Use of Dry Inoculum to Evaluate Beans for Resistance to Anthracnose and Angular Leaf Spot

D. A. INGLIS, Former Research Associate, D. J. HAGEDORN, Emeritus Professor, and R. E. RAND, Research Specialist, Department of Plant Pathology, University of Wisconsin-Madison, 53706

ABSTRACT

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A simple method was developed for testing beans in the field for resistance to *Colletotrichum lindemuthianum*, cause of anthracnose, and *Phaeoisariopsis griseola,* cause of angular leaf spot. Dry inoculum prepared from diseased leaves of greenhouse-inoculated beans and from fungal cultures grown on perlite-cornmeal V-8 juice agar medium was dried, pulverized, and then dusted onto moistened bean plants. Data from field plots indicated that dry inoculum of *C. lindemuthianum* and *P. griseola* was as effective as an aqueous conidial suspension for inoculating beans. Mean leaflet ratings increased throughout the growing season, and inoculated treatments had significantly lower yields than noninoculated treatments.

Anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. and angular leaf spot caused by *Phaeoisariopsis griseola* (Sacc.) Ferraris are foliar diseases on bean (*Phaseolus vulgaris* L.) and are distributed worldwide in many tropical and temperate regions (2,3). Development of resistant varieties is an effective strategy for controlling both diseases (10). Disease screening usually takes place in the greenhouse or field using an aqueous suspension for inoculum, prepared with conidia grown on an artificial medium (9). Field testing with a conidial suspension is difficult, however, when field plots are extensive and require large volumes of inoculum, and if the plots are a considerable distance from the laboratory. In addition, conidial suspensions do not store well and may be difficult to transport, so they must be prepared just before inoculation.

Dry inoculum preparations are potentially simple and convenient to produce, store, and transport, and are an efficient way of disseminating *C. lindemuthianum* and *P. griseola* conidia across large field plots. But, generally, they have not been used to inoculate leaves with foliar fungal pathogens (except obligate parasites, i.e., rusts, smuts, powdery mildews, etc.). However, Hooker (5) used dry inoculum of *Exserohilum* (=*Helminthosporium*) *turcicum* (Pass.) Leonard & Suggs to inoculate corn foliage in the field. Infection of various hosts was obtained by Kreitlow and Sherwin (6), who dusted finely pulverized dry inoculum of *Rhizoctonia* DC. ex Fr., *Sclerotinia* Fuckel, *Cercospora* Fres., and *Corynespora* Güssow over moistened leaves. Even so, the efficacy of dry inoculum versus aqueous conidial suspensions in disease screening nurseries has not been compared directly for any foliar fungal pathogen. This study evaluated different forms of dry inoculum of *C. lindemuthianum* and *P. griseola*, in comparison with liquid inoculum, for disease screening of beans in the field.

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MATERIALS AND METHODS

Anthracnose plots. The experiment designed was a randomized complete block with four treatments replicated four times. Plots were in two rows 1 m apart by 10 m long with 4.5-cm seed spacing. Half of each two-row plot was planted to the susceptible cultivar, Bountiful, and the other half to the resistant cultivar, Topcrop. Each plot was surrounded by a 1-m-wide planting of corn to limit cross-plot contamination. The entire plot was surrounded on all sides by a 4-m-wide planting of corn to minimize the entry of other foliar pathogens. Plots were established in 1983 and 1984 at the University of Wisconsin experimental farm at Hancock in areas isolated from other bean fields. Supplemental overhead irrigation was used as needed.

The first season, dry inoculum was prepared from diseased bean leaves collected the previous year from a field nursery and from leaves of diseased greenhouse-inoculated plants. Dry inoculum was prepared the second season from diseased leaves of greenhouse-inoculated plants and from *C. lindemuthianum* grown in glass jars on perlite-cornmeal V-8 juice agar medium, following the method of Miles and Wilcoxson (7). All diseased leaves and the fungus from the cultured perlite medium were dried immediately without heat in front of an electric fan, pulverized in a Wiley Mill, sized in an 18-mesh sieve to < 1 mm, and stored in double plastic bags at 5–10 C. The concentration of conidia per gram of dried leaves or of the fungus from the perlite medium was estimated by diluting the inoculum 1:100 with water and counting the number of conidia per milliliter with a hemacytometer. The final concentration of field-collected dry leaf inoculum was approximately 1.2×10^4 conidia per gram; greenhouse-prepared dry leaf inoculum was approximately 1.3×10^7 and 6.5×10^7 conidia per gram in 1983 and 1984, respectively; and laboratory-prepared dry perlite inoculum was approximately 2.9×10^6 conidia per gram. A conidial suspension in water was prepared in the traditional manner by suspending conidia collected from *C. lindemuthianum* cultures grown on V-8 juice agar medium. The concentration was adjusted with a hemacytometer to approximately 7.6×10^5 and 2.8×10^5 conidia per milliliter in 1983 and 1984, respectively. Application rates were adjusted so that for each type of inoculum, whenever possible, approximately 1.0×10^5 conidia were applied per plant. Due to the initial low concentration of conidia in the field-collected leaf inoculum, only 1.0×10^3 conidia per plant could be applied.

When they had two sets of fully expanded trifoliolate leaves (3–4 wk after planting) plants were inoculated early in the evening following irrigation to ensure that moist conditions prevailed throughout the infection period. Plants receiving dry inoculum were first wetted with water containing a sticking agent (12% potassium resinate and 2.5% potassium oleate a.i.) at a concentration of approximately 250 mg/ml. Each 5-m row was sprayed with 0.5 L of this solution. A measured amount of dry inoculum was shaken gently over the leaves so the distribution would be as uniform as possible. Conidia were suspended in the water-sticker solution and sprayed onto the plants immediately after suspension.

The number of diseased plants from one row of each plot (approximately 100

Table 1. Percentages[x] of plants of bean cultivars[y] with symptoms of anthracnose and angular leaf spot 2 wk after inoculation with dry inoculum and a conidial suspension of *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*

Treatment	Anthracnose plot				Angular leaf spot plot			
	1983		1984		1983		1984	
	Bountiful	Topcrop	Bountiful	Topcrop	Montcalm	California Dark Red	Montcalm	California Dark Red
Conidial suspension	100	8	100	0	26	30	100	100
Laboratory-prepared dry perlite inoculum	...[z]	...	100	1	...	...	100	98
Greenhouse-prepared dry leaf inoculum	100	0	100	2	98	80	99	99
Field-collected dry leaf inoculum	100	96	...	...	...	...	...	...
Noninoculated	15	0	2	0	8	6	4	5

[x] Means of four replicates, 100 plants per replicate, except for the 1984 angular leaf spot plot where percentages are the means of three replicates.
[y] Bountiful and Montcalm = susceptible, Topcrop = resistant, California Dark Red = tolerant.
[z] Treatment not done.

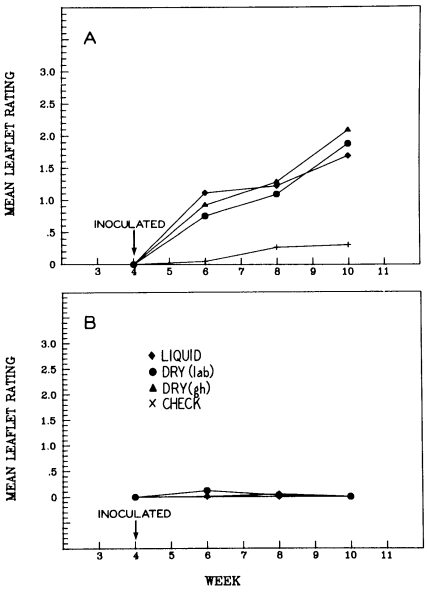

Fig. 1. Mean leaflet ratings from the 1984 anthracnose plot for **(A)** Bountiful and **(B)** Topcrop 2, 4, and 6 wk after inoculation with dry (laboratory- and greenhouse-prepared) inoculum and a conidial suspension (liquid) of *Colletotrichum lindemuthianum*. Means are of all two-thirds to fully expanded leaves from four replicates (six plants per replicate).

plants) was counted 2 wk after inoculation to determine the infective efficacy of each type of inoculum. Thereafter, the mean disease rating for leaflets (MLR) from six plants of each treatment was assessed at 2-wk intervals to evaluate leaf-to-leaf spread of *C. lindemuthianum*. Leaflets were scored by collecting all two-thirds to fully expanded trifoliolate leaves and rating them 0 = no disease, 1 = 1–10% veins with lesions, 2 = 11–25% veins and veinlets with lesions, and 3 = 26% or more veins and veinlets with lesions. Yield was evaluated for the 1984 plot by harvesting the pods from 3 m of a treatment row 10 wk after planting.

Angular leaf spot plots. The experimental design was the same as that used for the anthracnose field trials, except that eight-row plots, each 5 m long, were planted to cultivar Montcalm or California Dark Red kidney beans. Field observations have indicated that Montcalm is less tolerant to *P. griseola* than California Dark Red (D. J. Hagedorn, *personal communication*). Plots were established in 1983 and 1984 at the University of Wisconsin experimental farm at Arlington. Areas isolated from other bean plantings were chosen for planting. Supplemental overhead irrigation again was available.

Plants were inoculated 3 wk after planting with inoculum, prepared, and adjusted as for the anthracnose experiment. Application methods also were the same. The concentration of greenhouse-prepared dry inoculum was 4.2×10^7 and 8.4×10^5 conidia per gram of dried leaves in 1983 and 1984, respectively. Laboratory-prepared dry perlite inoculum had 3.1×10^6 conidia per gram. Application rates were adjusted so that for the dry inoculum approximately 1.0×10^5 conidia were applied to each plant, if possible. The conidial suspension contained 5.8×10^4 and 1.1×10^4 conidia per milliliter in 1983 and 1984, respectively. Approximately 1.0×10^4 conidia were applied per plant.

The percentage of diseased plants 2 wk after inoculation, the mean number of leaflets per plant, and the MLR of each treatment at 2 wk intervals following inoculation was assessed both years. Disease severity was rated on a scale of 0 = no disease, 1 = 1–10% leaflet area with lesions, 2 = 11–25% leaflet area with lesions, 3 = 26–50% leaflet area with lesions and limited chlorosis, 4 = 50% or more of the leaflet area with lesions and extensive necrosis, and 5 = defoliation. This rating scale is similar to the one used by Moreno (8). At seed maturity, yield was evaluated for 3 m of a row.

RESULTS

Anthracnose plots. The percentage of plants with anthracnose 2 wk following inoculation is reported in Table 1. All inoculum types were effective in establishing infection. Topcrop was not resistant to the *C. lindemuthianum* race of the field-collected dry leaf inoculum. Thereafter, field-collected dry inoculum was not used in order to eliminate the possibility of introducing other pathogens. In spite of efforts to limit plot-to-plot spread of *C. lindemuthianum*, 15% of the noninoculated plants of the susceptible cultivar were diseased after 2 wk.

The way in which MLRs increased during the growing season was similar both years, and is reported for the 1984 plot (Fig. 1). The mean disease rating for leaflets of Bountiful were similar for the three inoculum treatments, indicating that leaf-to-leaf spread of the disease occurred as readily for dry forms of inoculum as for the conidial suspension. None of the inoculum treatments altered the expected reaction of the resistant cultivar, although it was noted that some pods of Topcrop developed small anthracnose lesions late in the season following inoculation with the conidial suspension.

All inoculated plots of the cultivar Bountiful yielded significantly less than the noninoculated check plots, although no yield differences were observed among inoculum treatments (Table 2). There were no significant differences in yield between treatments for Topcrop.

Angular leaf spot plots. The percentage of plants with angular leaf spot 2 wk after inoculation is reported in Table 1. The conidial suspension was ineffective in causing infection in 1983, possibly because moist conditions following inoculation did not prevail for a long enough period of time and the foliage dried. However, in 1984 the conidial suspension and the dry inoculum were equally effective in establishing infection, and the percentage of plants of Montcalm and California Dark Red that were infected was about the same.

The mean disease rating for leaflets increased similarly during both growing seasons, and are shown for the 1984 plot (Fig. 2). As was the case for the *C. lindemuthianum* plots, MLRs between inoculum treatments differed only slightly (Fig. 2A,B). Symptoms of angular leaf spot were not visibly severe until about the fifth week after inoculation when sporulating lesions on oldest

(inoculated) leaves and wind-driven rain storms coincided to cause movement of the fungus within the canopy. Defoliation then began to occur for Montcalm (Fig. 2C). Defoliation of California Dark Red also began to occur about 5 wk after inoculation, except for plants in 1984 that were inoculated with dry inoculum. These began to defoliate 7 wk after inoculation (Fig. 2D). Because there was a slight wind when this dry inoculum was applied, perhaps the actual number of spores reaching some of the plants was diminished and the disease was slower to develop on the more tolerant California Dark Red. Indeed, throughout the season plants of Montcalm generally had higher MLRs and fewer number of leaflets than plants of California Dark Red, verifying past observations that Montcalm is less tolerant than California Dark Red to *P. griseola*. Yields of inoculated plants were significantly lower than those of noninoculated plants (except for those inoculated with the conidial suspension in 1983) in both years for both cultivars (Table 2).

DISCUSSION

Simple inoculation procedures are needed when screening large numbers of plants for resistance to foliar diseases (5). Results of the 1983 and 1984 trials show that dry inoculum, whether prepared from diseased leaves from greenhouse-inoculated plants or from fungus grown on perlite medium cultures, is as effective as an aqueous conidial suspension for inoculating the foliage of beans with *C. lindemuthianum* and *P. griseola*. Symptoms of both anthracnose and angular leaf spot developed on almost all inoculated plants within 2 wk of inoculation, indicating a high degree of infection efficiency. Secondary spread of the pathogen was evidenced by the steady increase in MLRs during the growing season. Red kidney beans inoculated with *P. griseola* began to lose leaves 5–7 wk after inoculation, and were nearly defoliated by the season's end. Border plots of corn failed to prevent spread of either disease from inoculated plots to adjacent noninoculated plots. However, inoculated plants had significantly lower yields compared with noninoculated plants. With the exception of the field-collected dry leaf *C. lindemuthianum* inoculum used in 1983, none of the inocula altered the expected reactions of the resistant or tolerant cultivars. Therefore, these techniques could likely be adapted for use in other field-testing programs.

Each type of dry inoculum tested had different requirements for preparation, quantification, and application. Preparation of dry inoculum was simplest from field-collected, dried, diseased leaves. This inoculum has successfully been used for large-scale field screening of bean cultivars for bacterial brown spot resistance (4). However, the method was unsuitable for *C. lindemuthianum* and *P. griseola* inoculation because the concentration of conidia per gram was too low, too few conidia were viable, and the risk of introducing contaminants into the plot was too great. Furthermore, the conidia in field-collected dry inoculum were much more difficult to count, because of the presence of numerous other fungal spores. These problems were overcome by preparing dry inoculum from diseased leaves of greenhouse-inoculated plants. However, this inoculum preparation required considerable greenhouse space, was somewhat time-consuming, and necessitated the use of mist chambers to ensure infection and induce sporulation. Quantification also was tedious unless the concentration of conidia per gram was relatively high. The cornmeal-perlite V-8 juice agar medium was successfully used to prepare dry inoculum. Probably many other types of media could be used for this purpose. Quantification was done by counting the number of conidia in a diluted water suspension using a hemacytometer, although dilution plating onto V-8 juice agar medium with antibiotic amendments probably could also be done.

Applying dry inoculum to foliage in

Table 2. Yields for bean plants inoculated with dry inoculum and a conidial suspension of *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*[v]

	Anthracnose plot[w]		Angular leaf spot plot[x]			
	Bountiful	Topcrop	Montcalm		California Dark Red	
Treatment	1984	1984	1983	1984	1983	1984
Conidial suspension	31.05 a[y]	44.74 a	12.7 b	6.31 a	14.5 b	6.71 a
Laboratory-prepared dry perlite inoculum	31.92 a	45.73 a	...[z]	7.31 a	...	7.24 a
Greenhouse-prepared dry leaf inoculum	32.17 a	49.01 a	9.5 a	7.92 a	11.2 a	7.5 a
Noninoculated	39.61 b	46.23 a	13.2 b	10.95 b	15.4 b	11.59 b

[v] Means are average of four replicates (each replicate was all plants from 3 m of row), except for the 1984 angular leaf spot plot where the means are the average of three replicates.
[w] Weight (g/plant) of green pods.
[x] Weight (g/plant) of dried seed.
[y] Means in the same column followed by the same letter are not significantly different ($P = 0.05$), as determined by Student-Newman-Keuls test.
[z] Treatment not done.

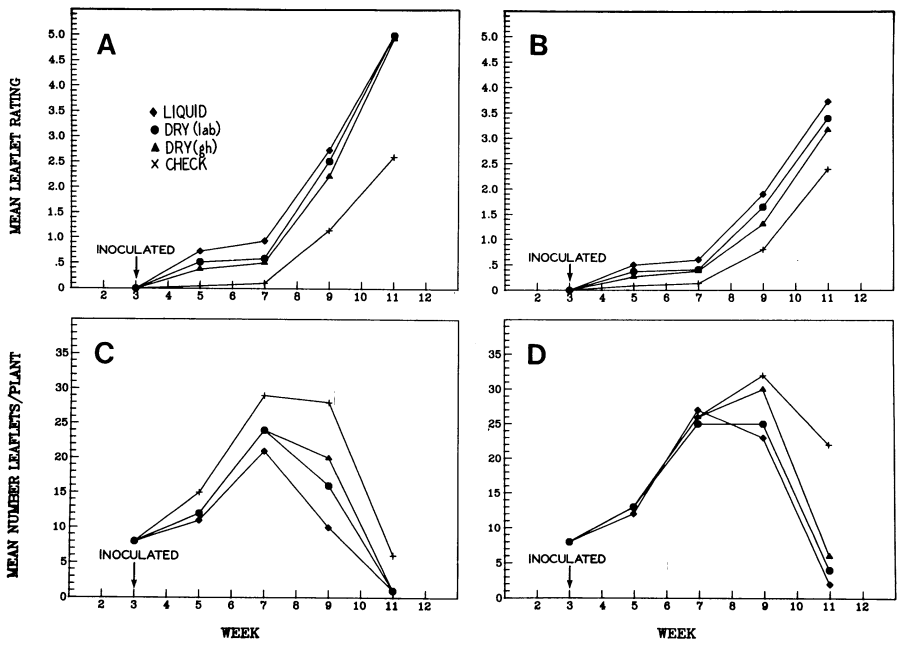

Fig. 2. (A) Mean leaflet ratings and **(C)** mean number of leaflets per plant from the 1984 angular leaf spot plots for Montcalm, and **(B)** mean leaflet ratings and **(D)** mean number of leaflets per plant from the 1984 angular leaf spot plots for California Dark Red 2, 4, 6, and 8 wk after inoculation with dry (laboratory- and greenhouse-prepared) inoculum and a conidial suspension (liquid) of *Phaeoisariopsis griseola*. Means are of all two-thirds to fully expanded leaves from four replicates (six plants per replicate).

the field is superior to applying a conidial suspension in terms of both space and time. Since dry inoculum can be stored in the refrigerator, it can be prepared well in advance (1–2 yr) of the anticipated inoculation date. Since it occupies very little space, it is not difficult to transport to field plots located far from the laboratory. Once at the field plot, dusting dry inoculum onto leaves moistened with water containing a sticking agent is no more difficult than applying a conidial suspension. Dry inoculum should not be applied when it is windy, however, because wind will interfere with inoculum deposition on target bean leaves and may disperse conidia to adjoining plots. Since conidia in dry inoculum have not yet begun to germinate (as they do in a suspension of water), dry forms of inoculum can theoretically persist for a longer time in the field after inoculation than conidia added to an aqueous suspension and then transported. This is advantageous when proper environmental conditions for infection and disease development do not prevail immediately following inoculation, a situation that may have occurred in the 1983 *P. griseola* plot.

Aqueous conidial suspensions will probably remain the inoculum of choice for foliar pathogens like *C. lindemuthianum* that are fast-growing and relatively easy to isolate, maintain, and culture, providing laboratory expertise and facilities are available and field plots are not too far away. Dry inoculum is probably advantageous to conidial suspensions for foliar pathogens like *P. griseola*, however, that are extremely slow-growing (1) (colony diameter may increase less than 1 millimeter per week on many media, including V-8 juice agar [Inglis, *unpublished*]), even when field plots are near the laboratory. The greatest advantage for using dry inoculum, however, may be that loss of virulence, associated with growing and repeatedly transferring a pathogen on artificial media in the laboratory, is circumvented.

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