Epiphytic Populations of *Pseudomonas syringae* pv. *syringae* on Snap Bean and Nonhost Plants and the Incidence of Bacterial Brown Spot Disease in Relation to Cropping Patterns

Julianne Lindemann, Deane C. Arny, and Christen D. Upper

First, second, and third authors, Department of Plant Pathology, University of Wisconsin, Madison 53706; third author, Plant Disease Resistance Research Unit, Agricultural Research Service, USDA, Department of Plant Pathology, University of Wisconsin, Madison 53706.

Present address of first author: Advanced Genetic Sciences, Inc., 6701 San Pablo Avenue, Oakland, CA 94608.

Cooperative research supported by the College of Agriculture and Life Sciences, University of Wisconsin, Madison, Project 2238, and Agricultural Research Service, U.S. Department of Agriculture.

We thank Dawn Enge and Lesly Segal for technical assistance and S. A. Vicen for assistance in preparation of the figures.

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply approval to the exclusion of other products or vendors that also may be suitable.

Accepted for publication 12 June 1984.

ABSTRACT

Lindemann, J., Arny, D. C., and Upper, C. D. 1984. Epiphytic populations of *Pseudomonas syringae* pv. *syringae* on snap bean and nonhost plants and the incidence of bacterial brown spot disease in relation to cropping patterns. Phytopathology 74:1329-1333.

Plots were established at 11 locations on a 64-km east-west transect through the major bean-growing area of central Wisconsin. Bacterial brown spot disease epidemics occurred in four of six plots located in the bean-growing area and in none of five plots outside the bean-growing area even though the bean seedlot was naturally infested with the pathogen. Epiphytic populations of *Pseudomonas syringae* pv. *syringae* pathogenic to bean (Psb) were greater on symptomless bean leaflets and corn leaves from the bean-growing area than from the eastern and western portions of the transect where there was no commercial snap bean production. The pathogen was detected on hairy vetch samples from the bean-growing area only. Leaves of oak, black locust, rye, and sow thistle near commercial snap bean fields supported epiphytic populations of Psb. Differences in brown spot disease incidence on beans and differences in epiphytic populations of Psb on both host and nonhost plants in different portions of the transect are probably the result of the intensive cropping of snap beans in the central part of the transect.

Bacterial brown spot disease of bean (*Phaseolus vulgaris* L.) incited by *Pseudomonas syringae* pv. *syringae*, causes serious losses to bean growers in Wisconsin (8,16). Some reports have attributed brown spot disease epidemics entirely to infected seed sources (7,8,19), while others have attributed the epidemics to inoculum that overwinters in debris from infected plants (5) or to inoculum present as epiphytes on nonhost leguminous plants (4). All three sources may contribute inoculum to some extent in Wisconsin, but their relative importance is not known.

In the central sands area of Wisconsin, bacterial brown spot disease is more severe in late than in early planted fields (5). Disease also is severe when beans follow beans or peas in the same field the same year or in consecutive years (5,16). Thus, local increases in brown spot disease incidence during a single growing season and from one season to the next may be favored by intensive cropping practices. The presence of *P. syringae* pv. *syringae* pathogenic to bean (Psb) was detected on hairy vetch plants adjacent to bean fields without a history of brown spot disease epidemics (4). Similarly, *P. syringae* pv. *tomato* was detected as an epiphyte on foliage of weeds and nonhost crop plants in areas with no history of tomato culture (21). To our knowledge, it has not been determined whether cropping history patterns in an area have any quantitative effect on epiphytic populations of plant pathogenic bacteria, although the increased concentration of susceptible host plants in an area has been reported to increase disease incidence of some fungal and viral diseases of plants (3).

The objectives of this research were to determine the abundance of epiphytic Psb populations on both host and nonhost plants, and the incidence of the brown spot disease in relation to the intensive cropping of beans.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Experimental transect. Epiphytic populations of *P. syringae* and Psb on leaves of several plant species were quantitated at several locations along a 64-km east-west transect through central Wisconsin. Within the 21-km central portion of the transect, an average of 10% of the total land area is planted to snap beans annually (Wisc. Dept. Agric., Statistical Reporting Service, *unpublished*). There were no commercial plantings of snap beans prior to or during this experiment in the portions of the transect 16 km west and 27 km east of this bean-growing area.

In a preliminary survey in 1978, bean leaflets were sampled from 12 sites and corn leaves from 17 sites along the transect. Since samples were taken from available plantings, plant cultivars, planting dates, size of fields, and crop management practices differed from site to site. In 1979 and 1980, 11 plots were planted on private land by individual cooperators (Fig. 1). Six of the plots (plots 3 to 8) were located in the bean-growing area. Five plots were either west (plots 1 and 2) or east (plots 9, 10, and 11) of this area. The plots were planted from one seedlot of the snap bean cultivar Eagle, which is highly susceptible to infection by the pathogen, and one seedlot of the sweet corn hybrid Merit. Each plot had ~500 bean plants and 100 corn plants, each in 27 m of row, most commonly arranged as six 4.5-m-long rows. In 1979, the corn was planted during the third week of May and the beans were planted during the first week of June. In 1980, both corn and beans were planted during the first week of June. No pesticides were applied to the plots. In 1979, none of the plots were irrigated. In 1980, because of dry conditions, plots 2, 4, 5, 6, 7, 9 and 11 were irrigated one or more times at the discretion of the cooperator.

Assessment of bacterial brown spot disease incidence on snap beans. In 1978, brown spot disease incidence was not quantitated at the sampling sites, but a qualitative assessment of brown spot was

made by isolating Psb from presumptive brown spot lesions. In 1979 and 1980, brown spot incidence (percentage of bean leaflets in the top third of the plant canopy showing lesions) in the experimental plots was assessed when the beans were ready for harvest. At each sample location, presumptive brown spot disease etiology was substantiated by isolating Psb from several lesions.

Sampling procedure. In 1978, three to five samples each of two corn leaves or two symptomless bean leaflets were taken from each site for quantitation of epiphytic bacterial populations. Bean leaflets were taken from the top third of the plant canopy during the pod-setting stage of plant development. Corn leaves were taken at ear height. In 1979 and 1980, samples consisted of individual symptomless bean leaflets taken from the top third of the bean canopy or individual corn leaves from the middle third of the corn canopy. In 1979, bean and corn leaves were sampled three times during the growing season. In 1980, bean leaflets were sampled five times and corn leaves three times during the growing season. The number of replicate samples per plot differed on the different sampling dates and are indicated in the results. The other plant species that were sampled occasionally were *Vicia villosa* L. (hairy vetch), *Sonchus arvensis* L. (sow thistle), *Quercus* spp. L. (red or black oak), *Secale cereale* L. (winter rye), and *Robinia pseudoacacia* L. (black locust). Each sample was sealed in a plastic bag and stored in an ice chest until it could be processed. In 1978 and 1979, samples were processed the same day they were picked. In 1980, samples were stored overnight at 4 C and processed the next day.

Leaf sample processing procedure. Samples were weighed, cut into 5-cm^2 pieces with sterile scissors and placed in an Erlenmeyer flask containing a known volume (~10 ml per gram of leaf) of washing buffer (6.25 g of KH_2PO_4, 8.75 g of K_2HPO_4, and 1 g of Bacto-peptone [Difco]) per liter of glass distilled water, adjusted to pH 7.0 (15). The flasks were shaken for 2–4 hr at room temperature (24–27 C) on a rotary shaker (~250 rpm). The undiluted wash and serial 10-fold dilutions of the leaf wash in sterile distilled water were plated (0.1 ml per plate) onto Medium B of King et al (10) supplemented with 100 mg of cycloheximide per liter. Plates were incubated 3–4 days at room temperature before the number of total and fluorescent (fl^+) colonies were counted. Estimates of bacterial populations for each sample were transformed to $\log_{10}$ colony-forming units (cfu) per gram (cfu/g) fresh weight before calculating mean populations at the sampling sites. Bacterial population sizes are expressed as log transformed (eg, as $\log_{10}$ [measured value]) throughout this paper (9).

Isolation procedure. In 1978 and 1979, up to five fl^+ bacterial colonies per sample were isolated. In 1980, cytochrome oxidase tests (ox) were performed on all fl^+ colony-types present on the dilution plates and a maximum of four fl^+ ox^- colonies per sample were isolated. The number of isolates was weighted to approximately represent the major fl^+ colony-types present on plates with from 20 to 100 fl^+ colonies whenever possible. Each isolate was purified by restreaking several times from well-separated colonies onto nutrient glycerol agar (NGA: 3.3 g of Bacto-peptone, 2.7 g of nutrient broth, 2.0 g of yeast extract, 25 ml of glycerol, and 15 g of Bacto-agar per liter of glass distilled water [15]) and thereafter maintained on NGA slants at 4 C.

Estimating population levels of *P. syringae* and *Psb*. Purified field isolates identified as *P. syringae* produced a fluorescent pigment on Medium B of King et al (10) and were cytochrome oxidase negative (ox^-) and arginine dihydrolase negative (arg^-) (2). *P. syringae* was distinguished from *P. viridiflava* on the basis of colony morphology and color of fluorescence. A subset of 100 $fl^+ox^-arg^-$ isolates was tested for Gram differentiation (20). In 1978 and 1979, estimates of population sizes of *P. syringae* were made from the fraction of fl^+ isolates per sample that were determined to be *P. syringae* multiplied by the population of fl^+ bacteria per sample. In 1980, populations of *P. syringae* were assumed to be adequately estimated by the number of fl^+ ox^- colonies per sample, since all fl^+ ox^- isolates were determined to be *P. syringae* when further characterized.

All isolates of *P. syringae* were tested for pathogenicity to bean by using a pod inoculation assay (18) with pods of the susceptible cultivars Tenderwhite (1978) or Eagle (1979 and 1980). Pods were produced on plants grown in a greenhouse and harvested when 10–15 cm long. They were surface disinfested by soaking for 2 min in 0.525% (w/v) sodium hypochlorite (Chlorox diluted 1:10 with water) and then rinsed ten times in deionized water. Duplicate pods or pod halves were inoculated with 5-μl droplets of a bacterial cell suspension from a 24-hr-old culture on NGA, adjusted to 0.1–0.2 OD units at 600 nm (~10^8 cfu/ml). Pods were wounded through the inoculum droplets with a sterile 26- or 27-gauge hypodermic needle, and then incubated at room temperature (17–20 C) in petri plates lined with moist sterile filter paper. Pathogenic isolates caused a markedly sunken and water-soaked green lesion on the pods within 3 days and nonpathogenic isolates caused either a small brown lesion or no visible reaction. The Psb populations per sample were estimated from the fraction of the isolates of *P. syringae* that were pathogenic to bean by the pod assay. When none of the isolates of *P. syringae* from a sample were pathogenic to bean, or when no *P. syringae* was isolated, the log Psb population was arbitrarily assigned a value of 2.0, which is approximately the lowest bacterial population normally detectable by this dilution plate method (one colony per plate seeded with the undiluted leaf wash).

RESULTS

Incidence of bacterial brown spot. In the 1978 preliminary survey in commercial fields brown spot disease lesions were present at all eight sites in the bean-growing area (Oasis and Leola townships), but were detected at only one of four sites outside the bean-growing area (one of two sites in Rome township, and neither of two sites in Bloomfield township). In the plot studies in 1979 and 1980,

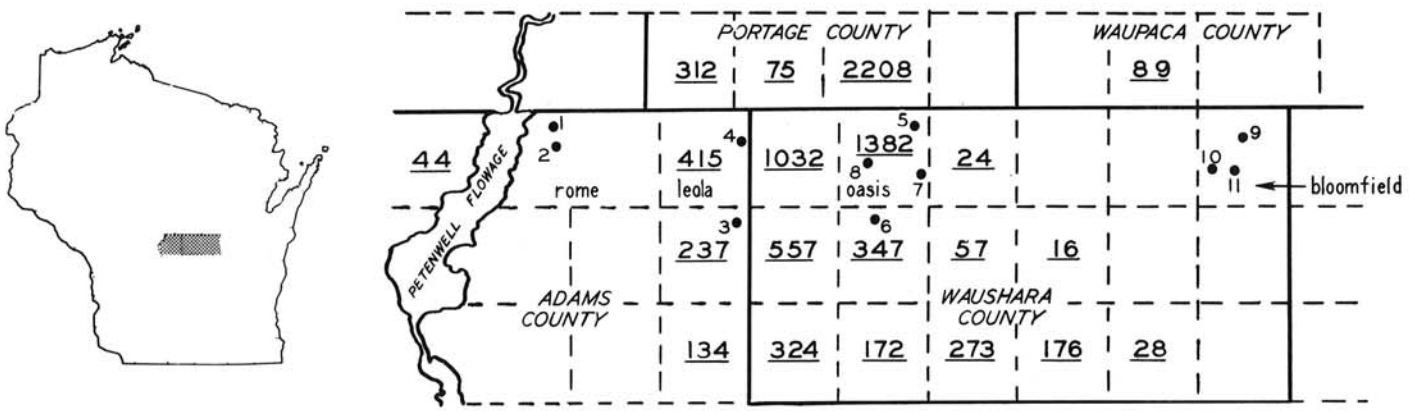

Fig. 1. Locations in the central Wisconsin transect of the 11 experimental plots (●) relative to the commercial production of snap beans (underlined numbers = hectares harvested in 1979). Solid lines delineate counties. Dashed lines delineate townships (standard townships = 93.24 km^2). Names in lowercase letters are townships where plots were located. Shaded area in the inset is the study area.

moderate to severe brown spot disease epidemics (23–99% incidence) occurred in the same four plots (plots 4, 5, 6, and 7), all of which were located in the bean-growing area. Each year five of the other plots had no detected brown spot disease, and incidence in the two remaining plots did not exceed 5% (see Table 1 in cited reference 13).

Epiphytic populations of Psb on bean leaflets. In preliminary studies in 1978, estimates of mean log Psb populations were 5.04 ± 0.2 on bean leaf samples from eight commercial fields in the bean-growing area, and 2.37 ± 0.2 on bean leaf samples from four private gardens outside the bean-growing area. The presence of Psb was detected on 38 of 40 (95%) samples from the bean-growing area, but on only 4 of 12 (33%) samples from outside the bean-growing area.

At the 11 experimental plots, log Psb populations on individual, symptomless bean leaflets ranged from none detected to 6.7 in 1979, and from none detected to 7.8 in 1980. Estimates of mean Psb populations at the individual plots were accompanied by large variances (means were probably overestimates and variances underestimates of the true values, because Psb was detected on <50% of all leaflets sampled and leaflets on which no Psb were detected were arbitrarily assigned log population values of 2.0). In 1979, there were no significant differences among Psb populations at the 11 plots at flowering or harvesttime. In 1980, mean Psb populations on bean leaflets at all four diseased plots (plots 4, 5, 6, and 7) were not significantly different from mean Psb populations at all the nondiseased plots (Table 1, Fig. 2). When the Psb population data were grouped by sampling area for analysis, mean Psb populations were greater in the bean-growing area than in the nonbean-growing area at four of five sampling dates in 1980, but at none of the dates in 1979.

The high frequency of leaflets on which no *P. syringae* or Psb were found may have contributed substantially to the lack of significant differences between mean log population sizes when comparing the individual plots in 1979 and 1980 and when comparing the two sampling areas in 1979. Such data sets are not readily amenable to analysis of variance, unless some approach is taken that minimizes the effect of the censored points.

Differences between the two sampling areas were significant with respect to the frequency of detection of Psb on individual bean leaflets. In 1979, the fraction of leaflets on which Psb was detected was greater in the bean-growing area than outside of it on two of three sampling dates, ie, 0.42 vs 0.3 at flowering time and 0.45 vs 0.2 at harvest. In 1980, the fraction of leaflet samples with detected Psb was consistently greater in the bean-growing area (Table 2). In addition, the frequency of high Psb populations (log cfu/g ≥4.0) was greater in the bean-growing area at flowering time in 1979 and at four of five sampling dates in 1980.

Epiphytic populations of Psb on corn leaves. In 1978, Psb was detected on 7 of 50 (14%) of corn leaf samples from 10 commercial fields in the bean-growing area, but on only 1 of 32 samples (3.1%) of corn leaves from five commercial fields and two private gardens outside the bean-growing area.

In 1979, log Psb populations on individual corn leaves ranged from none detected to 7.40. Overall, Psb was detected on 22 of 88 corn leaf samples (25%) from the bean-growing area and on none of 90 corn leaf samples from outside the bean-growing area. All of the corn leaf samples on which Psb was detected were in plots 5, 6 and 7 where Psb was detected on 22 of 36 corn leaf samples (61%). Mean

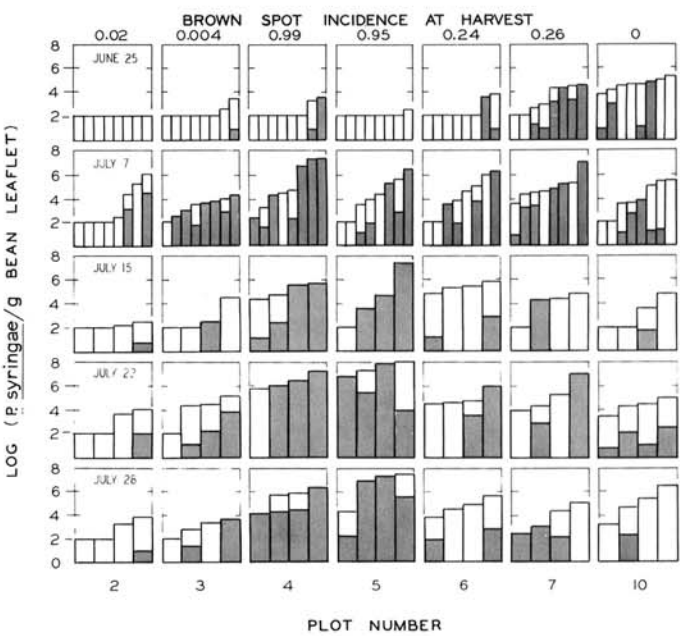

Fig. 2. Epiphytic populations of *Pseudomonas syringae* (log cfu/g) on cultivar Eagle bean leaflets on five sampling dates in 1980 at seven of the 11 experimental plots in the central Wisconsin transect. Each bar represents the population of *P. syringae* on an individual symptomless leaflet. The fraction of each bar that is shaded represents the fraction of the isolates of *P. syringae* from each leaflet that was pathogenic to bean (Psb).

TABLE 1. Epiphytic populations of *Pseudomonas syringae* pv. *syringae* pathogenic to bean (Psb) on cultivar Eagle bean leaflets at 11 experimental plots in the central Wisconsin transect in 1980

Plot[b]	Mean[a] epiphytic Psb (log cfu/g fresh weight)				
	25 June[c]	7 July	15 July	22 July	28 July
1	2.00[d]	2.00	2.00	2.00	2.13
2	2.00	2.76	2.00	2.43	2.27
3	2.10	3.29	2.15	3.78	2.50
4	2.32	4.61	4.90	5.44	5.40
5	2.00	3.67	4.39	7.42	6.32
6	2.38	3.25	3.47	3.63	3.23
7	3.27	4.00	2.56	3.81	2.84
8	2.00	2.00	2.00	3.22	2.00
9	2.00	2.00	2.00	2.00	2.00
10	3.03	3.30	2.34	3.88	2.58
11	2.00	2.00	2.00	2.00	2.00
LSD ($P = 0.05$)	0.57	1.31	1.41	2.18	1.19

[a] Mean of the logarithm of colony-forming units per gram (fresh wt) (log cfu/g) values from 8 (25 June and 7 July) or 4 (15, 22, and 28 July) individual, symptomless leaflets.
[b] Plots 3 through 8 are located in the bean-growing area.
[c] Plants were at the first trifoliolate leaf stage, full flower, and ready for harvest on 25 June, 15 July, and 28 July, respectively.
[d] When no Psb was detected, the log of the Psb population was arbitrarily assigned a value of 2.00.

TABLE 2. Frequency of detection of *Pseudomonas syringae* pv. *syringae* pathogenic to bean (Psb) on symptomless bean leaflets in the two sampling areas of the central Wisconsin transect in 1980

Character	Sampling date, 1980				
	28 June	7 July	15 July	22 July	28 July
Bean-growing area					
Fraction of leaflets with detected Psb[a]	0.23	0.60	0.46	0.63	0.63
Fraction of leaflets with log Psb populations ≥4.0	0.08	0.33	0.33	0.58	0.33
Nonbean-growing area					
Fraction of leaflets with detected Psb[a]	0.10	0.18	0.10	0.25	0.15
Fraction of leaflets with log Psb populations ≥4.0	0.08	0.10	0.00	0.10	0.05

[a] (Number of samples with detected Psb)/(Total number of samples).

log Psb populations on corn leaves were 4.8 (plot 5), 5.65 (plot 6) and 3.06 (plot 7) when the bean plants were at full flower in 1979.

Overall, in 1980, Psb was detected on 16 of 72 corn leaf samples (22%) in the bean-growing area and on 6 of 60 corn leaf samples (10%) outside the bean-growing area. Since Psb was detected on such a small fraction of the samples, estimates of mean Psb populations were strongly influenced by censored data points as discussed above. Thus, there were no significant differences between mean Psb populations on corn in the bean-growing area and the nonbean-growing area. However, Psb was detected at high populations (log cfu/g $\geq$4.0) on corn at all three sampling dates in the bean-growing area, but only on the last sampling date (2 September) outside of the bean-growing area. Thus, based on both frequency of isolation of Psb and the frequency of detection of high Psb populations over a 3-yr period, Psb was more abundant on corn leaf samples from the bean-growing area than from the two adjacent nonbean-growing areas.

Relative abundance of Psb and heterologous *P. syringae* on bean and corn leaves. A large proportion (44%) of the isolates of *P. syringae* from symptomless bean leaflets was not pathogenic to bean (Table 3). Psb was frequently present on bean leaflets together with populations of heterologous *P. syringae* (non-Psb). In 1980, at least 25% of the leaflets per location per sampling date with any detected *P. syringae* had both Psb and non-Psb (Fig. 2). In plots in which bean leaflets supported epiphytic populations of Psb but in which brown spot epidemics did not develop (plots 1, 2, 3, 8, and 10 in 1980), 72% of the leaflets had mixed populations of *P. syringae* whereas 47% of the leaflets in plots where brown spot epidemics occurred (plots 4, 5, 6, and 7) had mixed populations of *P. syringae*. Since a considerable degree of competition probably occurs between conspecific individuals, epiphytic populations of heterologous *P. syringae* on bean leaflets may naturally reduce brown spot incidence.

In each of the 3 years, 1978, 1979, and 1980, the percentage of the total isolates of *P. syringae* that was Psb was lower on corn than on symptomless bean leaves (Table 3). In addition, in 1979 and 1980 the percentage of individual corn leaves on which Psb was detected (12 and 17%) was lower than on apparently healthy bean leaflets (30 and 33%). This suggests that bean leaflets may be more favorable than corn leaves as hosts for the epiphytic growth of Psb relative to heterologous *P. syringae*.

Epiphytic populations of Psb on other nonhost species. In 1979 and 1980, Psb was detected on 5 of 12 (42%) samples of hairy vetch (*Vicia villosa*) from the bean-growing area, but on 0 of 7 samples from the nonbean-growing area (Table 4). Similarly, 13 of 24 (54%) isolates of *P. syringae* from hairy vetch in the bean-growing area were Psb, but 0 of 18 isolates of *P. syringae* from vetch in the nonbean-growing area were Psb.

All four of the other plant species that were sampled in the bean-growing area in 1978 and 1979 also had detectable Psb populations; oak, black locust, winter rye, and sow thistle (Table 4). To our knowledge, these plants as well as corn have not been reported previously to harbor Psb as an epiphyte. The oak, locust, sow thistle, and hairy vetch sampled were located near large commercial bean fields, and winter rye is commonly planted as a cover crop in bean fields. All four of these nonhosts possibly may serve as overwintering sites for Psb, and all may contribute inoculum for brown spot disease epidemics.

TABLE 3. Isolates of *Pseudomonas syringae* obtained from bean leaflets and corn leaves in the central Wisconsin transect during 1978, 1979, and 1980

Crop and year	Bean area		Nonbean area		Total	
	Isolates (no.)	Psb (%)	Isolates (no.)	Psb (%)	Isolates (no.)	Psb (%)
Bean						
1978	183	94	24	21	207	86
1979	88	67	93	30	181	46
1980	456	55	159	26	615	48
Total and mean (%)	727	66	276	27	1,003	56
Corn						
1978	16	67	2	50	18	65
1979	124	39	19	0	143	36
1980	131	35	77	25	208	31
Total and mean (%)	271	38.5	98	20	369	35

[a] Psb = *P. syringae* pv. *syringae* pathogenic to bean.

DISCUSSION

Within the experimental transect, such gross physical environmental variables as temperature, rainfall, and length of growing season varied little between locations. Thus, the primary factor associated with the occurrence of brown spot epidemics was the location of plots in areas where snap beans are extensively cultivated. If seedborne Psb, which was detected on greenhouse-grown plants from the cultivar Eagle seed source, had been the only important inoculum source, then disease development should have been independent of plot location. This location effect was apparent not only with respect to brown spot disease incidence, but also in relation to epiphytic populations of Psb on host and nonhost plants. On the basis of the frequency of Psb isolation, leaves of bean, corn, and hairy vetch harbored greater Psb populations in the bean-growing area than in two adjacent nonbean-growing areas. Psb was found on at least one sample of every plant species that was sampled in the bean-growing area. Four of the five plant species that are newly reported to harbor Psb were not legumes. Thus, the range of plant species that support the epiphytic growth of Psb is much broader and less specific than has been assumed, and it is possible that other plant species in the area might also harbor Psb. These results are consistent with the argument that local epiphytic Psb populations are a sufficiently important inoculum source that brown spot disease epidemics in central Wisconsin are unlikely in their absence and that the presence of these large local populations is associated with (and probably the result of) the intensive, frequent cropping of beans.

The long-term effect of intensive cropping of large acreages of beans may be to favor the growth of Psb while slowly reducing populations of heterologous *P. syringae*. If bean acreage is directly

TABLE 4. Detection of *Pseudomonas syringae* pv. *syringae* pathogenic to bean (Psb) on leaves of five nonhost plant species along a transect in central Wisconsin during 1978, 1979, and 1980

Plant species	Township[a]	Date sampled	Samples (no.)	*P. syringae* isolates (no.)	Psb detected[b] (log cfu/g mean ± SE)
Vicia villosa L.	Rome	June 1979	1	0	ND[c]
		Sept 1980	3	15	ND
	Leola	June 1979	3	9	3.68 ± 0.71
		Sept 1980	6	15	4.54 ± 0.24
	Oasis	Sept 1980	3	0	ND
	Bloomfield	Sept 1980	3	3	ND
Secale cereale L.	Leola	June 1979	5	21	4.01
	Oasis	June 1979	3	7	ND
Sonchus arvensis L.	Oasis	June 1979	2	2	2.57
Quercus sp. L.	Oasis	July 1978	2	5	3.36
Robinia pseudoacacia L.	Oasis	July 1978	2	11	3.98 ± 0.49

[a] Rome is 16 km west and Bloomfield is 27 km east of the bean-growing area (Leola and Oasis townships).

[b] Mean populations of Psb determined only from those samples in which Psb was detected.

[c] ND = none detected.

related to local increases in Psb populations, then Psb should have some selective advantage over other bacteria when they are competing on bean leaves. Two lines of indirect evidence from this reseach suggest that some selection for Psb on bean leaves probably occurs: the percentage of leaves with detectable Psb was greater on beans than on two nonhosts, corn and hairy vetch; this difference was more pronounced where the selection pressure should have been greatest, ie, in the bean-growing area.

In their recent review, Burdon and Chilvers (3) state that increasing the acreage of susceptible host plants in an area should result in increased disease incidence if the pathogen is wind dispersed, but not if the pathogen depends on splash dispersal since the additional host plants must be within dispersal range to influence the distribution of the pathogen. Since *P. syringae* is dispersed during dry, sunny weather (11,14) as well as in aerosols and splash droplets during rain (4,23) and since airborne *P. syringae* can successfully land on, colonize, and infect leaves of a susceptible host (22), the results presented here are consistent with Burdon and Chilver's (3) analysis. In addition, bacterial aerosols are generated during mechanical harvesting (17) and such a dispersal mechanism may be consistent with the large Psb populations detected on corn in September 1980, as well as for large Psb populations reported in autumn on hairy vetch (4).

Results of the present study provide indirect evidence for the possible role of wind dispersal of Psb in the observed location effect. Brown spot disease incidence on plants in plots within the bean-growing area was not related specifically to the proximity of a plot to a commercial bean field or to hairy vetch plants from which Psb could have been dispersed by splashing rain, although rain splash dispersal of Psb from other nearby plant species cannot be ruled out. In 1979, plots 5, 6, 7, and 8 were adjacent to (within 0.16 km of) bean fields, but plots 3 and 4 were not, and in 1980, plots 3, 5, 7, and 8 were adjacent to bean fields, but plots 4 and 6 were not. The two nonepidemic plots, 3 and 8, were surrounded by trees which may have reduced the amount of airborne Psb that was dispersed to these plots from adjacent bean fields or plants harboring epiphytic populations of Psb (1,6) or may have altered the microclimate of the plots so that it was not favorable for the epiphytic growth of Psb.

In this study, we have detected differences in epiphytic Psb population sizes based on the frequency of their detection in spite of the lack of significant differences between mean Psb populations by analysis of variance. In addition, we have compared the frequency of individual leaflets with high Psb populations to the incidence of bacterial brown spot disease with quite favorable results (12,13).

LITERATURE CITED

1. Belot, Y., and Gauthier, D. 1975. Transport of micronic particles from atmosphere to foliar surfaces. Pages 583-591 in: Heat and Mass Transfer in the Biosphere. I. Transfer processes in the plant environment. D. A. de Vries and N. H. Afgan, eds. Scripta Publishing Co., Washington, DC.
2. Buchanan, R. E., and Gibbons, N. E. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. The Williams & Wilkins Co., Baltimore, MD.
3. Burdon, J. J., and Chilvers, G. A. 1982. Host density as a factor in plant disease ecology. Annu. Rev. Phytopathol. 20:143-166.
4. Ercolani, G. L., Hagedorn, D. J., Kelman, A., and Rand, R. E. 1974. Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. Phytopathology 64:1330-1339.
5. Hagedorn, D. J., and Patel, P. N. 1965. Halo blight and bacterial brown spot of bean in Wisconsin in 1964. Plant Dis. Rep. 49:591-595.
6. Hagedorn, D. J., and Wade, E. K. 1964. Bacterial blight of Wisconsin canning peas in 1963. Plant Dis. Rep. 48:318-320.
7. Harrison, D. E., and Freeman, H. 1965. Bacterial brown spot (*Pseudomonas syringae*) of French bean. J. Agric. Victoria 63:523-533.
8. Hoitink, H. A. J., Hagedorn, D. J., and McCoy, E. 1968. Survival, transmission and taxonomy of *Pseudomonas syringae* van Hall, the causal organism of bacterial brown spot of bean (*Phaseolus vulgaris* L.). Can. J. Microbiol. 14:437-441.
9. Hirano, S. S., Nordheim, E. V., Arny, D. C., and Upper, C. D. 1982. Lognormal distribution of epiphytic bacterial populations on leaf surfaces. Appl. Environ. Microbiol. 44:695-700.
10. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluoresin. J. Lab. Clin. Med. 44:301-307.
11. Lindemann, J., Arny, D. C., Hirano, S. S., and Upper, C. D. 1981. Dissemination of bacteria, including *Pseudomonas syringae*, in a bean plot. (Abstr.) Phytopathology 71:890.
12. Lindemann, J., Arny, D. C., and Upper, C. D. 1981. Epiphytic *Pseudomonas syringae* population size greater than a threshold level is predictive of brown spot incidence on snap beans. (Abstr.) Phytopathology 71:890.
13. Lindemann, J., Arny, D. C., and Upper, C. D. 1984. Use of an apparent infection threshold population of *Pseudomonas syringae* to predict incidence and severity of brown spot of bean. Phytopathology 74:1334-1339.
14. Lindemann, J., Constantinidou, H. A., Barchet, W. R., and Upper, C. D. 1982. Plants as sources of airborne bacteria, including ice nucleation-active bacteria. Appl. Environ. Microbiol. 44:1059-1063.
15. Lindow, S. E., Arny, D. C., and Upper, C. D. 1978. Distribution of ice nucleation-active bacteria on plants in nature. Appl. Environ. Microbiol. 36:831-838.
16. Patel, P. N., Walker, J. C., Hagedorn, D. J., Garcia, C. D., and Teliz-Ortiz, M. 1964. Bacterial brown spot of bean in central Wisconsin. Plant Dis. Rep. 48:335-337.
17. Pérombelon, M. C. M., Fox, R. A., and Lowe, R. 1979. Dispersal of *Erwinia carotovora* in aerosols produced by the pulverization of potato haulm prior to harvest. Phytopathol. Z. 94:249-260.
18. Ribeiro, R. 1978. Characterization of the bacteria inciting bean wildfire and corn chocolate spot. Pathogen variability and disease resistance studies on bean yellows. Ph.D. thesis, University of Wisconsin, Madison. 147 pp.
19. Rudolph, K., Delgado, M., and Baykal, N. 1973. Pathological aspects of *Pseudomonas syringae* van Hall on bush bean spp. Pages 44-53 in: Synopsis Int. Soc. Plant Pathol. Working Party on *Pseudomonas syringae* group. Inst. Nat. Rech. Agron., Angers, France.
20. Ryu, E. 1938. On the Gram-differentiation of bacteria by the simplest method. J. Jpn. Soc. Vet. Sci. 17:31.
21. Schneider, R. W., and Grogan, R. G. 1977. Bacterial speck of tomato: sources of inoculum and establishment of a resident population. Phytopathology 67:388-394.
22. Surico, G., Kennedy, B. W., and Ercolani, G. L. 1981. Multiplication of *Pseudomonas syringae* pv. *glycinea* on soybean primary leaves exposed to aerosolized inoculum. Phytopathology 71:532-536.
23. Venette, J. R., and Kennedy, B. W. 1975. Naturally produced aerosols of *Pseudomonas glycinea*. Phytopathology 65:737-738.