Nonoverwintering of Xanthomonas Bean Blight Bacteria in Michigan

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ABSTRACT

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A series of studies was conducted over 10 yr to determine survival of *Xanthomonas campestris* pv. *phaseoli* (*X. c.* pv. *phaseoli*) in Michigan. Twenty isolates of *X. c.* pv. *phaseoli* and 10 bean genotypes were used to minimize possible isolate and host effects. Pathogenic *X. c.* pv. *phaseoli* were never isolated from 191 separate tissue samples that overwintered (October through April) during the periods 1972–1973, 1975–1977, 1977–1979, and 1980–1982. Thus crop debris infected with *X. c.* pv. *phaseoli* does not constitute a source of primary inoculum for the bean common blight disease in Michigan.

Contaminated seed is the most important source of inoculum for the bean common bacterial blight disease, caused by *Xanthomonas campestris* pv. *phaseoli* (*X. c.* pv. *phaseoli*) (14). Thus the use of pathogen-free seed has been the main method used to control the disease in most bean production areas of the United States. However, outbreaks of the disease occasionally occur even when pathogen-free seed is planted, suggesting the existence of other sources of *X. c.* pv. *phaseoli* inoculum.

One possible alternate source of primary inoculum could be bacteria surviving in infected crop debris; however, overwintering studies of *X. c.* pv. *phaseoli* have produced conflicting results (1,4–8,10,12,15,16). In two early studies, *X. c.* pv. *phaseoli*-like bacteria were recovered from overwintered infected plant debris, but the isolates were not identified or tested for pathogenicity (7,8).

Schuster and Coyne (11) buried and then recovered *X. c.* pv. *phaseoli* as long as 22 mo later; survival of *X. c.* pv. *phaseoli* was better when bean and weed residues remained on the soil surface than when buried in the soil (10,11). Wimalajeewa and Nancarrow (15) were unable to isolate pathogenic *X. c.* pv. *phaseoli* from infected tissue left longer than 11 wk on the soil surface or buried 3 wk in the soil. In both studies, the bacteria isolated were tested for pathogenicity.

The ability of *X. c.* pv. *phaseoli* to overwinter has also been studied by sowing pathogen-free bean seed of susceptible cultivars into soil infested the previous season(s) with infected crop debris. Lack of typical symptoms of disease on the subsequent bean crop led to the conclusion that *X. c.* pv. *phaseoli* did not overwinter (1,4,12). Little is known, however, of the factors governing infection of healthy plants from overwintered inoculum; inoculum loads may have been too low to initiate infection, yet viable pathogen cells may have been present.

These examples of the disparity of results regarding overwintering of *X. c.* pv. *phaseoli* may be explained in part by the use of different procedures for isolating and detecting the pathogen in infected plant debris. Also, isolates of *X. c.* pv. *phaseoli* may differ in their ability to overwinter. Moreover, host genotype may influence survival (3); this factor has not been considered previously. We have attempted to address, over a number of years, the effects of such factors as site of placement of infected tissue, host genotype, and pathogen isolate on survival of *X. c.* pv. *phaseoli* (Table 1). During these studies, we used several techniques to isolate and identify the *X. c.* pv. *phaseoli* pathogens. We now present the results of these studies, which indicate that *X. c.* pv. *phaseoli* does not overwinter in Michigan.

MATERIALS AND METHODS

Sites of placement. Three distinct ecological sites were chosen to represent places where bean plant tissues might normally occur under standard agricultural practices, namely plants left standing in the field, tissue remaining on the soil surface, and tissue at a depth of about 25 cm (normal plowing depth). Tissue samples placed on and beneath the soil surface were contained in nylon mesh stockings knotted or sewn at both ends; samples were placed in duplicate so that one each could be retrieved after 1 and 2 yr of overwintering. Samples were generally placed in the field during the normal harvest period of September to November.

Placements were established in agricultural fields near the cites of East Lansing, St. Louis, and Saginaw, MI. The rationale for using such diverse locations over a number of years was to obtain a wide variation in year-to-year environmental effects of overwintering on *X. c.* pv. *phaseoli*.

Host genotypes and pathogen isolates. During this study, we used infected tissues from numerous dry bean cultivars showing various disease reactions to *X. c.* pv. *phaseoli* (namely, susceptible, tolerant, and resistant). A total of 20 isolates of *X. c.* pv. *phaseoli* were used to represent both the standard *X. c.* pv. *phaseoli* type and the *X. c.* pv. *phaseoli* fuscans variant, which produces a brown diffusible pigment in certain culture media. In several cases, rifampin-resistant mutants of *X. c.* pv. *phaseoli* were employed in efforts to enhance the efficiency of pathogen recovery from infected tissues. Specific details regarding the tissue samples were as follows.

Study A. Leaves were collected in July 1972 from rows of Seafarer navy beans showing severe common blight symptoms. The plants had been inoculated three times during the growing season with a mixture of 13 *X. c.* pv. *phaseoli* isolates. Leaves were cut into squares 1–2 cm, air-dried, and 100-mg samples placed inside nylon packets sown together with nylon thread.

Study B. Stems and leaves naturally infected with *X. c.* pv. *phaseoli* isolates R10, Ra, or R10-S6 were collected from plants grown in the field. All three isolates were resistant to the antibiotic rifampin, and R10-S6 was also resistant to streptomycin (13). Tissue was wrapped in double-layered, fine-mesh nylon. Seafarer beans were grown in a 37-m plot at the East Lansing farm and inoculated with *X. c.* pv. *phaseoli* isolate R10 at 12 days after planting. By the end of the growing season, 85% of the plants were systemically infected; the dead, mature plants were left standing in the plot over the winter.

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Study C. Leaves infected with *X. c.* pv. *phaseoli* isolates R15-1 and R17 (3) were collected from resistant (tepary bean, *Phaseolus acutifolius* cv. Arizona-buff), tolerant (Great Northern Nebraska 1 Selection 27 and MSU-51319), and susceptible (Seafarer) bean genotypes grown in the greenhouse and the field. Samples of pulverized dried tissue were wrapped in fine-mesh nylon bags tied with nylon thread.

Study D. Tissue samples consisted of infected stems of the bean genotypes Tuscola and Pinto UI-114 (susceptible), Great Northern Valley (tolerant), and Great Northern Nebraska 1 Selection 27 (resistant). Infected stems were obtained from greenhouse-grown plants that had been injected when 10 days old with bacterial suspensions (10^8 cells per milliliter) of each isolate. Stem segments were excised 1 cm above and below the injection points and allowed to air-dry at room temperature. Stem segments were tied in nylon mesh bags, five segments per bag.

Positive controls. Each study included positive controls, which consisted of identical infected tissue samples that were stored in a laboratory incubator (4 C) for a period identical to that of the infected field samples. All studies also included negative controls, which consisted of healthy plant tissue placed within 3 m of the infected samples. Such negative controls served to detect possible background or natural contamination of the site by *X. c.* pv. *phaseoli.*

Methods of detection. Attempts to recover *X. c.* pv. *phaseoli* from overwintered plants and tissue samples involved an extraction process whereby tissue was incubated directly, or after trituration with a mortar and pestle, in liquid solutions. The solutions were then plated directly on standard or antibiotic-amended media or injected into young kidney bean seedlings as described under individual studies.

Study A. Tissue was removed from the packets and transferred aseptically to a flask containing 10 ml of buffered saline (0.01 M phosphate buffer, pH 7.2, and 0.85%, w/v, NaCl). After incubation for 2 hr, a sample of the liquid was injected into the primary leaf node of 10-day-old red kidney bean seedlings (cultivar Manitou) (9). A second 1-ml sample was transferred into a flask containing 50 ml of sterile buffered yeast extract (BYE) (1 g of yeast extract and 1 L 0.01 M phosphate buffer, pH 7.2), incubated for 18 hr on a rotary shaker, and injected into bean seedlings.

Study B. To recover viable cells of R10 and Ra, a portion of the tissue was homogenized in a mortar and pestle in 0.01 M phosphate buffer, pH 7.2, and samples were plated on rifampin agar medium with rifampin (100 μg/ml), cycloheximide (200 μg/ml), and PCNB (100 μg/ml) (13); streptomycin sulfate (250 μg/ml) was also added when attempting to isolate R10-S6. The remaining portions of tissue were first incubated in BYE supplemented with these antibiotics, then plated on solid media; suspect bacteria were tested for pathogenicity by seedling injection.

Study C. Tissue samples were homogenized in phosphate buffer and appropriate serial dilutions of each sample plated on YCA (10 g of yeast extract, 2.5 g of $CaCO_3$, 15 g of agar, and 1 L of distilled H_2O) supplemented with 150 μg/ml of rifampin, 100 μg/ml of cycloheximide, and 100 μg/ml PCNB. Other portions of the sample were initially incubated in BYE with antibiotics before being plated on solid media and infiltrated into bean leaves. Comparison tissue samples were maintained at room temperature in the laboratory and assayed at 6-mo intervals over a 2-yr period.

Study D. The five stem sections in each sample were triturated in 5 ml of sterile buffered saline, and samples of the liquid loop were streaked onto YCA to isolate *X. c.* pv. *phaseoli* 11 and 15, and onto YCA plus 100 μg/ml of rifampin to isolate *X. c.* pv. *phaseoli* Ra. Suspect bacteria were purified by single-colony transfer and tested for pathogenicity by the seedling injection method. An additional 1-ml sample of the liquid was placed into liquid media of the same type and injected into bean seedlings after 96 hr on the rotary shaker.

RESULTS

Positive and negative controls. In all cases, pathogenic *X. c.* pv. *phaseoli* cells were recovered from positive control tissue samples maintained at 4 C in the laboratory for the same periods of time as those placed in the field. In study C, for example, positive control samples of

Table 1. Sites of placement, genotypes, and pathogen strains used in studies on overwintering of *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) in Michigan

Study	Site of placement: Standing plants	Soil surface	Buried 25 cm	Location (date)[a]	Host genotypes and disease reactions to *Xcp*[b]	Pathogen isolates[c]	No. of samples assayed	Method of detection used[d]
A	No	Yes	Yes	EL (1972–1973)	Seafarer (S)	7 *Xp*: 897-1, 897-2, 1205-2, 1307 BBL-25, Pinto, I_2 6 *Xpf*: M, 988-2, 1020-1 I_2 1376-2, 1253-1	3	Enrich + SI
	No	Yes	Yes	Sag (1972–1973)	Seafarer (S)	7 *Xp*: 897-1, 897-2, 1205-2 1307 BBL-25, Pinto, I_2 6 *Xpf*: M, 988-2, 1020-1 I_2 1376-2, 1253-1	3	Enrich + SI
B	Yes	Yes	Yes	El (1975–1976)	Seafarer (S) Sani Lac (S)	*Xpf* R10, *Xp* Ra, *Xpf* R10-S6 *Xpf* R10, *Xp* Ra, *Xpf* R10-S6	18	RMS + Enrich + SI
C	Yes	Yes	Yes	EL (1977–1978)	Seafarer (S) GN Nebr. 1 Sel. 27 (R) Tepary (R)	*Xp* R15-1, *Xpf* R17 *Xp* R15-1, *Xpf* R17 *Xp* R15-1, *Xpf* R17	36	RSM + Enrich + SI
	No	Yes	Yes	Sag (1977–1978)	Seafarer (S) GN Nebr. 1 Sel. 27 (R) Tepary (R)	*Xp* R15-1, *Xpf* R17 *Xp* R15-1, *Xpf* R17 *Xp* R15-1, *Xpf* R17	36	RSM + Enrich + SI
	No	Yes	Yes	St. Louis (1977–1978)	Seafarer (S) GN Nebr. 1 Sel. 27 (R) Tepary (R)	*Xp* R15-1, *Xpf* R17 *Xp* R15-1, *Xpf* R17 *Xp* R15-1, *Xpf* R17	36	RSM + Enrich + SI
D	No	Yes	Yes	EL (1980–1982)	Tuscola (S) UI-114 (S) Valley (T) GN Nebr. 1 Sel. 27 (R)	*Xp*11, *Xp* 15, *Xp* Ra *Xp*11, *Xp* 15, *Xp* Ra *Xp*11, *Xp* 15, *Xp* Ra *Xp*11, *Xp* 15, *XP* Ra	33	RSM + Enrich + SI
	No	Yes	Yes	Sag (1980–1982)	Tuscola (S) UI-114 (S) Valley (T)	*Xp*11, *Xp* 15, *XP* Ra *Xp*11, *Xp* 15, *XP* Ra *Xp*11, *Xp* 15, *XP* Ra	26	RSM + Enrich + SI

[a] EL = East Lansing, Sag = Saginaw.
[b] Disease reactions: S = susceptible, T = tolerant, R = resistant.
[c] *Xp* = *X. campestis* pv. *phaseoli*, *Xpf* = *X. campestis* pv. *phaseoli*, fuscans variant (produces brown, diffusible pigment).
[d] Enrich = Enrichment in liquid nutrient medium, SI = seedling injection (a), and RSM = rifampin-selective media (13).

infected Seafarer tissue yielded 2.8 (10^7), 2.6 (10^7), and 1.5 (10^7) colony-forming units (cfu) of *X. c.* pv. *phaseoli* R17 per gram dry weight of tissue after 0, 12, and 24 mo of storage, respectively. *X. c.* pv. *phaseoli* populations were not determined in studies A, B, and D; however, *X. c.* pv. *phaseoli* was easily recovered qualitatively on basal and antibiotic-amended media as well as by seedling injection. Although comparative tests have not been conducted, studies during the development of the rifampin-selective media (RSM) system indicate that those media can detect *X. c.* pv. *phaseoli* in bean tissue homogenates at densities as low as 10^2–10^3 cfu/ml; RSM detected down to 10 *X. c.* pv. *phaseoli* per 0.3 g fresh weight of root tissue, even in the presence of adhering soil particles (13). Negative controls from all sites failed to yield *X. c.* pv. *phaseoli* when isolations were attempted.

Standing plants. No bacteria of *X. c.* pv. *phaseoli* isolate R10 were isolated from systemically infected plants left standing during the winter after the 1976 growing season, and beans planted in the same plot the following season did not show symptoms of bean blight (study B). On the other hand, *X. c.* pv. *phaseoli* isolate R15-1 was recovered from samples of infected plants left standing in the field after the 1978 growing season in October, November, and December 1978, but no viable pathogenic bacteria were recovered in January, February, March, and May 1979 (study C).

Tissue samples on or beneath soil surface. *X. c.* pv. *phaseoli* was never recovered from overwintered infected tissue samples placed on the soil surface or at a depth of 25 cm. In most instances, duplicate samples were assayed after 1 and 2 yr of overwintering.

DISCUSSION

Our studies demonstrate that *X. c.* pv. *phaseoli* does not survive Michigan winters, and thus infected crop debris constitutes no hazard as a source of primary inoculum. These conclusions are based on our inability to isolate pathogenic *X. c.* pv. *phaseoli* from 191 separate tissue samples over 6 yr of study during 1972–1982. Placement of tissue samples on the soil surface and at plowing depth (20–30 cm) gave similar results. Neither the *X. c.* pv. *phaseoli* isolates nor host genotypes used affected the results. In our studies, the maximum time that *X. c.* pv. *phaseoli* could be isolated from standing plants was until December, 3 mo after normal harvest.

Schuster and Coyne (11) reported that *X. c.* pv. *phaseoli* survived as long as 22 mo in Nebraska. Several factors could account for the apparent discrepancy between our results.

First, *X. c.* pv. *phaseoli* isolates could differ in survivability. Schuster and Coyne suggest that with the other bean bacterial pathogens, *Pseudomonas syringae* pv. *phaseolicola* (halo blight) and *Corynebacterium flaccumfaciens* (wilt), the more virulent isolates are better adapted for survival (11). We have no evidence that such a correlation between virulence and survivability occurs with *X. c.* pv. *phaseoli*; a total of 20 pathogenic isolates of *X. c.* pv. *phaseoli*, including the brown pigment-producing fuscans variant, were employed in our study with identical negative results. Of the 20 isolates, 15 were wild type and 5 were naturally occurring mutants resistant to antibiotics. We noted no difference in survivability between the two groups.

Second, previous studies have shown that host genotype greatly influences *X. c.* pv. *phaseoli* growth and survival in various bean tissues. Schuster and Coyne showed reduced survival of *X. c.* pv. *phaseoli* in tissue of tolerant cultivars of Great Northern beans compared with susceptible ones. In our study, we included tolerant Valley Great Northern and resistant Great Northern 1 Selection 27 and obtained identical negative results.

Third, environmental conditions in Nebraska may in fact differ in some way from those in Michigan to allow *X. c.* pv. *phaseoli* to survive over the winter.

Certainly, additional studies are needed relative to survivability of *X. c.* pv. *phaseoli*, which is an efficient colonizer of both host and nonhost crop species during the growing season (2). Moreover, it is a paradox that *X. c.* pv. *phaseoli*, which can survive as long as 35 yr in infected bean seed in the laboratory (A. W. Saettler, *unpublished*), cannot survive even one winter in the field. Research endeavors toward understanding the physiological properties associated with bacterial survival should lead to new approaches to the control of phytobacterial diseases.

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LITERATURE CITED

1. Burkholder, W. H. 1930. The bacterial diseases of the bean: A comparative study. N.Y. (Cornell) Agric. Exp. Stn. Mem. 127. 88 pp.
2. Cafati, C. R., and Saettler, A. W. 1980. Role of nonhost species as alternate inoculum sources of *Xanthomonas phaseoli*. Plant Dis. 64:194-196.
3. Cafati, C. R., and Saettler, A. W. 1980. Effect of host on multiplication and distribution of bean common blight bacteria. Phytopathology 70:675-679.
4. Hedges, F. 1946. Experiments on the overwintering in the soil of bacteria causing leaf and pod spots of snap and lima beans. Phytopathology 36:677-678.
5. McCready, S. B. 1911. Bacterial Blight. Ont. Agric. Coll. Exp. Farm Annu. Rep. (1910) 36:46.
6. Muncie, J. H. 1914. Two Michigan bean diseases. Mich. Agric. Exp. Stn. Spec. Bull. 68. 12 pp.
7. Patel, M. K. 1929. Viability of certain plant pathogens in soils. Phytopathology 19:295-300.
8. Rapp, C. W. 1920. Bacterial blight of beans. Okla. Agric. Exp. Stn. Stillwater Bull. 131. 39 pp.
9. Saettler, A. W. 1971. Seedling injection as an aid in identifying bean blight bacteria. Plant Dis. Rep. 55:703-706.
10. Schuster M. L. 1967. Survival of bean bacterial pathogens in the field and greenhouse under different environmental conditions. Phytopathology 57:830.
11. Schuster, M. L., and Coyne, D. P. 1974. Survival mechanisms of phytopathogenic bacteria. Annu. Rev. Phytopathol. 12:199-221.
12. Wallen, V. R., and Galway, D. A. 1979. Effective management of bacterial blight of field beans in Ontario—a 10-year program. Can. J. Plant Sci. 1:42-46.
13. Weller, D. M. 1978. Ecology of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* in Navy (pea) beans (*Phaseolus vulgaris* L.). Ph.D. thesis. Michigan State University, East Lansing. 137 pp.
14. Weller, D. M., and Saettler, A. W. 1980. Evaluation of seedborne *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* as primary inocula in bean blights. Phytopathology 70:148-152.
15. Wimalajeewa, D. L. S., and Nancarrow, R. J. 1980. Survival in soil of bacteria causing common and halo blights of French bean in Victoria. Aust. J. Exp. Agric. Anim. Husb. 20:102-104.
16. Zaumeyer, W. J. 1930. The bacterial blight of beans caused by *Bacterium phaseoli*. U.S. Dep. Agric. Tech. Bull. 186. 36 pp.