Evaluation of the "Dome Test" as a Reliable Assay for Seedborne Bacterial Blight Pathogens of Beans

L. K. LAHMAN and N. W. SCHAAD, Department of Plant, Soil, and Entomological Sciences, University of Idaho, Moscow 83843

ABSTRACT

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Samples of 15 bean seed lots were assayed by "dome test" procedures for *Pseudomonas syringae* pv. *syringae* (*P. syringae*), *P. syringae* pv. *phaseolicola* (*P. phaseolicola*), *Xanthomonas campestris* pv. *phaseoli* pv. *phaseoli*), and *X. campestris* pv. *phaseoli* var. *fuscans*. Some seed lots were chosen because they were positive in dome tests elsewhere. Bacteria were isolated from lesions of bean seedlings in a dome test. Bacteria producing a white, flat, nonmucoid fluorescent colony or yellow-mucoid colony on King et al's medium B and yeast extract-dextrose calcium-carbonate agars, respectively, were purified. Identities of possible pseudomonad blight pathogens were verified by utilization of manitol, sorbitol, and lactate, levan formation, oxidase reaction, and pathogenicity. Possible xanthomonads were verified by pathogenicity tests. Thirty-one fluorescent colonies and 66 yellow-pigmented colonies were isolated from 11 and 8 of the 15 seed lots, respectively. Neither *P. phaseolicola* nor *X. phaseoli* were identified in the 15 seed lots, and *P. syringae* was identified in only one of the 15 seed lots. However, six pseudomonads that failed standard pathogenicity tests produced water-soaking when injected into seedlings kept under dome test conditions. These results suggest that the dome test is not a reliable assay procedure for issuing phytosanitary certificates.

Pseudomonas syringae pv. *syringae* (*P. syringae*), the causal agent of brown spot; *P. syringae* pv. *phaseolicola* (*P. phaseolicola*), the causal agent of halo blight; and *Xanthomonas campestris* pv. *phaseoli* var. *fuscans* (*X. phaseoli* var. *fuscans*), the causal agent of foscous blight, are serious seedborne pathogens of beans worldwide (27). Detection of these pathogens in seed is essential for effective disease control. In Idaho (24) and Michigan (3) and in member countries of the European and Mediterranean Plant Protection Organization (2), a zero tolerance for *X. phaseoli* and *P. phaseolicola* is required in laboratory and/or field tests for seed certification. However, such standards are only as effective as the sensitivity and specificity of the certification procedure (14,19). In Idaho, a zero tolerance for *P. syringae, P. phaseolicola, X. phaseoli*, and *X. phaseoli* var. *fuscans* is required in field inspections to certify bean seeds for plantings in the state. Michigan allows 0.005% blight in field inspections (3) but requires zero tolerance in a laboratory assay. Seeds are soaked for 48–72 hr in a liquid semiselective medium for *X. phaseoli* and *X. phaseoli* var. *fuscans*, and the concentrated liquid is tested by Ouchterlony agar gel double diffusion (20). Asgrow Seed Company (Twin Falls, ID) tests for blight pathogens by soaking 25-kg random samples of cull seeds for 3–6 hr in water (24). The liquid is then sprayed onto susceptible bean seedlings previously wounded with sand blown from a paint sprayer (24). In England, the seed is either ground to a flour in a hammer mill and soaked in water or placed in a Stomacher (16,17). Dilutions of the liquid are plated onto King et al's medium B (KB) agar (9), and fluorescing colonies are purified and identified by phage or immunofluorescence. Another test developed in North Dakota and currently used to evaluate bean seeds for blight pathogens is the "dome test" (21). In this test, seeds are soaked for 24 hr, vacuum-infiltrated with the same solution, and grown in a humid chamber until symptoms appear within 7–12 days. Because the dome test has been used to rate Idaho seed (Rodgers Bros. Seed Co. and Idaho Seed Bean Co., Twin Falls, ID, *personal communication*) and because of a lack of published data on its specificity, we have investigated the reliability of the assay for detection of bacterial blight pathogens. A brief report of this research has been published (10).

Present address of first author: Monsanto Corp., 800 N. Lindberg, St. Louis, MO 63167.

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MATERIALS AND METHODS

Dome test procedure. Fifteen bean (*Phaseolus vulgaris* L.) seed lots (most produced in Idaho and rated positive in dome tests elsewhere) were assayed according to a modified dome test procedure (22). First, bean seeds (350 g) were agitated for 20–30 sec in 400 ml of tap water plus two or three drops of phosphate-free detergent (Liqui-nox, Alconox, Inc., New York, NY) and rinsed three times in tap water. Next, seeds were surface-disinfested in 300 ml of a solution

of one part chlorine bleach (5.25% NaOCl) to four parts water for 3 min and rinsed three times in sterile distilled water (SDW). After soaking 8 min in 600 ml of 1,000 mg/ml $Na_2S_2O_3 \cdot 5H_2O$, the seeds were rinsed three times in SDW, and all but 50–100 seeds were soaked for 24 hr in 600 ml of SDW at room temperature. Concurrently, the 50–100 seeds were imbibed for 24 hr on wet filter paper in sterile petri dishes. Seeds in the soak solution were discarded, and the 50–100 imbibed seeds were then placed in 50–100 ml of the seed-soak solution under vacuum (11.25 kg/2.5 cm^2) for 230 sec. The infiltrated seeds were placed in an upside-down clear plastic cake dome (Centennial Mills, Inc., Spokane, WA) containing 60 g of coarse, sterile vermiculite moistened with 400 ml of sterile maneb solution (150 mg/ml of maneb, 80% active, du Pont de Nemours & Co., Wilmington, DE). Additional vermiculite was added to cover the seeds and another plastic dome was placed right side up, making a chamber. Rims of the cake domes were sealed together around the perimeter with transparent tape. The chambers were placed under constant light provided by four cool-white fluorescent lamps (Sylvania CW/VHO 115W) 20 cm above the top dome at room temperature (20–25 C). After 5–14 days, isolations were made from lesions in the resulting seedlings by grinding diseased primary leaves or hypocotyls in 1 ml of SDW using a sterile mortar and pestle. A flamed loop was used to streak samples onto KB and yeast-extract-dextrose calcium carbonate (YDC) agars (26). Single colonies fluorescing on KB agar or producing yellow-pigmented, mucoid colonies on YDC agar were purified and stored on slants of nutrient glucose (13) or YDC agar, respectively.

Characterization of strains isolated in dome tests. Tests used for identification of strains fluorescing on KB agar included the oxidase test, utilization of L-lactate, D-manitol, and D-sorbitol, formation of levan, and pathogenicity (12). *P. phaseolicola* C-199 (J. W. Guthrie, University of Idaho, Moscow) and *P. syringae* C-203 (D. M. Webster, Asgrow Seed Co., Twin Falls, ID) were used for comparisons. Colony morphology on YDC agar was used to identify possible strains of *X. phaseoli* or *X. phaseoli* var. *fuscans* (5). Pale yellow, mucoid, convex colonies also producing a brown water-soluble pigment were considered possible *X. phaseoli* var. *fuscans*.

Pathogenicity was determined for all suspected strains of *Pseudomonas* and *Xanthomonas*. Fluorescent strains were grown for 15–18 hr in liquid medium 523 (8) at 100 rpm on a New Brunswick model G-25 incubator shaker at 26 C. Inoculum for each strain was adjusted to 10^7 colony-forming units per milliliter (cfu/ml) using a colorimeter at 540 nm, serially diluted, and plated onto nutrient agar to determine the actual number of colony-forming units (23). Bean seeds (cultivar Eagle) were planted in 10-cm-diameter pots containing sterile soil mix of sand-peat (1:1, v/v) and grown for 10–12 days in the greenhouse at 25 ± 5 C. A modified multiple-needle inoculator (1) was made by attaching 15 minuten (0.15-cm-diameter) insect mounting pins (Danni Butterfly Enterprises, Cleveland, OH) to a 1.5-cm-diameter cork. The inoculator pins were dipped into inoculum and then pierced through the adaxial surface of a partially expanded primary leaf supported from below with a hand. Two inoculations were made on each side of the midrib of each leaf of four plants. The insect pins were dipped in 95% ethanol and flamed between different inocula. Plants were covered with platic bags and placed in a Conviron E8M environmental chamber at 20 ± 1 C with cool-white fluorescent illumination at 16,000 lux. After 24 hr, the bags were removed and the plants left in the chamber for 5–7 days before recording symptoms.

Yellow-pigmented strains were tested for pathogenicity as described for pseudomonads, except the inocula were adjusted to 10^8 cfu/ml and infiltrated with a 3-cc syringe into trifoliolate leaves (14–21 days old) of the cultivar Gala. The plants were incubated in a greenhouse at 25 ± 5 C.

Detection of known levels of *P. phaseolicola* in seed-soak solution by dome test procedures. The effects of seed-soak solution and SDW containing *P. phaseolicola* C-199 at 10^5 cfu/ml infiltrated into bean seedlings were compared with solutions not containing *P. phaseolicola*. All solutions were diluted and plated onto KB agar before infiltration of seeds to determine the actual number of bacteria present. A seed lot of cultivar Eagle was chosen for the study in which no fluorescing bacteria were observed in previous plating of seed-soak solutions on KB agar. Seeds (350 g) from this seed lot were prepared for the dome test as described. However, instead of infiltrating only seed-soak solution, other additional solutions were compared. Each of eight sets of 35 seeds was separated from the bean seed-soak solution and infiltrated with one of the following solutions: 1) seed-soak solution after 0 hr of incubation, 2) seed-soak solution after 24 hr, 3) seed-soak solution after 0 hr plus *P. phaseolicola* added after 0 hr, 4) seed-soak solution after 24 hr plus *P. phaseolicola* added after 24 hr, 5) seed-soak solution after 24 hr plus *P. phaseolicola* added at 0 hr, 6) sterile water, 7) sterile water after 0 hr plus *P. phaseolicola* added after 0 hr, and 8) sterile water after 24 hr plus *P. phaseolicola* added after 0 hr. The seeds were placed in domes as described, and symptoms of seedlings were recorded 7 days after sowing. Isolations from water-soaked tissue were made 7 and 12 days after sowing as previously described.

Symptom development in seedlings in cake domes resulting from saprophytic bacteria. Four fluorescent and two yellow-pigmented strains isolated from seedlings in dome tests of seed lots 2, 4, 5, and 10, and 3 and 5, respectively, were tested for symptom development in cake domes. These strains had failed to result in symptoms in standard pathogenicity tests. Each strain was grown for 18 hr at 27 C in liquid medium 523 and then adjusted to 10^9–10^{10} cfu/ml. Hypocotyls of 6-day-old bean seedlings (cultivar Eagle) growing in cake domes were injected with the liquid inoculum with a 3-cc syringe and 25-gauge needle. The inoculated seedlings were incubated in the domes and symptoms were observed after 5–7 days.

Table 1. Identification of fluorescent and yellow-pigmented bacteria isolated from dome test seedlings of commercial bean seeds[a]

Seed lot	Cultivar	Number of strains obtained	
		Fluorescent bacteria[b]	Yellow-pigmented bacteria[c]
1	Nw 410	4	24
2	Nw 59	6	0
3	Viva	0	10
4	G.N. Ui 36	2	1
5	Nw 590	4	6
6	G.N. Ui	0	10
7	Nw 63	3	12
8	Gala	0	0
9	Pinto	1	1
10	Fiesta	6	2
11	Admiral	0	0
12	Seafarer	1	0
13	Great Northern	1	0
14	Gala	2	0
15	Zircon	1	0

[a] Seeds assayed in dome test as described by Venette (21), consisting of vacuum-infiltrating bean seeds with seed-soak solution and planting them in a humid chamber.
[b] Determined on King et al's medium B (9).
[c] Determined on yeast-extract-dextrose calcium carbonate agar (26).

RESULTS

Characterization of bacteria isolated from commercial seed lots in dome tests. Symptoms observed on bean seedlings growing in cake domes included: 1) water-soaked spots on primary leaves and cotyledons, 2) brown, curled tissue at leaf margins and/or brown flecking of leaf tissue, 3) general and local leaf chlorosis, 4) reddening of leaf veins and hypocotyls, and 5) soft rotting of hypocotyls. Many seedlings with soft rot collapsed during the 2-wk test.

Fluorescent and yellow-pigmented bacteria were isolated from 11 and 8 of 15 seed lots, respectively (Table 1). The fluorescent strains were grouped by similar reactions in biochemical and pathogenicity tests (Table 2) as follows: group I—strain from seed lot 10; group II—from seed lots 1, 2, 4, 7, 10, and 12; group III—strains from seed lots 1 and 9; group IV—strains from seed lots 1, 2, 4, 5, 7, 10, and 13; and group V—strains from seed lots 10 and 15. Strain 10r-1, isolated from a single seedling in seed lot 10, was phenotypically identical to *P. syringae* C-203 (Table 2). This strain also resulted in lesions indistinguishable from those produced by C-203.

None of 66 yellow bacteria isolated from the eight seed lots were typical of *X. phaseoli* or *X. phaseoli* var. *fuscans* on YDC agar. These colonies were either flat and nonmucoid or slimy with nondiscrete margins. Furthermore, no strains produced lesions in pathogenicity tests.

Detection of known *P. phaseolicola* in seed-soak solutions and dome test seedlings. All treatments, including the water control, resulted in leaves with water-soaking and/or constriction of the hypocotyls. All concentrations of *P. phaseolicola* dropped 100-fold in the seed-soak solution but less than 10-fold in water only (Table 3).

Seedling symptoms produced by saprophytic strains. All strains tested produced water-soaked leaf tissue in seedlings growing in domes. In addition, the strains from seed lots 4 and 5 produced soft rotting of hypocotyls. No water-soaking or stem rotting was observed in the water controls, but the control did have some leaf curling and constricted stem tissue, which is commonly observed in seedlings during dome tests. All bacteria isolated from tissues of such seedlings were nonpathogenic when subjected to standard pathogenicity tests.

Table 2. Phenotypic properties of *Pseudomonas syringae* pv. *syringae* C-203, *P. syringae* pv. *phaseolicola* C-199, and unidentified fluorescent strains isolated from seedlings in dome tests[a]

	Known strains		Unidentified fluorescent strains (dome test)				
Trait[b]	C-199 ($n = 1$)	C-203 ($n = 1$)	I ($n = 1$)	II ($n = 8$)	III ($n = 3$)	IV ($n = 17$)	V ($n = 2$)
Utilization of							
D-Sorbitol	−[c]	+	+	+	+	−	+
D-Manitol	−	+	+	+	+	+	+
L-Lactate	−	+	+	+	+	+	+
Oxidase reaction	−	−	−	+	−	−	−
Levan formation	+	+	+	−	−	−	−
Pathogenicity[d]							
Water-soaking, brown lesions with halos restricted 1–5 mm around lesion	−	+	+	−	−	−	−
Water-soaking with halos expanding into areas greater than 5 mm of the leaf	+	−	−	−	−	−	−

[a] Seeds assayed in dome test as described by Venette (21), consisting of vacuum-infiltrating bean seeds with seed-soak solution and planting them in a humid chamber. Symptoms on seedlings consisted of brown lesion, water-soaking, chlorosis, and soft rotting of the hypocotyl and reddening of leaf veins.

[b] As described by Sands et al (12).

[c] + = Positive reaction or growth; − = negative reaction or no growth.

[d] Unexpanded primary leaves of 10- to 12-day-old beans (cultivar Eagle) were inoculated with a set of 15 insect pins previously dipped in bacterial suspensions adjusted to 10^7 cfu/ml. Inoculated plants were grown in an environmental chamber at 20 ± 1 C and constant light for 5–7 days as described.

Table 3. Comparison of colony-forming units per milliliter (cfu/ml) of *Pseudomonas syringae* pv. *phaseolicola* and other bacteria recovered in dilution plating of solutions at the time of infiltration into bean seeds[a]

	cfu/ml	
Treatment[b]	*P. phaseolicola*	Other bacteria
1. Soak solution after 0 hr of incubation	0	6.5×10^1
2. Soak solution after 24 hr of incubation	0	3×10^3
3. Soak solution after 0 hr of incubation + *P. phaseolicola* added at 0 hr	4×10^5	−
4. Soak solution after 24 hr of incubation + *P. phaseolicola* added at 24 hr	7×10^5	−
5. Soak solution after 24 hr of incubation + *P. phaseolicola* added at 0 hr (incubated together)	3×10^3	1×10^3
6. Sterile water only after 0 hr of incubation	0	0
7. Sterile water + *P. phaseolicola* added after 0 hr of incubation	2×10^5	0
8. Sterile water after 24 hr of incubation + *P. phaseolicola* added at 0 hr (incubated together 24 hr)	1×10^5	0

[a] *P. phaseolicola* colonies were determined based on their ability to fluoresce on King et al's medium B (9). A seed lot was chosen for the study if no fluorescing bacteria were observed in preliminary screening. In the study, all colonies that fluoresced were considered to be *P. phaseolicola* C-199, and all nonfluorescing bacteria were considered to be other bacteria.

[b] Dome test, consisting of vacuum-infiltrating bean seeds with seed-soak solution and planting them in a humid chamber (21), was modified to include solutions such as water and soak solutions amended with *P. phaseolicola* infiltrated into individual sets of 35 bean seeds.

DISCUSSION

Of more than 200 seed lots assayed by dome tests in previous studies, only two were found free of *Pseudomonas* and *Xanthomonas* bean blight pathogens (26). In contrast, of 15 commercial and breeders' seed lots, including six identified elsewhere as containing bacterial blight pathogens in dome tests, only one lot was contaminated with a bean blight bacterium. This bacterium, from seed lot 10, was identified as *P. syringae* following the methods of Sands et al (12). Seedlings in dome tests from all the seed lots we tested were commonly observed with water-soaked, brown, and/or soft rotted lesions. However, neither *X. phaseoli* nor *P. phaseolicola* were isolated from such lesions. Instead, various saprophytic pseudomonads and yellow-pigmented bacteria were isolated.

The most plausible explanation for differences in the test results of the same seed lots is that the organisms causing symptoms in seedlings in the dome test are easily misidentified. Our identifications were based on recommended biochemical and pathological tests (5,12). In our pathogenicity tests, symptoms resulting from *P. syringae, P. phaseolicola*, and *X. phaseoli* were characteristic (27) and easily distinguished from reactions caused by bacteria isolated from seedlings in dome tests. Initial symptoms (at 4–5 days) produced by *P. syringae* were small water-soaked spots.

After 7–10 days, lesions enlarged and became brown and brittle in the center surrounded by small, restricted, yellow halos 1–5 mm in diameter. *P. phaseolicola* also produced water-soaked spots within 4–5 days, but by 7 days showed general chlorosis extending in an area greater than 5 mm around the water-soaked tissue. On the other hand, of six dome test strains tested, all caused water-soaking when injected at high concentrations into bean seedlings maintained in the humid environment of the domes, but none were positive in controlled pathogenicity tests.

One of the major problems with the dome test is that high concentrations of saprophytic bacteria can produce lesions when the environment favors the saprophytes over the host. This is demonstrated by the many types of symptoms including water-soaking, dry brown leaf tissue, and soft rotting observed on seedlings in the dome test. Similar symptoms were observed on leaves of tomato plants, which were induced to natural water-soaking by high humidity and then exposed to high levels of saprophytic bacteria such as *Escherichia coli, P. fluorescens*, and *Enterobacter aerogenes* (7). The domes in which the seedlings are incubated during dome tests are at a high relative humidity, and condensation is evident on the sides of the domes throughout the test. This could result in formation of water films on seedling surfaces that could then favor the activity of soft rot organisms. Maher and Kelman (11) report that water films on tissue surfaces can reduce oxygen levels that in turn favorably influence growth of pectolytic bacteria. Soft rotting observed in the cake domes could be caused by organisms such as *B. subtillis* (4), *P. fluorescens* (6), or *P. viridiflava* (25). These organisms are found as part of normal bean microflora (15,16). The humid environment of the domes and the high level of saprophytes are not the only factors that put the host seedlings at a disadvantage. Sterile water vacuum-infiltrated into bean seeds resulted in water-soaking of seedlings kept in domes, whereas water injected into seedlings with a syringe did not result in water-soaked lesions. Thus, infiltrating bean seeds with seed-soak solution containing high levels of saprophytic bacteria must further place the host at a disadvantage. In an effort to simplify the test, some laboratories use a rating scale to evaluate dome test seedlings (22) and they omit isolation and pathogenicity test information. Use of the dome test in this way is based on the premise that most seed lots are contaminated to some degree with blight pathogens (22). Our investigations suggest this omission leads to inaccurate, unreliable results. Furthermore, even with pathogenicity tests, the proliferation of saprophytic bacteria during incubation could obscure the presence of actual pathogens.

Additional problems exist with the seed-soak solution. Taylor reported that *P. phaseolicola* can be extracted from seeds only after they have soaked for 24–48 hr, by which time contaminating bacteria make isolation of the pathogen difficult (16). Schaad (14) recommends that if an enrichment technique is necessary, it should include antibiotics or chemicals that reduce or eliminate growth of contaminants. When we monitored the bacteria in soak solutions, we found that populations of known *P. phaseolicola* were reduced, possibly by other antagonistic bacteria. One saprophyte associated with beans, *P. fluorescens*, is reported to be antagonistic to *P. phaseolicola* (18). It is possible that this or other antagonists exist in the bean seed-soak solutions that hinder the recovery of low levels of the blight pathogens.

The dome test was first reported 5 yr ago in a trade journal (21), but the method, along with sensitivity and field data, have never been published to support the test's effectiveness. This test should not be recommended for seed testing purposes nor should phytosanitary certificates be issued on the basis of dome test results.

LITERATURE CITED

1. Andrus, C. F. 1948. A method of testing beans for resistance to bacterial blights. Phytopathology 38:757-759.
2. Anselme, C., and Mathys, G. 1977. Seed health protection on an international level; technical, legislative and commercial aspects. ISTA Congress, 18th. Madrid, Spain. 25 pp.
3. Copeland, L. O., Adams, M. W., and Bell, D. C. 1975. An improved seed programme for maintaining disease-free seed of field beans (*Phaseolus vulgaris*). Seed Sci. Technol. 3:719-724.
4. Doudoroff, M., and Palleroni, N. J. 1974. Genus I. *Pseudomonas*. Pages 217-243 in: Bergey's Manual of Determinative Bacteriology. R. E. Buchanan and N. E. Gibbons, eds. William & Wilkins, Baltimore. 1,246 pp.
5. Dye, D. W. 1980. *Xanthomonas*. Pages 45-49 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
6. Hagar, S. S., and McIntyre, G. A. 1972. Pectic enzymes produced by *Pseudomonas fluorescens*, an organism associated with "pink eye" disease of potato tubers. Can. J. Bot. 50:2479-2488.
7. Johnson, J. 1937. Relation of water-soaked tissues to infection by *Bacterium angulatum* and *B. tabacum* and other organisms. J. Agric. Res. 55:599-618.
8. Kado, C. I., and Heskett, M. G. 1970. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas, Xanthomonas*. Phytopathology 60:969-976.
9. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Med. 44:301-307.
10. Lahman, L. K., and Schaad, N. W. 1983. Reliability of 'Dome' test as an assay for bean blight pathogens. (Abstr) Phytopathology 73:805.
11. Maher, E. A., and Kelman, A. 1983. Oxygen status of potato tuber tissue in relation to maceration by pectic enzymes of *Erwinia carotovora*. Phytopathology 73:536-539.
12. Sands, D. C., Schroth, M. N., and Hildebrand, D. C. 1980. *Pseudomonas*. Pages 36-44 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
13. Schaad, N. W. 1980. Initial identification of common genera. Pages 1-11 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
14. Schaad, N. W. 1982. Detection of seedborne bacterial plant pathogens. Plant Dis. 66:885-890.
15. Schnathhorst, W. C. 1954. Bacteria and fungi in seeds and plants of certified bean varieties. Phytopathology 44:588-592.
16. Taylor, J. D. 1970. The quantitative estimation of the infection of bean seed with *Pseudomonas phaseolicola* (Burkh.) Dowson. Ann. Appl. Biol. 66:29-36.
17. Taylor, J. D. 1978. Report on Sixteenth International Workshop on Seed Pathology. International Seed Testing Association, Zurich, Switzerland.
18. Teliz-Ortiz, M., and Burkholder, W. H. 1960. A strain of *Pseudomonas fluorescens* antagonistic to *Pseudomonas phaseolicola* and other bacterial plant pathogens. Phytopathology 50:119-123.
19. Tempe, J. de 1970. Routine methods for determining the health condition of seeds in the seed testing station. Proc. Int. Seed Test. Assoc. 35:1-42.
20. Trujillo, G. E., and Saettler, A. W. 1980. A liquid semi-selective medium for *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans*. Res. Rep. Mich. State Univ. Agric. Res. Stn. 411. 8 pp.
21. Venette, J. R. 1978. Disease and bean seed certification. N.D. Seed J. (12):3.
22. Venette, J. R. 1983. Detecting seed-borne bacterial pathogens in bean seed: A semi-quantitative method. Mich. Dry Bean Dig. 7:2.
23. Vidaver, A. K. 1980. *Corynebacterium*. Pages 12-15 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
24. Webster, D. M., Atkin, J. D., and Cross, J. E. 1983. Bacterial blights of snap beans and their control. Plant Dis. 67:935-940.
25. Wilkie, J. P., Dye, D. W., and Watson, D. R. W. 1973. Further hosts of *Pseudomonas viridiflava*. N.Z. J. Agric. Res. 16:315-323.
26. Wilson, E. E., Zeitoun, F. M., and Fredrickson, D. L. 1967. Bacterial phloem canker, a new disease of Persian walnut trees. Phytopathology 57:61621.
27. Zaumeyer, W. J. and Thomas, H. R. 1957. A monographic study of bean diseases and methods for their control. U.S. Dep. Agric. Tech. Bull. 868. 255 pp.