Effect of Host Genotype on Multiplication of *Pseudomonas phaseolicola*

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ABSTRACT

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We studied the multiplication of *Pseudomonas phaseolicola* (*Pp*) in seedlings of halo blight susceptible Charlevoix and tolerant Montcalm and Seafarer bean cultivars. Strain R13 of *Pp*, which is resistant to rifampin, was used to facilitate isolation of the bacteria from field material. Dilutions of ground (total) and rinsed (surface) first, third, or fifth trifoliolate leaves were plated on King's Medium B containing 50 μg/ml each of rifampin and cycloheximide. Increase of *Pp* R13 in leaves of cultivar Charlevoix followed a typical bacterial growth curve. Populations of *Pp* in the stationary phase remained high for up to 15 days on all leaves of Charlevoix; on third trifoliolate leaves, 27% of the total population was surfaceborne. Lower numbers of *Pp* R13 were detected in leaves of cultivar Montcalm and 5% was surfaceborne. Seafarer supported slightly higher numbers of *Pp* R13 than Montcalm, and 8% of the total was surfaceborne. Tolerant bean cultivars may serve as carriers of *Pp*, and secondary spread from them may occur before visible symptoms develop.

Halo blight, which is caused by *Pseudomonas phaseolicola* (Burk.) Dows. (*Pp*), is a serious disease of bean (*Phaseolus vulgaris* L.) whenever infested seed is planted in environments favorable for disease development. Although foliage sprays effectively control secondary disease spread, the use of resistant cultivars and planting of disease-free seed are the most practical and effective control measures (9,13). Cultivar Montcalm is a halo blight-tolerant, dark-red kidney bean that was developed for commercial production in the humid Great Lakes area (9). During the past several years, however, *Pp* has been isolated from pod samples of Montcalm grown for certified seed. This suggested that Montcalm plants might be carriers of *Pp* and provide a source of inoculum for secondary disease spread to neighboring fields of susceptible cultivars.

The purpose of the research reported here was to examine the multiplication and distribution of *Pp* in plants of susceptible Charlevoix and tolerant Montcalm and Seafarer cultivars.

MATERIALS AND METHODS

Field design. Certified, disease-free seed of susceptible Charlevoix, and tolerant Montcalm and Seafarer was planted in the field. The 1978 plots were four rows 5 m long, and 1979 plots were six rows 3 m long. In both years, rows were 50 cm apart, and seed spacing in the row was 9 cm. All treatments were replicated three times.

Bacterial isolate and inoculation. Spontaneous mutant R13 of *P. phaseolicola,* which is resistant to 50 μg/ml rifampin and as virulent as the parental wild type (Shiels 2), was obtained by conventional selective plating methods. Inoculum in 1978 was prepared by rinsing R13 grown for 24 hr on plates of King's Medium B (KMB) (modified to contain 3 g instead of 6 g of $MgSO_4 \cdot 7H_2O$ [6]) into sterile distilled water. Cell concentration was adjusted to 6×10^8 cells per milliliter as determined by standard turbidimetric and dilution plate techniques. Plants were inoculated when 22 days old by gently spraying the bacterial suspension on the upper and lower leaf surfaces of expanded primary and first trifoliolate leaves until they were thoroughly wetted. No water-soaking of leaf tissue was observed. Inoculation procedures in 1979 were similar to those described for 1978 except the inoculum was prepared in sterile 0.01 M PO_4 buffer, pH 7.2; the inoculum contained approximately 1.5×10^7 cells per milliliter; and the plants were 27 days old when inoculated.

Determination of bacterial populations. Total numbers of bacteria were determined on first, third, and fifth trifoliolate leaves of each bean cultivar. Each leaf was assayed for the presence of R13 for up to 20 days after emergence of the leaf. Three samples of seven leaves each (21 leaflets) were randomly selected from each cultivar and homogenized for 3 min with 0.01 M PO_4 buffer (pH 7.2) in a Waring Blendor. One milliliter of buffer was added for each 6 cm^2 of leaf tissue. Serial dilutions were prepared and plated on KMB supplemented with 50 μg/ml each of rifampin and cycloheximide (KMBR). Plates were incubated for 4 days at room temperature and colonies were counted. Numbers of bacteria are expressed as the logarithm of the number of colony forming units (CFU) per 30 cm^2 of leaf tissue (the average size of one leaflet). Leaf area was measured with a Li-Cor area meter (Model 3000, Lambda Instruments Corp., Lincoln, NE) by using leaf tracings on paper.

Stems were sampled to determine internal presence of *Pp* R13. Stem sections from the first through third node (~ 3 cm in length) were excised from 12 plants of each genotype, and surface sterilized in 2.5% NaOCl for 3 min (three replications per genotype). All sections from one genotype were collectively rinsed in running distilled water for 1 min and in sterile distilled water for 1 min, homogenized in 200 ml of 0.01 M PO_4 buffer (pH 7.2) for 3 min, and serial dilutions were plated on KMBR plates. Numbers of bacteria were expressed as CFU per stem section.

In 1979, numbers of surface (external) bacteria were determined on the third trifoliolate leaf by gently shaking seven leaves for 2 min with 150 ml of sterile PO_4 buffer in flasks and plating the rinse solutions on KMBR. The rinsed leaves were homogenized to determine internal bacteria; total bacterial populations were the sum of surface plus internal populations.

Flower blossoms were assayed for the presence of R13 by macerating 20 freshly opened blossoms in 50 ml of PO_4 buffer in a Waring Blendor and plating serial dilutions on KMBR; each

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genotype was replicated three times. Numbers of bacteria were expressed as CFU per blossom (one fresh blossom = 0.135 g fresh weight).

Numbers of *Pp* R13 on the surfaces of mature pods were determined by rinsing 21 pods in 150 ml of PO_4 buffer (pH 7.2) for 2 min and plating serial dilutions of the rinse solution on KMBR. Populations were expressed as CFU per pod.

Disease evaluation. Disease severity on the three bean genotypes was determined periodically on the first, third, and fifth trifoliolate leaves throughout the growing season. Individual leaflets obtained from 21 plants were sampled and rated for percent leaflet infection 13 times.

Bacteria were isolated from leaves of Montcalm and Seafarer, which exhibited only atypical symptoms. Lesions were aseptically excised, rinsed in sterile distilled water, and homogenized in 1 ml of sterile PO_4 buffer in sterile mortars and pestles. Streaks for single colonies were made on KMBR.

Statistical analysis. Analysis of bacterial numbers in the three bean genotypes was by a method appropriate for a split-plot design with bean genotypes as the whole plots and time as the subplots. Significant differences between the populations on the three genotypes were estimated using least significant ranges (L.S.R.) obtained from Tukey's *w*-procedure (11).

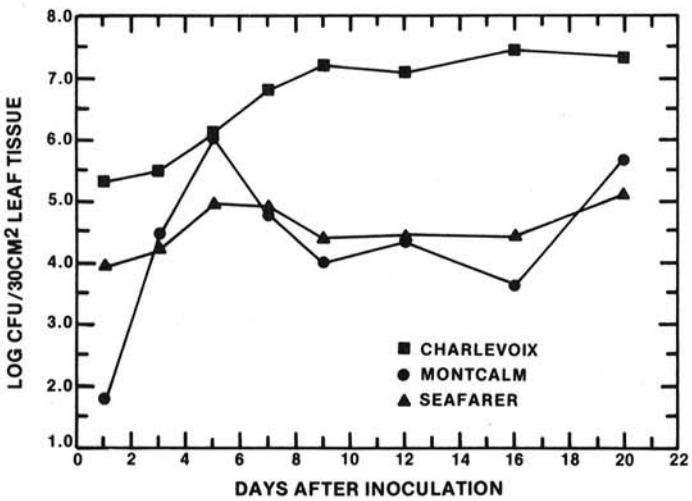

Fig. 1. Total numbers of *Pseudomonas phaseolicola* (R13 mutant) in first trifoliolate leaves of susceptible (Charlevoix) and tolerant (Montcalm and Seafarer) bean cultivars. Twenty-two-day-old plants were inoculated by gently spraying upper and lower leaf surfaces with a bacterial suspension containing 6×10^8 cells per milliliter. Values are averages of three replicates.

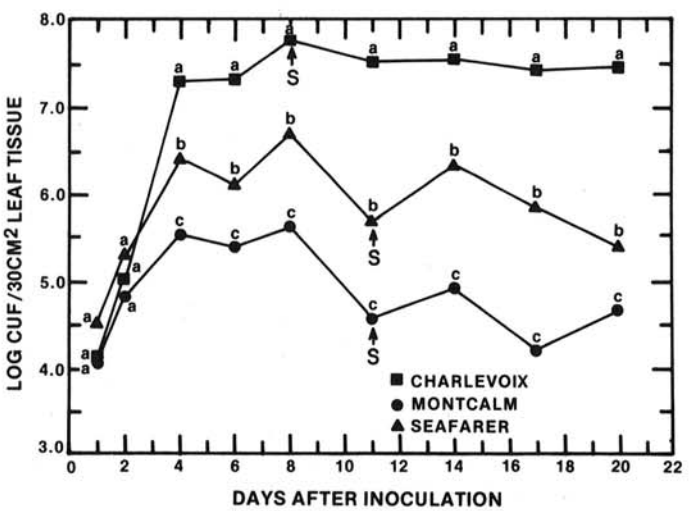

Fig. 2. Total numbers of *Pseudomonas phaseolicola* (R13 mutant) in the first trifoliolate leaves of susceptible (Charlevoix) and tolerant (Montcalm and Seafarer) bean cultivars. Twenty-seven-day-old plants were inoculated by gently spraying upper and lower leaf surfaces with a bacterial suspension containing 1.5×10^7 cells per milliliter. Values are averages of three replicates. Means for the same day with the same letter are not significantly different at $\alpha = 0.05$ by Tukey's *w*-procedure. S = symptoms.

RESULTS

Field study. Trends in populations of *Pp* R13 in inoculated first trifoliolate leaves are shown in Fig. 1; similar trends were noted in uninoculated third and fifth trifoliolate leaves. In 1978, numbers of R13 on inoculated first trifoliolate leaves increased slowly and stabilized 9 days after inoculation at about 5×10^7 CFU per 30 cm^2 of leaf tissue. Lower numbers of *Pp* R13 were associated with leaves of the tolerant cultivars, and levels fluctuated between 10^4 and 10^6 CFU/cm^2 leaf tissue. Population trends were similar on third and fifth trifoliolate leaves.

Lesions were observed on susceptible Charlevoix leaves as small water-soaked areas along the lower edges 5 days after inoculation when numbers of *Pp* reached approximately 10^6 CFU per leaflet. Symptoms were present on Charlevoix throughout the growing season with severity greatest on the young, later-emerging leaves. Although moderate numbers of bacteria were associated with leaves of tolerant cultivars, lesions were detected on Montcalm and Seafarer only sporadically. Very few lesions developed on the uninoculated third and fifth trifoliolate leaves of Montcalm, and lesions on Seafarer were atypical, consisting of hypersensitive-type spots without water-soaking. Symptom appearance in Montcalm and Seafarer did not appear to be associated with particular numbers of bacteria.

In 1979, controlled spray techniques resulted in more even distribution of primary inoculum on primary and first trifoliolate leaves. Population trends (Fig. 2) of *Pp* R13 in all cultivars consisted of a 4-day exponential phase followed by a stationary phase in which populations remained relatively stable or declined gradually. About 5×10^7 CFU per 30 cm^2 occurred on Charlevoix leaves 4 days after inoculation and remained at this level for 16 days. Significantly lower numbers of R13 were detected on tolerant cultivars compared to susceptible Charlevoix; significantly higher numbers were detected on Seafarer than on Montcalm from 4 to 20 days after inoculation.

Isolate *Pp* R13 colonized the third trifoliolate leaves of Charlevoix soon after leaf emergence (Fig. 3) and approximately 5×10^7 CFU per leaflet were detected in most samples. Surfaceborne bacteria comprised about 27% of the total population on Charlevoix leaves, but numbers on tolerant Seafarer and Montcalm were significantly lower (8 and 5% of total, respectively); Seafarer leaves supported significantly higher numbers of bacteria

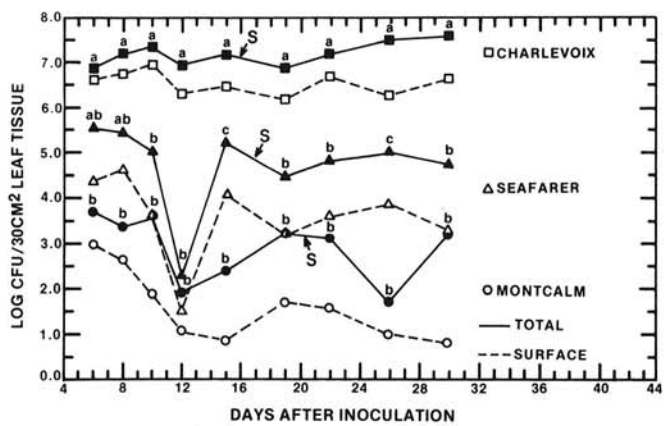

Fig. 3. Total and surface numbers of *Pseudomonas phaseolicola* (R13 mutant) in and on the third trifoliolate leaves of susceptible (Charlevoix) and tolerant (Montcalm and Seafarer) bean cultivars. Twenty-seven-day-old plants were inoculated by gently spraying upper and lower leaf surfaces with a bacterial suspension containing 1.5×10^7 cells per milliliter. Values are the averages of three replicates. Means for the same day with the same letter are not significantly different at $\alpha = 0.05$ by Tukey's *w*-procedure. S = symptoms.

TABLE 1. Numbers of *Pseudomonas phaseolicola* (*Pp*) (R13) in newly opened blossoms and on pods of field-grown bean cultivars Charlevoix, Montcalm, and Seafarer[a]

Cultivar	D.R.[b]	CFU/blossom[c]	CFU/pod[d]
Charlevoix	S	3.7×10^4	8.6×10^7
Montcalm	T	0.0	9.7×10^1
Seafarer	T	1.5×10^1	3.1×10^1

[a] Plants were inoculated at seedling stage with a suspension of *Pp* R13 at 1.5×10^7 cells per milliliter. Blossoms were sampled 23 days after inoculation. Surface numbers on pods were determined 65 days after inoculation.
[b] Disease reaction: S = susceptible, T = tolerant.
[c] Twenty blossoms of each cultivar were macerated in a Waring Blendor with 50 ml of 0.01 M PO_4 buffer (pH 7.2). Bacterial numbers are expressed as colony forming units (CFU) per blossom.
[d] Twenty-one pods of each cultivar were shaken for 2 min in 150 ml of 0.01 M PO_4 buffer (pH 7.2). Bacterial numbers are expressed as CFU per pod.

than Montcalm at 15 and 26 days after inoculation.

Significantly higher numbers of *Pp* R13 were detected on the fifth trifoliolate leaves of Charlevoix compared to leaves of tolerant cultivars (Fig. 4). Bacterial numbers on Montcalm and Seafarer also increased gradually, but never exceeded 10^4 CFU per leaflet.

Disease symptoms were less severe in 1979 than in 1978. In 1979, almost 10% of first trifoliolate leaves of susceptible Charlevoix were infected by the end of the sampling period. Halo blight was less severe on third and fifth trifoliolate leaves of Charlevoix than on the first trifoliolate leaf, and symptoms were variable on plants of cultivars Montcalm and Seafarer. Lesions on tolerant cultivars were observed only occasionally and were atypical. Isolations from suspect lesions of Montcalm yielded R13 bacteria in only 45% of the cases, suggesting that half of such 'atypical' lesions were caused by factors other than halo blight bacteria.

Halo blight bacteria were isolated from the inside of first, second and third internode sections of Charlevoix (1.9×10^7 CFU per section), Montcalm (5.2×10^2 CFU per section), and Seafarer (1.9×10^3 CFU per section) cultivars; no disease symptoms were visible in the stem sections of tolerant cultivars.

Blossoms of Charlevoix were colonized with blight bacteria, (3.7×10^4 CFU per blossom) (Table 1); numbers were less (15 CFU per blossom) in Seafarer blossoms and R13 was not detected in Montcalm blossoms.

The surface of mature pods of susceptible Charlevoix supported high numbers of bacteria (Table 1), whereas numbers on pods of tolerant cultivars were much lower.

DISCUSSION

Under conditions simulating a natural halo blight infection, *P. phaseolicola* R13 grew faster, and higher total numbers were detected in leaves of susceptible Charlevoix than in leaves of tolerant Montcalm and Seafarer. This difference was apparent in the first trifoliolate leaves of all three cultivars that had been inoculated simultaneously with the same bacterial suspension. Isolate R13 grew exponentially on all cultivars for about 4 days. However, final populations in Charlevoix were 10 times higher than those in Seafarer, and 100 times those in Montcalm. Omer and Wood (8) reported similar results with susceptible and resistant beans in the greenhouse. A similar pattern was also observed when *P. phaseolicola* was inoculated into a natural host (bean) and into a nonhost (cherry) (4). Population studies of other bacterial diseases of bean and soybean have yielded similar results (1–3,7,10). In general, bacteria are detected soon after inoculation irrespective of host genotype, but final bacterial numbers are lower in resistant than susceptible tissue.

Bacterial numbers were high in both the third and fifth trifoliolate leaves of susceptible Charlevoix shortly after emergence. This suggests early colonization of the apical bud by the pathogen.

Symptom development in the form of water-soaking in susceptible tissues coincided with bacterial numbers of $\sim 10^6$ CFU per 30 cm^2 of leaf tissue. Bacteria were also detected on leaves

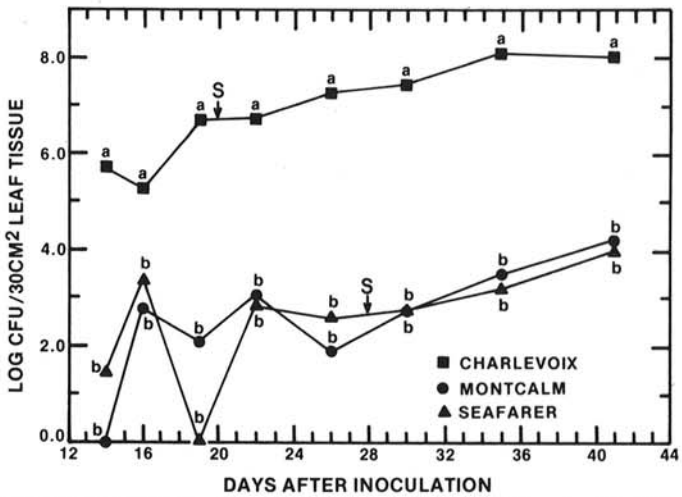

Fig. 4. Total numbers of *Pseudomonas phaseolicola* (R13 mutant) in fifth trifoliolate leaves of susceptible (Charlevoix) and tolerant (Montcalm and Seafarer) bean cultivars. Twenty-seven-day-old plants were inoculated by gently spraying upper and lower leaf surfaces with a bacterial suspension containing 1.5×10^7 cells per milliliter. Values are the average of three replicates. Means for the same day with the same letter are not significantly different at $\alpha = 0.05$ by Tukey's *w*-procedure. S = symptoms.

of the tolerant cultivars, but levels were significantly lower and symptoms on these cultivars were frequently atypical. Both tolerant cultivars could serve as carriers of *P. phaseolicola* and provide a source of inoculum likely to spread to adjacent susceptible cultivars.

Numbers of *Pp* associated with leaf surfaces of susceptible cultivar Charlevoix comprised a greater percentage of the total population than in tolerant cultivars Montcalm and Seafarer. Weller and Saettler (12) recovered similar percentages of *Xanthomonas phaseoli* and *X. phaseoli* var. *fuscans* from leaf surfaces of susceptible bean cultivars as in the present study. On the other hand, Cafati and Saettler (2) detected higher surface populations on leaves of resistant cultivars and suggested that *Xp* did not extensively colonize the internal tissues of the resistant genotypes. According to our data, lower numbers of bacteria develop in tolerant cultivars and, therefore, the ability of such cultivars to serve as inoculum sources is reduced. Our data expand the work of Katherman et al (5) who demonstrated a reduced ability of inoculated resistant cultivars compared to susceptible cultivars, to serve as inoculum sources for secondary spread of *Pp*.

The high numbers of *Pp* in flower buds are correlated with bacterial spread to developing pods. Low numbers of *Pp* in blossoms and on surfaces of pods further illustrate the limited ability of the pathogen to colonize the tolerant cultivars. High numbers of bacteria found inside stem sections of susceptible cultivar Charlevoix demonstrated that the pathogen can develop systemically.

Resistant cultivars historically have been developed on the basis of disease symptoms. The ability of a 'resistant' cultivar to support the growth of the pathogen in the absence of symptoms is an obvious danger. Breeding programs devoted to the development of halo blight resistance should include tests to detect populations of the pathogen in segregating and advanced genotypes.

LITERATURE CITED

1. Allington, W. B., and Chamberlain, D. W. 1949. Trends in the populations of pathogenic bacteria within leaf tissue of susceptible and immune plant species. Phytopathology 39:656-660.
2. Cafati, C. R., and Saettler, A. W. 1980. Effect of host on multiplication and distribution of bean common blight bacteria. Phytopathology 70:675-679.
3. Daub, M. E., and Hagedorn, D. J. 1980. Growth kinetics and interactions of *Pseudomonas syringae* with susceptible and resistant bean tissue. Phytopathology 70:429-436.
4. Ercolani, G. L., and Crosse, J. E. 1966. The growth of *Pseudomonas*

phaseolicola and related pathogens in vivo. J. Gen. Microbiol. 45:429-439.
5. Katherman, M. J., Wilkinson, R. E., and Beer, S. V. 1980. The potential of four dry bean cultivars to serve as sources of *Pseudomonas phaseolicola* inoculum. Plant Dis. 64:72-74.
6. King, E. O., Ward, M. K. W., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
7. Mew, T. W., and Kennedy, B. W. 1971. Growth of *Pseudomonas glycinea* on the surface of soybean leaves. Phytopathology 61:715-716.
8. Omer, M. E. H., and Wood, R. K. S. 1969. Growth of *Pseudomonas phaseolicola* in susceptible and in resistant bean plants. Ann. Appl. Biol. 63:103-116.
9. Saettler, A. W., and Andersen, A. L. 1978. Bean diseases and their control. Pages 172–179 in: L. S. Robertson and R. D. Frazier, eds. Dry Bean Production—Principles and Practices. Ext. Bull. E-1251, Michigan State University, East Lansing. 225 pp.
10. Scharen, A. L. 1959. Comparative population trends of *Xanthomonas phaseoli* in susceptible, field tolerant, and resistant hosts. Phytopathology 49:425-428.
11. Steel, G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 pp.
12. Weller, D. M., and Saettler, A. W. 1980. Colonization and distribution of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* in field-grown beans. Phytopathology 70:500-506.
13. 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. 255 pp.