Presumptive Procedure (Dome Test) for Detection of Seedborne Bacterial Pathogens in Dry Beans

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ABSTRACT

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An assay of bean seed lots for pathogenic bacteria, referred to as the dome test, is described. Seeds are soaked in water, the soaked bean infusion is vacuum-infiltrated into pregerminated seeds from the same lot, and symptoms are observed on the seedlings grown in high humidity. In tests of 233 lots of bean seeds, all of the lots produced at least some seedlings with water-soaked spots on primary leaves. Intensity of water-soaking varied among seed lots. Plant pathogens were isolated from lesions on plants from 118 of 139 lots and were characterized by biochemical, physiological, morphological, and pathogenicity tests as *Pseudomonas syringae* pv. *syringae* or pv. *phaseolicola* or *Xanthomonas campestris* pv. *phaseoli*.

Bacterial diseases adversely affect dry edible bean production in North Dakota. In seasons with abundant rainfall, entire fields have been destroyed because of severe bacterial disease. Common blight, caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, has been the most prevalent disease, but halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.) Young, Dye, & Wilkie, has been devastating, especially when the crop was attacked early in the season. A third disease, bacterial brown spot, caused by *P. s.* pv. *syringae* van hall, recently has caused losses, particularly on kidney beans.

Survival of bacterial pathogens in the seeds of beans has been well established. Both surface contaminant and internally cloistered bacteria may serve as sources for disease onset (9). Detecting seedborne bacteria is difficult, especially if the incidence and/or severity of contamination is low. Direct isolation, serological procedures, bacteriophage, pathogenicity tests on bacteria from soaked bean infusion, and growing-on of contaminated seeds have been used (32). Each test varies in sensitivity, specificity, and complexity; none has been widely accepted as a standard seed assay (32). Few of the tests are quantitative, and the more specific tests are designed to detect a single pathogen. The objective of the studies reported here has been to develop a simple, rapid, and reliable presumptive test for determination of the probable risk of bacterial disease associated with planting commercial seed lots. Bacterial diseases of beans are highly contagious, and most bean seed certification standards have required no detectable bacterial pathogens ("zero tolerance" or "none"). Therefore, a test that could access the relative amount of contamination of seeds by any pathogen alone or in combination with other pathogens would be highly useful.

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MATERIALS AND METHODS

The dome procedure. Tests were made on about 500 g of seed taken from about 1-kg samples submitted by growers or collected from various sources. About 100 undamaged seeds from each lot were washed in cold tap water with detergent to remove soil, immersed in 1% sodium hypochlorite for 2–3 min, then rinsed in an aqueous solution of 6×10^{-3} M sodium thiosulfate. Next, the seeds were rinsed three or four times in sterile deionized water (SDW), placed on sterile paper towels or plastic-covered absorbent pads (commercial baby diapers) moistened with a maneb suspension (150 μg/ml), and incubated for 48–72 hr at 27–33 C. The maneb was found to retard growth of fungal contaminants (J. R. Venette, *unpublished*). After incubation, 30 germinated seeds selected for radicles through the seed coats but less than 2 cm long were carefully transferred to a clean Erlenmeyer flask. An infusion (prepared from the respective seed sample by soaking 400 ml [800–1700 count or 325 g] of seeds, treated with sodium hypochlorite and sodium thiosulfate as before, in 1,800 ml of SDW in a 2-L flask on a shaker for 18–24 hr at room temperature) was added to the germinated seeds. The flask was sealed with a sterile fitting connected to a vacuum line, then vacuum (380–510 mm Hg) was applied for 20–25 sec and rapidly released. The infiltrated seedlings were removed from the infusion and evenly distributed on the surface of autoclaved fine vermiculite previously moistened with an aqueous suspension of maneb (150 μg/ml) and firmly packed into a 30-cm plastic dome (Cako Development Corp., Lonsdale, MN). The seedlings were then covered with 2 cm of a similarly moistened layer of vermiculite. A second dome was inverted over the base and attached by staples or tape. No attempt was made to form a tight seal. The assembled domes were incubated in the laboratory under continuous light (43 μmol m^{-1} sec^{-2}) from cool-white fluorescent lights suspended 15 cm above the dome top for 5–14 days. Ample air circulation was provided so that temperature increases within the dome were limited. Temperatures were monitored with standard laboratory thermometers and occasionally an electronic sensor accurate to 0.1 C. In three domes, relative humidity was measured with a hygrometer.

Assays. Initially, 135 samples of beans taken from seeds harvested the previous year were assayed in the dome test. Eighty-four of these samples were certified, nine were registered, two were foundation, three were breeders' stock, two were affidavit, and the balance were of commercial or unknown status. Seeds from Idaho made up 53 of the samples: three from California, 18 from Wyoming, 49 from North Dakota, two from Michigan, one from Japan, and the rest of unknown origin. Major bean types included pinto (102 lots) and navy (18 lots) beans.

Bacteria were isolated from water-soaked lesions on unifoliolate (primary) leaves from the plants in the domes. Two techniques were used. In the first, leaves with the lesions were removed and rinsed in 1% sodium hypochlorite for 1 min, then three times in SDW. Portions of lesioned tissue were macerated with sterile forceps, then streaked across the surface of King's medium B (KB) 31, nutrient agar (NA) (31), or D4 medium (35) in petri dishes. With the second technique, portions of plants or whole plants were triturated in 1 ml of SDW

with a mortar and pestle. A loopful of the macerate was streaked as before. Plates were incubated at 27 C for 48–96 hr and examined for colonies characteristic of the bean pathogens. Sterile swabs (27) wetted with sterile deionized water were touched to the suspect colonies, then gently rubbed on the adaxial surface of water-congested expanding primary Manitou red kidney bean leaves. The area of water congestion was achieved by intromission of SDW through an airbrush at 776 mm Hg pressure. The inoculated plants were immediately placed in a humid chamber for 16–18 hr at 20 C, then transferred to a greenhouse for 2 wk at 22–25 C. The colonies selected on the plates were marked, and the plates were stored. Colonies associated with a pathogenic response were recovered from the original plates and repeatedly streaked for isolation. Strains were then grown at 27 C for 48 hr on 5% sucrose-amended nutrient agar (NAS) (6), on KB, or on yeast dextrose carbonate (YDC) medium (31), depending on the suspected identity of the pathogen. Identification of bacterial strain was based on Gram stain reaction, production of fluorescent pigments, oxidase, levan, colony coloration, and pectolytic enzymes on crystal violet pectate medium (31). Generally, two suspect pathogens from each seed lot were retested for pathogenicity. An artist's airbrush (17) was used to force an aqueous suspension of bacteria, about 1×10^7 colony-forming units per milliliter (cfu/ml), into young expanding primary leaves of Manitou light red kidney bean leaves. Water and known pathogenic strains (Table 1, strains 3, 5, and 12) were included as controls.

In a second assay, 10 unidentified navy bean seed lots from the 1985 crop grown near Casselton, ND, were obtained from a local elevator, and 10 seed lots from the 1981 crop were obtained from the North Dakota State Seed Department. The dome assay was conducted as before, except the concentration of sodium hypochlorite was reduced to 0.1% and dilute HCl was added to reduce pH to 7.5. The sodium thiosulfate rinse was not used. In addition, the soak infusion was plated directly and duplicate domes were prepared from each seed sample. The temperature of incubation was slightly higher at 26 C. Three or four leaves with water-soaked spots on unifoliolates were aseptically removed, weighed, and collectively ground in 1 ml of SDW. The homogenate was serially diluted in 10-fold dilutions of SDW, and 0.1-ml samples were spread on the surface of nonselective medium KB or NA and on selective media named KBBC (22) and MXP (1). Another selective medium, SNBC (22), specific for *P. s.* pv. *syringae*, was used in the assay of seeds from Casselton. In the latter, three or four additional diseased leaves were sampled 10–12 days after the tests began and handled as described. In the tests on state seed samples, four or five diseased cotyledons were assayed on the selective media and on NA. In all assays, known strains of *P. s.* pv. *syringae, P. s.* pv. *phaseolicola*, and *X. c.* pv. *phaseoli* were streaked on separate plates of media to provide a comparative basis for colony morphology.

For phenotypic characterization, colonies resembling those of the plant pathogens were selected from the plates and restreaked on selective media to ensure colony type. Single, well-separated colonies were restreaked on NA or KB to ensure purity. Pseudomonads were characterized by fluorescence on KB (30), Gram reaction (Hucker method) (37), shape, oxidase reaction (ASM method 1) (37), motility (4), levan reaction (30), potato soft rot reaction (6), fermentative-oxidative ability (Hugh and Liefson medium) (37), arginine dihydrolase reaction (Thornley's method) (37), β-glucosidase (positive for *P. s.* pv. *syringae*, negative for *P. s.* pv. *phaseolicola*) (13), hypersensitive reaction (HR) on tobacco cultivar White Burley (30), pectolytic reaction at pH 5 and 8.5 (negative for *P. s.* pv. *syringae*, positive at low pH for *P. s.* pv. *phaseolicola*) (30), growth on sorbitol, mannitol, inositol, erythritol (positive for *P. s.* pv. *syringae*, negative for *P. s.* pv. *phaseolicola*) (30), growth on selective media KBBC or SNBC for *P. s.* pv. *syringae* (22), and pathogenicity on red kidney beans in greenhouse tests. Xanthomonads were characterized by colony color and mucoid growth on YDC (5), motility, Gram reaction, shape, hydrolysis of starch (6) and Tween 80 (6), production of acid from glucose, mannose, and arabinose (5) but not inositol or adonitol (6), oxidative-fermentative reaction, catalase reaction (ASM method 1) (37), citrate utilization (37), oxidase reaction, production of H_2S from peptone (34), milk proteolysis (purple milk) (5), nitrate reduction (5), and HR on tobacco. Xanthomonads were tested for pathogenicity on red kidney beans and for growth on selective medium MXP (1).

For pathogenicity tests, red kidney beans (Charlevoix) were grown in the greenhouse from surface-sterilized seeds planted singly in 5-cm-square pots containing pasteurized greenhouse mix. Uniform, undamaged seedlings were inoculated on the unifoliolate leaves within 48 hr after leaves had flattened. Inoculum, taken from 24- to 48-hr cultures of bacteria and adjusted in SDW to a concentration of $1–3 \times 10^8$ cfu/ml using absorbance measurement in a Spectronic 20 at 600 nm, was diluted 100-fold in SDW. Inoculum was sprayed through an artist's airbrush at a line pressure of 776 mm Hg onto the lower surfaces of the unifoliolate leaves until water congestion appeared. The airbrush was rinsed in 70% ethanol and SDW between inoculations. Water checks and reference strains of *P. s.* pv. *syringae, P. s.* pv. *phaseolicola*, and *X. c.* pv. *phaseoli* were included with each group of suspect pathogens. Inoculations were made outside the greenhouse to minimize spray and aerosol contamination. Four spots were inoculated on each leaf, and four leaves on two plants were always tested.

Inoculated plants were returned to the greenhouse, and the pots were arranged in steamed wooden flats so that plant contact was not possible. The flats were then filled with pasteurized sand so that the sand covered the pots by 1–2 cm. The sand served as a moisture reservoir, stabilized the growing conditions of the plants, and made watering of the plants without wetting the foliage practical. Plants inoculated with pseudomonads were incubated under greenhouse conditions (25 ± 5 C, 12-hr light/dark period). Plants inoculated with xanthomonads were incubated in a moist chamber for 16–20 hr before being incubated in the greenhouse.

Bacteria that caused water-soaking, necrosis, reddened lesion margins,

Table 1. Pathogenic bacteria, designators, and origins of reference strains used in these studies

Pathogen	No.	Designator	Origin
Pseudomonas syringae pv. *syringae*	1	Pss82 JL	Vidaver, U. Nebraska
	2	Pss2771	Hagedorn, U. Wisconsin
	3	Pss-Field	Field, N. Dakota
P. s. pv. *phaseolicola*	4	Psp83 K2	Vidaver, U. Nebraska
	5	Psp V	Field, N. Dakota
	6	PspL-4-2	Field, N. Dakota
	7	Psp G50	Patil, U. Hawaii
	8	Psp CH21	Saettler, Michigan State U.
	9	Psp HB38	Saettler, Michigan State U.
	10	Psp19304	ATCC
Xanthomonas campestris pv. *phaseoli*	11	Xcp DRC	Vidaver, U. Nebraska
	12	Xcp F10	Field, N. Dakota
	13	Xp6-1	Roth, U. Wyoming
	14	Xp-10	Roth, U. Wyoming
	15	Xp-9	Roth, U. Wyoming
	16	Xp-11	Roth, U. Wyoming
	17	Xp1	Roth, U. Wyoming
	18	Xpf	Saettler, Michigan State U.

reddened veins, tissue collapse, and/or tissue browning on greenhouse-grown plants were considered pathogenic (23). Strains that caused indistinct reactions were retested. If indistinct reactions occurred on retesting, leaf sections from the original zone of inoculation were triturated in SDW and the plant sap was reinoculated into leaves as previously described. If symptoms failed to occur after reinoculation, the strain was considered nonpathogenic.

In a third, more extensive assay, 78 lots of pinto, red kidney, and navy beans submitted to the State Seed Department in 1985–1986 were tested as described.

RESULTS

The plastic cake domes proved inexpensive, disposable, and readily available and prevented cross-contamination among seed lots. The domes were large enough so that a plant did not touch the top or walls of the container and individual plants could be evaluated for disease. The number of pregerminated seeds added to each dome was limited to 30 to ensure that each plant had about 24 cm^2. Condensed moisture was visible on walls of the dome but seldom on the top. Relative humidity was measured at 94–96%. Leaves did not appear to possess visible surface moisture except when they occasionally touched the sides of the dome. Temperatures within the domes fell within +2 C of room temperature. Mean differences of +0.3 and +0.8 C were recorded in two different experiments.

Plant emergence ranged from 20 to 100% (30 seeds). Generally, high-quality seed from western growing regions had emergence of 80%. Most plants in the dome appeared normal except for a few that became inverted when the hypocotyl hook straightened and the roots were lifted from the vermiculite. Emergence occurred 2–3 days after planting. Seed coats split normally and were usually cast without difficulty. Mechanically damaged plants did not appear with greater frequency than observed in greenhouse plantings. Initial symptoms of disease were observed about 5–7 days after emergence. Spots of water congestion were often observed on cotyledons. Their number varied with the quality of the seed lot. Surface colonies of opalescent or yellow bacteria were associated with many of the water-soaked spots. Sometimes the spots remained small. In contrast, others enlarged, coalesced, and the cotyledons became a yellow softened mass. Some lesions were dark, sunken, and surrounded by a white to light green halo. Bleached tissue often extended through one-half of the cotyledon.

The primary root, when visible, was often dark, and the dark tissue extended onto the base of the hypocotyl. An extensive set of adventitious roots generally formed in the vermiculite. These adventitious roots were normal in appearance. The hypocotyls generally appeared sound. On heavily infected plants, opalescent or yellow bacterial matrix often oozed from water-soaked lesions on the stems.

Frequently, a portion (1–2 cm) of the epicotyl just below the primary node became water-soaked and then collapsed. The tissue generally became darkened, shriveled, and the stem wilted. When this occurred, tissues distal to the collapsed stem became darkened. There did not seem to be a strong association between water-congested spots on the leaves and stem collapse on the same plant, although epicotyl collapse seemed to occur as cotyledons began to shrivel.

Various symptoms occurred on the primary leaves. Initially, tiny water-soaked spots were observed on the lamina. Numbers and sizes of spots varied. When spots were numerous, leaf malformations, especially a rolling and twisting of the leaf blade, occurred. Spot aggregation occurred at different places on leaves, and in some cases, lesions covered the entire leaf. After several days, tissue in these areas became necrotic and medium brown to brownish green. Water-congested tissue was still observable in these darkened areas. Microscopic observations of symptomatic leaves sectioned through fresh lesions and mounted in water showed clear bacterial flow from the margins of the lesions. On some primary leaves, leaf veins became darkened, necrotic, and appeared shriveled. In these cases, water-congested tissue occurred near the veins.

Sometimes, flattened primary leaves would expand and leaf margins would develop a distinct scorched appearance on the outer edge of the leaf blade. When infection was not severe, plants would produce expanded, albeit dwarfed, primary leaves and the first set of trifoliolate leaves would begin to expand. There was no apparent effect of the vacuum-infiltration process on the beans when seedling beans infiltrated with SDW were compared with those simply soaked in water for an equivalent period of time.

Assays. Symptoms appeared on at least some plants from all 135 lots of beans evaluated in the initial test. Number, intensity, and types of symptoms varied among the lots, but water-soaked lesions on the unifoliolate leaves and epicotyl collapse were common to all the tests. Plants from western-grown, high-quality seed had the fewest symptoms, whereas plants from commercial lots of beans grown in North Dakota had the most numerous and severe symptoms.

Table 2. Dome assay of 10 navy bean seed lots from the 1985 crop grown near Casselton, ND

Lot	Av. no. lesions/leaf[a]	Av. emergence[b]	Cfu/g 7-day-old leaf tissue on medium[c]				Recovery of pathogens[d]	
			KB	KBBC[e]	SNBC[e]	MXP[f]	Soak	Older leaves
1	34 ± 2	22.5 ± 0.5	3.4×10^9	5.9×10^6	0	0	···	*Pss*
2	33 ± 1	30	5.0×10^9	0	0	0	···	···
3	22 ± 4	28 ± 2	1.5×10^9	0	0	0	···	*Pss*
4	27 ± 6	29	2.1×10^{10}	1.4×10^9	0	0	*Pss*	*Pss*
5	38 ± 9	30	2.0×10^8	2.6×10^6	2.3×10^6	0	*Pss*	*Pss*
6	36 ± 8	27.5 ± 0.5	3.0×10^9	0	0	0	···	*Xcp*
7	54 ± 14	22.5 ± 1.5	9.5×10^9	0	0	4.6×10^6	*Xcp*	*Xcp*
8	63 ± 6	26	1.3×10^{10}	5.9×10^4	6.2×10^4	0	···	*Pss*
9	47 ± 5	25 ± 2	4.8×10^9	3.3×10^6	0	0	*Pss*	*Pss*
10	34 ± 3	25.5 ± 2.5	1.5×10^9	1.2×10^8	0	0	*Pss*	*Pss*, *Xcp*

[a] Lesions were counted on both primary leaves of each plant and averaged over all emerged plants in duplicate domes. Counts were made after 7 days of incubation.

[b] Emergence was based on 30 seeds per dome.

[c] KB is King's medium B, KBBC and SNBC are named media selective for *Pseudomonas syringae* pv. *syringae* (*Pss*), and MXP is a named medium selective for *Xanthomonas campestris* pv. *phaseoli* (*Xcp*). Values are based on fresh weights of three or four representative leaves per dome.

[d] Duplicate platings of 0.1 ml each were made from a 1,800-ml soak of bean suspension onto all of the listed media. As a confirmatory test, three or four leaves from plants incubated 10–12 days in the domes were assayed on all of the listed media. Colonies characteristic of the pathogens were identified by biochemical and pathogenicity tests.

[e] Colonies from KBBC and SNBC were identified as *P. s.* pv. *syringae*.

[f] Colonies from MXP were identified as *X. c.* pv. *phaseoli*.

Pathogenic bacteria were isolated from 41 severely infected lots from the initial assay. Pathogenic bacteria caused water-soaking, chlorosis, and restricted necrosis on greenhouse-grown plants. Some strains caused reddened margins of lesions, expanded necrosis, and reddened veins.

Pathogenic bacteria were gram-negative and rod-shaped. Colony morphologies were similar to those of known pathogenic strains used as controls. Those pathogenic gram-negative bacteria that produced cream-colored colonies on NA were oxidase-negative and did not pit CVP, fluoresced on KB, and produced a positive levan reaction were presumptively identified as pseudomonads. The pathogenic gram-negative bacteria that produced slow-growing, yellow, rough to smooth edged colonies on NA and mucoid colonies on NAS were nonpectolytic on CVP were identified as xanthomonads. Forty of the lots contained both xanthomonads and pseudomonads, and one contained only pseudomonads.

Plants grown from each of 10 lots of navy beans from the 1985 crop had water-soaked lesions on the primary leaves (Table 2). Depending on the seed lot, the average number of spots per leaf in each dome ranged from 22 to 63. Plant-pathogenic bacteria were isolated from leaves in nine of the 10 lots. Seven of the lots had *P. s.* pv. *syringae*, and three had *X. c.* pv. *phaseoli*. Pathogens were recovered from the soak infusion from five of the 10 lots. The KBBC medium was more efficient for isolation of *P. s.* pv. *syringae* than was SNBC. Numbers of bacteria on these media were always lower than numbers on KB.

Water-soaked spots were observed on seedlings from five of the 10 lots of seed from the 1981 crop (Table 3). When pathogens were isolated from the infusion, higher numbers of the same pathogens were isolated from leaves and cotyledons of plants in the respective dome. The five seed lots that were negative for bacterial pathogens were assayed again and, as before, appeared free of pathogenic bacteria.

All of the pseudomonads were gram-negative rods from cream-colored colonies that fluoresced on KB. They were motile, levan-positive, oxidative rather than fermentative, oxidase-negative, and unable to rot potato slices. They caused HR on tobacco, were nonpectolytic at pH 5.0 and 8.5, were arginine dihydrolase-negative, and were positive for β-glucosidase. All grew on sorbitol, mannitol, inositol, erythritol, and selective medium KBBC and were pathogenic on greenhouse-grown bean seedlings. The *P. s.* pv. *phaseolicola* reference strain (83K2) was unable to grow on the polyhydric alcohols and was pectolytic at pH 5.0. Characteristics of the isolates matched those of *P. s.* pv. *syringae* strain 2771 used as a reference. Strains isolated from plants in domes appeared less virulent than the reference strains in some tests. Xanthomonads isolated from plants in domes were generally yellow and mucoid on YDC; one strain was white and nonmucoid. The xanthomonads hydrolyzed starch and Tween 80 and produced acid from glucose, mannose, and arabinose, but the production of acid from arabinose was slower than that of the reference strain, Xcp-DRC. They were catalase-positive, H_2S-positive, oxidative, and oxidase-negative. Only one strain produced HR on tobacco. All were pathogenic on greenhouse-grown bean seedlings and grew on MXP, but all strains but one were less virulent than the reference strain. Because the characteristics matched those of the reference, the isolates were identified as strains of *X. c.* pv. *phaseoli*.

Pathogenic bacteria were isolated from 63 of the 78 state seed lots. Strains identified as *X. c.* pv. *phaseoli* were isolated from 41 seed lots, *P. s.* pv. *syringae* from 44, and *P. s.* pv. *phaseolicola* from five (Tables 4 and 5). When *P. s.* pv. *phaseolicola* was isolated, no other pathogens were detected. Most of the strains of *P. s.* pv. *syringae* (77%) and *P. s.* pv. *phaseolicola* (60%) were identical to the characteristics of reference strains in the physiological tests (Table 4). Minor variations occurred between characteristics of *X. c.* pv. *phaseoli* reference strains and those of strains isolated from plants grown in domes and identified as *X. c.* pv. *phaseoli* (Table 5).

DISCUSSION

Use of a dome test to detect seed from pathogens in part or in whole is not without precedent. Similar tests have been devised for detecting bacterial pathogens in soybeans (19,25), cotton (12,14), and tomatoes (20). To detect bacterial pathogens in *Phaseolus* beans, Grogan and Kimble (9) planted contaminated bean seeds on moist vermiculite and beneath a deep layer of dry vermiculite in a large pot. After 2 days,

Table 3. Isolation of bacteria from 10 lots of beans from the 1981 crop[a]

Lot[b]	Medium[c]	Bacterial populations			Pathogen identification	
		Soak (cfu/ml)	Leaves (cfu/g)[d]	Cotyledons (cfu/g)	Soak	Cotyledons or leaves
Lot 1	NA	1.2×10^8	8.7×10^8	2.9×10^9	···	···
Fleetwood	KBBC	NG[e]	NG	NG	···	···
Navy	MXP	NG	NG	NG	···	···
Lot 2	NA	5.5×10^4	6.7×10^7	8.5×10^7	···	···
Seafarer	KBBC	1.8×10^4	4.0×10^7	7.9×10^7	*Pss*	*Pss*
Navy	MXP	NG	NG	NG	···	···
Lot 3	NA	1.0×10^6	7.6×10^8	5.4×10^7	···	···
Nodak	KBBC	4.6×10^1[f]	TNTC[5g]	TNTC	···	···
Pinto	MXP	NG	NG	NG	···	···
Lot 4	NA	1.8×10^3	8.5×10^8	7.4×10^8	···	···
Olathe	KBBC	5.6×10^2	TNTC	TNTC	*Pss*	*Pss*
Pinto	MXP	NG	NG	NG	···	···
Lot 5	NA	6.0×10^6	5.8×10^9	5.6×10^8	···	···
···	KBBC	2.5×10^3	TNTC	1.4×10^7	*Pss*	*Pss*
Navy	MXP	NG	NG	NG	···	···
Lot 6	NA	7.6×10^3	5.6×10^8	6.7×10^6	···	···
Nodak	KBBC	NG	NG	NG	···	···
Pinto	MXP	NG	NG	NG	···	···
Lot 7	NA	1.6×10^8	3.1×10^9	8.5×10^8	···	···
C20	KBBC	NG	NG	NG	···	···
Navy	MXP	1.8×10^3	4.1×10^6	2.3×10^6	*Xcp*	*Xcp*
Lot 8	NA	1.0×10^6	1.2×10^9	1.3×10^8	···	···
Upland	KBBC	NG	NG	NG	···	···
Navy	MXP	NG	NG	NG	···	···
Lot 9	NA	2.7×10^6	5.0×10^9	9.8×10^7	···	···
Pindak	KBBC	NG	NG	NG	···	···
Pinto	MXP	NG	NG	NG	···	···
Lot 10	NA	4.9×10^6	No leaves	9.5×10^9	···	···
···	KBBC	NG	No leaves	NG	···	···
Pinto	MXP	3.6×10^2	No leaves	3.8×10^5	*Xcp*	*Xcp*

[a] Assays were made on the infiltrating infusion (soak) and on leaves and cotyledons after 7 days of incubation.
[b] Lots are identified by cultivar, if known, and by market class.
[c] NA is nutrient agar (Difco), KBBC and MXP are named media selective for *Pseudomonas syringae* pv. *syringae* (*Pss*) and *X. c.* pv. *phaseoli* (*Xcp*), respectively.
[d] Based on the fresh weights of three or four leaves or three or four cotyledons.
[e] NG = no growth.
[f] Not characterized as *Pss*.
[g] TNTC = Too numerous to count.

the pot was flooded with water for 30 min. Pots were drained and moistened periodically so that plants were exposed to high relative humidity as they emerged. A fungicide was applied to retard fungal contamination. The plants that emerged had water-soaked spots on the cotyledons and primary leaves.

Parker and Dean (26) planted bacterially contaminated bean seeds in deep vermiculite in pots and incubated the pots in plastic-covered frames on top of water-soaked burlap pads to keep relative humidity high. They found leaf, petiole, and stem lesions usually accompanied by bacterial exudate on infected plants with as many as 65% of the seedlings being infected.

Use of pregerminated seeds. Jensen and Livingston (15) used pregerminated bean seeds to test pathogenicity of *P. s.* pv. *phaseolicola* strains. The germinated seeds were soaked in bacterial suspension and planted in pots in the greenhouse. Water-soaked spots and cankers were observed on cotylendons and stems, respectively.

Use of infusion. An enrichment technique is employed in most current assays for seedborne bacterial pathogens of beans and appears essential for effective detection if bacteria infect a low percentage of seeds (3). Infusion prepared by soaking seeds in water is a simple enrichment technique. Saettler (28) suggested soaking beans in water, water with yeast extract (29), and in later studies (39), a semiselective medium to enhance detection of blight bacteria. In Idaho, Guthrie et al (11) soaked beans in SDW before doing serological tests. Guthrie (10) noted that contamination by saprophytes obscured test results and recommended soaking beans in 0.03% crystal violet solution to reduce gram-positive bacterial contamination. Wilson (42) showed soaking bean seeds in water for less than 24 hr before planting increased the number of infected seedlings and accelerated symptom expression. Webster et al (41) inoculated bean plants with infusion from soaked beans and observed symptoms as a method to detect seedborne bacteria.

Vacuum infiltration. Goth (8) used vacuum infiltration to introduce virulent bacterial pathogens into bean seeds. Symptoms on inoculated plants were equivalent to those produced from naturally infected seed. Vacuum infiltration has been used to inoculate bean leaves with various pathogens for growth-kinetic (2) and physiological studies (21,24).

Pathogenicity tests. Most strains of pathogenic bacteria isolated from plants grown in the domes were less virulent than reference strains of the pathogens. Those bacteria that cause characteristic and diagnostic symptoms of disease in beans have been well described as being variable in virulence. Bacterial pathogens with differing virulence have been isolated from bean seeds (36).

In our laboratory, the most virulent strains of bean bacterial pathogens caused typical symptoms when inoculated onto primary leaves that were more than one-half expanded. Less virulent strains caused less distinct reactions on older leaves but caused typical symptoms when inoculated onto leaves shortly after leaf

Table 4. Characterization of pathogenic pseudomonads isolated from 49 of 78 dome tests on seed submitted to the North Dakota State Seed Department for quality assessment

	No. of strains[a]											Ref. strains	
Pseudomonads	**34**	**3**	**2**	**3**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	***Pss*[b] (2)**	***Psp*[c] (7)**
Fluorescent on King's medium B	+	+	w[d]	w	+	+	w	w	+	w	w	+	+
Gram reaction	−	−	−	−	−	−	−	−	−	−	−	−	−
Rod-shaped	+	+	+	+	+	+	+	+	+	+	+	+	+
Levan	+	+	w	+	+	w	w	w	w	w	+	+	+
Oxidase	−	−	−	−	−	−	−	−	−	−	−	−	−
Potato soft rot	−	−	−	−	−	−	−	−	−	−	−	−	−
Arginine dihydrolase	−	−	−	−	−	−	−	−	−	−	−	−	−
Tobacco hypersensitivity	+	−	+	+	+	+	−	+	−	−	−	+	+
Pectolytic activity													
pH 5.0	−	−	−	+	+	−	−	+	−	−	−	−	+
pH 8.5	−	−	−	−	−	−	−	−	−	−	−	−	−
Sole carbon source utilization													
Mannitol	+	+	+	−	+	+	+	−	+	+	+	+	−
Sorbitol	+	+	+	−	+	+	+	−	+	−	+	+	−
Erythritol	+	+	+	−	+	+	+	−	+	−	+	+	−
Inositol	+	+	+	−	+	+	+	−	+	−	+	+	−
Presence of β-glucosidase	+	+	+	−	+	+	+	−	+	−	+	+	−
Motile	+	+	+	+	+	+	+	+	+	+	+	+	+
Pathogenic on kidney beans	+	+	+	+	+	+	+	+	+	+	+	+	+
Identification	s	s	s	p	s	s	s	p	s	p	s	s	p

[a] Each strain represents bacteria isolated from plants in a single dome.
[b] Reference strains 1 and 2 from Table 1 (*Pseudomonas syringae* pv. *syringae*).
[c] Reference strains 4–10 from Table 1 (*P. s.* pv. *phaseolicola*).
[d] w = Weak reaction.

Table 5. Characterization of pathogenic xanthomonads isolated from 39 of 78 dome tests on seed submitted to the North Dakota State Seed Department for quality assessment

	No. of strains[a]													Ref. strains		
Xanthomonads	**12**	**10**	**4**	**2**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**(6)[b]**	**(1)[c]**	**(1)[d]**
On YDC																
Yellow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mucoid	w[e]	w	+	+	+	w	w	w	w	−	w	w	w	+	w	−
Gram reaction	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Rod-shaped	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose																
Oxidative	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fermentative	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Acid produced from																
Glucose	+	+	+	+	+	+	+	w	+	+	w	+	+	+	+	+
Arabinose	w	+	+	+	w	w	+	w	+	w	+	w	+	w	+	+	w
Mannose	+	+	+	+	+	w	+	+	+	+	+	+	+	+	+	+
Adonitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Inositol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Nitrate reduction	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Milk proteolyses	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Motile	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Simmon's citrate	+	+	+	−	+	+	−	−	+	+	+	+	w	w	+	+	−
Hydrolysis																
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
H_2S from peptone	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pathogenic on kidney beans	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a] Each strain represents a strain isolated from a single dome.
[b] Strains 11, 12, and 15–18 from Table 1 (*Xanthomonas campestris* pv. *phaseoli*).
[c] Strain 14 from Table 1 (*X. c.* pv. *phaseoli*).
[d] Strain 13 from Table 1 (*X. c.* pv. *phaseoli*).
[e] w = Weak but positive reaction.

flattening. From plants with indistinct reactions, virulent bacteria were often detected by reinoculation of susceptible leaves with slurry taken from the tissue with indistinct reaction.

Bacteria in seeds. Numerous bacterial species, including both saprophytes and parasites, have been isolated from bean seeds and from bean plants. Saprophytic bacteria in seeds have made direct isolations of pathogens difficult (33,38). High numbers of saprophytes on plants growing in the domes complicated the identification of pathogens when nonselective media were used (7) (Tables 2 and 3). Saprophytic bacteria may outnumber pathogens by up to 1,000:1 (40). Use of selective media markedly increased successful isolation of plant-pathogenic bacteria.

Occasionally, pathogenic bacteria were not isolated from diseased leaves from the domes. The reasons for this are not known. Sometimes, antagonistic bacteria were isolated from infected leaves, and they may have influenced recovery of the pathogens. Also, an efficient selective medium for *P. s.* pv. *phaseolicola* is not available, and the halo blight pathogen may have been undetected. This would explain the low recovery (3/49) of *P. s.* pv. *phaseolicola* from the different seed lots (Table 4). Commonly isolated saprophytes from bean seeds, *Erwinia herbicola* pv. *herbicola* (Lohnis) Dye and *Pseudomonas fluorescens* (Trevisan) Migula did not cause water-soaked plants in domes when suspensions of these bacteria were used in lieu of the infusion.

Use of dome test for regulatory purposes. Workers in Idaho (18) criticized the dome test because they felt it led to declaration of false positives (seed lots free of pathogens declared contaminated). They also suggested the dome test should not be used for regulatory purposes because test sensitivity and field data were not correlated. Sensitivity of the test was reported (16). Data correlating seed test results and disease in the field are difficult to obtain because environmental conditions largely determine the amounts and kinds of bacterial disease that develop as well as symptoms that occur (43). The dome test represents an estimate of initial inoculum. The correlary measurement of amount of field disease may not satisfactorily estimate the amount of initial inoculum for a number of reasons including the problems of infected seeds decaying before emergence or yielding plants with masked symptoms. In favorable weather, the diseases can spread rapidly from a few infected plants and the amount of initial seedborne inoculum may be overestimated. If growing-on reliably estimated the amount of contamination in a seed lot, it could be used as a definitive test and would obviate the need for any other testing procedure.

For use as a method to assess the relative risk of bacterial disease from a lot of seeds, a measure of bacterial contamination is determined by counting water-soaked spots on unifoliolate leaves of plants in domes. Western-grown, high-quality seed generally has yielded plants with fewer than 12 lesions per leaf. Seeds from fields with bacterial disease have often produced plants with more than 50 lesions per leaf. Any seed lot that produces plants with symptom intensities equivalent to those of high-quality, western-grown seed is considered to be low-risk. For comparative purposes, tests on lots of seed known to contain bacterial pathogens should be made concurrently.

It is our contention that diseased plants in the domes constitute a pathogenicity test. Isolating and retesting bacteria for pathogenicity serve as confirmatory procedures. When isolation and pathogenicity tests were carefully made on seed from the previous year's crop, 90% of the domes in one assay and 81% of the domes in another assay contained plants from which pathogenic bacteria were characterized. As selective media, especially for *P. s.* pv. *phaseolicola*, are improved, recovery may be even higher. In a subsequent report, we will show that isolations combined with serological testing of infected plant material identified pathogens from more than 96% of the domes. With such a high association between diseased plants and detection of plant-pathogenic bacteria, the dome test may be useful in itself as a presumptive procedure to detect bacterial pathogens of beans.

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