Evaluation of Seedborne *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* as Primary Inocula in Bean Blights

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The roles of several types of seedborne *Xanthomonas phaseoli* (*Xp*) and *X. phaseoli* var. *fuscans* (*Xpf*) were evaluated in the epidemiology of common (caused by *Xp*) and fuscous (caused by *Xpf*) bacterial blights of navy beans. Seeds externally infested with blight bacteria were a source of primary inoculum, and 14% of commercial navy bean seed lots were so contaminated. Surface populations of *Xp* and *Xpf* ranged from 0 to 4×10^4 bacteria per seed; minimal populations of 10^3–10^4 bacteria per seed were required for production of infected plants under field conditions. Symptomless seeds internally contaminated with *Xp* or *Xpf* were identified as potential primary inoculum sources. Seeds with visible symptoms always were associated with visibly infected pods and pod infection resulting from systemically borne bacteria often caused hairline suture lesions which are difficult to detect.

Additional key words: Phaseolus vulgaris.

Common and fuscous bacterial blights, which are incited by *Xanthomonas phaseoli* (E. F. Sm.) Dows (*Xp*) and *X. phaseoli* var. *fuscans* (Burkh.) Starr and Burkh. (*Xpf*), respectively, are major diseases of navy beans in Michigan. Internally infected seed is the main source of primary inoculum (11,12) and at times Michigan seed stocks have been heavily contaminated with *Xp* and *Xpf* (2,9). Disease control traditionally has relied on seed certification to maintain clean seed stocks. Epiphytotics of common, fuscous, and halo (*Pseudomonas phaseolicola* (Burkh.) Dows.) blights in the 1960s prompted expansion of the bean seed certification program in Michigan (2,5,6). Certification involves both a visual field inspection for blight symptoms and a laboratory seed test for internal bacterial contamination. Although this program has reduced seedborne *Xp* and *Xpf* in navy beans, disease outbreaks persist and some seed fields are rejected annually for certification. The chronic occurrence of blight in Michigan suggests that possibly the certification program does not detect low levels of infection. Other possible sources of primary inocula include externally contaminated seed and symptomless seed. Although the epidemiological significance of *Xp* and *Xpf* as seed surface contaminants has not been critically investigated, field observations and research on halo blight (3,4) suggest a possible role for such inoculum.

The purpose of this study was to determine the relative importance of various possible sources of seedborne primary inocula in the epidemiology of common and fuscous bacterial blights in Michigan navy beans.

MATERIALS AND METHODS

Surface sterilization of bean seed. Navy beans were surface-sterilized by gentle agitation in a 1:1 commercial bleach-distilled water solution (2.6% NaOCl) for 60 sec. To test the reliability of the procedure, autoclaved navy beans were artificially contaminated with blighted bean dust, treated with bleach, and individual seeds incubated in tubes of buffered-yeast extract (BYE = 10 g yeast extract per 1,000 ml of 0.01 M phosphate buffer, pH 7.2) for 120 hr. Of 300 tubes containing infected, surface-sterilized seeds none showed growth, whereas all tubes containing seeds whose surfaces were not sterilized did. The number of units forming colonies was an estimate of the number of bacteria in a sample.

Externally contaminated bean seed. Navy beans were artificially infested with bean blight bacteria by shaking 100 g of seed in a flask with 0.1 ml water and 0.1 g of pulverized, dried bean leaf tissue infected with either three wild-type isolates each of *Xp* and *Xpf* or rifampin-resistant mutants of *Xpf* and *Xp*, bacterial blight isolates R10 and Ra, respectively (10). Externally infested seeds were assayed for use as primary inoculum sources by planting two rows 6 m long, of infested or clean seed in a randomized block design replicated four times; each replication was separated by a row of corn. Disease was rated 49 and 56 days after planting.

Minimal population of blight bacteria on seeds necessary for successful seedling infection was determined by infesting seeds with varying levels of R10 and Ra ranging from 0 to 1.6×10^5 bacteria per seed. We planted 100 seeds in a 0.4 m^2 mound and arranged treatments in a randomized block design with at least 2.1 m between plots. To test for seed transmission, leaves and stem were monitored for R10 or Ra at bloom. Experiments were conducted both at the Botany and Plant Pathology Farm, Michigan State University, East Lansing, and the Saginaw Valley Bean and Beet Farm, Saginaw, Michigan.

Assays for external contamination by *Xp* and *Xpf* were conducted on samples of commercial seed lots obtained from the Michigan Department of Agriculture (MDA), Michigan Crop Improvement Association (MCIA), or personally collected from Michigan bean growers. Seed samples (125 g) were shaken in 40 ml of BYE for 1 min, the washings were incubated in 125-ml flasks on a rotary shaker at 25 C for 48 to 72 hr, then centrifuged at 10,000 *g* for 15 min. The pellet was resuspended in 0.25 ml of the original volume of the supernatant and injected into 15-day-old Manitou seedlings (9). Isolations were made from all plants 30 to 50 days after injection regardless of the presence of symptoms. Seed samples were also tested by the MDA Seed Testing Laboratory for internal blight contamination (Table 3, footnote b).

Experimental lots of externally infested seed were collected by mechanically threshing mature plants infected with common or fuscous blight. Populations of *Xp* or *Xpf* on the surface of bean

seed were determined by shaking 50 seeds in 50 ml of 0.01 M phosphate buffer (pH 7.2), one seed per milliliter of buffer and plating on yeast extract-calcium carbonate agar (YCA) + cycloheximide or rifampin agar medium (RAM) + cycloheximide (10). For samples with low surface populations, the washings were passed through a 0.22 μm Millipore filter and the bacteria resuspended in 1 ml buffer before plating.

Internal seed infection. Several methods were used to internally inoculate seeds. Half-filled green pods were scratched along the dorsal suture or the side with a syringe containing 10^8 *Xp* or *Xpf* cells per ml, or the bacteria were injected into the stem at the point of pedicel attachment. Strains R10 and Ra were established systemically in bean stems by injecting 10^8 cells per milliliter of suspension into the cotyledon node of 20-day-old seedlilngs. Pods from all inoculated plants were hand-harvested and individually opened. Visibly infected seeds were rated for disease severity on a scale of 1 to 4 in which 1 = darkening in the hilum region (hilum-spotted seed) and 4 = complete butter-yellow discoloration and shrivelling of the seed. Populations of R10 and Ra in infected seeds were determined by individually grinding five seeds of each infection type with mortar and pestle with phosphate buffer and subsequently plating serial dilutions on RAM. Data were based on an average seed weight of 0.18 g. The number of colony-forming units was an estimate of the number of bacteria in a sample.

Symptomless, internally infected seed. To assay for Ra or R10 in seed without symptoms, hand-harvested pods were wiped with a damp cloth to remove the dust, individually opened, and the seeds were removed with sterile forceps and placed in sterile test tubes. Individual seeds or all seeds from a single pod were surface sterilized, placed in 25 × 150 mm culture tubes containing 10 ml of BYE, supplemented with rifampin and cycloheximide, and incubated for 5–7 days on a rotary shaker at 25 C. Bacteria from tubes that became turbid were streaked on RAM to confirm the presence of R10 or Ra. During these studies R10-infested seed was used as a control to ensure the reliability of the surface sterilization technique.

RESULTS

Surface-infested seed as a source of primary inoculum. In 1975, plants grown from seed naturally and artificially infested with *Xp* and *Xpf* were significantly ($P = 0.01$) more diseased, 12 and 19.5% foliage infection, than plants grown from uninfested seed, 3.8 and 7.1% infection, when rated 48 and 55 days after planting, respectively. In 1976, similar results were obtained using R10-infested seed. In 1977, experiments were conducted at two locations to determine the inoculum load necessary to yield infected plants (Table 1). Surface populations of 10^3 to 10^4 bacteria per seed were required for plant infection. Results were similar in greenhouse studies.

Commercial and experimental navy bean seed lots were assayed to determine the populations of *Xp* and *Xpf* established on seed surfaces by mechanical threshing (Table 2). Populations of surface-borne blight bacteria ranged from 0 to more than 4×10^4 bacteria per seed; generally surface populations increased with the level of disease detected in the field prior to harvest. The bacterial

TABLE 1. Relationship between surface populations of bacterial blight pathogen isolates R10 and Ra on navy bean seed and the development of blighted plants[a]

R10			Ra		
No. bacteria/seed[b]	Bean cultivar	Infected plots/plots planted	No. bacteria/seed	Bean cultivar	Infected plots/plots planted
0	Tuscola	0/8	0	Tuscola	0/4
0	Seafarer	0/8	0	Seafarer	0/4
0-10^1★	Seafarer	0/8	5.00×10^1	Tuscola	0/4
10^1 - 10^2★	Seafarer	0/4	1.28×10^2	Seafarer	0/4
6.25×10^1	Seafarer	0/4	5.52×10^2	Seafarer	0/4
7.23×10^2	Tuscola	0/4	1.03×10^3	Tuscola	1/4
7.90×10^2	Seafarer	0/4	1.48×10^3	Seafarer	1/4
3.15×10^3	Tuscola	1/8	1.04×10^5	Tuscola	2/4
5.70×10^3	Seafarer	1/4	4.45×10^5	Seafarer	3/4
3.39×10^4	Tuscola	1/8			
7.80×10^4	Tuscola	2/4			
1.60×10^5	Seafarer	2/4			

[a] Data of Ra and R10 are pooled from experiments conducted at the Saginaw Valley Bean and Beet Farm, Saginaw, Michigan, and Department of Botany and Plant Pathology Farm, East Lansing, Michigan. One hundred seeds were planted in 0.4 m^2 plots and treatments were arranged in a randomized block design with at least 2.1 m between each plot.

[b] ★Indicates seed naturally infested by mechanical threshing; all other seed were infested with 0.1 g of R10 or Ra-infected bean dust per 100 g seed and 0.1 ml H_2O to promote sticking.

TABLE 2. Surface populations of blight bacteria *Xanthomonas phasioli* (*Xp*) and *X. phaseoli* var. *fuscans* (*Xpf*) on mechanically threshed navy bean seed isolate R10 and *X. phaseoli* isolate Ra on navy bean seedling growth[a]

	No. bacteria/seed[a]					Av. foliage infection	Isolate
Seed lot	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	%	
Experimental:							
Seafarer (1974)	0.0	2.9×10^4	6.6×10^2	1.3×10^3	0.0	80-90	*Xp-Xpf*
Seafarer (1975)	4.0×10^4	4.7×10^2	0.0	0.0	0.0	80-90	*Xpf*
Seafarer[b] (1976)	0.7	2.4	2.9	2.2	87	10	*Xpf* R10
Seafarer[b] (1976)	0.6	0.3	3.3	0.1	2.4	5	*Xpf* R10
Sanilac (1977)	2.5×10^3	0.0	0.0	0.0	3.6×10^2	20-40	*Xpf* R10
Sanilac (1977)	2.0×10^2	0.0	1.3×10^4	7.0×10^2	0.0	30-60	*Xp* Ra
Commercial:							
Seafarer (1976)	5.0×10^3	0.0	0.0	0.0			*Xpf*
Sanilac (1977)	0.0	1.4×10^4	9.2×10^2	0.0	0.0		*Xp*

[a] Surface populations were assayed by shaking 50 seeds in 50 ml phosphate buffer for 2 min and plating the washings on YCA + cycloheximide (25 μg/ml) or RAM + cycloheximide (25 μg/ml).

[b] Indicates samples where washings were concentrated by filtering through a 0.22 μm Millipore filter and resuspended in 1 ml of buffer.

populations were quite variable among several samples from a single seed lot; apparently bacteria were not uniformly distributed over the seeds.

Of 192 commercial seed lots from three growing seasons, 26 lots were externally infested with *Xp* and *Xpf* and six with *P. phaseolicola* (*Pp*) (Table 3). Results of tests for external blight bacteria in MDA and MCIA samples from 1976 and 1977 were compared to tests for internally borne bacteria by the MDA Seed Testing Lab in the same samples; 14% of the lots were externally infested with *Xp* or *Xpf* whereas only 11% were positive for blight by the MDA tests; 2% of the samples reported internally blighted by the MDA were not externally infested. The MDA seed testing does not distinguish between *Xp, Xpf,* and *Pp* infection. Two certified seed samples from 1976 and one from 1977 reported blight-free by the MCIA were externally infested, and one of the samples from 1976 produced blighted plants when planted in the field in 1977.

Navy bean seeds became externally contaminated during pod infection as well as during mechanical threshing. When isolates R10 or Ra were inoculated to the dorsal pod sutures of Seafarer and Sanilac beans, some seeds appeared healthy except for a faint yellow halo around the hilum. The halo varied in intensity from quite to barely visible under a dissecting microscope. Of 75 Seafarer seeds with the halo, R10 was isolated from 43 seeds; surface-sterilization eliminated surface-borne bacteria. Strain Ra was isolated from 20 of 30 Sanilac seeds exhibiting the halo but was not isolated from surface-sterilized seed. From seed pressed on RAM, bacteria were isolated only from the halo region; bacterial populations of 2.6×10^4 to 4.5×10^5 were associated with the halo. Seeds with masses of bacterial ooze adhering to the seed coat also were found in pods inoculated in the suture or side and from commercial and experimental seed lots. The bacterial ooze was easily scraped from the seed coat and its bacterial populations usually ranged from 10^5–10^7 per seed. Seed apparently became contaminated as a result of inner pod infection. When the above-mentioned types of seed were planted in the field or greenhouse, both produced infected plants.

Internal seed infection and bacterial populations. Internal infection of seed by *Xp* and *Xpf* caused a variety of symptoms. Severity ranged from a slight darkened spot in the hilum region (hilum spot) to complete butter-yellow discoloration and shrivelling of the seed coat. Populations of *Xp* and *Xpf* inside infected seeds increased with the severity of seed symptoms. The mean bacterial populations ± standard error of the mean per seed with disease ratings of 1 to 4 were $1.0 \times 10^6 \pm 4.0 \times 10^5$, $3.4 \times 10^8 \pm 1.1 \times 10^8$, $1.5 \times 10^9 \pm 3.6 \times 10^8$, and $4.2 \times 10^8 \pm 1.3 \times 10^8$ for R10, respectively; and $8.5 \times 10^5 \pm 3.1 \times 10^5$, $2.7 \times 10^8 \pm 8.9 \times 10^7$, $9.0 \times 10^8 \pm 2.5 \times 10^8$, and $4.4 \times 10^8 \pm 1.5 \times 10^8$ for Ra, respectively. A population of 1.8×10^5 bacteria was the lowest detected in hilum-spotted seed. In 30 commercial and experimental seed lots, hilum-spotted seed was the predominant type of infection; 123 seeds had hilum spots and only 17 had butter-yellow seed coat discoloration. R10 and Ra were isolated from 100% of seed with butter-yellow discoloration selected from inoculated pods, and from 60 and 64% respectively, of hilum-spotted seed. The germination rate and quality of the seedlings produced were affected by severity of

TABLE 3. Frequency of surface contamination by bacterial blight pathogen *Xanthomonas phaseoli* (*Xp*), *X. phaseoli* var. *fuscans* (*Xpf*), and *Pseudomonas phaseolicola* (*Pp*) in commercial navy bean seed lots

Year	Source[a]	Total samples	No. externally infested by *Xp*	*Xpf*	*Pp*	Internally[b] infected	Internally infected but not externally infested[c]
1975	Personally collected	24	1	1	0	Not Tested	Not Tested
1976	MDA	58	8	4	3	11	3
	MCIA	46	2	1	2	1	0
1977	MDA	30	4	2	1	4	0
	MCIA	34	2	1	0	2	1

[a] All samples were from commercial seed lots; MDA = samples submitted to Michigan Department of Agriculture; MCIA = samples collected by Michigan Crop Improvement Association from fields for which certification had been requested.
[b] The Michigan Department of Agriculture's (MDA) standard test for internal seed-borne blight bacteria involves: (i) surface sterilization of 2.2 kg seed for 10 min in 2.6% NaOCL; (ii) rinsing in sterile water; (iii) incubation of seed for 24 hr in sterile water containing 10 g yeast extract/1, and (iv) injection of a sample of liquid surrounding the seeds into the primary leaf node of young kidney bean seedlings.
[c] No. of samples determined positive for internal blight bacteria by MDA seed test and negative by the assay for external blight bacteria.

TABLE 4. Effect of seed infection by *Xanthomonas phaseoli* var. *fuscans* isolate R10 and *X. phaseoli* isolate Ra on navy bean seedling growth[a]

Infection[b] type	No. of seeds planted R10	Ra	Germination (%) R10	Ra	Seedlings[c] deformed (%) R10	Ra	Av. fresh wt of seedling (g) R10	Ra	Seedlings[d] infected (%) R10	Ra
Uninfected	40	33	100	97	0	0	1.21	1.27	0	0
1	40	30	95	97	3	3	1.06	1.16	60	50
2	34	9	91	100	65	45	0.83	0.68	100	90
3	35	11	63	64	71	57	0.56	0.58	90	100
4	25	8	28	13	100	100	0.33	0.46	100	100

[a] Seeds were planted in flats containing a 1:1 mixture of greenhouse soil and vermiculite; data were collected 7 days after planting. Data are pooled from three separate experiments.
[b] Infection types: 1 = seed with discoloration in the hilum region (hilum-spotted seed); 2 = seed with less than 10% butter-yellow discoloration; 3 = seed with 11–100% butter-yellow discoloration and no shrivelling; 4 = complete butter-yellow discoloration and partial seed shrivelling.
[c] Deformed seedlings were stunted and had no or smaller primary leaves.
[d] Infected seedlings were identified by individually homogenizing 10 seedlings grown from each infection type and planting on RAM + cycloheximide.

TABLE 5. Detection of bacterial blight pathogen isolates R10 (of *Xanthomonas phaseoli* var. *fuscans*) and Ra (of *X. phaseoli*) in symptomless navy bean seed[a]

Cultivar	Blight isolate	Site of inoculation[b]	No. seeds tested	No. infected
Seafarer (1976)	R10	S	200	3
Tuscola (1977)	R10	S	56	1
Seafarer (1977)	Ra	S	105	0
Sanilac (1977)	Ra	S	150	2
Seafarer (1978)	R10	C	310	1
Seafarer (1978)	Ra	C	250	1

[a] Seeds were assayed by surface-sterilizing individual or all seeds from a single pod and then incubating them in 25 × 150 mm culture tubes containing 10 ml of BYE + rifampin (50 μg/ml) on a rotary shaker for 5 to 7 days.
[b] S = seeds from pods inoculated with R10 or Ra along the suture of half-filled green pods; C = seeds from pods of plants inoculated at the cotyledon node with R10 or Ra 20 days after planting.

seed infection (Table 4). Hilum-spotted seed (type 1) was similar to uninfected seed in germination and the seedlings produced were generally normal in appearance. Most seed with type 2–3 infection produced abnormal seedlings, characterized by reduction in seedling size and absence or reduction in size of primary leaves; type 4 seeds that germinated were always seriously deformed. Isolate R10 was recovered from 43% of 28 seedlings grown from hilum-spotted seed planted in the field.

That seeds must have minimal populations of blight bacteria before they show symptoms suggests that symptomless navy bean seed could be infected with low levels of blight bacteria. Both Ra and R10 were isolated from inside seeds that showed no symptoms or discoloration; the seeds were from pods that had been inoculated with R10 and Ra along the suture or from symptom-free pods that had been produced on systemically infected plants (Table 5).

Pod symptoms and seed symptoms. Bacterial blight lesions developed on navy bean pods infected either by external inoculum or by systemically borne bacteria. Visibly infected seeds were produced only in pods bearing some form of lesions (Table 6). Pods infected by external inoculum exhibited typical irregular, water-soaked lesions. Only when the lesion included a portion of the dorsal suture were seeds with the butter-yellow discoloration produced. Pods on plants that were systemically infected developed either watersoaked lesions in the suture region or minute hairline lesions confined entirely to the suture (Table 6). The hairline symptoms were characterized by a narrow band of dark tissue several millimeters long, along the side of, or in, the dorsal suture. When the suture was split, the darkening was more evident on the inner surface of the suture. Extremely close visual examination with a dissecting microscope was sometimes required to detect the discoloration. For example, in one Tuscola pod no discoloration was seen on the outside of the suture and only a small spot was seen on the inside. The hairline lesion was generally confined to the pedicel end of the pod and the hilum spot was on the first or second seed in the pod.

Pod symptoms of any type were difficult to see when mature pods remained in the field for several days during cool, moist weather because fungi readily colonized the pod surface and caused it to darken.

DISCUSSION

Seeds with visible blight symptoms generally have been considered to be the main sources of primary inocula. In Michigan, hilum-spotting is the most important symptom of internally infected seed. In our sample lots of beans, hilum-spotted seed was more common than was severely infected seed. Furthermore, bacteria often were transferred from hilum-spotted seed to seedlings. Patterns of germination of this type of seed and resulting seedling growth were similar to those of uninfected seed. On the other hand, more severely infected seed (types 2 to 4) germinated at low rates and produced deformed seedlings; thus, this type of seed would not be an effective primary inoculum source.

Seeds externally infested with *Xp* or *Xpf* also could be sources of primary inocula. Infestation occurs during threshing when bacteria from dried bean tissue become air-borne in bean dust. Isolate R10 was routinely isolated on RAM when plates of this medium were placed around the thresher during threshing of R10-infected plants. The source of the bacterial contamination is mainly stems and pods, and to a lesser extent leaves, because most of the foliage drops prior to harvest. Minimal inoculum levels of 10^3–10^4 bacteria per seed were required for transfer of the bacteria from seed to seedlings; however, the natural minimal threshold might be lower, because, in this study, seeds were uniformly coated with bacteria. On the other hand, bacterial populations detected in replicate samples of a seed lot varied considerably, suggesting that blight bacteria were not evenly distributed among the seeds and were concentrated on smaller areas of the seed coats. In addition to contamination from mechanical threshing, seed surfaces might become contaminated during pod infection; infestation appears as light yellow halos around the hilum or crusts of yellow bacterial ooze on the seed coat. Whereas 14% of commercial seed lots tested were externally contaminated with *Xp* or *Xpf*, only 11% of the same seed lots were reported by the MDA Seed Testing Laboratory to be internally infected. This suggests a role for external contamination in the epidemiology of blight in Michigan.

Navy beans are susceptible to halo blight in the greenhouse (7), but the disease has not been observed in Michigan navy bean fields (9). The present study indicates that navy beans might be a source of *Pp* and the bacteria could spread to susceptible bean cultivars.

The Michigan seed certification program is based on the close correlation between pod symptoms and internal seed infection, and rejection of seed fields with visible pod blight is expected to eliminate seed infection. On the other hand, Burkholder (1) detected typical common blight-infected seed in symptomless pods. In this study, analysis of seed from hundreds of navy bean pods from four growing seasons showed that visible pod infection is a prerequisite for production of visibly infected seed. The relationship between pod infection and visible seed infection is most apparent when pods are infected by external inocula; seeds under the lesion often become visibly infected. However, when *Xp* and *Xpf* infect the suture systemically, the relation between seed and pod symptoms is less obvious. Systemically infected pods may display only a faintly darkened hairline lesion along the suture that in normal field inspections would seldom be detected. Furthermore, discoloration from molding of pods during cool, moist conditions at harvest probably would mask even distinctive pod blight symptoms.

TABLE 6. Relation between pod symptoms and seed symptoms caused on navy bean plants by bean blight pathogens *Xanthomonas phaseoli* (*Xp*) and *X. phaseoli* var. *fuscans* (*Xpf*)

Cultivar	Isolate	No. pods sampled	Site inoculated[a]	No. pods with lesions	No. infected pods with visibly infected seed	No. pods with hair-line suture lesions[b]	No. symptomless pods with visibly infected seed
Seafarer (1975)	*Xpf*	428	Pedicel	13	6	3	0
	Xp	400	Pedicel	15	4	1	0
	Xpf	431	Suture	431	401	0	0
	Xp	413	Suture	413	396	0	0
Seafarer (1976)	*Xpf* R10	219	Pedicel	5	2	1	0
	Xpf R10	186	Suture	180	133	0	0
Tuscola (1977)	*Xp*	364	Natural infection	15	6	3	0
Seafarer (1978)	*Xp* Ra	293	Cotyledon scar	20	2	1	0
	Xpf R10	375	Cotyledon	12	3	1	0

[a] Pedicels were inoculated by injecting a 10^8 cells/ml suspension of blight bacteria into the stem where the pedicel attaches to the stem; sutures were inoculated by scratching along the dorsal pod suture with the tip of a syringe containing 10^8 cells/ml; common blight in the 1977 Tuscola beans developed from natural infection; cotyledon scars of 20-day-old plants were inoculated with a 10^8 cells/ml suspension of bacteria using a syringe.

[b] Hairline suture lesions indicate pods with visible symptoms only along the suture that appeared as fine, short bands of darkened tissue; some of the lesions were only detected by close examination with a dissecting microscope.

Symptomless navy bean seed containing low populations of blight bacteria were detected and might serve as a source of primary inoculum. Such seed was produced not only in visibly infected pods but also, in two cases, in symptomless pods as a result of systemic bacterial movement from the stem. Bacterial populations could not be determined accurately in symptomless seed, but probably were less than 10^5 cells per seed. Detection of infection in symptomless seed would be almost impossible by current testing methods because of their low frequency in seed lots and the low populations within individual seed. Blight bacteria in symptomless seed were detected only by assaying seeds from plants that had been inoculated with rifampin-resistant strains R10 or Ra which could be monitored on a selective medium.

The results of this study indicate that the occurrence of common and fuscous blights in Michigan navy beans results from several types of externally and internally infected seed. Owing to the existence of multiple sources of seed inocula and to the difficulty in detection of *Xp* and *Xpf* in visual field inspection and in seed lots, chronic outbreaks of blight probably are inevitable in Michigan. Current seed testing techniques (8) can detect only a minimal threshold of infection in any given seed lot.

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