Epiphytic Survival of Pseudomonas syringae on Hairy Vetch in Relation to Epidemiology of Bacterial Brown Spot of Bean in Wisconsin

G. L. Ercolani, D. J. Hagedorn, A. Kelman, and R. E. Rand

Visiting Professor, Professors, and Specialist, respectively, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison. A contribution of Project 232.

Appreciation is expressed to J. W. Thomson for identification of hairy vetch, and P. H. Williams for assistance in the experiments with ultraviolet light. The assistance of S. Vicen in preparing the photographs and G. Weis for field experiments is also gratefully acknowledged.

Accepted for publication 14 May 1974.

ABSTRACT

The bacterial brown spot pathogen, *Pseudomonas syringae,* highly virulent (Vi^+) for bean, survived overwinter on leaves of hairy vetch in Wisconsin. Under natural conditions, these bacteria were the main components of the gram-negative epiphytic microflora on hairy vetch leaves throughout the year, except in April and from late June to mid-August. A correlation was established between the presence of high epiphytic populations of Vi^+ bacteria on hairy vetch in June and subsequent outbreaks of bacterial brown spot in adjacent bean fields. High numbers of Vi^+ bacteria can be spread from hairy vetch plants to bean crops during rainstorms early in the summer. Since hairy vetch is widespread as a weed in bean-growing areas in central Wisconsin, its inhabitation by Vi^+ bacteria is important to the epidemiology of bacterial brown spot of bean in this region. Greenhouse studies confirmed the ability of Vi^+ *P. syringae* to prosper as an epiphyte on hairy vetch.

Phytopathology 64:1330-1339.

Additional key words: Epiphytic pathogen, primary inoculum source, *Vicia villosa.*

The bacterial brown spot disease (*Pseudomonas syringae* van Hall) of bean (*Phaseolus vulgaris* L.) in Wisconsin is commonly serious, even in the absence of detectable seed transmission of the inciting bacterium. Since Patel et al. (18) first discovered the disease in this state in 1963, it has become progressively more widespread and economically important. Previous attempts, including one by Hoitink et al. (7), to establish the source of primary inoculum have not been successful. The purpose of this investigation was to determine whether *P. syringae* might occur on volunteer leguminous and nonleguminous plants growing in the vicinity of bean fields where bacterial brown spot was serious. A preliminary report has been made (5).

MATERIALS AND METHODS.—*Isolation procedures.*—Isolations were attempted from the following symptomless plants collected in the bean-growing area of central Wisconsin: alfalfa (*Medicago sativa* L.), lilac (*Syringa vulgaris* L.), locust (*Robinia hispida* L.), hairy vetch (*Vicia villosa* L.), red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), and yellow sweet clover [*Melilotus officinalis* (L.) Lam.].

Isolations were made in November, 1970, February and November, 1971, and January, 1972, from lilac and locust samples collected at eight locations. Alfalfa, red clover, white clover, and yellow sweet clover samples were collected late in the fall of 1970 and 1971 and during the spring of 1971 and 1972 at eight locations. These collections were made where bacterial brown spot was severe the previous summer. Additional samples of yellow sweet clover were collected in September, 1971, and throughout the summer of 1972, near several bean fields severely affected with bacterial brown spot. Samples of hairy vetch were collected once a month at three locations (plots A, B, C) from November, 1970, through June, 1971, and at six locations (plots A, D, E, F, G, H) from June, 1971, through August, 1972. These eight plots were located near bean fields where bacterial brown spot had been severe during the growing season preceding the earliest date of sampling. Additional samples of hairy vetch were collected in 1971 and 1972 at over 70 locations in central Wisconsin, near healthy bean fields and near bean fields with a history of bacterial brown spot.

Ten to 12 grams of plant material; i.e., dormant buds of lilac or locust opened by hand, or leaves of other plants, were placed in 300 ml of sterile deionized water (SDW) in 500-ml flasks and shaken for 2 h on a reciprocal shaker (120 strokes per min) at room temp. Tenfold dilutions of the supernatant fluid were pipetted in 1.0-ml samples into 10-cm diam petri dishes to which the two components of the plating medium (MVSA)—0.2 ml of a sterile 21% (w/v) solution of manganous sulphate (16) and 20 ml of molten (48 C) nutrient agar containing 5% sucrose and 6 μg/ml of crystal violet (1)—were added. The plates were surface-dried with the lid removed at 40 C for approximately 30 min, then closed and incubated at 26 C.

After 3 days, levan-forming and nonlevan-forming colonies were examined under a dissecting microscope and counted in sufficient numbers to estimate the number of bacteria in the original suspension with a precision of between 3 and 4% (15). Since evidence was available to indicate that isolates of *P. syringae* virulent for bean invariably produced levan (see Discussion), 100 levan-forming colonies from each plant species were selected at random, purified by restreaking on fresh plates of nutrient agar containing 5% sucrose (NAS) and maintained on slants of nutrient agar containing 2% glycerol (NAG) at 4 C.

Within 2 mo of isolation, levan-forming bacteria were examined for oxidase (11), arginine dihydrolase (27), pectolytic activity (2), production of a fluorescent pigment (8), and ability to induce a hypersensitive reaction in tobacco leaves (9). Isolates that gave a positive reaction in the last two tests only were regarded as potentially *P. syringae* (6, 17, 22, 23) and were further tested for virulence on beans.

Pathogenicity tests.—Preliminary attempts were made to determine the pattern of development and range of symptoms obtainable on test beans inoculated with *P. syringae* in the greenhouse. These studies included 52 isolates of *P. syringae* maintained in the culture collection of the Department of Plant Pathology of the University of Wisconsin, Madison; i.e., 42 isolates from bean leaves affected with bacterial brown spot in central Wisconsin and two isolates from each of the following hosts: apple (*Malus sylvestris* Mill.), lilac (*Syringa vulgaris* L.), peach [*Prunus persica* (L.) Batsch], pear (*Pyrus communis* L.) and sour cherry (*Prunus cerasus* L.). In addition, 82 fresh presumptive isolates of *P. syringae* from hairy vetch were tested also.

To prepare the inocula, the bacteria were grown on NAG slants at 26 C for 24 h, quickly suspended in SDW, adjusted turbidimetrically to 10^8 viable cells/ml and diluted to give 10^7 and 10^3 viable cells/ml, respectively. Each of these two suspensions was sprayed by means of a paint spray gun (Ceccato 2701-2711 fitted with a 1.5 nozzle) operated under a pressure of 0.703 kg/cm^2 at 25.4 cm from the upper and lower surface of the foliage of four bean plants of cultivar Tenderwhite. These inoculations were made when the second trifoliate leaf was approximately one-third expanded. Under these conditions, the centerline velocity of the suspension-carrying stream of air was approximately 9.14 dm/sec at the moment of impact on the leaves.

The bean isolates invariably induced typical brown spot symptoms 3-5 days after inoculation at either concn of inoculum, whereas isolates from other plants (except vetch and yellow sweet clover) induced necrotic flecks within 2 days of inoculation at the higher concn of inoculum only. These data were similar to those of Saad and Hagedorn (20, 21). The virulence of most isolates from hairy vetch was comparable to that of typical bean isolates (Vi^+, or "compatible" isolates). The isolates that induced necrotic symptoms were designated Vi^-, or "incompatible" isolates.

As an extensive range of physiological, nutritional, and biochemical tests failed to differentiate between Vi^+ and Vi^- isolates, a modification of the bean pod injection technique (10) used by Saad and Hagedorn (20, 21) was adopted for rapid screening purposes. The bacteria were grown on NAG and suspended in SDW as described above to give five dilutions between 10^8 and 10^4 viable cells/ml. A droplet of each of these was injected at each of five different places on one side of each of two surface-sterilized Tenderwhite bean pods at an early stage of seed enlargement. After a 3-day incubation at room temp, symptomatology was invariably correlated with virulence.

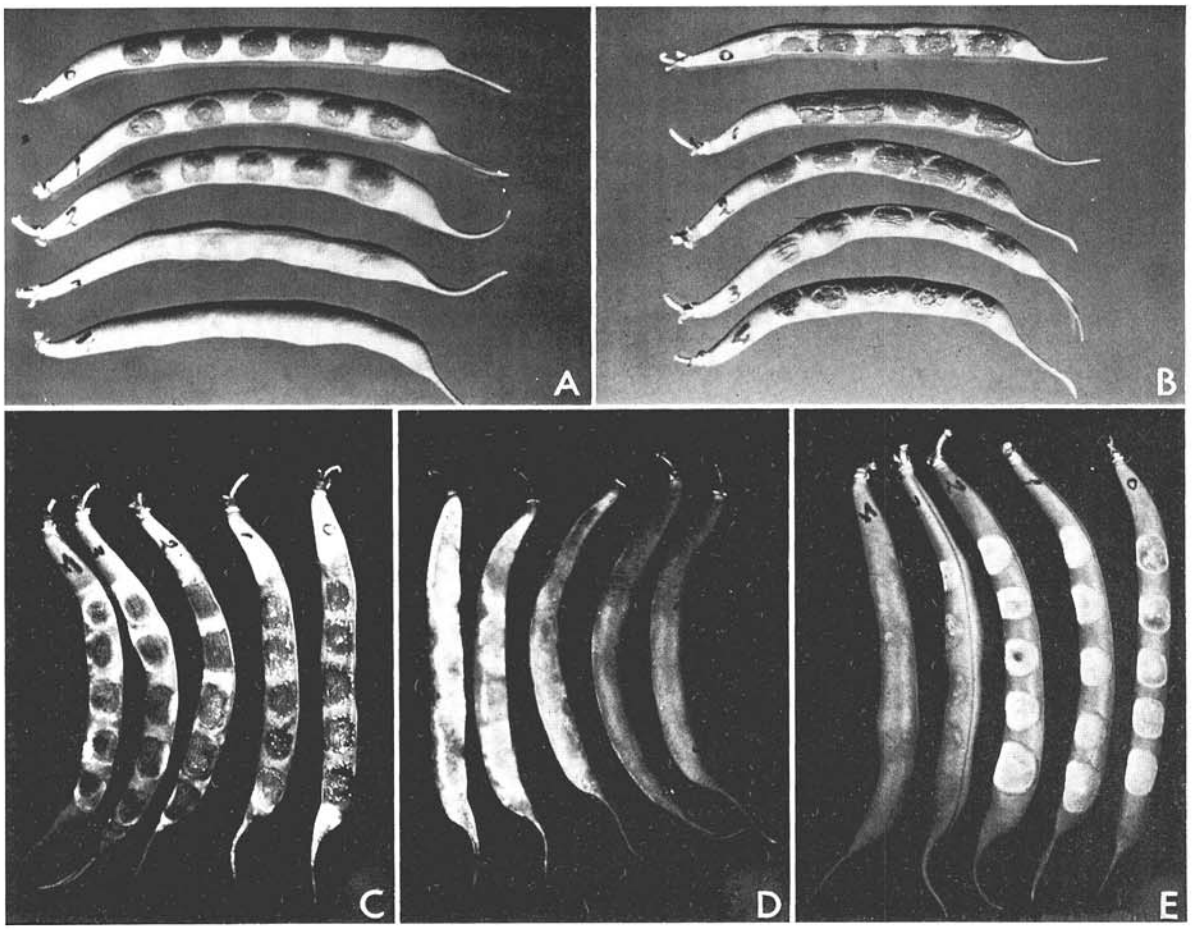

Fig. 1. Reaction of bean pods to inoculation with compatible (Vi^+) and incompatible (Vi^-) isolates of *Pseudomonas syringae* from hairy vetch. Individual pods in each set were injected subepidermally at five replicate points with one of five bacterial suspensions containing, respectively, 10^8 (0), 10^7 (1), 10^6 (2), 10^5 (3), and 10^4 (4) viable cells/ml, and photographed after a 5-day incubation at room temp. **A, E)** Inoculated side of one set of pods injected with Vi^- bacteria and photographed under visible (A) and ultraviolet (E) light. **B, C, D)** Inoculated (B, C) and noninoculated (D) side of one set of pods injected with Vi^+ bacteria and photographed under visible (B) and ultraviolet (C, D) light.

With Vi^+ isolates, inocula containing between 10^8 and 10^6 viable cells per ml always induced green, sunken, water-soaked lesions which spread beyond the injected area and were often accompanied by droplets of bacterial exudate at their surface, whereas inoculation with 10^4 viable cells/ml always resulted in sunken, dry, necrotic, light-brown lesions which never became extended beyond the injected areas; individual areas injected with 10^5 viable cells/ml showed either type of lesion or an intermediate lesion which was sunken and green with a brown border (Fig. 1-B). At this stage, the tips of the pods inoculated with between 10^8 and 10^6 viable cells/ml, as well as most of the tissues between the areas injected with 10^6 viable cells/ml or less, and, occasionally, some of the necrotic tissues themselves, showed a bright blue-green fluorescence when viewed under ultraviolet light (254 nm) (Fig. 1-C). When inoculated pods were further incubated for 48 h, little or no change occurred in visible symptoms, but the blue-green fluorescence extended to previously symptomless tissues on the noninoculated side of pods injected with 10^8, 10^7 and, occasionally, 10^6 viable cells/ml (Fig. 1-D).

With Vi^- isolates, bacterial concns between 10^8 and 10^6 viable cells/ml induced, after 2 days, sunken, dry, necrotic, dark-brown lesions which exhibited a bright blue-green fluorescence under UV light. Superficial light-brown to purplish discolorations of the injected area, which did not fluoresce under ultraviolet light, were typically produced by doses of 10^5 viable cells/ml, whereas no symptoms were normally observed following injection at the lowest dosage tested (Fig. 1-A, E).

Dissemination of P. syringae in the field.—To determine the relationship between occurrence of *P. syringae* on hairy vetch and inoculum potential of the bacterial brown spot pathogen in the field, a trap was designed to collect splashing raindrops. This consisted of a 23.8 × 2.5 cm glass test tube attached to a 10-cm glass funnel at an angle of 120° (Fig. 2). Sterile traps were placed vertically into the soil so that only the funnel remained above ground, facing the prevailing wind. The

traps were opened at the onset of rainstorms and plugged with rubber stoppers when the rain stopped, at which time they were taken to the laboratory. Water and other suspended materials caught in the traps were examined by a poured-plate technique as described above for leaf washings.

Experiments were made in 1971 and repeated in 1972 in commercial bean fields adjacent to a roadside occupied largely by hairy vetch plants harboring *P. syringae*. Samplings were restricted to two bean fields leeward of the road (plots M and N) in 1971, but were extended to include the opposite fields windward of the road (plots M′ and N′) in 1972. The two facing plots, M and M′, were sown with the same bean varieties on 13 June 1972, thus allowing a direct comparison of the distribution of Vi^+ bacteria in different directions away from the hairy vetch. In 1972, additional observations were made in a bean field where the corners were thickly covered with hairy vetch naturally contaminated with *P. syringae*.

In each field, as the beans were sown, the traps were placed at the edge of the roadside or field corner vegetation and also away from these plants at distances of 9.1, 18.2, and 30.5 meters (in 1971) or 9.1, 45.7, and 91.5 meters (in 1972) along a bean row.

Additional tests for the presence of *P. syringae* were made on soil from around the traps, and on buds and stipules from bean plants, by using the procedure described by Leben et al. (14), except that the medium used by these authors was replaced by MVSA.

Multiplication of P. syringae on hairy vetch.—The ability of *P. syringae* to colonize hairy vetch plants was studied in a greenhouse at a relative humidity which varied somewhat, but was generally about 60%. Experiments were made to compare the multiplication on hairy vetch of a strain of *P. syringae* highly virulent for bean (isolate Y 30) and that of a virulent isolate of *P. phaseolicola* (Burkh.) Dowson (Y 63). In each experiment, six groups of 6-wk-old hairy vetch plants, grown two to a 10-cm pot, were sprayed with a suspension containing 10^4, 10^5 or 10^6 viable cells/ml, of either *P. syringae* or *P. phaseolicola*. Three groups of comparable plants were sprayed with corresponding concns of a 1:1 mixture of the two bacteria, and a further group of control plants was sprayed with SDW. On the day after inoculation, and at weekly intervals for 7 wk thereafter, leaf washings of the plants from two pots were examined. On washings from plants sprayed with a mixture of *P. syringae* and *P. phaseolicola*, differential tests following isolation included, besides colony type, inoculation of bean pods and replica platings for gelatinase activity (24), hydrolysis of aesculin (25) and utilization of erythrytol (22). Control experiments indicated that neither bacterium was inhibited by the other during growth on isolation plates.

In another experiment, the multiplication of three isolates from leaf washings of hairy vetch, HVA 27 (Vi^+), HVW 3 (Vi^-) and HVA 14 ("*P. marginalis*" type), was compared. The inocula consisted of suspensions containing 10^3, 10^5 or 10^7 viable cells/ml of one isolate, a mixture of any two, or all three, isolates in equal proportions.

RESULTS.—*Recovery of P. syringae from hairy vetch.*—Bacterial populations recovered from hairy vetch leaves were largest (over 10^7 viable cells/g fresh wt) in May and June and smallest (10^4-10^5 viable cells/g fresh wt) in late July through mid-August in 1971-1972; a peak

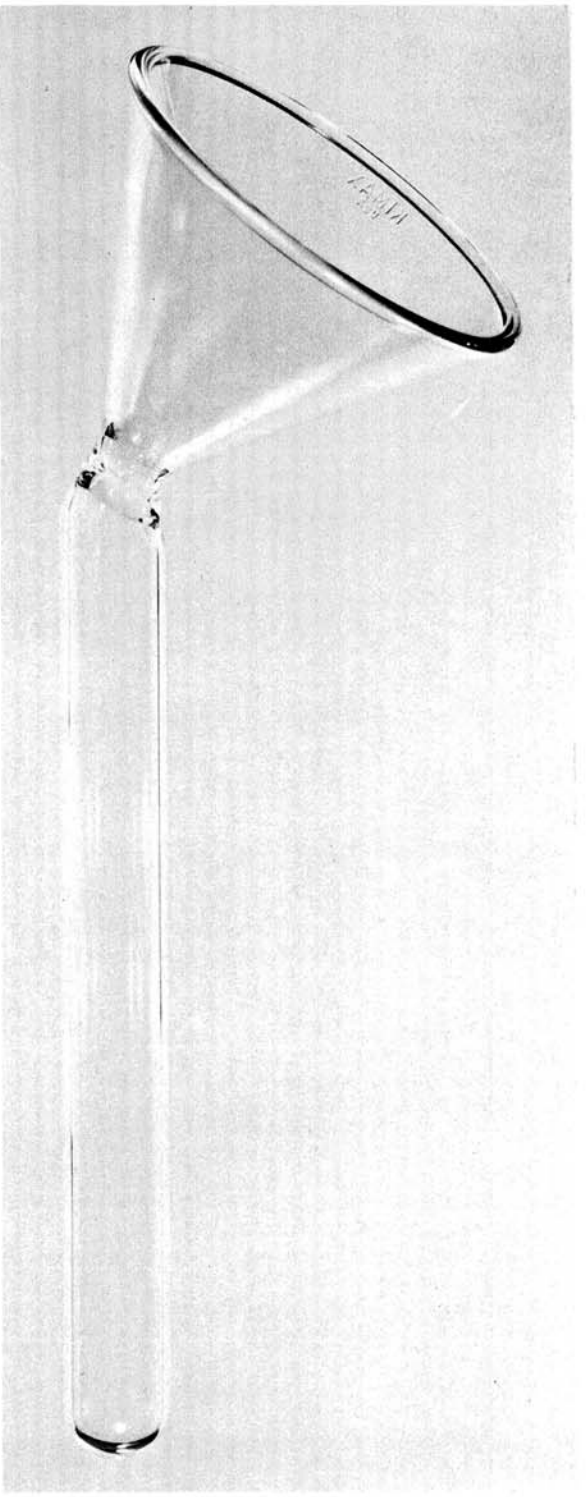

Fig. 2. Glass trap used for collecting splashing raindrops in the field.

TABLE 1. Viable counts of levan-forming (lev$^+$) and nonlevan-forming (lev$^-$) bacteria recovered from hairy vetch in plot A in 1970-71. For the levan-forming colonies, the percentage of Vi$^+$, Vi$^-$[a] and other bacteria is also given

Date of sampling	Lev$^+$ bacteria				Log number lev$^-$ bacteria per gram fresh wt
	Log number per gram fresh wt	Vi$^+$ (%)	Vi$^-$ (%)	Other bacteria (%)	
11/16/70	6.50	68	26	6	5.10
12/23/70	6.05	71	22	7	4.87
1/18/71	6.89	78	18	4	5.81
3/3/71	6.31	72	22	6	5.51
4/7/71	6.08	12	11	77	5.15
5/11/71	7.49	96	3	1	5.69
6/15/71	7.30	94	2	4	6.78
6/29/71	6.90	94	5	1	6.31

[a]Vi$^+$ were *P. syringae* highly virulent (compatible) on bean; Vi$^-$ were incompatible.

TABLE 2. Viable counts of levan-forming (lev$^+$) and nonlevan-forming (lev$^-$) bacteria recovered from hairy vetch in plot D in 1971-72. For the levan-forming colonies, the percentage of Vi$^+$, Vi$^-$[a] and other bacteria is also given

Date of sampling	Lev$^+$ bacteria				Log number lev$^-$ bacteria per gram fresh wt
	Log number per gram fresh wt	Vi$^+$ (%)	Vi$^-$ (%)	Other bacteria (%)	
6/29/71	6.65	92	4	4	5.79
7/14/71	5.89	84	14	2	5.36
7/22/71	4.48	1	83	16	4.64
8/5/71	4.63	0	95	5	5.27
8/18/71	5.28	25	67	8	4.89
8/30/71	7.83	84	12	4	6.18
9/21/71	6.93	68	22	10	6.10
10/31/71	7.50	71	16	13	6.01
11/30/71	7.71	75	23	2	6.31
12/13/71	6.57	64	25	11	5.38
1/10/72	6.46	72	15	13	5.45
3/23/72	6.93	78	11	11	4.98
4/18/72	6.83	18	12	70	5.75
5/25/72	7.32	95	2	3	5.68
6/20/72	7.67	93	6	1	5.34
7/11/72	6.38	79	15	6	5.21
7/26/72	4.75	0	91	9	5.11
8/3/72	4.29	0	88	12	4.65
8/23/72	6.46	64	30	6	6.28

[a]Vi$^+$ were *P. syringae* highly virulent (compatible) on bean; Vi$^-$ were incompatible.

of more than 10^7 viable cells/g fresh wt was recorded in the fall of 1971. Data from two plots are given in Tables 1 and 2. Samples collected during the rest of the year yielded between 10^6 and 10^7 viable bacteria/g fresh wt.

Levan-forming isolates constituted the majority of the bacteria except from late July to mid-August. There were mostly soft-rot pseudomonads in April, Vi$^-$ isolates of *P. syringae* in late July through mid-August, and Vi$^+$ isolates of *P. syringae* during the rest of the year. Vi$^+$ bacteria were isolated frequently in May (10^7 viable cells/g fresh wt), but were seldom, if ever, detected in late July through mid-August. Their counts were high again (well over 10^6 viable cells/g fresh wt) in September and remained approximately constant through the winter into the following spring, except for a transient reduction in April.

Additional samples of hairy vetch collected in 1971 and 1972 near bean fields with a history of bacterial brown spot, provided comparable results. Samples from fields where bacterial brown spot of bean was not severe the year before were apparently free of Vi$^+$ bacteria in April and only occasionally yielded Vi$^+$ bacteria in May. In June, however, Vi$^+$ bacteria were common (10^4-10^5 viable cells/g fresh wt) on hairy vetch in areas where bacterial brown spot of bean was not severe before. A close correlation was found between the presence of patches of hairy vetch harboring high numbers of Vi$^+$ bacteria in June and later occurrence of severe outbreaks of bacterial brown spot in adjacent bean fields.

Vi$^+$ bacteria were detected very rarely on hairy vetch near healthy bean fields in September, and then only at population levels not exceeding 10^3 viable cells/g fresh wt. Occasionally, low numbers of *P. phaseolicola* ($<10^3$ viable cells/g fresh wt) were also recovered from hairy vetch in the summer and in the fall.

The following evidence indicated that the bacteria

TABLE 3. Recovery of Vi$^+$ isolates of *Pseudomonas syringae* from rain splashes trapped at different distances leeward (plot M) and windward (plot M′) of hairy vetch plants naturally contaminated with the bacteria in the field

Date of rain	Amount of rain (mm)	Log number viable bacteria per ml of rain trapped at the indicated distance (in meters) from the hairy vetch							
		Plot M				Plot M′			
		0	9.1	45.7	91.5	0	9.1	45.7	91.5
6/28/72	6.75	4.40	3.65	<1	<1	4.64	2.93	<1	<1
7/2/72	16.27	4.30	4.54	2.33	<1	3.91	3.50	<1	<1
7/9/72	14.23	4.85	4.19	3.60	<1	3.81	3.47	<1	<1
7/17/72	4.83	4.53	3.84	3.50	<1	4.34	2.94	<1	<1
7/23/72	8.14	4.19	3.73	4.63	3.84	4.73	3.50	2.90	<1
7/26/72	14.49	3.87	4.30	3.92	3.62	4.59	4.02	3.62	<1
8/2/72	8.14	4.81	4.73	4.10	4.57	4.40	4.58	3.14	2.74
8/14/72	26.40	4.30	4.85	3.66	4.71	4.64	4.17	3.99	4.81

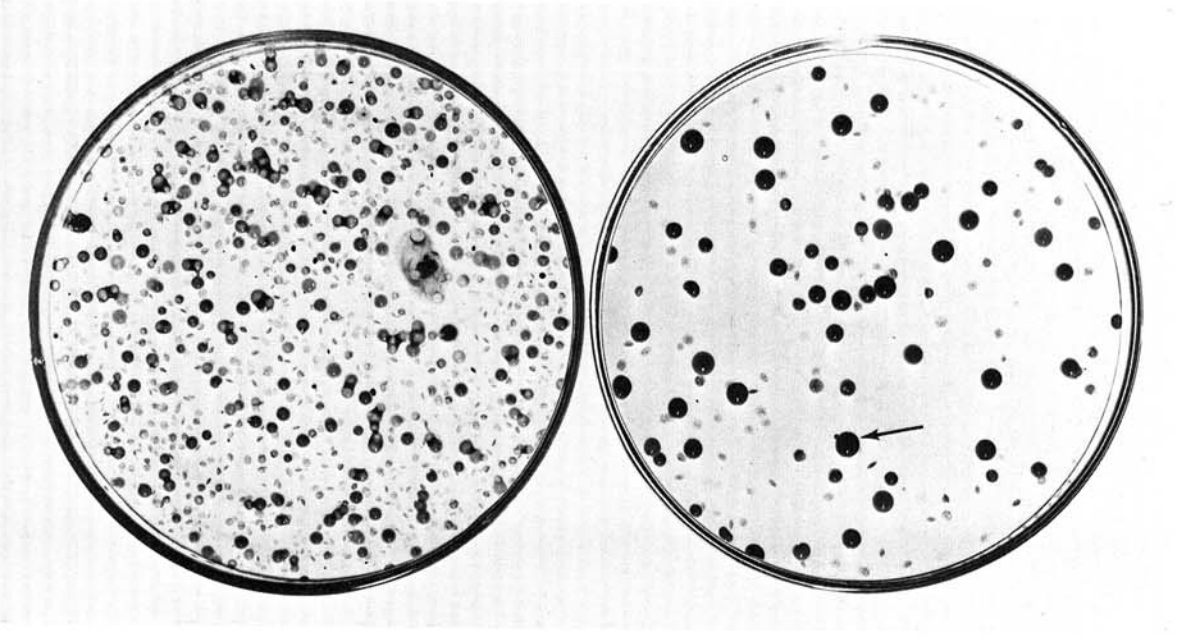

Fig. 3. Platings of a 10^{-2} (left) and a 10^{-3} (right) dilution of rain splashes collected in July in a bean field leeward of an extensive patch of hairy vetch plants naturally contaminated with *Pseudomonas syringae*. Most of the levan-forming colonies (arrow) are Vi$^+$ isolates of *P. syringae*.

recovered from hairy vetch were located at the plant surface:

(i) Microscopic observations of bleached, stained leaves (3) showed short chains and microcolonies of rod-shaped bacteria, similar in size to *P. syringae* cells, along with other microorganisms, on the leaf surface. A correlation existed between total counts of these bacteria and viable counts of *P. syringae* and other levan-forming bacteria from washings of comparable leaves.

(ii) When transverse sections of leaves known to harbor *P. syringae* were stained with carbol-thionin followed by orange G (26), the internal tissues invariably appeared to be free of bacteria. The planting of suspensions from whole ground leaves did not result in viable counts significantly greater than those obtained by the plating of leaf washings of comparable leaves.

(iii) Isolations from leaves using the balloon print technique (19) yielded *P. syringae* colonies in numbers similar to numbers of discrete chains and microcolonies of rod-shaped bacteria observed on bleached, stained leaves.

(iv) Exposure of leaves to UV light resulted in a dramatic decrease in the number of bacteria recovered by plating either leaf washings or ground leaves. Also, when a suspension of *P. syringae* was sprayed onto hairy vetch leaves under sufficient pressure to infiltrate it into the intercellular spaces, subsequent exposure of the leaves to UV affected the numbers of bacteria recovered from the leaf surface, but not from within the leaf.

Recovery of P. syringae from other plants.—Vi$^+$ bacteria were common on yellow sweet clover during summer and early fall, when they reached population

levels of 10^7 viable cells/g fresh wt. Alfalfa, red clover, white clover, locust, and lilac samples yielded only Vi^- bacteria consistently in fall and sporadically in spring.

Dissemination of P. syringae in bean fields.—The data for plot M show that Vi^+ bacteria were detected almost exclusively near the hairy vetch until the end of June, but occurred with progressively increasing frequency at other locations thereafter and could be isolated in approximately equal numbers from splashing rain in any of the traps by the end of July (Table 3). At that time, platings typically resulted in a predominance of levan-forming colonies (Fig. 3), most of which proved to Vi^+ *P. syringae*. Symptoms of bacterial brown spot were observed on beans within 18.2 m of hairy vetch during the first week in July, up to 61 m from hairy vetch at the end of July, and throughout the plot by mid-August.

An inverse relationship between distance of the traps from the hairy vetch and numbers of Vi^+ bacteria was also found in plot M′. However, traps equidistant from the hairy vetch invariably yielded less Vi^+ isolates in plot M′ than in plot M until mid-August. Symptoms of bacterial brown spot of comparable severity occurred in various parts of plot M′ approximately 2 wk later than at corresponding locations in plot M.

Bacterial populations on the hairy vetch windward of plot M and leeward of plot M′ did not differ significantly from those in plot D (Table 2).

The occurrence of a rather large sampling error prevented a reliable estimate of the density of Vi^+ bacteria on apparently healthy bean leaves. Roughly, the number of Vi^+ bacteria per leaf were within a ten-fold factor of the number recoverable from 1.0 ml of rain splashes at the same location.

Vi^+ bacteria were isolated only occasionally from the soil and from the buds and stipules of symptomless bean plants, but could easily be isolated from symptomless buds and stipules of bean plants infected with bacterial brown spot.

Multiplication of P. syringae on hairy vetch.—Spraying *P. syringae* alone at 10^4, 10^5, and 10^6 viable cells/ml resulted in counts after one day of 2.25×10, 2.78×10^2 and 4.40×10^3 viable cells/g fresh wt, respectively (Table 4). These population levels increased to values exceeding 10^6 viable cells/g fresh wt within the following 2 wk and then remained quite constant. With *P. phaseolicola,* the number of viable cells per gram fresh wt was approximately the same as with *P. syringae* one day after spraying and then increased for 4 or 5 wk, but final populations comparable in size to those of *P. syringae* were produced only when the initial suspension contained 10^6 viable cells/ml. When both bacteria were sprayed together, they were recovered in approximately the same proportion one day after spraying, but *P. syringae* was dominant in all isolations made at later dates; nevertheless, counts of *P. phaseolicola* were higher, with only a few exceptions, on plants sprayed with 10^4 or 10^5 viable cells/ml of a mixed, rather than a pure, suspension.

The multiplication on leaves of the three isolates from hairy vetch differed considerably (Table 5). When the bacteria were sprayed separately, viable counts for isolate HVA 27 (Vi^+) resembled those obtained with comparable concns of isolate Y 30. Isolate HVW 3 (Vi^-) gave results similar to isolate HVA 27 at the highest concn of inoculum only; when the initial suspension contained 10^5 and 10^3 viable cells/ml, the number of bacteria recovered from the hairy vetch continued to increase after inoculation, but final populations were approximately one-tenth and one-hundredth of those of isolate HVA 27, respectively. Isolate HVA 14 was detected occasionally on plants sprayed with 10^3 viable cells/ml, and the number of viable cells recovered from plants given higher doses decreased with time after spraying. The results of mixed inoculation experiments confirmed the data reported above, with the exception that isolate HVW 3 was detected in relatively lower numbers on plants sprayed with 10^5 viable cells/ml of suspensions containing this isolate in addition to isolate HVA 27, or isolates HVA 27 and HVA 14. Higher numbers of isolate HVA 14 were detected on plants where it had been sprayed together with isolate HVA 27 in a suspension containing a total of 10^7 viable cells/ml.

Control plants did not become contaminated in the greenhouse experiments.

DISCUSSION.—The results of this study indicate that

TABLE 4. Viable counts of *Pseudomonas syringae* isolate Y 30 and *P. phaseolicola* isolate Y 63 recovered from hairy vetch plants sprayed with either bacterium or with a mixture containing equal proportions of each in the greenhouse

Bacterial isolates	Log number bacteria per ml of inoculum	Log number bacteria per gram fresh wt after indicated number of wk				
		0 (1 day)	1	2	4	7
Y 30	4	1.35	4.83	6.65	6.74	6.72
	5	2.44	5.95	6.62	5.92	5.79
	6	3.64	5.45	6.44	6.91	6.41
Y 63	4	1.69	2.13	3.53	4.67	3.75
	5	2.84	2.88	4.64	4.49	4.62
	6	3.72	3.91	4.18	5.94	5.92
Y 30 + Y 63	4	1.65[a]	3.36	6.25	6.61	6.62
		(0.56; 0.44)	(0.99; 0.01)	(0.95; 0.05)	(0.96; 0.04)	(0.98; 0.02)
	5	2.45	5.35	6.30	5.44	6.50
		(0.55; 0.45)	(0.98; 0.02)	(0.93; 0.07)	(0.95; 0.05)	(0.95; 0.05)
	6	3.95	5.44	6.14	6.55	6.35
		(0.48; 0.52)	(0.94; 0.06)	(0.97; 0.03)	(0.94; 0.06)	(0.94; 0.06)

[a]The data for mixed inoculation experiments give the total viable counts for both bacteria together and also (in parentheses) the proportion of Y 30 and Y 63 in this order, in a random sample of 100 colonies from the isolation plates.

hairy vetch serves as a source of primary inoculum of the bean bacterial brown spot bacterium, *P. syringae,* in Wisconsin. Several hundred isolations from typical and atypical bacterial brown spot lesions on bean leaves and pods yielded *P. syringae* isolates which invariably produced levan colonies on 5% sucrose nutrient agar. The possibility that nonlevan-forming isolates of *P. syringae* are active in the induction of bacterial brown spot of bean in Wisconsin cannot be ruled out. However, the evidence indicates that the criteria adopted in this study for the screening of colonies on isolation plates were not likely to result in an underestimation of the number of colonies of Vi^+ bacteria. Since "colony-forming units" of *P. syringae* usually consisted of one or a few cells, errors arising from the bacteria forming tight chains or clumps of cells were not important.

The plating medium used allowed plating of Vi^+ and Vi^- isolates from suspensions containing 1:1 to 1:100 mixtures of *P. syringae* and other bacteria with a very high efficiency. The medium, however, was not selective for *P. syringae,* and viable numbers of *P. syringae* in samples containing low population levels may have been underestimated. This could have affected the samplings made in April when the addition of manganous sulphate to the medium did not appear to inhibit soft-rotting pseudomonads to a satisfactory extent.

The finding that VI^+ types of *P. syringae* are largely dominant among bacterial isolates from hairy vetch throughout the year, except in April and late July to mid-August, is perhaps correlated with climatic and vegetative conditions prevailing at different times of the year. The sampling from selected patches of hairy vetch during the winter indicated that the plants were apparently normal with healthy green leaves under the snow cover, which ranged up to 1.0 meter in depth from January through mid-March. In April, however, as a result of the snow melting away and the direct exposure to light, the hairy vetch was prostrate and the top layer of leaves extensively

TABLE 5. Viable counts of *Pseudomonas syringae* HVA 27 (Vi^+), *P. syringae* HVW 3 (Vi^-) and *Pseudomonas* sp. HVA 14 ("marginalis" type) recovered from hairy vetch plants sprayed with each bacterium separately or with mixtures containing equal proportions of two or all three bacteria in the greenhouse

Bacterial isolates	Log number bacteria per ml of inoculum	Log number bacteria per gram fresh wt after indicated number of wk				
		0 (1 day)	1	2	4	7
HVA 27	3	<1	3.11	5.33	5.81	6.83
	5	2.59	5.86	6.76	5.82	6.43
	7	5.32	6.25	6.87	6.27	6.82
HVW 3	3	<1	<1	1.43	1.82	4.23
	5	2.64	2.45	2.81	3.05	5.85
	7	5.82	6.31	6.72	6.42	6.52
HVA 14	3	<1	<1	<1	<1	<1
	5	2.70	1.88	2.22	<1	<1
	7	5.36	5.23	4.16	3.24	2.79
HVA 27 + HVW 3	3	<1[a]	3.24	5.45	6.33	6.47
		---	(1.00; 0.00)	(1.00; 0.00)	(1.00; 0.00)	(1.00; 0.00)
	5	3.13	6.13	6.43	6.75	6.65
		(0.48; 0.52)	(1.00; 0.00)	(1.00; 0.00)	(0.98; 0.02)	(1.00; 0.00)
	7	5.85	6.46	6.64	6.37	6.65
		(0.46; 0.54)	(0.42; 0.58)	(0.53; 0.47)	(0.56; 0.44)	(0.53; 0.47)
HVA 27 + HVA 14	3	<1	3.11	5.28	6.23	6.53
		---	(1.00; 0.00)	(1.00; 0.00)	(1.00; 0.00)	(1.00; 0.00)
	5	2.61	6.15	6.34	6.34	6.12
		(0.53; 0.47)	(1.00; 0.00)	(1.00; 0.00)	(1.00; 0.00)	(1.00; 0.00)
	7	5.57	6.36	6.48	6.23	6.34
		(0.44; 0.56)	(0.86; 0.14)	(0.90; 0.10)	(1.00; 0.00)	(1.00; 0.00)
HVA 27 + HVW 3 + HVA 14	3	<1	3.37	5.34	6.45	6.44
		---	(1.00; 0.00; 0.00)	(1.00; 0.00; 0.00)	(1.00; 0.00; 0.00)	(1.00; 0.00; 0.00)
	5	2.66	6.03	6.37	6.69	6.34
		(0.36; 0.30; 0.34)	(1.00; 0.00; 0.00)	(1.00; 0.00; 0.00)	(1.00; 0.00; 0.00)	(1.00; 0.00; 0.00)
	7	5.46	6.58	6.46	6.64	6.64
		(0.29; 0.37; 0.34)	(0.48; 0.44; 0.08)	(0.46; 0.54; 0.00)	(0.54; 0.46; 0.00)	(0.44; 0.56; 0.00)

[a]Cumulative viable counts for all the bacteria sprayed, the data for the mixed inoculaiton experiments give (in parentheses) the proportion of each bacterium, in the same order as in the first column, in a random sample of 100 colonies from the isolation plates.

rotted. Although care was taken to sample the underlying green leaves, most of the isolates from leaf washings at this time were soft-rotting pseudomonads of the *P. marginalis*-type. At the other time when Vi^+ isolates were in a minority (i.e., during part of July and August) a drop in their population levels may have been caused by some unfavorable change in the composition of leaf exudates on most plants on which seeds were ripening.

Greenhouse experiments indicated that *P. syringae* can multiply on leaf surfaces of healthy hairy vetch plants. Population levels reached by *P. syringae* Vi^- on hairy vetch in the greenhouse were of the same order as those found in the field, but were lower for Vi^+ types. As the latter usually contributed the bulk of viable counts in samplings from the field, maximum bacterial populations supported by hairy vetch plants in the greenhouse were approximately one order of magnitude lower than those detected on plants from the field; i.e., $<10^7$ vs. $\sim 10^8$ viable cells/g fresh wt. In this respect, it is interesting to note that Vi^+ isolates approached the stationary level at 10^7 viable cells/g fresh wt within 2 to 3 wk after inoculation at any concn between 10^3 and 10^7 viable cells/ml, whereas Vi^- isolates did so only when the initial inoculum contained 10^6 or 10^7 viable cells/ml. This suggests that Vi^+ are more efficient than Vi^- bacteria in colonizing the leaf surface of hairy vetch plants.

Greenhouse experiments indicate that *P. syringae* may have a "resident phase" (12, 13) on hairy vetch. Results obtained with Vi^+ isolates provide another example of a recognized bacterial plant pathogen surviving on a nonhost plant (4). As the host range of Vi^- isolates was unknown, the epidemiological significance of their natural occurrence on hairy vetch remains undetermined. The finding that *P. phaseolicola* can multiply to some extent on hairy vetch is also difficult to correlate with current epidemiological knowledge of the halo blight pathogen.

As the multiplication of *P. syringae* was not measured under monoxenic conditions in this study, the rate of growth of individual V_i^+ and V_i^- isolates on hairy vetch could not be estimated accurately. From an epidemiological standpoint, bacterial colonization of leaf surfaces in the field is unlikely to occur as a pure culture. Therefore, monoxenic conditions in the laboratory may not reflect the field situation with more accuracy than do open bench conditions in the greenhouse.

The data gathered from the sampling of rain splashes in bean fields bordered by hairy vetch suggest that significant numbers of Vi^+ bacteria are disseminated from contaminated hairy vetch plants to bean crops early in the summer. This may account for outbreaks of bacterial brown spot which cannot be traced to other sources of primary inoculum; i.e., to the bean seed itself (7). It would, thus, appear that the bacteria may go through at least two phases, a resident phase on hairy vetch and a parasitic phase on bean, in their life cycle in Wisconsin.

It would be desirable to know to what extent the epidemiology of bacterial brown spot of bean may be affected by the resident phase of the causal bacteria on hairy vetch. Clearly, this depends upon, among other factors, the frequency and extent of occurrence of hairy vetch near bean fields. Field trips to bean-growing areas in Adams, Marquette, Portage, and Waushara Counties in central Wisconsin in 1971 and 1972 indicated that hairy vetch is very widespread, especially along roadsides and in field corners, and that it is favored by certain agricultural practices. For instance, ordinary types of wheeled self-propelled sprinkler irrigators frequently leave unirrigated corners which are often occupied completely by hairy vetch. Also, hairy vetch is a common contaminant of the seed lots used to plant winter rye. When this crop is plowed up the following spring to plant beans, strips of rye are sometimes left standing to act as windbreaks, and this often results in nearly continuous belts of hairy vetch stretching across the fields. Thus, existing conditions in central Wisconsin suggest easy cross-contamination of bean and hairy vetch plants by Vi^+ bacteria.

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