20 | 15 |
| Rogers City | 18 | 14 |

[a]Five monthly intervals in which samples were tested.

[b]Tested samples included pod and stem pieces from infected Montcalm plants allowed to stand under field conditions during the 1983-1984 Winter at East Lansing and Rogers City.

[c]Number of samples on which the presence of synnemata bearing spores of *I. griseola* was observed. Tissue samples were tested by incubating 7 days in a mist chamber under high humidity conditions, and followed by microscopic examination.

Table 13. Detection of I. griseola in infected tissue placed 2 to 5 cm and 30 cm below the soil surface

| Location | Tissue Sample | | | |
|---|---|---|---|---|
| | buried 2 to 5 cm | | buried 30 cm | |
| | No. pieces of tissue tested[a] | No. of pieces with I. griseola[b] | No. pieces of tissue tested[a] | No. of pieces with I. griseola[b] |
| East Lansing | 30 | 6 | 30 | 1 |
| Rogers City I | 30 | 0 | 30 | 0 |
| Rogers City II | 30 | 0 | 20 | 0 |
| Rogers City III | 30 | 0 | 30 | 0 |

[a]Number of pod and stem pieces recovered from nylon bags which had been buried below soil surface at East Lansing and Rogers City. Three replications of 10 pieces each were tested for the presence of I. griseola by incubating the samples in a mist chamber.

[b]Number of pieces in which formation of synnemata and spores were observed.

The results shown in Table 14 indicate that the pathogen survived on the infected tissue buried at the two different depths.

Symptoms of ALS developed on Montcalm plants inoculated with suspensions prepared from all retrieved tissue samples except for the one sample placed 2 to 5 cm at the Rogers City I location (Table 14). It should be noted, however, that symptoms developed on Montcalm plants inoculated with macerated tissue prepared from a sample buried 30 cm below soil surface at the same location.

In all the cases, plants showing symptoms were incubated in a mist chamber at 100% RH. Formation of synnemata and spores on lesions was always observed, indicating that symptoms were indeed those associated with ALS.

In summary, these studies indicate that I. griseola may survive from one season to another either on debris left on the soil surface, or on debris buried when plowing down the soil. However, results shown in Tables 12, 13, and 14 seem to indicate that the pathogen loses viability more rapidly when infected tissue is buried below the soil surface than when on the surface.

## 4.5 Variability of the pathogen

From preliminary inoculation and screening tests, a group of 12 cultivars was selected to test for pathogenic variability in the Michigan isolates of I. griseola. A small number of isolates received from different countries and described in Section 3.1.1 were also included. Inoculum of each isolate was consistently adjusted to a concentration of $2 \times 10^4$ spores/ml and the methodology for inoculation, incubation, and evaluation were as described in Sections 3.1.3 and 3.1.4.

Results are shown in Table 15. A total of 23 Michigan isolates were tested which included 22 new isolates plus the Michigan 1 isolate used in Section 4.1. Results indicate that 22 of the 23 isolates followed the same

Table 14. Overwintering of *I. griseola* in infected tissue placed 2 to 5 cm and 30 cm below the soil surface and tested by inoculating susceptible Montcalm plants with suspensions of macerated tissue[a]

| | Tissue Sample | | | |
|---|---|---|---|---|
| | Infected tissue placed on soil surface | | Infected tissue buried 30 cm | |
| Location | No. of plants inoculated[b] | No. of plants infected[c] | No. of plants inoculated | No. of plants infected |
| East Lansing | 26 | 7 | 26 | 13 |
| Rogers City I | 28 | 0 | 28 | 2 |
| Rogers City II | 29 | 8 | 14 | 1 |
| Rogers City III | 28 | 2 | 28 | 2 |

[a]Leaf, pod, and stem pieces that had overwintered 2 to 5 cm and 30 cm below the soil surface were macerated in a blender containing 100 ml of sterile, distilled water. Susceptible Montcalm plants containing two trifoliolate leaves were inoculated by immersing the primary and trifoliolate leaves in the suspension containing the macerated tissue. Plants were incubated under high humidity conditions after inoculation until initial symptoms were observed.

[b]Total number of plants inoculated in two replications.

[c]Total number of plants in two replications that showed angular leaf spot symptoms.

pattern of infection. The only exception was a recent isolate obtained from infected seed grown near Rogers City, Michigan (Michign 31); this isolate induced an intermediate reaction on cultivar G 05686.

The same four types of reactions observed in Section 4.1 were again induced by the new Michigan isolates tested. Navy bean cultivars C-20, Seafarer and Tuscola, black bean cultivar Cornell 49242, and the cultivars BAT 332, and Pompadour (Checa) exhibited immune reactions to all of the Michigan isolates. The dark and light red kidney cultivars Montcalm, Isabella, and Red Kloud exhibited very susceptible reactions. Cultivar G 05686 was immune to all of the Michigan isolates except isolate Michigan 31.

Interestingly, Cultivar C-15 exhibited a resistant (+) reaction to seven isolates, an intermediate reaction (++) to 10 isolates, and a susceptible (+++) reaction to six isolates. It suggested again that the disease reaction exhibited by this cultivar to infection by Michigan isolates of I. griseola is affected greatly by different factors as temperature or inoculum concentration (Section 4.1).

Cultivar Pinto UI-111 also exhibited a variety of reactions reported in Section 4.1. Lesions were quite small (1-3 mm diam), and did not increase in size. Chlorosis was not observed and defoliation was absent.

Inoculations of the same 12 bean cultivars with isolates from locations other than Michigan induced different patterns of reactions, except for the Wisconsin 3 isolate which was identical to the Michigan isolates (Table 15). Included isolates were those from Colombia (Col 10), Dominican Republic (DR), Puerto Rico (PR-2, PR-3), Malawi (MA-1) and Brazil.

Results (Table 15) indicate that these isolates induced susceptible reactions on a wider host range of cultivars than Michigan isolates. The Malawian isolate induced a very similar pattern of infection as most of the Michigan

isolates, however, the former isolate induced a susceptible reaction on cultivar Pompadour (Checa) while the latter did not. The Brazilian isolate was non-pathogenic on Tuscola, Cornell 49242, G 05686, and Pompadour (Checa). The two Puerto Rican isolates included followed similar patterns of infection and both severely infected cultivar C-20 (Figure 10), however, PR-2 appeared more virulent than PR-3. The Dominican Republic isolate, while similar to PR-2 and PR-3, did induce slight symptoms on cultivar G 05686. Isolate Col 10 did not infect cultivars BAT 332 and G 05686 and induced slight symptoms in plants of cultivar Pompadour (Checa).

Results from these inoculation tests, together with those in Section 4.1, indicate that I. griseola isolates differ in pathogenicity. The isolates were then separated into five different pathogenicity groups on the basis of reactions on a series of 12 host differentials (Table 16).

No cultivar tested was immune to all of the I. griseola isolates. However, cultivar G 05686 was immune to all except the DR and Michigan 31 isolates to which exhibited slight reactions.

Table 15. Disease reactions of 12 bean cultivars inoculated with Michigan and other isolates of I. griseola.

| Cultivars | Disease Reaction Induced by Isolates[ab] | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mich. 1 | Mich. 6 | Mich. 9 | Mich. 10 | Mich. 11 | Mich. 12 | Mich. 13 | Mich. 14 | Mich. 15 | Mich. 16 | Mich. 17 | Mich. 18 | Mich. 20 | Mich. 21 | Mich. 22 | Mich. 23 | Mich. 24 | Mich. 25 | Mich. 26 | Mich. 27 | Mich. 30 | Mich. 31 | Mich. 33 | Wis. 3 | Ma. 1 | Brazil | Col. 10 | PR-2 | PR-3 | D.R. |
| 1- C-15 | + | + | ++ | +++ | ++ | + | ++ | +++ | +++ | ++ | ++ | +++ | ++ | +++ | + | +++ | ++ | ++ | ++ | ++ | + | + | + | ++ | ++ | + | +++ | +++ | +++ | +++ |
| 2- C-20 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | +++ | +++ | +++ | +++ |
| 3- Seafarer | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | +++ | ++ | ++ | +++ |
| 4- Tuscola | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | +++ | +++ | +++ |
| 5- Cornell 49242 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | ++ | + | ++ |
| 6- Montcalm | ++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | +++ | ++ | + | ++ |
| 7- Isabella | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | + | ++ |
| 8- Red Kloud | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ++ | +++ | +++ | ++ | +++ |
| 9- Pinto 111 | -/+ | - | -/+ | -/+ | -/+ | -/+ | -/+ | -/+ | -/+ | - | -/+ | - | ++ | ++ | -/+ | + | -/+ | -/+ | ++ | ++ | - | - | - | - | + | ++ | +++ | +++ | +++ | +++ |
| 10- Bat 332 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | ++ | +++ | +++ |
| 11- G 05686 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - | - | - | - | - | - | - | + |
| 12- Pompadour (Checa) | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - | -/+ | +++ | + | + |

[a]Disease reactions: - = Immune reaction, no symptoms; + = resistant reaction. 1 to 15% of leaf area with angular leaf spot (ALS) lesions. ++ = intermediate reaction. 16 to 30% of leaf area covered with lesions. +++ = more than 30% of leaf area covered with lesions. Lesions increased in size and coalesced. Chlorosis was always present and defoliation was a common characteristic.

[b]I. griseola isolates from Michigan (Mich 1-33), Wisconsin (Wis 3), Malawi (Ma 1), Brazil, Colombia (Col 10), Puerto Rico (PR-2, Pr-3), and Dominican Republic (D.R.). A spore concentration of $2(10^4)$/ml was used for each isolate. Plants with one trifoliolate leaf were inoculated and incubated in a mist chamber at 100% RH for four days.

Table 16. Pathogenicity groups of I. griseola

| Cultivar | Isolates in Pathogenicity group[a] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | Michigan Wisconsin 3 | Malawi 1 | Brazil | Col 10 | PR-2 PR-3 DR |
| 1- C-15 | I[b] | I | R | S | S |
| 2- C-20 | R | R | S | S | S |
| 3- Seafarer | R | R | I | S | I-S[d] |
| 4- Tuscola | R | R | R | S | S |
| 5- Cornell 49242 | R | R | R | S | R |
| 6- Montcalm | S | S | I | S | R |
| 7- Isabella | S | S | S | S | R |
| 8- Red Kloud | S | S | S | S | I-S |
| 9- Pinto 111 | R | R | I | S | S |
| 10- Bat 332 | R | R | I | R | I-S |
| 11- G 05686 | R-I[c] | R | R | R | R |
| 12- Pompadour (Checa) | R | S | R | R | I-S |

[a] I. griseola isolates from Michigan, Wisconsin, Malawi, Columbia (Col), Brazil, Puerto Rico (PR), and Dominican Republic (DR).

[b] R = resistant reaction. Includes plant with immune reaction (-) and plants with resistant reaction (+) in which 1 to 15% of leaf area was covered with angular leaf spot (ALS) symptoms. I = intermediate reaction (++) where 16 to 30% of leaf area was covered with ALS symptoms. S = susceptible reaction (+++), more than 30% of leaf area was covered with ALS symptoms. Chlorosis always present, lesions increased in size and coalesced. Defoliation was present.

[c] Only one isolate (Michigan 31) induced an intermediate reaction (I).

[d] Variable reaction between intermediate (I) and susceptible (S).


Figure 10. Cultivar C-20, highly resistant to Michigan isolates of I. griseola is severely attacked by Puerto Rican isolates of the pathogen.

# V. DISCUSSION

Angular leaf spot had not previously been reported in Michigan until the disease was observed in numerous seed fields of the Montcalm red kidney bean cultivar during the 1982 and 1983 growing seasons. The presence of the pathogen in Michigan seed fields constituted a possible threat to the commercial dry bean crop. During the last decade ALS has been reported of economic importance in many parts of the world due to the severe losses in yield that it causes. The present study was conducted to obtain information about factors affecting the pathogen and epidemiology of the disease it causes under Michigan conditions. Attention was also directed to possible control measures that could be undertaken to reduce or eliminate the presence of the pathogen from bean crop areas.

Michigan is the largest dry bean producing state in the U.S.; production is concentrated in the thumb and Saginaw valley areas of the state.

It was important then to ascertain disease reactions of the most commonly grown dry bean cultivars to the I. griseola pathogen.

The results indicated that the kidney and cranberry bean types are very susceptible. On the other hand, navy bean cultivars which are the most prevalent bean types grown in Michigan were highly resistant to infection. A navy bean cultivar, C-15, exhibited intermediate reaction to most of the Michigan isolates. Interestingly the Pinto cultivars, especially cultivar UI-111 developed different reaction types when inoculated with a particular isolate at different times; the differences may have been due to environmental factors.

Standard methods reported in the literature for production of inoculum as well as inoculation techniques worked well in the present study. A high yield of conidia was obtained from cultures grown 9-12 days on V-8 agar at 20 C as

reported by Alvarez-Ayala (1). In addition, spraying plants processing one or two trifoliolate leaves with a spore suspension of the pathogen and incubating in a saturated mist chamber for four days was very practical for testing disease resistance in the greenhouse.

Disease reactions were highly dependent on such factors as inoculum concentration, temperature, host cultivar, and I. griseola isolate (Tables 2 and 4). Alvarez-Ayala (1) had found that reaction of a cultivar to infection by I. griseola is highly afffected by inoculum concentration. However, he did not make observations on cultivars with different levels of resistance.

In the present study, we found that reaction of a very susceptible cultivar as Montcalm, or very resistant as C-20, were not affected by inoculum concentration as occurred with the intermediate reaction of cultivar C-15 when isolates of I. griseola were used.

Temperature also appeared to be an important factor affecting the reaction of a cultivar to the pathogen. Even though many researchers have indicated 24 C to be an optimum temperature for infection and disease development (10,48), Inglis and Hagedorn (28) found that optimum infection occurred at 24 C but ratings for leaf chlorosis were higher and days to defoliation were fewer when the plants were incubated at 28 C after infection. In many of the cases during the development of this study, the temperature in the greenhouse was higher than inside the mist chamber, which could have been important in the reactions of C-15 and Pinto UI-111 to infection by I. griseola.

The effect of temperature on disease reaction is of importance to a breeding program. Cultivars with a more stable resistance over a wider range of temperature would be more useful than those cultivars showing reactions that are temperature dependent.

Attention was directed to finding out what sources of primary inoculum may have been responsible for the severe outbreaks of the disease reported during the 1982 and 1983 growing seasons.

In the present study we found that I. griseola was present in seeds harvested from infected pods. The presence of the pathogen was directly correlated with physical appearance of the seeds such as discoloration of the seed coat, malformation, and/or presence of a dark color around the hilum area. Cardona-Alvarez (10) did not find the pathogen in any of the seed samples he tested. In constrast, Orozco-Sarria and Cardona-Alvarez (35) were able to detect the pathogen in infected seed from breeding line 143. However, the same authors indicated that several other varieties and lines gave negative results.

Angular leaf spot symptoms were obtained in plants grown from these infected seeds in the greenhouse indicating that seed transmission may be an important source of ALS infection in the field. Favorable environmental conditions such as air currents and splashing water, as well as high humidity are often found in Michigan seed production areas. These factors would be very important in the dissemination of the pathogen from infected seed since they favor the production of spores on the seed coat of infected seed. After germination, the seed coat which harbors the fungal structures of the pathogen, remains at the soil level. In order for the spores to reach the primary or trifoliolate leaves, dissemination by splashing water and/or air currents is needed.

The fact that seed lots belonging to the seed fields of the Montcalm dark red kidney bean cultivar were found infected with I. griseola, indicates that seed transmission may be a source of primary inoculum for the ALS disease. Besides, the fungus was recovered from infected seed after 17 months of

storage at room temperature, although level of infection was reduced over time. Since a portion of this seed is planted in the commercial dry bean production areas of Michigan and other states, care should be taken to avoid dissemination of the fungus to pathogen-free areas.

Other reports seem to indicate that seed infection by I. griseola is different among cultivars (18,35,45). In the present study, the pathogen was recovered not only from seed of the Montcalm cultivar but also from seed of other cultivars. Moreover, the intensity of disease symptoms on pods of a cultivar seems to be related to severity of seed infection; differences among cultivars on the site of infection in the seed coat were observed. In every case, in naturally or artificially infected seed of Montcalm cultivar, the pathogen was associated with the hilum area, while in artificially inoculated plants of seed types different than red kidney, the pathogen could be isolated from different sites on the seed coat. In contrast, Orozco-Sarria and Cardona-Alvarez (35), Dinghra and Kushalappa (18), and Sohi and Sharma (53), always found the pathogen associated with the hilum area.

The overwintering studies revealed that the pathogen can survive at least one season in Michigan. Cardona-Alvarez (10) indicated that the pathogen may survive two successive winters in the debris of previously infected crops. These results indicate that the overwintering ability of the fungus constitutes also a source of primary inoculum in the field.

It should be noted that whereas large amounts of synnemata and spores were recovered from infected tissue overwintered above ground, no viable fungal structures were obtained on tissue burried below the soil surface at Rogers City, Michigan, and very few structures were formed on tissue recovered at East Lansing, Michigan. However, the pathogen appeared viable on the tissue buried below soil surface when suspensions of macerated tissue of these

samples from the two locations were used to inoculate plants of the Montcalm cultivar inducing the development of typical angular leaf spot symptoms.

Nelson (33) indicated that overwintering tissues may be heavily colonized by saprophytic microbial species, apparently because of the ability of those species to utilize the available degradation metabolites produced by the pathogen and other saprophytics. The external presence of saprophytes on tissues would reduce the ability of pathogens to sporulate, and in that sense, restrict their relative survival. A similar situation could have occurred on the plant tissue buried at Rogers City and East Lansing.

The fact that production of spores did not occur under favorable conditions in infected tissue overwintered below soil surface, indicates that the danger of this tissue as source of primary inoculum is less than infected tissue left above ground.

Strategies for control of ALS have included the use of fungicidal sprays and the breeding of disease resistant cultivars. Control measures have not specifically been directed to reduce or eliminate the sources of primary inoculum, such as infected seed. In the present study, fungal growth was inhibited on infected seeds treated with the fungicides Captan-Zineb or Benomyl. The former fungicide is known to diffuse into the seed coat where many of the seed-borne fungi are located, but not the bean cotyledons. The benomyl fungicide penetrates the seed coat and cotyledons of beans to provide control (22).

Captan-Zineb is an economically feasible chemical widely used to control other seed-borne fungal diseases (22). However, since the ALS pathogen may be located at different sites on seeds of different cultivars, further investigations including different seed types of beans would be necessary to determine if one fungicide is better than the other. Certainly, the present results prove that this method of control is satisfactory for red kidney bean types.

A source of primary inoculum is reduced by chemical seed treatment, as well as the possible introduction of the disease to pathogen-free areas avoided.

The fact that the pathogen has less ability to overwinter below the soil surface than above implies that plowing the soil down after harvesting would help to control and reduce this source of primary inoculum of ALS for the next growing season. Another possible procedure of field sanitation could be removal of plant debris and/or burning of trash to reduce the potential inoculum existing on the infected debris left after harvest.

The initial screening tests revealed that there are sources of resistance in numerous common commercial dry bean cultivars to Michigan isolates of I. griseola. However, resistance was associated primarily with navy and black bean types whereas all red kidney and cranberry bean types were susceptible. Because the disease is most serious in the red kidney bean cultivars, a large number of additional cultivars/lines were screened to identify sources of resistance.

Many sources of resistance were identified among the 115 cultivars tested. However, susceptibility was associated primarily with large and medium size seed types and no cultivar with resistance was of the same type of seed size and color as Montcalm, the most commonly grown dark red kidney bean in Michigan. Consequently, the process of breeding for resistance in the red kidney bean types is going to be more complex because other agronomic characters such as seed size and color, plant architecture, and other traits are associated with the sources of resistance and many of these traits are undesirable for a kidney bean cultivar. Another possible breeding complication is that crosses between Montcalm and some of the resistant navy bean types have yielded abnormal and sterile F1 progeny (personal communication of Dr. Jim Kelly).

Fortunately, resistance was observed in some of the accessions from Malawi and Colombia, which suggests that sources of resistance with kidney bean traits such as seed size and color are available in foreign germplasm.

Inasmuch as bean cultivars can develop different foliage and pod reaction to I. griseola, breeding programs should include evaluations during several stages of plant development. Coyne and Schuster (16) reported different leaf and pod reactions to bacterial blight infection by Xanthomonas phaseoli. They indicated that disease reactions on leaves and pods were controlled by different genes. A susceptible pod reaction would be disadvantageous since it is known that the pathogen is transmitted in the seed.

According to Schwartz et al (44), inherent pathogenic variation within populations of I. griseola complicated efforts to obtain sources of stable resistance. The existence of races has previously been suggested by Alvarez-Ayala and Schwartz (2) and Marin-Villegas (31), however, both reports suggested that additional studies were required for confirmation.

In the present studies, it was found that I. griseola exhibited pathogenic specialization or the existence of races. Michigan isolates of I. griseola possessed a relatively narrower host range among the isolates tested and pathogenic variation seemed to be related to diverse geographic regions. Twelve differential cultivars were used to divide 30 I. griseola isolates into five pathogenicity groups. Isolates from Latin America showed the widest host range. The data indicate that for development of red kidney bean types for Michigan inoculations should be made with Michigan isolates.

Michigan 31 was the only isolate able to infect the highly resistant cultivar G 05686. It would be important to continue to search different Michigan areas to see whether similar isolates exist or whether Michigan 31 was possibly a mutation.

Although no other types of variation among isolates were measured, differences in virulence and aggressiveness appeared to exist among I. griseola isolates. Some isolates required longer time periods to induce the same disease reaction on the same cultivar. More definitive studies of virulence might include data as to number of lesions per leaf, size of lesions, amount of chlorosis induced, and number of days to defoliation.

The present study emphasizes that the most effective control strategy for ALS in Michigan is the breeding of new disease resistant red kidney bean cultivars. Efforts should be intensified to locate sources of resistance to Michigan ALS isolates in germplasm agronomically similar to the kidney bean type. However, such breeding efforts are long term in nature. For this reason, there are several strategies that can be directed at the moment to eliminating possible sources of primary inoculum.

Testing seed to be planted for contamination with I. griseola in the laboratory would help to reduce a source of inoculum and avoid the introduction of the disease to pathogen-free areas. Chemical seed treatment is an excellent means for reducing seed borne primary inoculum. A third strategy of control would consist of plowing down the plant debris after harvest so that the pathogen's ability to produce spores the following spring is severely reduced. In addition, crop rotation will avoid possible direct contact of the plants with infected material existing in the soil.

Finally, care should be exercised to prevent the introduction of any I. griseola isolate to an area in which it does not exist. For example, I. griseola isolates from Latin America are virulent on most of the navy, black and pinto bean cultivars grown in Michigan and thus, pose a threat. At the least, seed being imported from Latin America should be treated with the appropriate fungicide to eliminate seed borne inoculum.

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