

Colonization and Distribution of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* in Field-Grown Navy Beans

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ABSTRACT

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Rifampin-resistant mutants of *Xanthomonas phaseoli* (*Xp*) and *X. phaseoli* var. *fuscans* (*Xpf*) were used to study the multiplication and distribution of inoculum of bean blight bacteria in field-grown navy (pea) beans (*Phaseolus vulgaris*). Increase of *Xp* and *Xpf* in bean leaves resembled standard bacterial growth curves in vitro. Mean doubling times of *Xpf* and *Xp* were 19.4 and 18.8 hr, respectively, during the exponential growth phase. Numbers of *Xpf* and *Xp* peaked during the stationary phase and remained stable until leaf abscission; a slow death phase accompanied leaf decomposition on the soil surface. Symptom development on leaves required an inoculum density of at least 5×10^6 bacteria per 20 cm² of leaf tissue, usually corresponding to the density in the early stationary phase.

The multiplication of *Xp* and *Xpf* in the leaf canopy from seedling to reproductive phases of plant development were described by a series of growth curves displaced over time, with each curve representing bacterial multiplication on individual leaves of the main stem. Correlation of the sequence of symptom expression with the increase of the bacteria explained the late disease development that is characteristic of common and fuscous blights. Both *Xpf* and *Xp* spread throughout the canopy by rain, bud colonization, and systemic movement. At least 10% of the *Xpf* and *Xp* leaf population was removed as secondary inoculum during rainfall. Isolates *Xpf* R10 and *Xp* Ra were systemic in bean stems and roots, with doubling times of 22.8 and 23.8 hr, respectively in stems.

Common blight and fuscous blight, which are incited by *Xanthomonas phaseoli* (E. F. Smith) Dows. (*Xp*) and *Xanthomonas phaseoli* var. *fuscans* (*Xpf*) (Burkh.) Starr and Burkh. (both synonymous with *Xanthomonas campestris* (Pammel) Dows according to Buchanan and Gibbons [2]), respectively, are serious diseases of commercial bean (*Phaseolus vulgaris* L.) particularly in Michigan on navy (pea) beans. Studies by Burkholder (3,4) and Zaumeyer (23,24) are the basis for present concepts about the disease cycle of common and fuscous blights. Infected seed is a source of primary inocula and seedlings subsequently become infected. Bacteria from lesions are splashed by rain to other parts of the plant, and initiate the secondary disease cycle (23,25). Pod and seed infection complete the disease cycle.

The appearance of bacterial blight in bean fields is closely related to stage of plant development. Blight symptoms sometimes appear on seedlings; however, during the vegetative stage, when the foliage is rapidly expanding, symptoms generally are not seen. Typical field symptoms occur during the reproductive stage (3,4,9,18). Michigan navy bean fields appear to be blight free until late July and early August, then suddenly become blighted. Gloyer (9) suggested that bean plants are less susceptible during the vegetative stage than during the seedling and reproductive stages; more recent studies have confirmed the slightly greater susceptibility of bean plants during the reproductive stage (5). Burkholder suggested that late symptom expression is due to unfavorable environmental conditions for disease development during the first 45 days of the growing season (3,4).

The epidemiology of common and fuscous blights in the field is still poorly understood, as indicated by the lack of a probable

explanation for late disease development. A quantitative study of the population dynamics of *Xp* and *Xpf* under field conditions never has been conducted but would contribute to an understanding of disease development.

This study was conducted to: describe the variation of the density of populations of *Xp* and *Xpf* in field-grown navy beans during the seedling, vegetative, and early reproductive stages of plant growth, and to relate bacterial multiplication and spread to the pattern of disease development in the field.

MATERIALS AND METHODS

Rifampin-resistant mutants of *Xp* and *Xpf*. Isolation of rifampin-resistant mutants Ra and R10, and screening for similarity to the respective wild types *Xp* 11 and *Xpf* 16 were previously described (21). Multiplication and disease production by Ra and R10 were identical to those by wild types in bean leaves and stems. A rifampin-streptomycin resistant mutant, designated R10-S6, was also similar to the parent R10 and original wild type *Xpf* 16 (20).

Inoculation of field-grown navy beans with isolates Ra, R10, and R10-S6. Studies were conducted with navy (pea) beans located at the Botany and Plant Pathology Farm, East Lansing, MI. Eight plots of cultivar Seafarer, and one plot each of cultivars Sanilac and Tuscola were planted. Plants in plots were inoculated by spraying to runoff with an aqueous suspension of 10^8 cells per milliliter, by injecting the bacterial suspension into the cotyledon scar with a syringe or by planting hilum-spotted seeds infected with 10^6 R10 or R10-S6 cells per seed. Inoculum was prepared from 48-hr-old cultures grown on plates of yeast extract-calcium carbonate agar (YCA:10 g yeast extract, 15 g agar, 2.5 g CaCO₃ and 1,000 ml of water).

Isolation of Ra, R10, and R10-S6 from plant tissue. Rifampin agar medium (RAM), used to isolate Ra and R10, was prepared as previously described (21) except that sometimes concentrations of

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rifampin and cycloheximide were increased to 100 µg/ml. For isolation of R10-S6, RAM + cycloheximide was supplemented with streptomycin sulfate at 250 µg/ml. When the mutants were isolated from roots, 100 µg/ml pentachloronitrobenzene (PCNB) was added to the media. Plating efficiency was not affected by the addition of the inhibitors. Other microflora was isolated on YCA.

Mutants Ra and R10 were isolated from bean tissue homogenized in 0.01 M phosphate buffer, pH 7.2, with a Waring Blendor or mortar and pestle (total bacteria), or shaken in phosphate buffer (external bacteria); 0.1 ml-portions of serial dilutions of homogenates or washings were spread on plates of the appropriate medium and incubated for 5 days. Each leaflet was homogenized in about 5 ml of phosphate buffer. In 1976, samples consisted of 12 primary leaves or 15 trifoliolate leaflets and were replicated four times. In 1977, samples consisted of 14 primary leaves or 21 or 42 trifoliolate leaflets and were replicated two or three times. Leaf populations of *Xp* or *Xpf* are expressed as number of viable cells per 20 cm² of leaf tissue, which is about the average size of a trifoliolate leaflet or a primary leaf.

For isolation of internally borne R10 and Ra in 1976, stems were washed in running distilled water for 5 min, soaked in 70% ethanol for 5 min, soaked in 2.5% NaOCl for 10 min, and rinsed in sterile distilled water before being homogenized.

Multiplication and spread of R10 and R10-S6 in navy bean seedlings. Seafarer navy beans internally infected with R10 or R10-S6 at 10⁶ cells per seed (20) were hand planted in the field. At various intervals after planting, seedlings were separated into anatomical parts and homogenized with a mortar and pestle with phosphate buffer to assay for blight bacteria.

Multiplication and spread of R10 and Ra in leaves and buds of field-grown beans during vegetative and early reproductive stages. Sixteen-day-old Sanilac bean seedlings with expanded primary leaves and half-expanded first trifoliolate leaves were spray-inoculated in 1977 with isolates R10 or Ra at 10⁸ cells/ml. The primary leaves through the eighth trifoliolate leaf of the main stem were monitored for the presence of the bacteria until mid-

reproductive phase of plant development; the second and third trifoliolate leaves were pooled in a single sample.

Multiplication and systemic movement of R10 and Ra in the stems of field-grown navy beans. Twenty-day-old Seafarer beans with half-expanded second trifoliolate leaves were injected in the cotyledon scar with a suspension of isolate R10 or Ra (10⁸ cells per milliliter), and stems and roots were monitored for bacteria.

Effect of washing on blight bacterial populations on navy bean leaves. The loss of blight bacteria from leaves was studied in the greenhouse by gently washing inoculated primary and first and second trifoliolate leaves from field-grown plants in 0.01 M phosphate buffer for 2 min.

Rain-trapped Ra from field-grown navy beans. Seafarer bean seedlings were inoculated when 11 days old with isolate Ra, and runoff water was collected after subsequent rainfalls which occurred at 5, 9, 12, and 14 days after inoculation. Three wax cups (11.5 cm diameter) were placed around the base of a plant with the lip of the cup resting against the stem and the bottom anchored in the soil. Runoff water in the cups was plated within 4 hr after each rainfall. The total population of Ra on the plants was determined by assaying all primary and trifoliolate leaves expanded at each sample no sooner than 6 hr before rainfall.

Detection of surface-borne blight bacteria. Direct leaf prints were made by gently pressing leaves onto plates of RAM + cycloheximide for 1 min. Indirect leaf prints were made by pressing a replica plater covered with Parafilm onto a leaf and then onto RAM + cycloheximide.

The relative frequency of colonization of upper and lower surfaces of leaflets was determined by direct printing of symptomless first and second trifoliolate leaflets 30 days after 11-day-old seedlings with half-expanded primary leaves were inoculated with R10 or Ra. Sixty leaves were allocated among four replications; one outer leaflet was printed with the upper surface down and the other outer leaflet was printed in the opposite way. Some leaves were surface-sterilized before printing by immersion and gentle agitation for 30 sec in 2.5% NaOCl or by UV irradiation for 20 min.

RESULTS

Growth of R10 and Ra associated with leaves of field-grown beans. Colonization of isolates R10 and Ra associated with

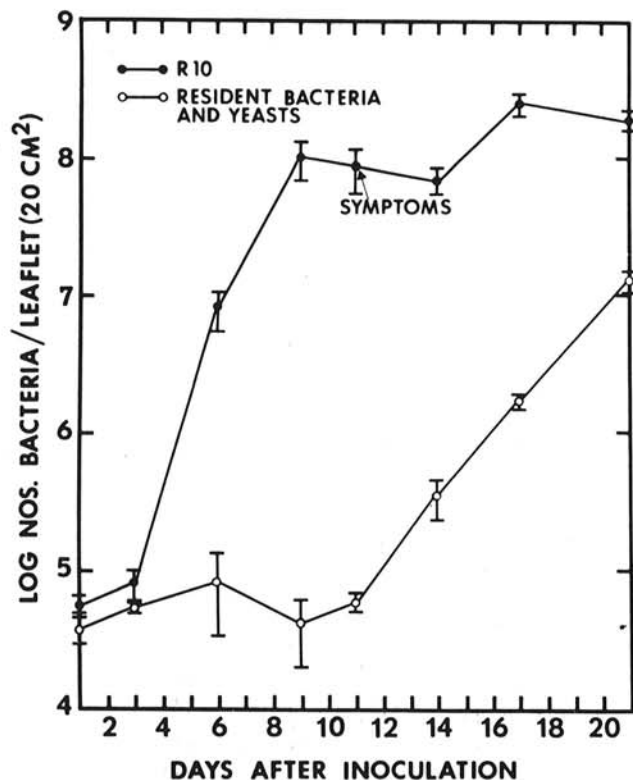


Fig. 1. Colonization by rifampin-resistant isolate R10 of *Xanthomonas phaseoli* var. *fuscans* and resident bacteria and yeasts of first and second trifoliolate leaves of Seafarer navy bean plants inoculated when 19 days old with an aqueous suspension of isolate R10. Data are means of four replications of 15 leaflets each, ± standard error.

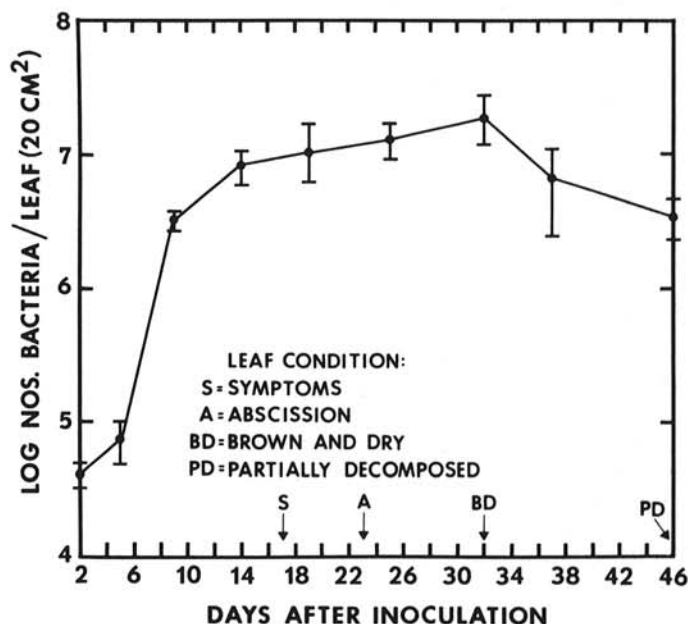


Fig. 2. Colonization by rifampin-resistant isolate Ra of *Xanthomonas phaseoli* of primary leaves of Seafarer navy beans inoculated when 11 days old with an aqueous suspension of isolate Ra. Data are means of two replications of 21 leaflets each, ± standard error.

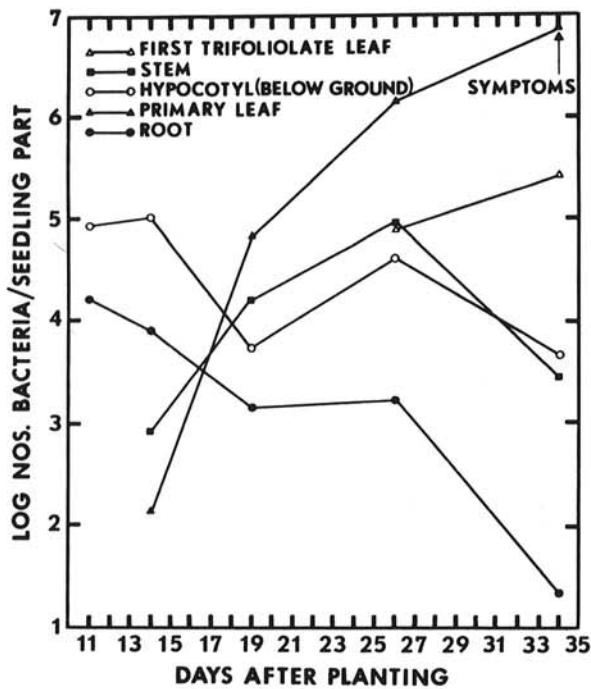


Fig. 3. Colonization by rifampin- and streptomycin-resistant isolate R10-S6 of *Xanthomonas phaseoli* var. *fuscans* of Seafarer navy bean seedlings. Hilum-spotted seeds, internally infected with R10-S6, were planted, and seedlings emerged after 12 days. Data are means of two replications, each with 10 primary leaves, 15 trifoliolate leaflets, and five other plant parts per replication. Primary leaves and trifoliolate leaves each averaged 20 cm²; stems and roots each averaged 0.3 g; and hypocotyls each averaged 0.1 g fresh weight.

primary leaves or first and second trifoliolate leaves of spray-inoculated bean plants followed typical sigmoidal growth curves with a 1- to 3-day lag phase and a 6- to 9-day exponential growth phase (Figs. 1 and 2). The mean doubling time for R10 was 18.3 hr on Seafarer in five experiments in 1976 (range, 13.6–23.8 hr) and 20.1 hr on Sanilac bean in one experiment in 1977. In 1977, the mean doubling time for Ra in one experiment was 19.8 hr on Sanilac and 17.7 hr on Seafarer. The R10 doubling times in hours (x) during the exponential growth phase, calculated from five experiments in 1976, were inversely linearly correlated with the mean temperature in degrees Celsius (y) by the following equation: $y = 76.1 - 29x$ ($r = 0.94$). Populations of R10 and Ra peaked during the stationary phase and remained stable until leaf abscission. A slow death phase accompanied decomposition of leaves on the ground, and 13 and 23 days after abscission the populations of Ra were only 58 and 30%, respectively, of the level on day 19 (Fig. 2).

Populations of saprophytic bacteria and yeast (YCA) ranged between 10⁴ and 10⁷ cells per 20 cm² of leaf tissue. They were generally stable while blight bacteria were in the exponential growth phase but increased as blight bacteria entered stationary growth phase (Fig. 1).

Multiplication and spread of R10 and R10-S6 in navy bean seedlings. Isolate R10 was associated with all seedling parts soon after seedling emergence after planting R10-infected seed. Primary leaves (average area 20 cm²), stems (average fresh weight 0.3 g), cotyledons, roots (average fresh weight 0.3 g), and terminal buds contained 7.3 × 10³, 1.0 × 10⁶, 7.4 × 10¹, 7.4 × 10⁴, and <20 bacteria per plant part, respectively, 6 days after planting, and 9.9 × 10³, 3.3 × 10⁶, 4.9 × 10³, 2.2 × 10⁵, and <20 bacteria per plant part, respectively, 9 days after planting. No symptoms were observed on these seedlings.

In a second study, a growth profile was developed for isolate R10-S6 in Seafarer seedlings (Fig. 3). Seedlings from seeds infected with R10-S6 emerged in 12 days because of cool weather following planting and the plants grew slowly. The greatest numbers of

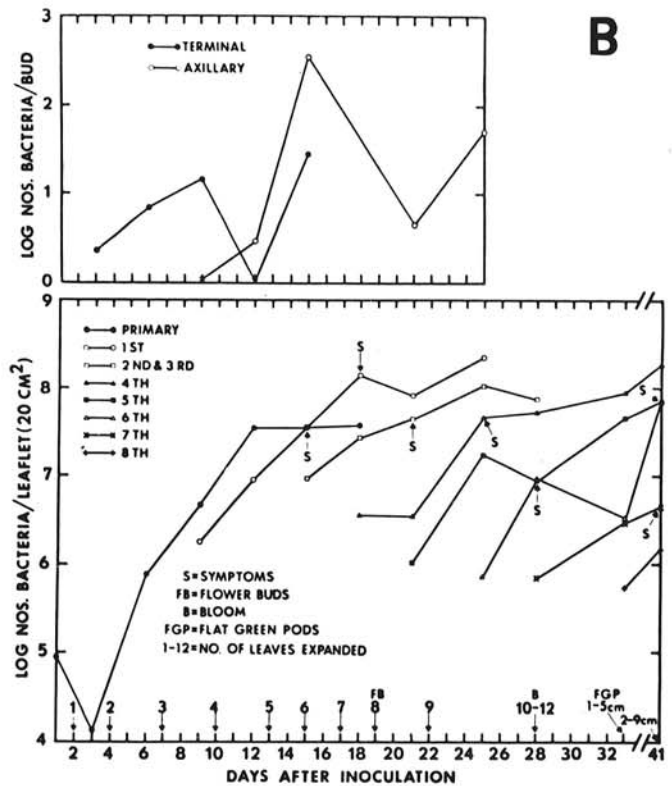
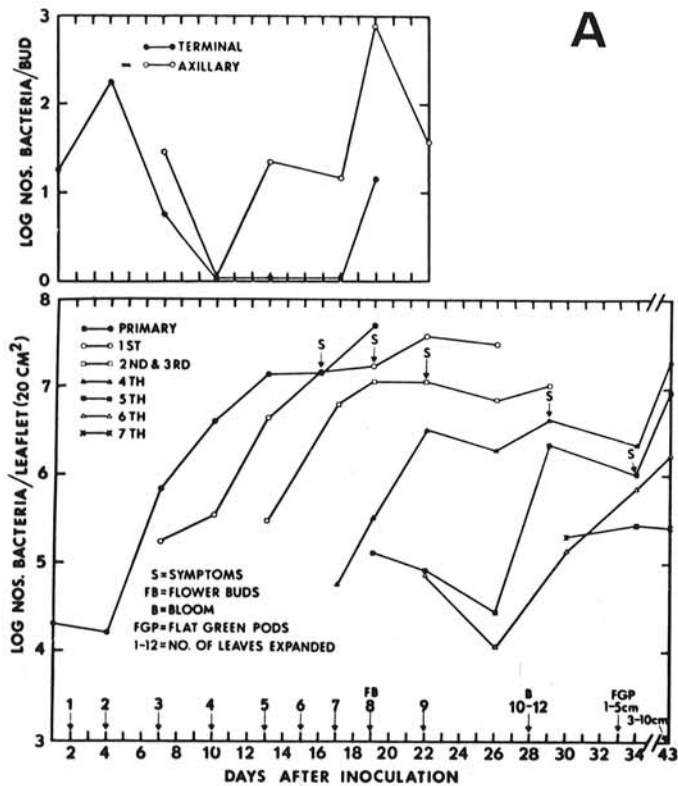


Fig. 4. Colonization by rifampin-resistant isolates **A**, R10 *Xanthomonas phaseoli* var. *fuscans* and **B**, Ra of *X. phaseoli* of the leaves and buds of Sanilac navy bean plants inoculated when 16 days old with aqueous suspensions of isolates R10 and Ra. Data are means of three replications, each with 14–21 leaflets, seven terminal buds, or 10–15 axillary buds. Bean plant developmental stages are defined as follows: "Flower buds," first noted as a cluster of swollen buds at the growing tip; "bloom," the stage in which all lower canopy flowers were open and some upper canopy flowers were closed; "flat green pods," pods with no visible seed filling.

bacteria were associated with the primary and first trifoliolate leaves. Bacteria also were isolated from stems, hypocotyls, and roots throughout the sampling period, but numbers on the roots declined slowly; cotyledons were not sampled. The number of R10-S6 cells that could be removed by washing root and hypocotyl tissue in phosphate buffer were similar to the number obtained by homogenizing samples. Isolate R10-S6 could easily be recovered from stems by printing on RAM.

Multiplication and spread of R10 and Ra in leaves and buds of field-grown beans during the vegetative and early reproductive stages. Growth profiles of Ra and R10 in the leaf canopy from seedling until early reproductive stages were described by a series of bacterial growth curves displaced over time, with each curve describing the state of bacterial growth on a given leaf in the canopy relative to the primary leaf node (Fig. 4A and B). Each leaf of the main stem was colonized by Ra or R10 as it unfurled, so that a gradient was established in the leaf canopy, with the oldest leaves supporting the highest numbers of bacteria. Symptoms first appeared on the primary leaves and then on the trifoliolate leaves from oldest to youngest. Their onset usually occurred during the stationary phase of bacterial growth on each leaf and required a minimum bacterial number as previously described. The sequence of symptom expression observed for the leaves of the main stem also was true for leaves of lateral stems. Pods were colonized in a manner similar to leaves. By bloom, symptoms were detected only up to the fourth (Fig. 4A) and sixth (Fig. 4B) trifoliolate leaves, even though R10 and Ra were detected by plate counts or leaf prints up to the top trifoliolate leaf.

Isolates R10 and Ra were associated with terminal and axillary buds (Fig. 4A and B) and new leaves less than 1 cm long were colonized. Numbers of Ra and R10 in flower buds were similar to numbers in vegetative buds. Up to 1×10^4 blight bacteria per flower were detected in unopened flowers, however, the populations were generally 10^1 to 2×10^2 per flower.

Relation of bacterial numbers and disease severity. In 1977, there was a significant ($P = 0.01$) correlation between the number of lesions and the numbers of R10 or Ra per leaflet (Fig. 5). The data

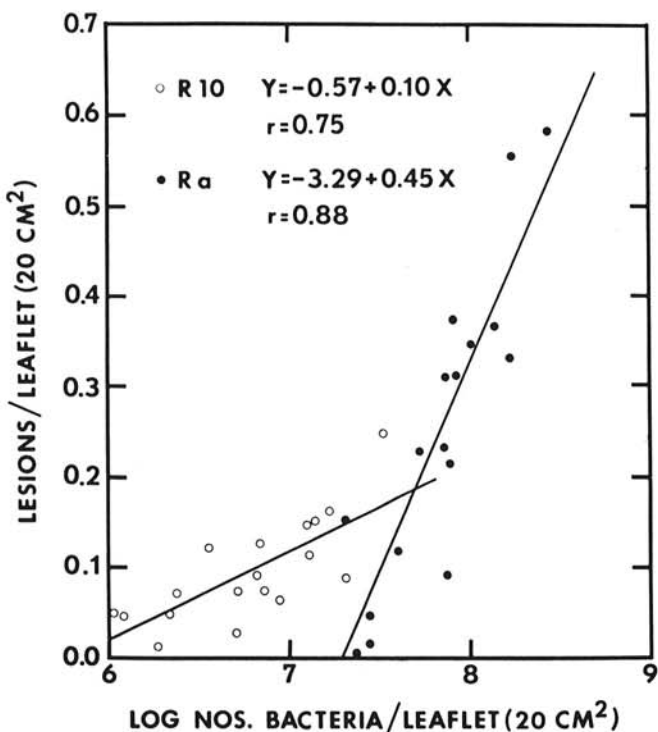


Fig. 5. Effect of numbers of rifampin-resistant isolates R10 of *Xanthomonas phaseoli* var. *fuscans* and Ra of *X. phaseoli* on the severity of disease in the second through the fifth trifoliolate leaves of the main stem of Sanilac navy bean plants inoculated when 16 days old with aqueous suspensions of each isolate. Data are means of three replications of 21 leaves each.

were collected from the same plants that yielded the data shown in Fig. 4(A and B), but only the second through fifth trifoliolate leaves were considered to insure that infection resulted from natural bacterial spread. Isolate Ra produced higher numbers of bacteria per leaflet, higher numbers before initial symptoms, and higher levels of disease than did isolate R10.

Multiplication and systemic spread of R10 and Ra in stems of field-grown navy beans. Ten days after syringe inoculation of navy bean cotyledon scars, isolates R10 and Ra were first detected internally in stems. At 13, 19, 26, and 31 days after inoculation, 20, 64, 71, and 85% of the stems, respectively, were internally infected with R10; at 12, 21, 28, and 31 days after inoculation, 19, 43, 62, and 80% of the stems, respectively, were infected with Ra. In 1976, 75% of Seafarer plants inoculated in the same manner were systemically infected with R10 at harvest, and in 1977, Tuscola plants were infected with R10 and Ra to a similar degree.

In 1978, numbers of R10 and Ra were monitored in stems after the bacteria had been injected into the cotyledonary node of Seafarer beans. Isolates R10 and Ra moved rapidly upward in the main stem. The numbers of R10 and Ra per unit of the main stem during the vegetative and reproductive stages were described by a series of growth curves representing bacterial multiplication in various sections of the main stem (Fig. 6); only the profile of Ra is shown since that of R10 was identical. The doubling time in stem tissue up to the fourth trifoliolate node averaged 22.8 hr (range 14.6–37.6 hr) and 23.7 hr (range 12.3–31.0 hr) for R10 and Ra, respectively. Stem lesions, initially observed as red discolorations, were formed when numbers reached about 10^7 – 10^8 R10 or Ra cells

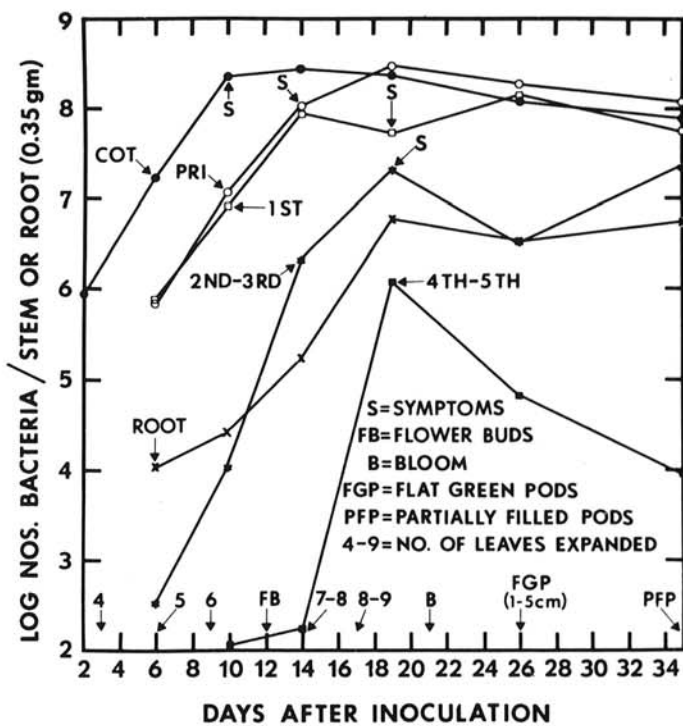


Fig. 6. Colonization by rifampin-resistant isolate Ra of *Xanthomonas phaseoli* of stems and roots of Seafarer navy bean plants inoculated when 20 days old by stabbing the cotyledon scar with a syringe containing a suspension of 10^8 Ra cells per milliliter. Data are means of two replications, each of five stems or roots. Sections of the main stem and root sampled are defined as follows: "root," both the tap and the fibrous roots; "cot," the cotyledon node and internode from the soil line to the primary leaf node; "pri," the primary node and the internode up to the first trifoliolate leaf node; "1st," the first trifoliolate leaf node and the internode up to the second trifoliolate leaf node; "2nd-3rd," the second and third trifoliolate leaf nodes and the internodes up to the fourth trifoliolate leaf node; "4th-5th," the fourth and fifth trifoliolate leaf nodes and the internodes up to the sixth trifoliolate leaf node. Bacterial numbers are based on a fresh weight of 0.35 g, which is approximately the weight of one single node plus internode. For definition of bean developmental stages, see legend to Fig. 3; in addition, "partially filled pods" represent green pods half filled with seeds.

per 0.35 g of stem tissue; lesions appeared first at the injection point and then moved progressively upward. Lesions were most pronounced at the nodes; however, symptoms also were observed at internodes. By bloom, lesions appeared only up to the second and occasionally the third trifoliolate leaf nodes, and they were not widely spread throughout the stem system until near pod maturity. In the leaf canopy, disease development resulting from systemic infection was similar to that detected in leaf studies and shown in Fig. 4A,B.

Isolates R10 and Ra multiplied in the roots (Fig. 6) and established a gradient with the lowest numbers in the lateral roots. No root lesions were detected and most of the bacteria were internal; about 0.03–0.8% of the total numbers of R10 and Ra were isolated from root surfaces in all samples by shaking the roots for 30 sec in water. Pressing roots on solid media also yielded R10 and Ra.

Effect of washing on numbers of blight bacteria from navy bean leaves. The number of blight bacteria recovered by washing was a fairly constant proportion of the total number on leaves throughout the 22-day study (Fig. 7). An average of 27 and 21% of the total R10 numbers on primary leaves of Seafarer was removed during the exponential and stationary phases of bacterial growth, respectively. In a similar experiment, 35 and 22% of the total numbers of R10 were washed from first and second trifoliolate leaves during the exponential and stationary phases, respectively. An average of 46 and 22% of the total numbers of Ra from primary leaves of Sanilac was removed during the exponential and stationary phases, respectively.

Rain-trapped Ra from field-grown navy beans. Numbers of Ra removed by rain decreased proportionally with time compared with the total numbers of Ra on the plants (Fig. 8). Five days after inoculation, 50% of the total blight bacteria associated with primary and first trifoliolate leaves were washed off; however, in this sample total numbers of bacteria from the leaf were determined after the wetting period. Nine and 14 days after inoculation, runoff water contained 16 and 12%, respectively, of the total numbers of Ra on leaves.

Rain runoff also was collected from Sanilac beans inoculated with R10 and Ra (those yielding the data shown in Fig. 4[A and B]). Sampling was not initiated until the leaf canopy was well expanded. At 19, 23, and 26 days after inoculation Ra was detected in runoff water at levels of 7.1×10^4 , 8.1×10^4 , and 2.8×10^4 cells per milliliter, respectively, and isolate R10 was detected at levels of 6.9×10^4 , 7.4×10^4 , and 3.0×10^3 cells per milliliter, respectively. Data collected represent means of three replications.

Detection of surface-borne blight bacteria. Isolates R10 and Ra were isolated from upper and lower leaf surfaces of inoculated and noninoculated leaves by direct and indirect leaf printing. Blight bacteria were detected on all parts of the leaf, but most frequently along the veins. A significantly ($P=0.05$) greater number of bottom surfaces than top surfaces (49 versus 32%) were colonized by R10, and more colonies usually developed from prints of lower surfaces. Similar results were obtained with leaves higher in the leaf canopy and with Ra. Surface disinfestation of symptomless leaves with NaOCl or UV irradiation reduced the Ra and R10 recovered about 20–40% below that of nontreated controls.

DISCUSSION

This study examined the variation of the density of populations of rifampin-resistant isolates of *Xp* and *Xpf* in Michigan navy (pea) beans. Numbers of R10 and Ra in leaves of field-grown plants were similar to those of *Xp* and *Xpf* in beans grown under controlled conditions (1,12) and to those for bacteria inciting other plant diseases (7,10,14,17,22). The exponential growth phase lasted for 6–10 days, and R10 and Ra had average doubling times of 19.4 and 18.8 hr, respectively. During the exponential phase the bacteria grew more slowly at lower temperatures. That symptom expression in bean leaves is a function of bacterial multiplication and requires minimum numbers of bacterial cells is consistent with the findings of Ercolani and Crosse (7).

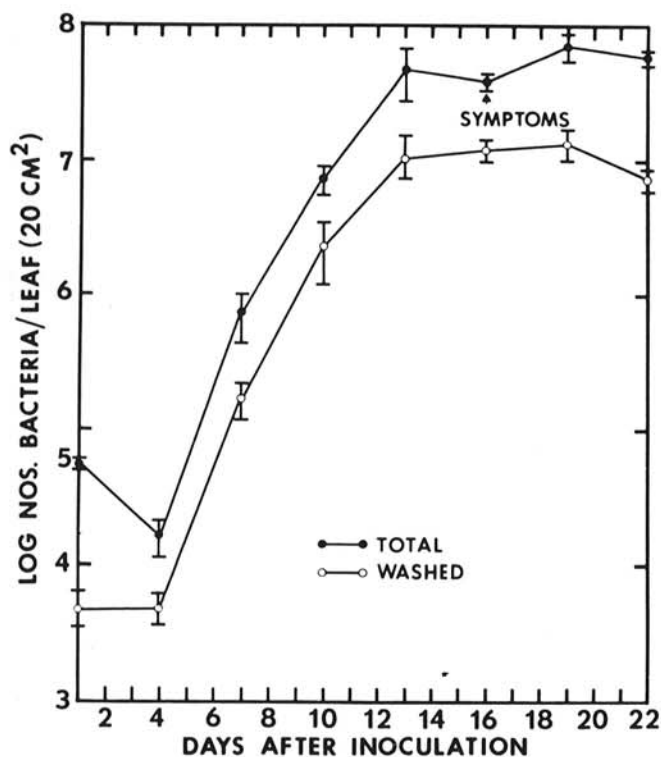


Fig. 7. Comparison of total numbers and numbers of rifampin-resistant isolate R10 of *Xanthomonas phaseoli* readily removed by washing associated with primary leaves of Seafarer navy bean plants inoculated when 11 days old with an aqueous suspension of isolate R10. Data are means of four replications, each of 12 leaves, \pm standard error.

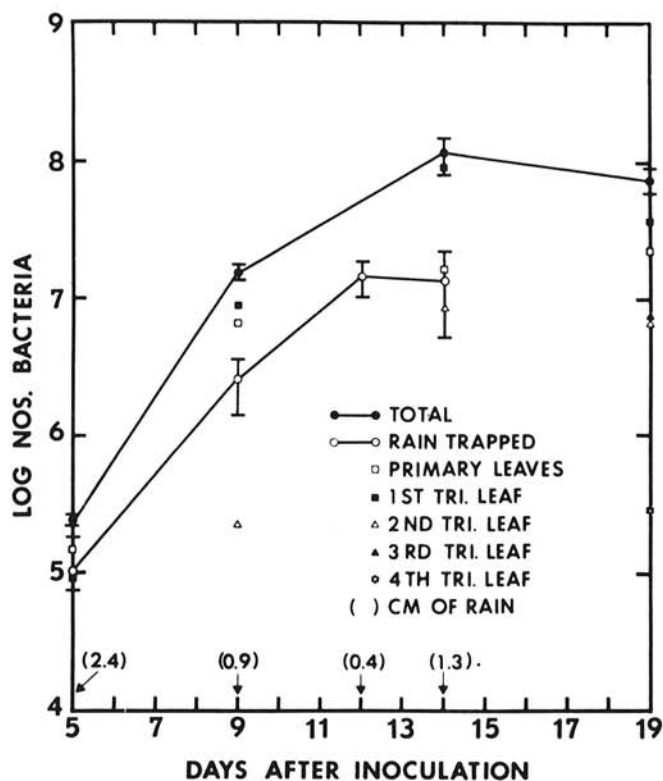


Fig. 8. Comparison of total numbers of rifampin-resistant isolate Ra of *Xanthomonas phaseoli* associated with Seafarer navy beans, and numbers of rain-trapped *X. phaseoli* in leaf runoff water. Eleven-day-old plants were sprayed with an aqueous suspension of isolate Ra. Total populations are means of two replications of seven plants each, \pm standard error, and rain-trapped populations are means of six replications of one plant each, \pm standard error.

The seedling phase of common and fuscous blights is critical to blight development, since inoculum units must become established to insure secondary spread. Primary leaves are the most important site for early bacterial multiplication and colonization of hypocotyls and cotyledons provides a reservoir of inoculum that insures infection of primary leaves. Cotyledons abscise rapidly, thus decreasing their importance as a source of secondary inocula. Initiation of the secondary disease cycle previously was linked to the appearance of oozing lesions on cotyledons and primary leaves (25); in this study, however, secondary spread occurred before seedling symptoms appeared.

Increases in colonization by R10 and Ra during the vegetative stage of plant growth are correlated with the stepwise spread of *Xp* and *Xpf* into the bean leaf canopy, and the correlation of particular bacterial numbers with the appearance of symptoms provides an explanation for the late field development of blight. Primary leaf infection initiates the sequence of upward and outward bacterial spread in the host. Each trifoliolate leaf is colonized by blight bacteria while it is expanding from the apical meristem and an inoculum gradient is established in the canopy. The prerequisite for minimum bacterial numbers before appearance of symptoms is manifested by a period of latent disease after a leaf is initially colonized. Thus, when bacterial populations in the lower leaves reached critical levels and symptoms appeared, the symptom-bearing leaves were hidden by newly expanded foliage. This "umbrella" effect occurred throughout the vegetative phase of plant growth, since rapid leaf expansion continued until bloom. This pattern of disease development was evident in Fig. 4(A and B) since at bloom, blight lesions were present only up to the fourth and fifth trifoliolate leaves, while the tenth to the twelfth trifoliolate leaves were expanded. Field inspection of the plots suggested that the plants were disease free, since only newly expanded leaves were observed. Because growth of navy bean plants is determinate, differentiation of new leaves from growing tips ceases at bloom. Numbers of bacteria in the outermost portion of the leaf canopy reach critical levels only after bloom. Thus, the sudden visibility of disease symptoms after bloom created the effect of a recent blight invasion. These results contradict assertions that unfavorable environmental conditions early in the growing season account for late development of blight (3,24). Isolates R10 and Ra multiplied, spread, and induced disease symptoms in the navy bean plants both before and after bloom.

Several mechanisms accounted for the stepwise spread of Ra and R10 within the bean canopy. Splashing rain is considered the most important dispersal mechanism, and lesions have been considered necessary for secondary spread (23,25) since bacterial ooze from blight lesions is washed away from leaves during rainfall. However, in this study, blight bacteria spread from symptomless leaves, as indicated by isolation of R10 and Ra from the upper leaf canopy before the lower inoculated leaves showed symptoms. Thus, blight bacteria may become widespread in a field before any symptoms appear. Laboratory measurements of leaf washings correlated with direct field measurements of rain-trapped bacteria; at least 10% of the total numbers of *Xp* and *Xpf* associated with leaves can be removed in rain runoff, suggesting the effectiveness of rain-splash dispersal. This figure corresponds to measurements of rain-splash and washing removal from plants infected with other plant pathogenic bacteria (6,9,14). That greater levels of *Xp* and *Xpf* are removed during the exponential than the stationary phase may be explained by the suggestion of Leben et al (14) that the bacteria are "glued down" in older lesions. Extracellular polysaccharide produced by the bacteria comprises a portion of the bacterial ooze covering lesions and in leaves polysaccharide levels would presumably be greatest when bacterial numbers were greatest. Bacteria would become more firmly bound to the leaf by the ooze as the infected tissue dried.

Epiphytic phases were demonstrated for many bacteria (6,8,13,16) but not conclusively for *Xp* and *Xpf*. This study indicates that a portion of the blight bacterial cells was present on the leaf surface, as indicated by leaf-print studies and the reduction in numbers of bacteria after treatment of leaves with bleach or UV light. The presence of epiphytic blight bacteria accounted in part

for the easy removal of bacteria before the appearance of symptoms.

Some pathogenic bacteria multiply in and spread from buds of annual plants to unfurling leaves (15,16); however this behavior has not been documented for *Xp* and *Xpf*. In the present study, bud colonization was an important mechanism for spread of *Xp* and *Xpf* into the leaf canopy. Although multiplication of R10 and Ra in the buds was detected, bud colonization was maintained primarily by rain and dew-trapped bacteria redistributed down the petioles and into the axils (20). Blight bacteria in flower buds and flowers were involved in the colonization of pods.

Burkholder (3) and Zaumeyer (23,24) characterized the systemic phase of *Xp*; however, the importance of systemic infection in the development of common and fuscous blights, especially in navy beans, is poorly understood. Haas (11) suggested that *Xp* does not systemically colonize the navy bean cultivars Sanilac, Seaway, and Seafarer. However, in the present study systemic colonization was an important mechanism for spread of blight bacteria.

The increase in numbers of stem-borne blight bacteria (Fig. 6) was similar to the increase in the tissues of the leaf canopy. Correlation of these increases of R10 and Ra in stems with symptom expression in the plant canopy supports the explanation for late disease development suggested by data on numbers of bacteria in leaves.

Root colonization by *Xp* and *Xpf* has not been fully confirmed. Burkholder (3) observed masses of bacteria in the tap and lateral roots; however, Zaumeyer (24) observed bacteria only in the region when xylem development changes from exarch to endarch type. Stanek and Lasik (19) reported that *Xpf* colonized the bean rhizosphere for 2 wk. Our study indicates that during the seedling phase the bacteria are mainly on the root surface and, with time, the population gradually decreased. A second phase of root colonization occurred after *Xp* and *Xpf* moved systemically into the stem and subsequently downward into the root. Blight bacteria eventually colonized the entire root system and numbers substantially increased. Although most of the bacteria associated with roots was internal during the vegetative and reproductive phases, a small portion was on the root surface throughout the growing season.

Multiplication of *Xp* and *Xpf* and symptom production in bean are affected by environmental conditions and host physiology. In this study, lower mean temperatures increased the doubling time of R10 and, in the stationary phase, indirectly reduced the numbers of bacteria in infected leaves. Further exponential growth of the bacteria was not sustained on senescing leaves. Thus, when minimal populations are present in the field, critical bacterial population levels may not be reached on leaves, and the bacteria appear in a resident phase (13) since no symptoms occur.

In general, the pattern of bacterial multiplication and spread was very similar for Ra and R10. However, Ra was a more aggressive colonizer of the foliage than R10, as indicated by its higher numbers in bean tissues (Fig. 4B versus Fig. 4A) and greater disease production (Fig. 5). In contrast, numbers of Ra and R10 cells were identical in stems. This difference relative to leaf and stem colonization suggests an ecological variation among blight isolates; ie, that isolates may vary in their ability to survive in, spread from, and colonize various bean tissues.

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