Angular Leaf Spot of Red Kidney Beans in Michigan

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ABSTRACT

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Numerous outbreaks of angular leaf spot (ALS), caused by *Phaeoisariopsis griseola*, were noted in Michigan fields of red kidney beans in 1982 and 1983. Variety tests revealed that navy, tropical black, and pinto beans were resistant, whereas red kidney and cranberry beans were susceptible. In some cases, foliage and pod reactions differed within the same cultivar. The pathogen survived at least two winters under Michigan conditions both in buried tissue and in infected standing plants. Strategies for control of ALS in Michigan should include crop rotation and incorporation of disease resistance into commercial red kidney bean cultivars.

Angular leaf spot (ALS), caused by the fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris (= *Isariopsis griseola* Sacc.), is a major disease of dry edible beans (*Phaseolus vulgaris* L.) in tropical and subtropical regions of the world (6). ALS has not been generally considered a disease problem in the United States, although sporadic outbreaks have been reported from Maryland (12), Pennsylvania (3), and Wisconsin (2,7). In 1982 and 1983, however, several Michigan seed growers experienced severe ALS on fields of the Montcalm dark red (8) kidney bean. The infected fields were located mainly in the northeastern lower peninsula of Michigan near Alpena and Rogers City. ALS had not previously been reported in Michigan, the largest dry bean-producing state in the United States. Thus, essentially nothing was known about the disease under Michigan conditions. The present study was conducted to obtain information on susceptibility of commercially grown dry bean cultivars to ALS and the ability of the ALS pathogen to survive overwinter in infected plant debris.

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MATERIALS AND METHODS

The pathogen was usually isolated from infected plant material by plating tissue pieces onto the surface of V-8 juice agar plates (200 ml of V-8 juice, 3 g of $CaCO_3$, 18 g of agar, and 800 ml of distilled H_2O) (1). In some cases, spores were removed from sporulating lesions and transferred directly to V-8 agar. Single-spore isolates were obtained by plating dilute spore suspensions in sterile distilled water onto the surface of potato-dextrose agar plates (PDA; 39 g of Difco PDA and 1,000 ml of distilled water). After 24 hr of incubation, individual germinated spores were located using the stereomicroscope and transferred to the surface of V-8 agar. Monosporic colonies that sporulated abundantly after 7–10 days at 20 C were maintained by periodic mass-transfer of spores to fresh V-8 agar plates.

Plant inoculations. Spores for inoculations were obtained by gently scraping the surface of sporulating colonies on V-8 plates incubated 10–12 days at 20 C. Final spore concentration as determined by hemacytometer was 10^4/ml of distilled water containing 0.05% (v/v) Tween 80 (polyoxyethylene sorbitan monooleate).

Inoculum was misted onto both surfaces of leaves on test plants possessing two trifoliolate leaves. Test plants with two fully expanded trifoliolate leaves were 15–18 days old. All tests included four replicates of each cultivar, and experiments were repeated three times. Plants were maintained in a moist chamber for 4 days at 22–28 C with periodic misting to maintain high relative humidity (near 100% RH). During summer months of 1983 and 1984, the maximum day temperature inside the mist chamber increased to 30–32 C; however, final disease reactions were not affected at these temperatures. Plants were then removed to greenhouse benches for an additional 6–10 days before they were rated for disease reactions. To examine pod reactions to *P. griseola*, plants in the mid-pod-filling stage of development (about 40 days after planting) were inoculated by spraying spore suspensions directly onto pods and incubating as previously described. In all cases, plants were grown from homogeneous seed stocks produced in the greenhouse; the stocks were derived from single-plant selections.

Overwintering studies (standing plants). Two groups of red kidney plants infected with *P. griseola* were allowed to stand in the field after the 1983 growing season: 1) plants of cultivar Montcalm that had been artificially inoculated at the Botany Farm at Michigan State University, East Lansing, and 2) plants of naturally infected Montcalm in a 10-ha field near Rogers City, MI. Beginning in December 1983, plants were collected randomly from the fields at monthly intervals for 5 mo.

Only pod and stem tissues were assayed for presence of viable pathogen. Leaves were lost at plant maturity. Assays were made on pod and stem pieces 1–2 cm long by placing the pieces on a screen and incubating them for 5–7 days at nearly 100% RH. Pieces were then removed, air-dried, and examined under the stereomicroscope for the presence of synnemata and spores. When spores were present, attempts were made to isolate *P. griseola* in axenic culture; isolates obtained were tested for pathogenicity on the universally susceptible cultivar Montcalm.

Survival in buried tissue. During August and September 1983, infected stems, leaves, and pods were collected from plants growing at both test sites. Sections (2 cm in diameter) were cut from infected leaves, whereas stems and pods were cut into pieces 1–2 cm long bearing typical lesions. The samples were air-dried at 20 C and stored at 4 C before use. During mid-December 1983, nylon bags were filled with 10 infected pieces and were buried in fields at two depths, 2–5 and 30 cm below the soil surface. Three bags were buried per site. One site was at the Botany Farm, and three sites were in the Rogers City area.

The buried samples were retrieved, one set in June 1984 and another set in June 1985, at each site. Two methods were used to test for *P. griseola* viability. The first method consisted of incubating half of the stem and pod pieces from a bag on a screen for 7 days in a moist chamber at 100% RH, then observing for fungal fruiting structures. Leaf sections had decomposed and were not recoverable from the bags. The second method involved inoculating plants with water suspensions prepared from the remaining half of the samples. Suspensions were prepared by macerating the tissues in a blender containing 100 ml of sterile distilled water. Fifteen Montcalm plants 15–18 days old were inoculated by briefly immersing the two trifoliolate leaves and the primary leaves in the suspension. Inoculated plants were incubated in a moist chamber at 100% RH until symptoms appeared, usually 7 days later. Control plants were inoculated by immersing leaves in sterile distilled water.

RESULTS

Pure cultures of *P. griseola* were easily obtained from plant tissue bearing sporulating lesions by direct transfer of spores to V-8 juice agar plates. Fungal growth was typically pigmented dark green, with sporulation developing after 6–8 days incubation at 20 C. After 10–15 days of incubation, however, tufts of gray mycelium developed on plates as newly formed spores germinated vegetatively. Routine transfers of cultures were made before regrowth appeared.

ALS symptoms appeared as small (1–2 mm in diameter) sunken lesions on leaves inoculated 10–12 days previously. Lesions gradually enlarged for several days; sporulation occurred only when plants were exposed to continuous moisture for 24–48 hr. Pod lesions were generally large (5–10 mm in diameter) with dark brown-red pigmentation.

Bean cultivar reactions. Isolates of the ALS pathogen from Michigan and Wisconsin appear almost identical in their host range among 24 of the major dry bean cultivars grown commercially in Michigan (Table 1). Kidney and cranberry bean cultivars were especially susceptible to infection with these isolates. Among the several navy cultivars tested, only Laker was fully susceptible. A large number of additional dry bean cultivars from other production areas of the United States were then tested for their reactions to the Michigan 5 isolate of ALS, with the following results: resistant—navy cultivars Admiral, Artic, Bunsi, Cumulus, Kentwood 83, Michelite, Midland, Sanilac, Wesland, and Zircon; tropical black cultivars B-190, Cornell 49242, Ebony, and T-39; pinto cultivars Agate, Ouray, and Pindak; and red mexican cultivar UI-37; intermediate—yelloweye cultivar Maine and red mexican cultivar Rufus; susceptible—navy cultivar Tall Bunyan; red kidney cultivars California Dark, California Light, Carmine, Manitou, Redkote, Royal Red, and Ruddy; cranberry cultivar UI-51; white kidney cultivar Kaboon; great northern cultivars Jules and Tara; yelloweye cultivars Kenearly and Steuben; and pink cultivar Viva.

Michigan 5 isolate of *P. griseola* was nonpathogenic to foliage of *Phaseolus acutifolius* 'Arizona Buff Tepary Bean A,' *Vigna unguiculata* 'Purple Hull' and 'Mississippi Silver' cowpea, and *Glycine max* 'Evans 82' soybean.

Pod reactions. Pod reactions were not necessarily positively correlated with reactions of foliage (Table 1), although only one isolate was tested on pods. Of 15 cultivars that showed foliage resistance to *P. griseola* (Michigan 5 isolate), five and six had susceptible and intermediate

Table 1. Foliage and pod reactions of commercial dry bean cultivars to several isolates of *Phaeoisariopsis griseola*

		Disease reaction induced by isolate[a]			
		Michigan 4	Michigan 5		Wisconsin
Class	Cultivar	Foliage	Foliage	Pod	Foliage
Navy	Aurora	–[b]	R	R	–
	C 20	R	R	I	R
	Fleetwood	R	R	S	R
	Laker	S	I	S	R
	Nep-II	R	R	I	R
	Neptune	R	R	I	R
	Seafarer	R	R	I	R
	Swan Valley	R	R	S	R
	Tuscola	R	R	I	R
Black	Black Beauty	R	R	I	R
	Black Magic	R	R	S	R
	Black Turtle Soup	R	R	S	R
	Domino	R	R	S	R
	Midnight	R	R	R	R
Kidney	Charlevoix	S	S	S	S
	Isabella	S	S	S	S
	Montcalm	S	S	S	S
	Redkloud	S	S	S	S
	Sacramento	S	S	–	S
Pinto	Olathe	R	R	–	R
	UI-III	R	R	–	R
Cranberry	Mich. Improved	S	S	S	S
	028	–	S	S	–
	Taylor Hort.	–	S	S	–

[a] Foliage: resistant (R) denotes 1 or 2, intermediate (I) denotes 3, and susceptible (S) denotes 4 or 5 on angular leaf spot disease rating scale of 1–5, where 1 = immune, no infection; 2 = 1–15% of leaflet area infected with angular leaf spot; 3 = 16–30% of leaflet area infected, lesions rarely surrounded by chlorosis; 4 = 31–50% of leaflet area infected, lesions surrounded with chlorosis; and 5 = more than 50% of leaflet area infected, lesions always surrounded with chlorosis, and defoliation common. Pod: resistant (R) denotes 1 or 2, intermediate (I) denotes 3, and susceptible (S) denotes 4 or 5 on an angular leaf spot disease rating scale of 1–5, where 1 = no infection, 2 = 1–25%, 3 = 26–50%, 4 = 51–75%, and 5 = greater than 75% of pod area covered with circular lesions.

[b] – = Not tested.

reactions, respectively. All cultivars with susceptible foliage reactions also showed susceptible pod reactions.

Overwintering studies (standing plants). *P. griseola* successfully overwintered in stem and pod tissues of infected Montcalm plants allowed to stand in the field during the winter of 1983–1984. Results shown in Table 2 indicate that the pathogen was readily recovered from tissue samples tested during five consecutive months starting in December 1983. Isolation and pathogenicity tests confirmed the identity of the isolates as *P. griseola*. The last sample was collected at about the same time (May) growers begin to plant beans in the spring.

Survival in buried tissue. Results on the formation of synnemata and spores by the pathogen on the buried samples retrieved after 1 yr are shown in Table 3. Few pieces (six of 30) of infected tissue buried 2–5 cm deep at East Lansing yielded fungal structures of the pathogen; even fewer pieces (one of 30) of infected tissue buried 30 cm at the same location yielded *P. griseola*. No synnemata or spores formed on samples retrieved from the three locations at Rogers City.

All samples collected after 1 and 2 yr were further tested by inoculating to susceptible Montcalm plants. Results (Table 4) indicate that pathogen survival depended on depth of burial of infected tissue; survival was greatest on tissue buried at 30 cm.

Symptoms of ALS developed on Montcalm plants inoculated with suspensions prepared from all retrieved tissue samples in 1984 except the one sample buried 2–5 cm deep at the Rogers City I location (Table 4). 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. Fewer samples tested in 1985 caused infection on Montcalm plants than samples tested in 1984.

When plants showing symptoms were incubated in a mist chamber at 100% RH, formation of synnemata and spores of *P. griseola* on lesions was always observed, indicating that symptoms were indeed those associated with ALS.

DISCUSSION

ALS had not previously been reported in Michigan until the disease was observed in numerous fields of the red kidney bean cultivar Montcalm during 1982 and 1983.

Standard methods for production of inoculum and for inoculation (1,2,4,7) 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). Greenhouse studies (4) showed that an inoculum concentration of 1×10^4–1×10^5 spores per milliliter was optimal for differentiating resistant, intermediate, and susceptible reactions of plants. In addition, spraying plants bearing one or two trifoliolate leaves with a spore suspension of the pathogen and incubating them in a saturated moist chamber for 4 days was a practical method for infection in the greenhouse.

Attention was directed to finding out whether overwintered inoculum of *P. griseola* may have been responsible for the severe outbreaks of the disease reported during 1982 and 1983. Over-

Table 2. Overwintering of *Phaeoisariopsis griseola* under field conditions

Date[a] / Location	No. of tissue samples Tested[b]	Positive for *P. 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] Samples included pod and stem pieces from infected Montcalm plants that stood under field conditions during the 1983–1984 winter at East Lansing and Rogers City, MI.
[c] Number of samples on which synnemata bearing spores of *P. griseola* was observed. Tissue samples were incubated 7 days in a moist chamber under high humidity conditions and examined microscopically.

Table 3. Detection of *Phaeoisariopsis griseola* after 1 yr in infected tissue placed 2–5 and 30 cm below the soil surface

Location	Tissue sample: No. pieces buried 2–5 cm Tested[a]	With *P. griseola*[b]	No. pieces buried 30 cm Tested[a]	With *P. 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 buried below the soil surface at East Lansing and Rogers City. Three replicates of 10 pieces each were tested for the presence of *P. griseola* by incubating the samples in a moist chamber. One bag was not recovered at Rogers City II (30-cm depth).
[b] Number of tissue pieces on which synnemata and spores formed after incubation.

Table 4. Overwintering of *Phaeoisariopsis griseola* in infected tissue placed 2–5 and 30 cm below the soil surface and tested by inoculating susceptible Montcalm plants with suspensions of macerated tissue[a]

Location	Year	Infected tissue buried 2–5 cm: Inoculated[b] (no.)	Infected[c] (no.)	Infected tissue buried 30 cm: Inoculated (no.)	Infected (no.)
East Lansing	1984	26	7	26	13
	1985	24	0	16	1
Rogers City I	1984	28	0	28	2
	1985	24	0	24	1
Rogers City II	1984	29	8	14	1
	1985	8	0	16	0
Rogers City III	1984	28	2	28	2
	1985	24	0	24	1

[a] Leaf, pod, and stem pieces that had overwintered 2–5 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 a suspension of macerated tissue. Plants were incubated under high-humidity conditions after inoculation until initial symptoms were observed, usually 10 days.
[b] Total number of plants inoculated.
[c] Total number of plants with angular leaf spot symptoms.

wintering studies revealed that the pathogen survived at least two consecutive seasons in Michigan and confirm the results of several previous studies. For example, Cardona-Alvarez (2) indicated that the pathogen may survive two successive winters in Wisconsin in the debris of previously infected crops. Two reports of survival from India indicate that *P. griseola* overwintered as stromatic tissue on debris (11) and that conidia survived up to 6 mo in plant debris under laboratory conditions and 8 mo under field conditions (10). These results indicate that overwintered fungus also constitutes a source of primary inoculum in the field.

It should be noted that synnemata and spores were readily recovered from infected tissue that overwintered above ground, but no viable fungal structures were observed on tissue buried below the soil surface at Rogers City and very few structures were formed on tissue recovered at East Lansing. The pathogen was recovered from debris when the indirect method of assaying on susceptible plants was used. The fact that production of spores did not occur under favorable conditions on infected tissue that had been buried over winter indicates that the inoculum potential of the pathogen from buried tissue may be less than that of the pathogen in debris left above ground.

Initial screening tests revealed that there are sources of resistance in numerous common commercial dry bean cultivars to *P. griseola.* Because the disease is most serious in the red kidney bean cultivars (4), a large number of additional cultivars/lines were screened to identify sources of resistance.

Resistance was found in navy, tropical black, and pinto beans. No resistance was found in lines with seed characteristics similar to Montcalm, the most commonly grown dark red kidney bean in Michigan. The process of breeding for resistance in red kidney beans will thus be complex.

Inasmuch as bean cultivars can develop different foliage and pod reactions to *P. griseola*, breeding programs should include evaluations of both the foliage and pods. A susceptible pod reaction would be disadvantageous because the ALS pathogen is known to be transmitted in the seed (4).

Although no other types of variation among isolates of *P. griseola* were measured, differences in virulence and aggressiveness have been observed (5). 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 indicates that an effective control strategy for ALS in Michigan would be the breeding of disease-resistant cultivars of red kidney beans. Efforts should be intensified to use reported sources of resistance (6,9) in germ plasm agronomically similar to the susceptible commercial cultivars.

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