Population Dynamics of *Erwinia carotovora* pv. *carotovora* in Potato Stems

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ABSTRACT

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Inoculum (2×10^3 cfu per plant) of *Erwinia carotovora* pv. *carotovora* micropipetted into a potato stem at a leaf axil increased logarithmically in the stem tissue of potato cultivar Norgold Russet, but increased less rapidly in similarly inoculated Russet Burbank potato plants. The population of a weakly virulent strain increased less rapidly than that of a highly virulent strain that caused rapid death of the plants. This weakly virulent strain caused only slight necrosis at the point of inoculation when inoculum of 2×10^7 cfu per plant was used. In infectivity titration tests, specific inoculum doses ranging from 10^8 to 10^2 cfu per plant were injected at a leaf axil of each plant by means of a capillary pipette. Probit analysis of quantal responses (number of stems showing soft rot) of potato cultivars to the highly virulent strain gave ED_{50} values of 1.27×10^5 for the susceptible Norgold Russet and 1.36×10^7 for the more resistant Russet Burbank. A soil strain, serogroup XXIX, was less virulent than a highly virulent strain isolated from a potato stem.

Since the mid-1970s it has been recognized in different geographic areas that *Erwinia carotovora* pv. *carotovora* could cause symptoms of blackleg and soft rot similar to those elicited by *Erwinia carotovora* pv. *atroseptica* in potato (*Solanum tuberosum* L.) stems (10,14,15,17,21–23).

Potato stems have been usually inoculated with soft rotting pathovars of Erwinia by inserting a sterile toothpick smeared with bacteria into the stem (5,9), or cutting off a leaf from the stem with a contaminated knife (2). However, since a standardized quantitative procedure has not been developed for the inoculation of potato stems with measured dosages of bacterial cells, little is known about population dynamics of strains of *E. carotovora* pv. *carotovora* in potato stems.

Recently a technique was developed by Lum and Kelman (13) to deliver a known quantity of inoculum using a calibrated micropipette. With this technique, resistance of tomato plants to *Pseudomonas solanacearum* E. F. Smith was evaluated and the virulence of the various strains of the pathogen was characterized. We used this technique in the present investigation to determine the population dynamics of different strains of *E. carotovora* pv. *carotovora* in stems of two cultivars of potato and to make a quantitative comparison of virulence. A preliminary report of this study has been presented (20).

MATERIALS AND METHODS

Plant culture. Seed tubers of cultivars Norgold Russet and Russet Burbank were obtained from a grower of certified potato seed, and maintained in cold storage at 5 C. Seed tubers to be planted were removed from storage and held at room temperature (24 ± 2 C) for 4–5 days. When treatment to break dormancy was needed, the tubers were treated with Rindite 2 wk before planting (12). Then the tubers were washed in running tap water for 10 min, surface disinfested in 0.5% NaOCl solution for 3 min, and again thoroughly rinsed in tap water. Pieces containing single eyes were removed with a melon-ball scoop and planted 3 cm deep in flats containing Terra-lite vermiculite (Horticultural Products, W. R. Grace & Co., New York, NY 10036). Flats were kept at 24 C in controlled environment growth chambers and watered daily. Hoagland's solution was applied at full strength twice a week. Three weeks after planting, seed pieces with uniform single sprouts were transferred to 12.5-cm-diameter plastic pots containing a 3:1 mixture of quartz sand and muck soil. Pots were then transferred to a greenhouse in which the temperature was maintained at 24 ± 3 C. Plants were inoculated 3 wk later when the stem length was about 30 cm.

Inoculum preparation. Three strains of *E. carotovora* pv. *carotovora*, hereafter referred to as *E. carotovora* strain MR 4, SR 102, or AK 241 (or simply, strain MR 4, SR 102, or AK 241), were used in these experiments. Strain MR 4 had been isolated from a potato stem at the Hancock Experimental Station in Wisconsin (19). Strains SR 102 and AK 241 were obtained from A. Kelman, Department of Plant Pathology, University of Wisconsin-Madison. The serogroups of the strains were determined by S. H. De Boer, Research Station, Vancouver, Canada. The serogroup of strain MR 4 differed from any that is currently known (6); and SR 102, which had been isolated from a potato plant, was of a serogroup similar to that of serogroup XIII (the strain resulted in a reaction of partial identity). These strains were selected for use in this research after being characterized in a preliminary test as weakly yirulent and highly virulent, respectively. Strain AK 241 was included because it was in serogroup XXIX, which had been isolated frequently from soil and plants in Wisconsin (14) and Oregon (18). The strains were maintained as suspensions in capped test tubes in sterile distilled water at 24 ± 2 C.

Bacterial inoculum consisted of 24-hr-old cultures growing on plates of casaminoacids-peptone-glucose (CPG) medium (Difco-glucose, 10 g; Bacto-peptone, 10 g; casaminoacids, 1 g; Bacto-agar, 18 g; and distilled water, 1,000 ml). Cell suspensions were prepared by adding sterile distilled water to each plate, agitating the plate by hand for a few minutes, then transferring the suspension to a sterile test tube. The cell density in this suspension was adjusted turbidimetrically with a Spectronic 20 colorimeter (Bausch & Lomb Co., Rochester, NY 14601) at a wavelength of 600 nm to a concentration of $\sim 2 \times 10^8$ colony-forming units (cfu) per milliliter. This suspension was serially diluted to the desired inoculum concentration. The cfu present in the original and the diluted bacterial suspension were determined by plating serial dilutions on crystal violet pectate (CVP) medium (3).

The desired dosage of *E. carotovora* in 100 µl of suspension was applied by means of a calibrated micropipette (Clay Adams, Division of Becton, Dickinson and Company, Parsippany, NJ 07054). Plants were inoculated at the axil of the sixth expanded leaf

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from the top of the plant. Prior to inoculation, the bud in the axil was removed and a wound, 3–5 mm deep, was made by insertion of the sharp end of a broken micropipette. The bacterial suspension was taken up by the plants within 4–12 hr. The micropipette was left in place. Sixteen plants were inoculated with each dosage level of bacteria.

Sampling method and analysis of the samples. Four of the inoculated plants were selected at random (plants were numbered and numbers selected at random) to be assayed on 1, 2, 4, or 8 days after inoculation. Stem sections 3 cm long were taken at the inoculation point, 2–5 cm above the inoculation point, and 2–5 cm below the inoculation point. When soft rot developed in the stem at the point of inoculation, the latter two stem sections were taken immediately above and below the soft rot areas. In order to determine the populations of *E. carotovora* in the stems, the stem sections were surface-sterilized in 0.5% NaOCl solution for 1 min; the middle 1 cm was then excised, cut into five pieces, and fragmented for 1 min in 10 ml of sterile distilled water in a 50-ml tube of an Omni-Mixer homogenizer (Ivan Sorvall, Inc., Norwalk, CT 06470). The homogenized suspension was diluted 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, and 0.5-ml aliquots of each of the resulting suspensions were then plated on CVP plates. Plates were incubated for 2 days at room temperature (24 ± 2 C); colonies that formed pits in the agar surface typical of those formed by *E. carotovora* were counted (3). The two remaining 1-cm pieces were dried for 24 hr at 105 C and the dry weight per unit length of stem was determined.

In order to statistically analyze the response to inoculum dosage, the probit of the proportion of plants showing soft rot was regressed onto log dosage by using the BMD and BMDP series computer program developed at the Health Sciences Computing Facilities, University of California, Los Angeles 90024. All the experiments were repeated once.

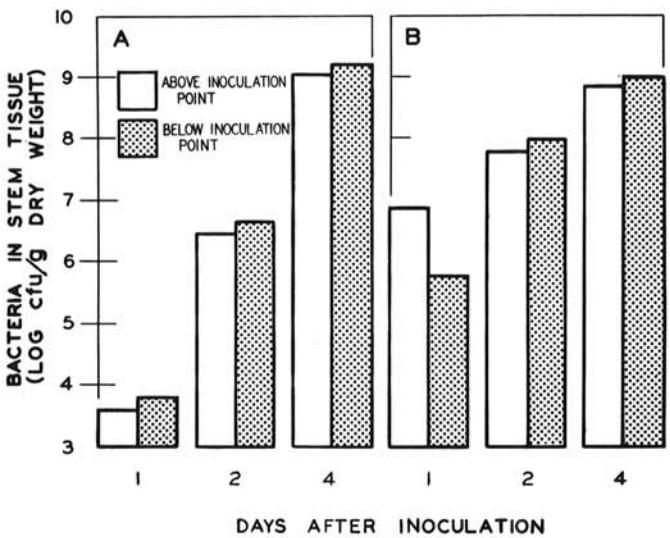

Fig. 1. Populations of *Erwinia carotovora* pv. *carotovora* (strain SR 102) in stems of Norgold Russet after inoculation with **A**, 2×10^3 and **B**, 2×10^7 cfu per plant. Data represent an average of four plants at each sampling time.

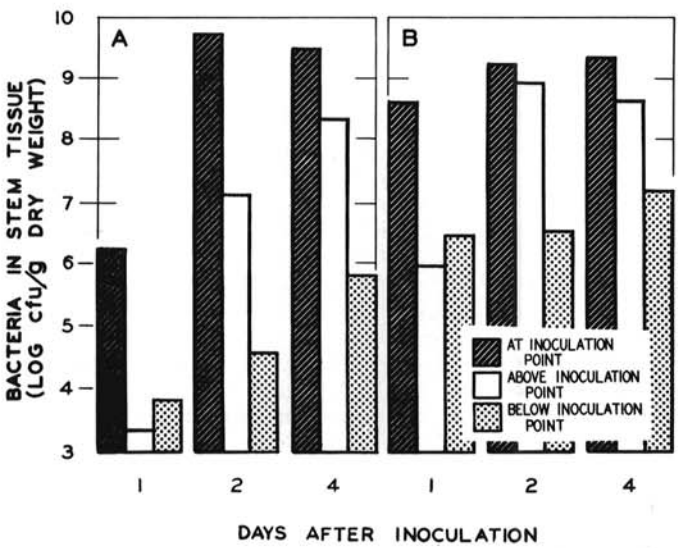

Fig. 2. Populations of *Erwinia carotovora* pv. *carotovora* (strain AK 241) in Norgold Russet stems after inoculation with **A**, 2×10^3 and **B**, 2×10^7 cfu per plant. Data represent an average of four replicate plants at each sampling time.

RESULTS

Plants of cultivar Norgold Russet showed symptoms of epinasty, yellowing, or wilting of the leaves, and discoloration of vascular tissue 2 days after inoculation with the highly virulent strain (SR 102). Soft rot began to appear in the stem tissue by the fourth day after inoculation. At this stage, all the leaves above the inoculation point were completely wilted, and some of the leaves below the inoculation point were either wilted or showed some degree of epinasty. The symptoms were similar, but less severe, in plants of Russet Burbank.

Population dynamics in the stem. Populations of strains SR 102 and AK 241 in the stem sections taken both above and below the inoculation point in Norgold Russet stems increased by a factor of 10^6 (Fig. 1) and 10^5 (Fig. 2), respectively, between days 1 and 4 following inoculation of plants with 2×10^3 cfu per plant. However, when an initial inoculum dosage of 2×10^7 cfu per plant of either SR 102 or AK 241 was used, the populations above the inoculation point increased only by a factor of 10^3. Thus, the population of both strains reached a maximum of 10^9–10^{10} cfu/g dry weight in Norgold Russet prior to tissue breakdown regardless of inoculum dosage (Figs. 1 and 2).

The weakly virulent strain MR 4 caused only necrosis at the inoculation point. With an initial inoculum dosage of 2×10^3 cfu per plant, the population of this strain both above and below the inoculation point increased by 10^3–10^4 between the first and the eighth day after inoculation. An inoculum dosage of 2×10^7 cfu per plant resulted in no greater population in plant tissue (10^7 cfu/g dry weight) than an inoculum dosage of 2×10^3 (Fig. 3). No soft rot developed in any plants inoculated with MR 4.

The population growth of *E. carotovora* in Russet Burbank was not similar to its population growth in Norgold Russet. None of the strains were able to reproduce well in Russet Burbank, even with a

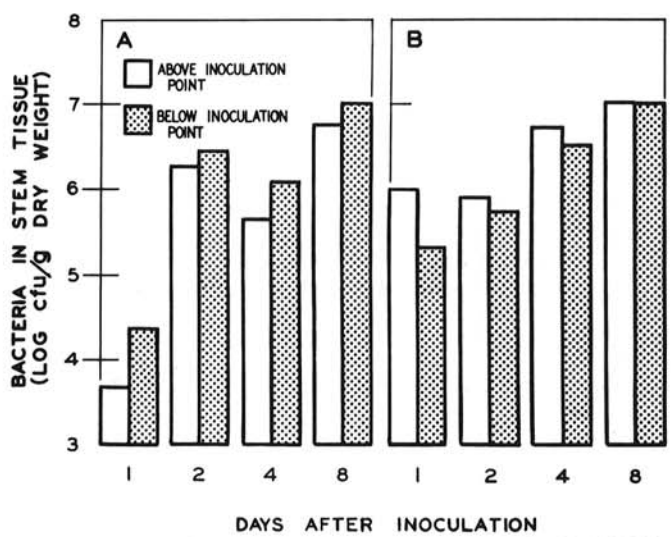

Fig. 3. Populations of *Erwinia carotovora* pv. *carotovora* (strain MR 4) in stems of Norgold Russet plants after inoculation with **A**, 2×10^3 and **B**, 2×10^7 cfu per plant. Data represent an average of four plants at each sampling time.

high inoculum dosage of 2×10^7 cfu per plant (Table 1). Population sizes of MR 4 and SR 102 actually decreased with time. Stem soft rot developed at the inoculation point only with the highly virulent strain SR 102 and in a small number of plants.

Virulence of the strains. To study the differences in virulence of strain SR 102 and strain AK 241 in cultivars Norgold Russet and Russet Burbank, plants were grown in a greenhouse in flats of 20 plants each in vermiculite at temperatures ranging from 24 ± 2 C. For each cultivar, 20 plants were inoculated by means of a micropipette with inoculum dosages ranging from 10^2 to 10^8 cfu per plant. Plants were watered every other day as needed and Hoagland's nutrient solution was applied at full strength twice a week.

Soft rot incidence was greater in stems in Norgold Russet than in those of Russet Burbank. Strain AK 241 was less virulent than SR 102 in both cultivars (Table 2). AK 241 was able to incite stem soft rot in either Norgold Russet or Russet Burbank only when the dosage was increased to 10^8 cfu per plant.

Probit regression analysis of the data showed that log ED_{50} (5.105 ± 0.449) for SR 102 in Norgold Russet was significantly lower than the log ED_{50} (7.133 ± 0.789) for this strain in Russet Burbank, $P = 0.05$. Slopes of probit regression lines for Norgold Russet and Russet Burbank were not significantly different, $P = 0.05$. Chi-square tests on the data for Norgold Russet ($\chi^2 = 1.035$) and for Russet Burbank ($\chi^2 = 0.432$) indicated no heterogeneity of departure of the observed values from the probit regression lines. Probit regression equations fitted the experimental data well, with coefficient values (r) of 0.96 and 0.97 for cultivars Norgold Russet and Russet Burbank, respectively.

DISCUSSION

A reliable standardized quantitative method is crucial for studying the infectivity of bacteria in host tissue. The method should be sufficiently precise that host resistance or susceptibility and bacterial virulence can be characterized quantitatively. Inoculation with a specific dosage of bacterial cells by means of a calibrated capillary micropipette provided a sensitive assay of the infectivity of strains of soft rotting *E. carotovora* pv. *carotovora* in potato stem tissue and a means of comparing rates of population growth following inoculation.

A comparison of the population growth of weakly virulent and highly virulent strains of *E. carotovora* in stems of the susceptible cultivar Norgold Russet demonstrated that the weakly virulent strain, MR 4, was able to increase only up to 10^7 cfu/g dry weight of the stem, even when a high dosage of inoculum was used, and was incapable of producing soft rot. In contrast, a highly virulent strain of the bacterium attained a population level of 10^9 cfu/g dry weight and caused tissue maceration even when inoculated at a low dosage.

Strain AK 241 (serogroup XXIX) was obtained most frequently in isolations from soil in Wisconsin (14). This strain was also the one obtained most frequently in isolations from stems of symptomless plants. Powelson (18) reported that serogroup XXIX was isolated from plants in certain commercial fields in Oregon. Our results indicate that this strain was less virulent in Norgold Russet and Russet Burbank than strain SR 102. This may explain why this strain frequently has been isolated from symptomless plants in the field (14).

Ercolani (8) successfully used infectivity titrations and probit analysis in the study of the dose-response relationships between phytopathogenic bacteria and their hosts. Lum and Kelman (13) used this system to distinguish between tomato lines resistant or susceptible to *Pseudomonas solanacearum*, and also to detect differences in virulence among strains of the bacteria from different geographic areas. The feasibility of using ED_{50} values to define susceptibility or resistance of host species was demonstrated for *Corynebacterium michiganense* on tomato (7) and for *Erwinia chrysanthemi* on corn (24).

On the basis of ED_{50} values obtained in this study significant differences in levels of host resistance or in levels of pathogen virulence were demonstrated. The difference between the ED_{50} values required for induction of stem rot in Norgold Russet and those required for Russet Burbank demonstrated the degree to which Russet Burbank is more resistant to *E. carotovora* than is Norgold Russet. The growth of the bacteria was restricted in cultivar Russet Burbank, whereas in Norgold Russet substantial populations of bacteria developed.

Erinle (9) also found that populations of *E. carotovora* pv. *carotovora* in potato cultivars Majestic and Red Craigs either remained stationary or decreased; as a result, it neither incited blackleg nor caused a rapid soft rotting (hollowing-out) of the stems. In contrast, he found that populations of *Erwinia carotovora* pv. *atroseptica* generally increased and incited blackleg in both of those cultivars. His results also indicate that cultivars that are resistant to *E. carotovora* pv. *carotovora* may not necessarily be resistant to *E. carotovora* pv. *atroseptica*. On the other hand, Munzert and Hunnius (16) found that there was no significant correlation between resistance to the soft rot in the tuber seed pieces and the occurrence of the blackleg in the stems. They suggested the existence of a cultivar-specific mechanism of resistance situated at the base of the stem. Their results also indicate the necessity of testing resistance to each of the characters separately.

The results obtained here demonstrate that *E. carotovora* is an effective stem colonizer; substantial growth of the pathogen occurred in the stem tissue accompanied and preceded by vascular discoloration and vascular damage. Hellmers and Dowson (11) found that when *E. carotovora* pv. *carotovora* and *E. carotovora* pv. *atroseptica* were introduced into the vascular bundles of potato stems, progressive decay of stem tissue followed movement of the pathogen up the stem in the vascular system. However, when inoculum was introduced into interfascicular (parenchyma) regions, it did not lead to infection (11). Histopathological studies of blackleg of potato (1) also indicated the presence and movement of soft rotting *Erwinia* spp. in vascular bundles of host plants. Further studies are needed to understand the development of populations of *Erwinia* spp. in the vascular tissue and the relationships of the *Erwinia* spp. to vascular pathogens such as *Fusarium roseum* (4) and *Verticillium dahliae* (19).

These experiments were conducted with a limited number of strains and a limited number of potato cultivars. A large-scale experiment that would include many cultivars and many bacterial strains would be required to provide an assessment of the range in

TABLE 1. Population sizes (log cfu/g dry weight) of three strains of *Erwinia carotovora* pv. *carotovora* in the stems of potato cultivar Russet Burbank plants after inoculation into the stems by means of a micropipette

Days after inoculation[a]	Sampling area and strain					
	Above[b]			Below[b]		
	MR 4	SR 102	AK 241	MR 4	SR 102	AK 241
1	7.3[c]	7.5	7.4	7.2	7.7	5.8
2	6.3	6.7	6.8	5.4	7.5	6.3
3	6.9	7.5	7.7	7.0	7.3	6.8
4	6.3	5.9	7.5	6.0	6.5	6.4

[a] Inoculum dosage was 2×10^7 cfu per plant.
[b] Two centimeters above or below the inoculation point, or beyond the soft rot area.
[c] Each value is the mean of populations in four plants.

TABLE 2. Percentage of plants with soft rot in stems of potato cultivars Norgold Russet and Russet Burbank inoculated with a range of dosages of two strains of *Erwinia carotovora* pv. *carotovora*

Cultivar	Bacterial strain	Inoculum dosage (log cfu/plant)					
		3	4	5	6	7	8
Norgold Russet	SR 102	0	30	50	70	75	90
	AK 241	0	0	0	0	0	20
Russet Burbank	SR 102	0	5	5	20	45	75
	AK 241	0	0	0	0	0	15

virulence of various strains of *E. carotovora* and of the range of resistance among the potato cultivars in commercial use in the USA.

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