The Use of Dry-Leaf Inoculum for Establishment of Common Bacterial Blight of Beans

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ABSTRACT

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Inoculum of *Xanthomonas campestris* pv. *phaseoli*, causal agent of common bacterial blight of beans, was prepared from dried diseased bean leaves collected from greenhouse- or field-grown plants. Viable colony-forming units in the dry-leaf inoculum were quantified on general and selective media. Pathogenic strains of *X. c.* pv. *phaseoli* were recovered from dry-leaf inoculum stored for up to 6 yr. The efficacy of dry-leaf inoculum for establishing common blight epidemics in the field was compared to that of an aqueous cell suspension in tests with a susceptible and resistant bean cultivar. Dry-leaf inoculum was applied to seed at planting or to plants at two stages of growth. Symptom severities in plants inoculated with the dry-leaf inoculum were similar to those of plants inoculated with aqueous cell suspensions. Symptom severity varied with inoculation method, but the expected cultivar reaction to the disease was observed after inoculation with dry-leaf inoculum or the aqueous cell suspension. Dry-leaf inoculum provides a simple, rapid, and effective technique for inoculating beans with *X. c.* pv. *phaseoli*, particularly where laboratory facilities are limited.

Common bacterial blight of beans caused by *Xanthomonas campestris* pv. *phaseoli* (E. F. Smith) Dye can be a major constraint on dry bean (*Phaseolus vulgaris* L.) production, particularly in tropical and subtropical countries (27). Most dry bean cultivars are susceptible to common blight, and the disease occurs even in areas where certified pathogen-free seed is used (28). Therefore, planting resistant cultivars is the most practical approach to managing common bacterial blight.

The evaluation of bean germ plasm for resistance to *X. c.* pv. *phaseoli* can be a tedious and technically difficult procedure. A common technique for inoculating beans in the field consists of spraying bacterial suspensions (approximately 1×10^8 colony-forming units [cfu]/ml in water or buffer) on plants. This procedure requires maintenance of bacterial cultures, preparation and quantification of bacterial suspensions, and inoculation of plants at least once during the growing season. Furthermore, successful inoculation requires favorable environmental conditions during and after inoculation for bacterial survival, population increase, and subsequent disease development.

In this paper we describe a rapid, simple technique for inoculating beans with *X. c.* pv. *phaseoli*. Research involved the development of methods for preparation of dry-leaf inoculum, quantification of inoculum, evaluation of application methods, and comparison of common bacterial blight development after inoculation with dry-leaf inoculum with that after inoculation with aqueous cell suspensions.

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MATERIALS AND METHODS

Dry-leaf inoculum production and storage. Dry-leaf inoculum was prepared from diseased bean leaves (cv. Topcrop or Bountiful) collected 14 days after greenhouse-grown plants had been razor blade-inoculated with a *X. c.* pv. *phaseoli* (Wisconsin strain, WT-1) suspension (1×10^8 cfu/ml). (In razor blade inoculation, incisions are made in leaves with a razor blade that has been dipped in an aqueous cell suspension of *X. c.* pv. *phaseoli* [18]). Diseased leaves were dried at 28–30 C for 24–48 hr, ground thoroughly in a Waring Blendor, sifted through a 32-mesh sieve (< 500 μm), and stored in plastic bags at 4 or −20 C. Inoculum was similarly prepared from field-collected leaves with common bacterial blight symptoms.

Inoculum quantification. A standard technique was developed in which a 1.0- or 0.1-g sample of dry-leaf inoculum was placed in 100 ml of sterile 0.01 M potassium phosphate buffer (PB) (pH 7.2) and sonicated 20 min in an ultrasonic cleaner. From this suspension, a dilution series was prepared and 0.1-ml aliquots were plated on three media: yeast-dextrose-carbonate agar (YDCA) (7), sucrose-peptone agar (SPA) (10), and MXP, a semiselective medium for *X. c.* pv. *phaseoli* (3). Counts were made after incubation for 3–5 days at 24 C. Bacterial populations (cfu/g of dry-leaf inoculum) were determined for inocula prepared from field-collected and greenhouse-inoculated leaves.

In 1986, dry-leaf inoculum from 1986 greenhouse-inoculated leaves was stored at four different temperatures (−20, 4, 24, and 32 C) over a 12-mo period, and *X. c.* pv. *phaseoli* survival was determined. After 6- and 12-mo storage, 0.1-g samples of inocula stored at different temperatures were assayed, as before, on SPA and MXP.

After all quantification experiments, five colonies were randomly selected and tested for pathogenicity by inoculating fully expanded trifoliolate leaves of 4- to 5-wk-old bean plants (cv. Topcrop) in a 24–28 C growth chamber using razor blade or Carborundum cotton swab inoculation (4). After 7–10 days, inoculated plants were evaluated for disease symptoms and isolations were made from leaves.

Field studies with dry-leaf inoculum. Field plots were established in 1985 and 1986 to evaluate two techniques for inoculating bean plants with dry-leaf inoculum. In one technique, an aqueous dry-leaf inoculum suspension was sprayed in the furrow on the seed or over the furrow soil surface immediately after planting. To prepare the suspension, dry-leaf inoculum (4 g) was mixed with 1.9 L of water plus three drops of spreader-sticker spray adjuvant in a pump sprayer. The suspension (1.9 L) was applied to a 4.6-m row (about 100 seeds) at low pressure (approximately 0.9 kg/cm^2) through the sprayer with the nozzle removed. In a second technique, dry-leaf inoculum (4 g/4.6-m row) was applied to bean plants at the V1-V2 growth stage (primary and first trifoliolate leaves developed) or R1 stage (after bloom, many trifoliolate leaves developed) (17). Leaves were injured with a 1% Carborundum suspension plus three drops of spreader-sticker with a CO_2-pressurized sprayer at 7.2 kg/cm^2 through hollow cone nozzles with tip-core size D4-13. Inoculum was applied with a flour sifter (a 9.5-cm-diameter aluminum can with a 1.5-mm-diameter pore size sieve bottom) to plants immediately after spraying. These techniques were compared with a liquid inoculation technique in which an aqueous bacterial suspension was prepared in PB from *X. c.* pv. *phaseoli*

grown on SPA for 24–48 hr and diluted to obtain an optical density of 0.2 at 600 nm in a spectrophotometer (model 340, Sequoia-Turner Corp., Mountain View, CA), which results in about 1×10^8 cfu/ml; 250 ml of this suspension was applied per 4.6 m of row with a Micro-ulva sprayer (Micron Sprayers Ltd., Broomyard, England). All foliar treatments were applied in the late afternoon of partly cloudy or sunny days with little wind, following a morning overhead irrigation. The control treatment was not inoculated.

In 1985, a plot of the susceptible cultivar Topcrop was established in Madison, WI, on 24 July (soil type: Kegona silt-loam, Mollic Hapludalf). The dry-leaf inoculum suspension was applied in the furrow or over the furrow soil surface. Treatment plots consisted of two 3-m rows spaced 0.9 m apart with about 80 seeds spaced 3.75 cm per row, and treatments were separated by four border rows of beans. Treatments were replicated four times in a randomized complete block design. In 1986, the Madison plot was the same as in 1985, except that dry-leaf inoculum was applied in the furrow only and was planted 26 July.

Another plot was established at Hancock, WI, at the UW Experimental Station on 27 June 1985 (soil type: Plainfield loamy sand, Typic Udipamment). All treatments described above except the aqueous cell suspension applied to V1-V2 stage plants were applied to two bean cultivars: the susceptible Topcrop and the resistant Great Northern (GN) Harris (5). Treatment plots consisted of two 9.2-m rows spaced 0.9 m apart with 200 seeds per row spaced 3.75 cm. In one row, Topcrop was planted in the first half row, then GN Harris. In the adjacent row, GN Harris was planted in the first half row, then Topcrop. Treatments were separated by six border rows of beans and were replicated four times in a randomized complete block design. In 1986, the Hancock plot was planted on 18 June. All treatments and the plot design were identical to those of 1985, except for addition of the aqueous cell suspension applied to plants at V1-V2 stage. Plants at V1-V2 stage were inoculated 16 and 20 days after planting and plants at R1 stage were inoculated 40 and 44 days after planting in 1985 and 1986, respectively.

Disease development was monitored in all plots throughout the growing season. Ten leaves showing symptoms were collected from each plot for each year and assayed for *X. c.* pv. *phaseoli*. From each leaf, three tissue sections were excised from the lesion margins and macerated in one drop of sterile H_2O on a microscope slide. A loopful of this suspension was diluted in another drop of sterile water and a loopful of this suspension was streaked on MXP. Plates were incubated 3–5 days at 24 C and then checked for xanthomonad colonies. Five colonies from each plot for each year were tested for pathogenicity as before.

Plants at R7 stage (full length pods and seeds) were rated for disease incidence and severity, and yields were determined at Hancock on 2 September 1985 and 1 September 1986. At Madison, plants were rated for disease on 7 October 1985 and 10 October 1986. Disease ratings were obtained from 20 randomly selected plants of each cultivar from one row of each treatment replicate (total of 80 plants per treatment). Plants were visually rated for disease based on a 0–5 scale (0 = no disease, 1 = 1–10% blight, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = >75%) (13). The total number of diseased plants and the number of diseased trifoliolate leaves and diseased leaflets per plant (from three randomly selected leaves per plant) were recorded. At Hancock, yields were determined in the remaining row of each treatment replicate. Plants from the inner 3 m were harvested, pods were removed from plants, and fresh weights were determined.

For each treatment replicate, means were determined for disease parameters and yield, and treatment means were calculated. For each of the variables (disease rating, yield, and percent diseased plants, leaves, and leaflets), an analysis of variance was performed on mean replicate values incorporating the effects of treatment, variety, and replication. Residuals from the fitted model were tested for normality with a Shapiro-Wilk W statistic (20). In no case was the hypothesis of normality rejected at the 10% probability level. Consequently, no transformation of the data was deemed necessary. Where the F statistic was significant, means were separated with a Fisher's least significant difference test.

RESULTS

Dry-leaf inoculum production. Viable bacteria were consistently recovered from dry-leaf inoculum prepared from greenhouse- or field-inoculated bean leaves (Table 1). The population of viable bacteria in the dry-leaf inocula was quantified using general (YDCA and SPA) and semiselective (MXP) media. Populations of *X. c.* pv. *phaseoli* from dry-leaf inoculum were readily determined by plating appropriate dilutions (usually −5, −6, or −7) of the dry-leaf inoculum-PB suspension that had been sonicated 20 min. Viable bacterial counts ranged

Table 1. Recovery of *Xanthomonas campestris* pv. *phaseoli* from dry-leaf inoculum[x]

Year produced/ year tested	Source[y]	Medium[z]	Colony-forming units per gram
1981/1987	Field	SPA	4.4×10^8
		MXP	5.4×10^8
1982/1985	Field	YDCA	1.9×10^9
		MXP	1.1×10^9
1982/1987	Field	SPA	2.8×10^9
		MXP	1.6×10^9
1985/1985	GH	YDCA	1.6×10^{10}
		MXP	1.4×10^{10}
1985/1985	GH-D	YDCA	8.1×10^9
		MXP	7.7×10^9
1985/1987	GH	SPA	1.2×10^9
		MXP	1.2×10^9
1985/1985	Field	MXP	7.3×10^9
1985/1987	Field	SPA	2.3×10^9
		MXP	2.7×10^9
1986/1986	GH	SPA	3.1×10^{10}
		MXP	2.7×10^{10}
1986/1987	Field	SPA	1.8×10^9
		MXP	1.5×10^9

[x] Inoculum (0.1–1.0 g) sonicated in 100 ml of sterile 0.01 M potassium phosphate buffer, *X. c.* pv. *phaseoli* colony-forming units quantified from dilution plate counts, and inoculum stored at 4 C.

[y] GH = Greenhouse-produced infected leaves, GH-D = greenhouse-produced infected leaves, diseased tissue only.

[z] SPA = Sucrose-peptone agar, MXP = *X. c.* pv. *phaseoli* semiselective medium, YDCA = yeast-dextrose-carbonate agar.

Table 2. Mean disease ratings and disease incidence for the *Xanthomonas campestris* pv. *phaseoli* inoculation plot at Madison, WI[y]

Treatment	Mean disease rating[z]	Disease incidence (%)		
		Plants	Leaves	Leaflets
1985				
Dry-leaf inoculum suspension				
In furrow	1.7 a	99 a	73 a	53 a
Over furrow	1.5 a	95 a	70 a	53 a
Control	0.1 b	10 b	4 b	3 b
1986				
Dry-leaf inoculum suspension				
In furrow	1.4 a	100 a	85 a	68 a
Control	0.0 b	0 b	0 b	0 b

[y] Means followed by the same letter are not significantly different at $P = 0.05$, according to Fisher's least significant difference test. Means represent values from 20 plants, 100 leaves, and 300 leaflets from each of four replicates per treatment.

[z] Based on a scale of 0–5, where 0 = no disease, 1 = 1–10% blight, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = >75%.

from 10^8 to 10^{10} cfu/g (Table 1), with greatest recovery from greenhouse-produced dry-leaf inocula less than 1 yr old. Viable bacterial counts decreased over time, but dry-leaf inoculum produced in 1981 and 1982 had 10^8 and 10^9 cfu/g, respectively, in 1987. Fewer bacteria were recovered from dried diseased tissue than from entire dried diseased leaves, and bacterial recovery from dry-leaf inoculum from field-collected leaves was similar to that from greenhouse-inoculated leaves (Table 1). Little contamination of inoculum with fungi or other bacteria was detected, regardless of isolation medium or dry-leaf inoculum source.

X. c. pv. *phaseoli* was recovered from dry-leaf inoculum stored at all four temperatures for 6 mo and 1 yr. Initial bacterial recovery from this dry-leaf inoculum was 3.2×10^9 cfu/g. After 12 mo of storage at −20, 4, 24, and 32 C, viable bacterial counts were 3.1×10^8, 4.2×10^7, 1.3×10^7, and 8.4×10^4 cfu/g, respectively.

All 50 xanthomonad colonies recovered from the dry-leaf inoculum that were tested for pathogenicity caused symptoms of common bacterial blight on beans, and *X. c.* pv. *phaseoli* was always reisolated from inoculated leaves.

Field studies. In all field trials, dry-leaf inoculum and/or aqueous cell suspension treatments resulted in significantly more common blight than in controls (Tables 2, 3, and 4). Plants from seed inoculated with dry-leaf inoculum suspensions had lesions on primary leaves 2–3 wk after planting. Plants inoculated with aqueous cell suspensions or dry-leaf inoculum had water-soaked lesions on primary and/or trifoliolate leaves 1–2 wk after inoculation. *X. c.* pv. *phaseoli* was consistently isolated from these lesions.

In the Madison plot, a moderate level of common blight occurred in dry-leaf inoculum treatments, with more disease detected where dry-leaf inoculum suspensions were applied directly over seed in 1985 (Table 2). In the Hancock trials, mean disease ratings (MDRs) in dry-leaf inoculum treatments were not significantly different from those of the aqueous cell suspension treatments for the same inoculation method in 1985 or 1986 (Tables 3 and 4). The greatest amount of common blight occurred when V1-V2 stage plants were inoculated (Tables 3 and 4). In 1985, GN Harris plants had a MDR of 2.4 when V1-V2 stage plants were inoculated, which was significantly greater than that for other GN Harris treatments and greater than that for Topcrop (Table 3). Mean disease ratings for Topcrop treatments in 1985 were not significantly different except for the dry-leaf inoculum suspension applied over the furrow soil surface, which was lower than dry-leaf inoculum applied to V1-V2 stage plants. In 1986, MDRs for GN Harris plants inoculated at V1-V2

Table 3. Mean disease ratings, disease incidence, and yields for the *Xanthomonas campestris* pv. *phaseoli* inoculation plot at Hancock, WI, in 1985[w]

Treatments[x]	Mean disease rating[y]	Disease incidence (%)			Yield[z] (g)
		Plants	Leaves	Leaflets	
Topcrop, susceptible bean cultivar					
Dry-leaf inoculum suspension					
In furrow	1.6 ab	99 a	56 b	38 b	3,292 a
Over furrow	1.2 b	90 a	41 c	20 c	3,351 a
Dry-leaf inoculum					
V1-V2 stage	2.0 a	100 a	74 a	53 a	2,613 b
R1 stage	1.5 ab	99 a	65 ab	39 b	3,224 a
Aqueous cell suspension					
R1 stage	1.5 ab	93 a	57 b	35 b	3,105 ab
Control	0.2 c	20 b	4 d	2 d	3,547 a
Great Northern Harris, resistant bean cultivar					
Dry-leaf inoculum suspension					
In furrow	1.3 b	81 a	38 b	24 b	3,150 a
Over furrow	0.6 cd	50 b	15 c	8 de	2,958 a
Dry-leaf inoculum					
V1-V2 stage	2.4 a	99 a	69 a	53 a	2,926 a
R1 stage	0.9 bc	78 a	28 b	13 cd	3,045 a
Aqueous cell suspension					
R1 stage	1.0 bc	75 a	32 b	17 c	3,047 a
Control	0.2 d	25 c	4 c	2 e	3,044 a

[w] Means followed by the same letter are not significantly different at $P = 0.05$, according to Fisher's least significant difference test. Means represent values from 20 plants, 100 leaves, and 300 leaflets from each of four replicates per treatment.

[x] V1-V2 = Primary and first trifoliolate leaves developed; R1 = after bloom, many trifoliolate leaves developed.

[y] Based on a scale of 0–5, where 0 = no disease, 1 = 1–10% blight, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = >75%.

[z] Mean yield of pod fresh weights from a 3-m row.

Table 4. Mean disease ratings, disease incidence, and yields for the *Xanthomonas campestris* pv. *phaseoli* inoculation plot at Hancock, WI, in 1986[w]

Treatments[x]	Mean disease rating[y]	Disease incidence (%)			Yield[z] (g)
		Plants	Leaves	Leaflets	
Topcrop, susceptible bean cultivar					
Dry-leaf inoculum suspension					
In furrow	2.7 a	100 a	87 a	68 a	2,628 bcd
Over furrow	2.0 b	96 a	76 ab	53 bc	3,059 abc
Dry-leaf inoculum					
V1-V2 stage	2.5 a	100 a	84 a	62 ab	2,389 cd
Aqueous cell suspension					
V1-V2 stage	2.5 a	100 a	86 a	65 a	2,145 d
Dry-leaf inoculum					
R1 stage	1.8 b	99 a	68 b	47 c	3,463 a
Aqueous cell suspension					
R1 stage	1.9 b	98 a	67 b	45 c	3,286 ab
Control	0.8 c	66 b	24 c	10 d	3,093 ab
Great Northern Harris, resistant bean cultivar					
Dry-leaf inoculum suspension					
In furrow	0.8 c	75 bc	35 b	18 b	2,957 a
Over furrow	0.5 d	51 d	17 c	8 c	2,588 a
Dry-leaf inoculum					
V1-V2 stage	1.4 a	99 a	65 a	42 a	2,514 a
Aqueous cell suspension					
V1-V2 stage	1.2 ab	95 a	64 a	39 a	2,690 a
Dry-leaf inoculum					
R1 stage	1.0 bc	90 ab	42 b	23 b	2,758 a
Aqueous cell suspension					
R1 stage	0.9 c	74 c	34 b	19 b	2,809 a
Control	0.2 e	16 e	3 d	1 c	2,871 a

[w] Means followed by the same letter are not significantly different at $P = 0.05$, according to Fisher's least significant difference test. Means represent values from 20 plants, 100 leaves, and 300 leaflets from each of four replicates per treatment.

[x] V1-V2 = Primary and first trifoliolate leaves developed; R1 = after bloom, many trifoliolate leaves developed.

[y] Based on a scale of 0–5, where 0 = no disease, 1 = 1–10% blight, 2 = 11–25%, 3 = 26–50%, 4 = 51–75%, and 5 = >75%.

[z] Mean yield of pod fresh weights from a 3-m row.

stage were significantly greater than those for any other GN Harris treatment. All MDRs for GN Harris were significantly less than those for Topcrop (Table 4). Topcrop MDRs for the dry-leaf inoculum suspension applied directly to seed and the dry-leaf inoculum and aqueous cell suspension applied to V1-V2 stage plants were significantly greater than the MDRs for other treatments (Table 4).

Dry-leaf inoculum suspensions applied directly over seed resulted in significantly more disease than when inoculum was applied over the furrow soil surface (Table 3). Application of these suspensions directly over seed resulted in disease levels similar to those in foliar treatments applied to R1 plants (Tables 3 and 4) and in 1986 resulted in MDRs significantly greater than those for plants inoculated at R1 stage.

There was significantly more disease on Topcrop plants than on GN Harris plants in only two treatments in 1985 (dry-leaf inoculum suspension applied to the furrow soil surface and dry-leaf inoculum applied to R1 stage plants). In 1986, Topcrop MDRs were significantly greater than for GN Harris for all treatments (Table 4).

There was no significant yield reduction in any GN Harris treatments either year. Significant yield reductions in Topcrop occurred when aqueous cell suspensions or dry-leaf inoculum were applied to V1-V2 stage plants in 1985 and 1986 (Tables 3 and 4).

DISCUSSION

A dry-leaf inoculum of *X. c.* pv. *phaseoli* was prepared from dried diseased bean leaves, and viable bacteria were recovered from this inoculum for at least 6 yr. Approximately 90% fewer colony-forming units were recovered from dry-leaf inoculum after 1 yr of storage. Two-year-old inoculum still contained 10^9 cfu/g and 6-yr-old inoculum contained 10^8 cfu/g. It is not surprising that *X. c.* pv. *phaseoli* could survive in dry-leaf inoculum given the ability of the pathogen to survive in infected bean seed for as long as 10 yr (28). In the Sudan, *X. c.* pv. *phaseoli* was isolated from dried dolichos bean leaves after 18 mo of storage at room temperature, and beans inoculated with bacterial suspensions prepared from dried leaves developed common blight (19). *X. c.* pv. *malvacearum* (Smith) Dye was recovered from dried infected cotton leaves stored at room temperature for 6 yr (1,22), but not after the seventh year (22). We have prepared dry-leaf inoculum of *X. c.* pv. *campestris* (Pammel) Dowson from infected cabbage leaves and recovered pathogenic cells from 2-yr-old inoculum (*unpublished*). Infectivity of *X. c.* pv. *glycines* (Nakano) Dye, which causes bacterial pustule disease of soybean, was reduced after a 30-mo storage period in frozen infected leaves (14), but leaves were not dried before storage, and drying appears to be critical for efficient long-term survival in plant material (24).

Storage of bacteria in dry-leaf inoculum circumvents storing cultures on agar media and periodically transferring cultures to prevent loss of pathogenicity. Xanthomonads are notorious for loss of pathogenicity after repeated subculturing (26). Dry-leaf inoculum can be used for long-term storage of *X. c.* pv. *phaseoli* (cells are readily recovered by sonicating and/or shaking inoculum in PB and streaking out the liquid onto media) or for storing large amounts of inoculum to be used for inoculating plants the following year. We routinely store *X. c.* pv. *phaseoli* in dried leaves, and survival is enhanced by storage at 4 C or below.

Inoculum can be produced from field-collected leaves instead of greenhouse-inoculated leaves. Diseased leaves in the field can be rapidly collected and large amounts of dry-leaf inoculum produced in a short period of time. Furthermore, it is not necessary to collect leaves with severe symptoms because the bacteria survive as epiphytes on bean leaves (2,8,19,21), and leaves with few symptoms can be heavily colonized. To reduce introduction of other indigenous pathogens, leaves with symptoms of other diseases must be avoided and/or discarded. Thus, the use of dry-leaf inoculum for *X. c.* pv. *phaseoli* can provide a method for plant breeders and pathologists to rapidly produce large amounts of inoculum for inoculating bean plants from one year to the next. Dry-leaf inoculum produced from field-collected diseased leaves has been used to inoculate plants with *Pseudomonas syringae* van Hall (9), *P. s.* pv. *phaseolicola* (Burk.) Young, Dye, & Wilkie (D. J. Hagedorn and R. E. Rand, *personal communication*), and *P. s.* pv. *lachrymans* (Smith & Bryan) Young, Dye, & Wilkie (25).

For dry-leaf inoculum to be of practical significance, there must be effective application methods. We found that dry-leaf inoculum could be applied to seed at planting time or after planting for establishment of common bacterial blight. Application of dry-leaf inoculum suspensions to seed in the furrow was more effective than application over the furrow soil surface. The bacteria rapidly colonized the emerging seedling (8) but did not kill the plant. This was a concern, for emerging seedlings from seed heavily infested with *X. c.* pv. *phaseoli* may be killed before or soon after emergence (27). Application of the dry-leaf inoculum in an aqueous solution allowed for relatively uniform application of inoculum to seed and may have enhanced bacterial colonization and/or survival by providing moisture. Furthermore, inoculating seed eliminates returning to the field later in the season to inoculate plants and resulted in disease levels similar to aqueous cell suspensions. The technique was successful in two different soil types, loamy sand at Hancock and silt loam at Madison. Inoculation of seed also provides a natural mode of infection for *X. c.* pv. *phaseoli* and emulates natural inoculum associated with bean seed or plant debris. Other inoculation techniques using high concentrations of inoculum introduced by wounding or water-soaking (27) are more severe than natural infection and may lead to artifacts. Cultivars with resistance, such as GN Harris, GN Tara, and GN Nebraska No. 1, are less rapidly colonized by *X. c.* pv. *phaseoli* (2,23,27) and may be considered susceptible in tests where severe methods of inoculation are used. Use of seed inoculation with dry-leaf inoculum may allow identification of such cultivars, i.e., those that sustain leaf symptoms, but at generally lower levels than susceptible cultivars, and yield well despite disease (16). There is no common blight resistance in *P. vulgaris* similar to that in *P. acutifolius* A. Gray (16,23), though the resistance of GN Harris was derived from interspecific *P. vulgaris* × *P. acutifolius* progeny (5,6,11).

Application of dry-leaf inoculum to plants was also an effective method for inoculating beans with *X. c.* pv. *phaseoli*, and disease levels were similar to those in aqueous cell suspension treatments. The high pressure spray of Carborundum before application of inoculum injured plants and provided initial moisture to help hold inoculum on leaves and may have been beneficial in establishment of the pathogen. Application of inoculum early in the development of the plant (V1-V2 stage) and enhancement of infection by wounding led to rapid disease development in plants. These treatments could be used when higher levels of disease are desired. Plants inoculated at R1 stage with aqueous cell suspensions or dry-leaf inoculum had less disease than plants inoculated at V1-V2 stage. When treatments were applied after bloom, MDRs were consistently lower for GN Harris than for Topcrop. Inoculation after bloom may be better for separation of resistant from susceptible cultivars. Mean disease ratings in dry-leaf inoculum treatments for both years were consistent with the expected results for the two cultivars and indicate that dry-leaf inoculum can be used for determining the reaction of bean cultivars to common blight.

Additional methods of applying dry-leaf inoculum could be devised, such as application to seed with a sticker (i.e., methyl cellulose), direct application to plants without a prior wounding treatment, or addition of dry-leaf inoculum to water or buffer to create a liquid inoculum that can be quantified

and sprayed on plants (12,15,25). Large amounts of inoculum of *X. c.* pv. *malvacearum* were produced by soaking infected cotton trash in water and using the filtrate to inoculate plants in the field. This technique was successfully employed in the Sudan to screen for bacterial blight resistance in cotton cultivars (15). This technique was considered adequate for screening cultivars for disease resistance but not for genetic analysis of host-pathogen interaction (12).

In some treatments, greater differences between GN Harris and Topcrop were seen in percent of plants, leaves, and leaflets infected. These parameters may be helpful in identifying cultivars with resistance when MDR differences are difficult to obtain (e.g., weather is unfavorable for disease development). In our study, these parameters reflected MDRs, but under other conditions these data may be very useful in determining disease reaction. Yield data are used to distinguish tolerant from susceptible cultivars and may be used to rate the relative severity of inoculation techniques. In 1985, GN Harris and Topcrop plants inoculated with dry-leaf inoculum at V1-V2 stage had similar MDRs, but only Topcrop plants had reduced yields. Thus, GN Harris appeared tolerant and the inoculation treatment was relatively severe. In 1986, once again only Topcrop yields were reduced in this treatment. Mean disease ratings for GN Harris were significantly lower than those for Topcrop, suggesting that GN Harris was resistant.

Dry-leaf inoculum of *X. c.* pv. *phaseoli* prepared from dried infected bean leaves can be stored for prolonged periods of time and used for field inoculations. Use of such inoculum may greatly simplify screening for disease resistance, particularly for researchers with limited access to laboratory facilities and materials and/or limited expertise in maintenance of bacterial cultures and preparation of aqueous cell suspensions. These restrictions exist in subtropical and tropical developing countries where common blight is a major constraint on bean production.

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