Longevity of *Xanthomonas campestris* pv. *phaseoli* in Naturally Infested Dry Bean (*Phaseolus vulgaris*) Debris

ELADIO ARNAUD-SANTANA and ESTELA PENA-MATOS, Arroyo Loro Experiment Station, San Juan de la Maguana, Dominican Republic; and DERMOT P. COYNE and ANNE K. VIDAVER, Department of Horticulture and Department of Plant Pathology, University of Nebraska, Lincoln 68583

ABSTRACT

Arnaud-Santana, E., Pena-Matos, E., Coyne, D. P., and Vidaver, A. K. 1991. Longevity of *Xanthomonas campestris* pv. *phaseoli* in naturally infested dry bean (*Phaseolus vulgaris*) debris. Plant Dis. 75:952-953.

In two experiments, initiated in 1986 and 1987 in the Dominican Republic, *Xanthomonas campestris* pv. *phaseoli*, the causal organism of the common blight disease of dry beans (*Phaseolus vulgaris*), survived in surface debris composed of diseased leaves. In contrast, leaves in plastic mesh bags located at a depth of 15 cm were apparently decomposed and devoid of the pathogen in less than 30 days. After extraction of bacteria from debris using 12.5 mM potassium PO_4 buffer (pH 7.1) with 1 mM $MgSO_4$, dilutions were made and plated onto MXP medium. The pathogen survived for 5 mo in the diseased debris on the ground surface in both experiments but was not detected at 6 and 7 mo in experiment II. The pathogen was isolated from debris placed underground for 24 hr but was not detected after 30 days in either experiment. Presumptive *X. c. phaseoli* recovered from debris was pathogenic on cv. PC-50.

Common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye is one of the major diseases of dry beans (*Phaseolus vulgaris* L.) in many production areas in the world (8,12). The pathogen can survive on seed (7,12) and is seed transmitted (11). It also has been shown to survive in dry leaves under laboratory conditions for at least 6 yr (4). Survival of *X. c. phaseoli* in bean debris from previous crops is known, but the period is subject to controversy (3,6,7,9,10). The pathogen was recovered from diseased bean debris buried for as long as 22 mo under field and greenhouse conditions in Nebraska (7). However, investigators in Michigan and Australia were unable to isolate the bacterium from diseased debris placed on and beneath the soil surface (6,10). Gilbertson et al (5) found that the bacterial organism survived in the field for at least 7 mo under nontillage conditions and for a maximum of 4 mo with tillage practices in Wisconsin. Saettler et al (6) explained that these conflicting results were attributable to differences in climate, geographic area, cultural practice, and, possibly, strains of *X. c. phaseoli*. Gilbertson et al (5) pointed out that the sensitivity of techniques used for bacterial recovery could also contribute to the varying results.

We report here the survival of *X. c. phaseoli* in diseased bean debris under tropical conditions in the Dominican Republic.

Published as Journal Series Paper 9304, Nebraska Agricultural Research Division.

Research was conducted under Title XII Bean/Cowpea CRSP (AID contract DAN-1310-G-SS-6008-00).

Accepted for publication 22 March 1991 (submitted for electronic processing).

MATERIALS AND METHODS

Seeds of dry bean cultivar PC-50, which is susceptible to common bacterial blight, were planted on 20 September 1986 and 18 September 1987 in a field of the Arroyo Loro Experimental Station, San Juan de la Maguana, Dominican Republic.

Natural infection of the plants occurred, and symptoms of common bacterial blight were observed 35 days after planting at flowering time in both years. Diseased leaves were collected from fully mature (ripe) plants and from different areas of the field on 30 November 1986 and 25 November 1987. These leaves were divided into 36 sets of 10 g each and enclosed in saran fiber polypropylene (1 × 2 mm mesh) bags. The bags were tied to stakes with fishing lines for easy retrieval and placed in the soil or on the soil surface. The same procedure was repeated on 25 November 1987, except 66 bags of leaves were used to extend the experiment to 10 sampling dates.

The experimental design was a split-plot with three replicates. The main plots were the location of bags—placed on the soil surface or at 15 cm depth. The bags were placed 75 cm apart in the field. The subplots were sampling times. The main plots were arranged in a randomized complete block design with 18 and 33 bags per block in experiments I and II, respectively. Samples were collected every month for 5 and 10 mo in 1986 and 1987, respectively. However, because the pathogen was not isolated from the sixth and seventh month samples in experiment II, no more samplings were made. The first samples were collected 24 hr after the experiment commenced.

Five grams of debris were taken from each bag, added to 50 ml of 12.5 mM potassium PO_4 buffer (PB) (pH 7.1) with 1 mM $MgSO_4$ in a beaker, and allowed to soak for 2 hr with occasional swirling. Tenfold dilutions were made (five in total), and 100 μl of each were plated onto MXP, a semiselective medium for *X. c. phaseoli* (2). The plates were incubated at 24–26 C and colonies were counted after 3–4 days. The colonies were pale, yellow, convex, mucoid, and surrounded by a zone of starch hydrolysis. Colonies consisted of large and small types in about a 3:1 ratio.

Presumptive typical *X. c. phaseoli* colonies were subcultured on MXP, transferred to nutrient broth (Difco), and incubated for 48 hr at 24–26 C. Cell

Table 1. Mean population levels of *Xanthomonas campestris* pv. *phaseoli* detected in infested dry bean debris placed on and in soil in two experiments (1986–1987 and 1987–1988), San Juan de la Maguana, Dominican Republic

Sampling date	Days (no.)	Placement of infested debris	
		On soil surface (cfu/g of debris)	In soil (cfu/g of debris)
Experiment I			
12/1/1986	1	$>10^9$[a]	$>10^9$
12/31/1986	30	$>10^9$	0
1/30/1987	60	$>10^9$	0
3/1/1987	90	$>10^9$	0
3/31/1987	120	1.3×10^8	0
4/30/1987	150	6.0×10^6	0
Experiment II			
11/26/1987	1	$>10^9$	$>10^9$
12/23/1987	30	$>10^9$	0
1/23/1988	60	$>10^9$	0
2/24/1988	90	$>10^9$	0
3/21/1988	120	1.3×10^8	0
4/20/1988	150	4.3×10^7	0
5/20/1988	180	0	...
6/19/1988	210	0	...

[a]Too many to count; $>10^9$ cfu/g of debris.

suspensions of both types of colonies were made by transferring colonies to glass tubes containing 25 ml of PB (pH 7.1) and diluted to read *A* at 0.1 on a Bausch and Lomb Spectronic 20 spectrophotometer set at 640 μm. The dilution was then transferred into a flask containing 250 ml of PB (pH 7.1) to give a concentration of 10^7 cfu/ml. The inoculum was introduced into the abaxial surface of fully expanded fourth trifoliate leaves of 5-wk-old PC-50 plants with the multiple needle inoculation method (1). Control plants were inoculated only with distilled water. Plants were examined for disease symptoms after 12 days based on the presence or absence of chlorosis, necrosis, and water-soaking within and/or at the margins of the inoculated areas. The following disease rating scale was used: 1 = no disease symptoms in the inoculated area, 3 = <30% inoculated area with disease symptoms, and 5 = >30% of inoculated area with disease symptoms.

RESULTS AND DISCUSSION

The survival of the bacteria depended on bag location (Table 1). For the first 90 days, large populations ($>10^9$ cfu/ml^{-1}) were recovered from all samples on the soil surface. In contrast, the pathogen was recovered only after 24 hr from the buried debris and was not detected in later samplings. Similar results were observed in experiment II where the bacteria were isolated after 6 and 7 mo from debris on the soil surface. The debris on the soil surface remained intact, and leaf forms could be recognized for the duration of both experiments. The buried debris was decomposed within 30 days.

Presumptive colonies of *X. c. phaseoli*, both large and small types, were pathogenic on PC-50. All inoculated plants developed necrotic tissues surrounded by water-soaking zones (disease rating = 5). These symptoms are typical of PC-50 when artificially inoculated with the common bacterial blight pathogen.

Our observations on bacterial survival are comparable to those of Schuster and Coyne (7,8) and Gilbertson et al (5), who found that *X. c. phaseoli* overwintered in bean debris on the soil surface in Nebraska and Wisconsin. Our results are also comparable to those of Saettler (6) and Wimalajeewa and Nancarrow (10), who found that the pathogen did not survive on buried dry bean debris.

Our results were different from those of Schuster and Coyne (8) and Gilbertson et al (5), who found that the bacteria survived in the soil for 22 and 4 mo, respectively. Differences in climatic and soil conditions affecting the debris decomposition could contribute to the shorter longevity of the bacteria in the buried debris in our experiments. The decline in bacterial populations in the debris on the soil surface after 4 and 5 mo in our experiments coincided with a large increase in rainfall. However, the rainfall did not affect the decomposition of the debris in either year. The rainfall for the months of November–April 1986–1987 was 61.7, 0.0, 11.4, 11.7, 34.2, and 150.0 mm, respectively (experiment I), and November–June 1987–1988 was 30.0, 40.0, 12.0, 4.9, 116.0, 38.2, 66.9, and 99.6 mm, respectively (experiment II). There was only a slight variation in monthly temperatures for the duration of both experiments (mean range 29.3–31.1 C for 1987–1988; 29.7–31.7 C for 1987–1988).

Because in some regions of the Dominican Republic, two successive bean crops are planted every year with a minimum of a 30-day period between crops (E. Arnaud-Santana, *personal observation*), bean debris should be plowed under or buried in the soil to reduce the amount of potential inoculum of *X. c. phaseoli* to cause common bacterial disease in bean.

ACKNOWLEDGMENTS

We thank the Title XII Bean/Cowpea CRSP (AID contract DAN-1310-G-SS-6008-00) and SEA-CESDA, Dominican Republic, for providing financial support for the research. We also thank the staff and technicians at the Arroyo Loro Experiment Station, San Juan de la Maguana, for their assistance.

LITERATURE CITED

1. Andrus, C. F. 1948. A method of testing beans for resistance to bacterial blights. Phytopathology 38:757-759.
2. Claflin, L. E., Vidaver, A. K., and Sasser, M. 1987. MXP, a semi-selective medium for *Xanthomonas campestris* pv. *phaseoli*. Phytopathology 77:730-734.
3. Fett, W. F. 1979. Survival of *Pseudomonas glycinea* and *Xanthomonas phaseoli* var. *sojensis* in leaf debris and soybean seed in Brazil. Plant. Dis. Rep. 63:79-83.
4. Gilbertson, R. L., Rand, R. E., Carlson, E., and Hagedorn D. J. 1988. The use of dry-leaf inoculum for establishment of common bacterial blight of bean. Plant Dis. 72:385-389.
5. Gilbertson, R. L., Rand, R. E., and Hagedorn, D. J. 1990. Survival of *Xanthomonas campestris* pv. *phaseoli* and pectolytic strains of *X. campestris* in bean debris. Plant Dis. 74:322-327.
6. Saettler, A. W., Cafati, R., and Weller, D. M. 1986. Nonoverwintering of Xanthomonas bean blight bacteria in Michigan. Plant Dis. 70:285-287.
7. Schuster, M. L., and Coyne, D. P. 1974. Survival mechanisms of pathogenic bacteria. Annu. Rev. Phytopathol. 12:199-221.
8. Schuster, M. L., and Coyne, D. P. 1975. Survival factors of plant pathogenic bacteria. Agric. Exp. Stn. Univ. Nebr. Lincoln Res. Bull. 268. 53 pp.
9. 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.
10. 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.
11. Yoshii, K. 1980. Common and fuscous blights, Pages 155-172 in: Bean Production Problems. H. F. Schwartz and G. E. Galvez, eds. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
12. 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. 225 pp.