A Bacteriophage-Typing System for Surveying the Diversity and Distribution of Strains of *Erwinia carotovora* in Potato Fields

D. C. Gross, M. L. Powelson, K. M. Regner, and G. K. Radamaker

First, third, and fourth authors: Department of Plant Pathology, Washington State University, Pullman 99164-6430; second author: Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331.

We thank P. Gray for assistance in phage typing and M. Arbogast for serotyping strains.
This research was supported in part by the Washington Potato Commission.
Plant Pathology New Series No. 0077, College of Agriculture and Home Economics Research Center, Project 5508, Washington State University, Pullman 99164-6420.
Accepted for publication 19 October 1990 (submitted for electronic processing).

ABSTRACT

Gross, D. C., Powelson, M. L., Regner, K. M., and Radamaker, G. K. 1991. A bacteriophage-typing system for surveying the diversity and distribution of strains of *Erwinia carotovora* in potato fields. Phytopathology 81:220-226.

A bacteriophage-typing system was developed and used to survey the diversity and distribution among strains of *Erwinia carotovora* subsp. *carotovora* and *E. c.* subsp. *atroseptica* from the rhizospheres and stems of potatoes grown in the Columbia Basin of the Pacific Northwest. With a phage enrichment method and strains of *E. carotovora* from 25 serogroups as hosts, 13 phages displaying distinct host-range activities were isolated from potato and soil samples. In addition, a potato strain of *E. chrysanthemi* was used to isolate phage N (Ech-3), which did not infect strains of *E. carotovora*. All strains of *E. c. atroseptica* were sensitive to at least one of five phages, and strains in both subspecies of *E. carotovora* were sensitive to phage isolates C (304-32), E (101-1), and J (465-2-3-6). In three commercial fields in 1981, the phage groups occurring at midseason were AEJ, E, and EJ for *E. c. atroseptica* and G, GI, EF, and F for *E. c. carotovora*; total rhizosphere and stem populations of *E. carotovora* from symptomless plants ranged from 2×10^3 to 3×10^6 cfu/g (fresh weight) at midseason. In 1982, numbers of *E. carotovora* recovered from stems and rhizospheres increased from low and sporadic levels in late May to over 10^5 cfu/g (fresh weight) by early July. Phage group EJ of *E. c. atroseptica* was predominant among the strains from Norgold Russet and Russet Burbank seed tubers, and it composed 35–43% of the total strains of *E. carotovora* recovered from rhizosphere and stem samples later in the season. No specific phage group was clearly associated with cultivar, date of isolation, and either rhizosphere or stem samples. Of the 389 strains of *E. carotovora* collected over a 2-yr period, 44% and 48% were typed, respectively, to one of 14 phage groups and one of nine serogroups. All strains of *E. c. atroseptica* were members of serogroup I, whereas IV and XXXVII were the two most common serogroups of *E. c. carotovora*. A few phage groups and serogroups were composed of the same strains, including phage group CDGIM and serogroup XVIII, phage groups E and EF and serogroup XXIX, and phage groups D and DL and serogroup XXXVII.

Additional keywords: bacterial soft rot, black leg, *Solanum tuberosum*.

Erwinia carotovora is endemic to the Columbia Basin of the Pacific Northwest where potatoes are grown. This bacterium causes a soft rot of both stems and tubers (4,29,31,35). It can also colonize the vascular tissues to cause wilting that may lead to potato early dying, a syndrome that is intensified by coinfection by *Verticillium dahliae* (29,32). Significant populations of *E. carotovora* have been recovered from symptomless plants and tubers in several major potato production areas of the United States, suggesting that the bacterium is widespread in potato fields (3,4, 13,35). It is common for rhizosphere populations in Washington to exceed 10^6 cfu/g (fresh weight) in samples that lack discernible soft rot or wilt symptoms (17). However, *E. carotovora* is not restricted to a plant source, since it can be recovered from surface- and well-water reservoirs that serve as sources of irrigation (4,19). Thus, *E. carotovora* can exist in disparate habitats, complicating disease control measures aimed at eliminating primary sources of inoculum.

Diverse strains of *E. carotovora* have been isolated from potatoes, reflecting possible differences in the field environment, aboveground or belowground locations, or the original inoculum source (4,9,10,26). Strains are readily identified as either *E. c.* subsp. *atroseptica* or *E. c.* subsp. *carotovora*, and can cause indistinguishable symptoms in susceptible potato cultivars. Nevertheless, *E. c. atroseptica* is associated more frequently with early-season black leg than is *E. c. carotovora*, reflecting its ability to parasitize at lower temperatures (27). *E. c. carotovora* is the predominant subspecies at midseason and composes approximately 80% of the strains recovered from potatoes grown in the Columbia Basin (28,35).

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De Boer et al (12) pioneered the use of the Ouchterlony double-diffusion test for categorizing strains of *E. carotovora* into serogroups based on similarities in the lipopolysaccharide (LPS) O-antigen (10,11). Serological relationships among strains of *E. carotovora* have been used since then to document strain heterogeneity within the species (14,15), and to explore the ecology of strains in potato fields (4,9,26,30). For example, strains of *E. c. carotovora* have been divided into over 40 serogroups, of which IV, V, XIV, XVIII, XXIX, XXXVII, and XXXIX are common to water, foliage, and diseased stems in the Columbia Basin in Oregon (4). Notably, serogroup XXIX was frequently identified in soil samples collected in Oregon and Wisconsin prior to planting (4,22); 15.2 and 5.3% of the strains from surface- and well-water sources in Oregon were classified as serogroups XXIX and XVIII, respectively (4). Serogroup III was prevalent on seed tubers grown in a mountain valley in British Columbia, and De Boer (9) concluded that this serogroup composes the major belowground population. A second population of serogroup XVIII is predominant on foliage, possibly originating from airborne sources. These observations and others (3,4,26) suggest that environmental factors contribute more to the success of specific serogroups than the inoculum source. However, a serious limitation to using the serological method for strain differentiation in ecological and epidemiological studies is the prevalence of strains of *E. c. carotovora* unreactive to available antisera. Cappaert et al (4) reported that only 20% of 614 strains of *E. c. carotovora* from stems with aerial soft rot symptoms in Oregon could be identified, and De Boer (10) estimated that only about two thirds of the strains from various sources are typeable with the present system. In contrast, *E. c. atroseptica* is highly serologically homogeneous, with about 98% of the strains belonging

to serogroup I and the remaining strains placed in only a few serogroups (10). Consequently, tracing the distribution of strains of *E. c. atroseptica* in nature is not feasible because of high serological homogeneity.

Virulent bacteriophages exhibit host specificity that has proven valuable in differentiating ecological types within a species (2). Although phage typing has been used extensively to type strains of medically important species, few phage-typing systems have been developed for phytobacteria (2). Liew and Alvarez (20) used five virulent phages, isolated from soils of cabbage fields and infected seed, to segregate strains of *Xanthomonas campestris* pv. *campestris* into two main lysotypes that were further divided into seven subtypes. Crosse and Garrett (6) used virulent phages as diagnostic aids to distinguish cherry and plum strains of *Pseudomonas syringae* pv. *morsprunorum*. A rapid presumptive test for *P. s.* pv. *tomato* based on sensitivity to pathovar-indicative phages was developed by Cuppels (7); it permits direct testing of bacteria in tomato leaf lesions without preliminary isolation and purification. Despite these examples illustrating the utility of phage typing in phytobacteriology, phages active against *E. carotovora* and *E. chrysanthemi* have rarely been isolated from soil or infected plant material (2,5). After failing to isolate virulent phages for *E. chrysanthemi* from soil and infected plants, Paulin and Nassan (25) used temperate phages with activity spectra that showed limited correlation with bacterial strain clusters originating from specific host plants.

The purpose of this study was to isolate virulent phages that would be useful for typing strains of *E. c. carotovora* and *E. c. atroseptica* recovered from potatoes grown in the Columbia Basin. Because a high proportion of strains of *E. carotovora* cannot be differentiated by current serological methods, the aim was to develop a phage-typing system that would augment identification methods used to trace strains in epidemiological studies.

TABLE 1. Bacteriophage types and serogroups of strains of *Erwinia carotovora* subsp. *carotovora*, *E. c.* subsp. *atroseptica*, and *E. chrysanthemi* used to isolate virulent phages from potato fields[a]

Strain no.	Species and subspecies	Serogroup	Phage type
SK31	*E. c. atroseptica*	I	AE
cc101	*E. c. carotovora*	III	E
cc302	*E. c. carotovora*	IV	None
cc102	*E. c. carotovora*	V	None
SK189	*E. c. carotovora*	VI	None
SK68	*E. c. carotovora*	VII	EKM
N20	*E. c. carotovora*	VIII	None
SK61	*E. c. carotovora*	X	None
cc106	*E. c. carotovora*	XI	GI
cc502	*E. c. carotovora*	XIV	G
SK65	*E. c. carotovora*	XIV	G
cc104	*E. c. carotovora*	XV	BM
414-3-1	*E. c. carotovora*	XVIII	CIM
465-2-3	*E. c. atroseptica*	XVIII	EJ
SK6	*E. c. atroseptica*	XVIII	C
cc505	*E. c. carotovora*	XXVII	None
cc304	*E. c. carotovora*	XXVIII	CFGIM
W3C33	*E. c. carotovora*	XXVIII	CM
cc501	*E. c. carotovora*	XXIX	EF
cc305	*E. c. carotovora*	XXXI	None
cc506	*E. c. carotovora*	XXXII	None
cc306	*E. c. carotovora*	XXXIII	CGI
cc503	*E. c. carotovora*	XXXIV	None
cc504	*E. c. carotovora*	XXXIV	EH
cc301	*E. c. carotovora*	XXXV	None
cc108	*E. c. carotovora*	XXXVI	None
cc303	*E. c. carotovora*	XXXVII	DL
W3C18	*E. c. carotovora*	XXXVII	DL
cc110	*E. c. carotovora*	XXXVIII	None
cc105	*E. c. carotovora*	XXXIX	None
cc103	*E. c. carotovora*	XL	F
W3CH1	*E. chrysanthemi*	None	N

[a] Bacteriophage types were determined by reactions to the lytic phages described in Table 2.

In order to obtain phages with a useful degree of specificity, soil and plant samples were collected from potato fields. Phages with distinctive host ranges were used to differentiate strains of *E. carotovora* recovered from potato over a 2-year period.

MATERIALS AND METHODS

***Erwinia* strains.** The 31 serologically characterized strains of *E. c. carotovora* and *E. c. atroseptica* used for phage enrichment to isolate phages are listed in Table 1 along with one strain of *E. chrysanthemi*. All strains of *E. c. carotovora* and *E. c. atroseptica*, except for W3C18 and W3C33, were obtained from M. L. Powelson. Strains cc101 through cc505 were isolated from potatoes grown in Oregon or from seed tubers. Strains 414-3-1 and 465-2-3 were isolated from well water and soil, respectively; strains SK6, SK31, SK61, SK65, SK68, and SK189 were described previously (14). Strains W3C18 and W3C33 of *E. c. carotovora* and strain W3CH1 of *E. chrysanthemi* were isolated from diseased potato stems collected in 1980 from commercial Russet Burbank potato fields near Quincy, Washington.

Isolation and purification of virulent phages. Stem tissue and rhizosphere soil used as sources of phages were collected from 15 commercial potato (*Solanum tuberosum* L.) fields in the Columbia Basin of Washington in 1980. In each field, stems and rhizosphere soil were collected from three plants with symptoms of potato early dying at scattered locations. Soil and stem samples were bulked separately, chilled on ice for transport to the laboratory, and stored at 4 C until they were used for isolation of phages.

In general, phage isolation and purification methods described by Billing (1) were used. The soft rot erwinias were grown at 25 C in 10-ml portions of nutrient broth-yeast extract (NBY) liquid medium (33) to 10^8 cfu/ml, as determined by optical density and comparison to a standard growth curve. For each strain, 1-g (fresh weight) portions of soil or stem tissue were added to a 10^8-cfu/ml log-phase culture and incubated overnight at 25 C with slow rotary shaking. After incubation, cells and debris were pelleted by centrifugation for 15 min at 11,700 *g* and discarded. One 5-ml portion of the supernatant was sterilized by the addition of 500 μl of chloroform, and the other 5-ml portion was sterilized by a standard membrane filtration method (1). Sterile suspensions were stored in sterile screw-cap tubes at 4 C until needed.

Both suspensions were tested for the presence of virulent phage by screening for lysis of the host bacterium. After reaching the exponential growth phase, bacteria were adjusted to 10^8 cfu/ml in NBY broth at 25 C. NBY agar plates were then overlaid with 2.5 ml of NBY soft agar (0.7% [w/v] agar) containing 0.1 ml of bacterial suspension. Duplicate plates were spot-tested (10 μl) with both sterile suspensions and incubated at 25 C for 16–24 hr. Positive reactions were observed as either clear (or turbid) plaques or zones of confluent lysis within the test area.

To obtain pure phage strains, suspensions yielding lytic zones were serially diluted in NBY broth and mixed (0.1 ml) with 2.5 ml of NBY soft agar containing 10^8 cfu/ml of the host strain. Individual plaques that formed on NBY agar were picked with a straight wire, mixed with 2.5 ml of NBY soft agar, diluted in NBY soft agar if necessary, and mixed with the host strain before pour plating onto NBY agar. This procedure of propagating isolated plaques was repeated a total of four times to yield phage isolates that exhibited uniform plaque morphology.

Propagation and preservation of bacteria and phages. Strains of *E. carotovora* and *E. chrysanthemi* were routinely cultured at 25 C on NBY agar and stored at 4 C. Reference stocks of bacterial strains were preserved by lyophilization. Long-term storage of field strains of *Erwinia* was in sterile distilled water at 4 C and in glycerol-minimal salts buffer (18) at −80 C. High-titer stocks of purified phages were prepared using a modification of the plate method described by Billing (1). NBY soft agar from a plate with confluent lysis was scraped into a sterile flask (250-ml Erlenmeyer) containing NBY liquid medium (10 ml) and slowly shaken (50 rpm) for 4 hr. After removal of agar and cells by centrifugation, the supernatant was mixed slowly with chloroform

(500 μl) in a screw-cap test tube and stored at 4 C. Stocks containing 10^9–10^{12} plaque-forming units (pfu) per milliliter were prepared for all phages. For long-term storage, phage preparations in NBY medium were mixed with an equal volume of glycerol and stored at −80 C.

Serological and phage typing. All field and reference strains of *E. carotovora* were serologically typed by the Ouchterlony double-diffusion technique (24). Antisera produced against 24 serogroups were used in immunodiffusion tests as previously described (30). The strains of *E. carotovora* representing various serogroups (Table 1), together with 389 field strains of *E. carotovora*, were grown for phage typing in NBY medium, adjusted to 10^8 cfu/ml, and overlaid on NBY agar to prepare a uniform bacterial lawn as described above. Each phage preparation was diluted to a routine test dilution that was 10 times the number of plaque-forming units per milliliter required for confluent lysis of the propagating strain; routine test dilution titers ranged between 10^4 and 10^6 pfu/ml, depending on the phage isolate. Duplicate plates were spot-tested (5 μl) with each of the 14 distinct phages (Table 2) at routine test dilution. After incubation for 16–24 hr at 25 C, sensitivities of bacteria to phages were recorded. All strains of *E. carotovora* were phage typed on at least two occasions to verify the phage group. Original propagating strains were routinely used to monitor activities of phage preparations.

Field sampling and enumeration of bacterial populations. Three commercial potato fields in the Columbia Basin of Washington planted with the cultivar Russet Burbank were sampled for *E. carotovora* in 1981 (fields A, B, and C), and two fields in 1982 (fields D and E). In addition, experimental plantings of the potato cultivars Russet Burbank and Norgold Russet at field F were made in 1982 as previously described (35). Samples of seed tubers used to plant field F were reserved for isolation of *E. carotovora.*

Fields in 1981 were sampled once at about midseason (23 July), whereas all fields in 1982 were sampled at approximately 3-wk intervals beginning 4–6 wk after planting. Four plants were sampled in different quadrants of each field in 1981, whereas three plants were sampled (35) at each of three sites in fields on all sampling dates in 1982. Only plants that did not show soft rot symptoms were sampled. Intact potato stems were bulked at each sampling site, and roots and loosely adhering soil were collected in separate bags. Plant samples from different locations within a field were bagged separately, labeled, and transported to the laboratory as described above. Field collections were stored at 4 C for 12–18 hr prior to assaying for bacterial populations.

Populations of *E. carotovora* and total bacteria were determined by plate counts on crystal violet-pectate agar (8) and tryptic soy agar (23), respectively, by methods essentially described by Xu and Gross (35). Bacteria were recovered from weighed root samples (~1.5 g) by washing (30 min, 250 rpm) in magnesium sulfate (100 mM, 10 ml). The wash solution was serially diluted in 12.5 mM sterile potassium phosphate buffer, pH 7.2, before plating. Stem samples were weighed (~1 g), briefly rinsed in sterile deionized water, ground in phosphate buffer (10 ml) with mortar and pestle, and serially diluted and plated. Stem sections were excised just above the soil line, halfway up the stem, and about 5–10 cm from the top of the stem. Samples from different parts of the stem were processed separately. Strains of *E. carotovora* were recovered from potato tubers as previously described (35); their populations were not determined.

Characterization of field strains of *E. carotovora.* Three to five colonies of *E. carotovora* per original isolation were streaked to fresh crystal violet-pectate agar and subsequently purified to colony homogeneity. The determinative tests described by De Boer et al (13) were used to distinguish strains of *E. c. atroseptica* from *E. c. carotovora*, and they included production of acid from α-methyl D-glucoside, production of reducing substances from sucrose, and growth at 36 C. A test for phosphatase production (16) was used to identify *E. chrysanthemi* (4).

RESULTS

Isolation and host range of virulent phages from potato. A total of 31 strains representing 25 serogroups of *E. c. carotovora* and *E. c. atroseptica* plus one strain of *E. chrysanthemi* were used as hosts to enrich for the isolation of virulent phages from potato stems and rhizosphere soils (Table 1). Only 52 of the ~960 enrichment cultures yielded phages after testing the 32 reference strains for sensitivity; 14 of these phages exhibited unique host ranges (Table 2 and Fig. 1). Phages were isolated from both stem and rhizosphere soil samples. Phages were not isolated for 13 strains of *E. c. carotovora*, and these bacteria proved to be insensitive to all preparations yielding phages (Table 1). Of the 25 serogroups of *E. c. carotovora* and *E. c. atroseptica* tested, strains from 13 serogroups were found to be sensitive to at least one of the 14 different phages.

Phages were isolated most frequently with strains SK31 of *E. c. atroseptica* and W3CH1 of *E. chrysanthemi* as hosts. Of the 52 total phage preparations, 14 and 8 were obtained with strains SK31 and W3CH1, respectively, as hosts. Furthermore, one third of the sampled potato fields yielded virulent phages that were restricted in host range to strain W3CH1 (Table 2). Phages isolated using strain SK31 as a host were all identical in host range to phage isolate A (I-4). Strains SK31 and W3CH1 were not observed to produce temperate phages.

The unique host ranges of the 14 virulent phages are listed in conjunction with their original propagative strains in Table 2. However, phage isolates D (303-20) and L (W3C18-1) were distinguished only with field strains of *E. carotovora* susceptible to phage isolate D (303-20) and not to phage isolate L (W3C18-1) (Fig. 1). Six of the phage isolates (A [I-4]; B [104-41]; H [504-35]; J [465-2-3-6]; K [SK68-1]; and N [Ech-3]) were restricted to the original propagative strain (Table 2). In contrast, phage

TABLE 2. Host range of virulent bacteriophages isolated from potatoes and field soil

Phage isolate	Bacterial strain	Phage sensitivity[a]														Phage type[b]
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	
A (I-4)	*E. c. atroseptica* SK31	+	−	−	−	+	−	−	−	−	−	−	−	−	−	AE
B (104-41)	*E. c. carotovora* cc104	−	+	−	−	−	−	−	−	−	−	−	−	+	−	BM
C (304-32)	*E. c. carotovora* cc304	−	−	+	−	−	+	+	−	+	−	−	−	+	−	CFGIM
D (303-20)	*E. c. carotovora* cc303	−	−	−	+	−	−	−	−	−	−	−	+	−	−	DL
E (101-1)	*E. c. carotovora* cc101	−	−	−	−	+	−	−	−	−	−	−	−	−	−	E
F (501-34)	*E. c. carotovora* cc501	−	−	−	−	+	+	−	−	−	−	−	−	−	−	EF
G (502-1)	*E. c. carotovora* cc502	−	−	−	−	−	−	+	−	−	−	−	−	−	−	G
H (504-35)	*E. c. carotovora* cc504	−	−	−	−	+	−	−	+	−	−	−	−	−	−	EH
I (414-3-1-1)	*E. c. carotovora* 414-3-1	−	−	+	−	−	−	−	−	+	−	−	−	+	−	CIM
J (465-2-3-6)	*E. c. atroseptica* 465-2-3	−	−	−	−	+	−	−	−	−	+	−	−	−	−	EJ
K (SK68-1)	*E. c. carotovora* SK68	−	−	−	−	+	−	−	−	−	−	+	−	+	−	EKM
L (W3C18-1)	*E. c. carotovora* W3C18	−	−	−	+	−	−	−	−	−	−	−	+	−	−	DL
M (W3C33-1)	*E. c. carotovora* W3C33	−	−	+	−	−	−	−	−	−	−	−	−	+	−	CM
N (Ech-3)	*E. chrysanthemi* W3CH1	−	−	−	−	−	−	−	−	−	−	−	−	−	+	N

[a] + = Sensitive; − = resistant. Phages were spotted at routine test dilution.
[b] Phage type shown for propagative strain.

isolate E (101-1) had the broadest host range, infecting strains of both subspecies of *E. carotovora*. The six strains susceptible to phage isolate E (101-1) were placed in six serogroups identified as I, III, VII, XVIII, XXIX, and XXXIV (Table 1). The host ranges of phages were somewhat restricted to one or the other subspecies of *E. carotovora* (Tables 1 and 2). Strains of *E. c. atroseptica* representing serogroups I and XVIII were susceptible only to one or more of phage isolates A (I-4), C (304-32), E (101-1), and J (465-2-3-6); isolates C (304-32), E (101-1), and J (465-2-3-6) were the only phages that could infect strains of both subspecies of *E. carotovora*. Phage isolate N (Ech-3) was restricted exclusively to the potato strain W3CH1 of *E. chrysanthemi*. Of the propagative strains, cc304 was susceptible to the most phages and was placed in phage group CFGIM (Table 2).

Most of the phages consistently produced clear plaques on lawns of susceptible strains. The notable exceptions were phage isolates A (I-4), B (104-41), H (504-35), and K (SK68-1), which consistently produced turbid plaques. Plaque size ranged from minute areas of lysis less than 1 mm in diameter, as observed for phage isolate F (501-34), to large plaques 2–3 mm in diameter, as observed for phage isolate C (304-32). Plaques were also examined for halos surrounding the areas of lysis and were noted to occur for phage isolates C (304-32), D (303-20), G (502-1), L (W3C18-1), and M (W3C33-1).

Phage types of *E. carotovora* isolated in 1981. Average middle stem and rhizosphere populations of *E. carotovora* in the three fields ranged from 2×10^3 to 3×10^6 cfu/g (fresh weight) (Fig. 2). None of the plants sampled on 23 July showed visible symptoms of early dying, even though relatively high populations of the bacterium were recovered at both points of sampling. Field A had the highest average populations of *E. carotovora*, and 67% of the strains were identified as *E. c. atroseptica*. In contrast, only 25 and 5% of the strains recovered from plants collected from fields B and C, respectively, were *E. c. atroseptica*. Regardless of the field, *E. c. atroseptica* was isolated from both stem and rhizosphere samples with no discernible tendency to be recovered from one sample source over another.

Approximately 20 strains from each of the three potato fields were phage typed. In field A, strains of *E. c. atroseptica* were placed in phage groups AEJ (11%), E (45%), and EJ (11%), whereas the remaining strains (33%) were *E. c. carotovora* and were insensitive to all phages. In field B, all strains of *E. c. atroseptica* were placed in phage group E (30%), whereas strains of *E. c. carotovora* were more diverse, representing phage groups G (15%), GI (5%), and EF (15%); the remaining strains (35%) could not be typed. As few as 20% of the strains from field C were sensitive to phages; 15% were identified as *E. c. carotovora* in phage group F, and the sole strain of *E. c. atroseptica* was typed to phage group EJ (5%). Regardless of the field, no specific phage group was clearly associated with samples collected from either potato stems or rhizospheres.

Phage types of *E. carotovora* isolated in 1982. Average seasonal populations of *E. carotovora* and total bacteria recovered from potato fields in 1982 at fields D and E did not differ notably from those shown for a Russet Burbank planting at field F (Fig. 3). Typically, numbers of *E. carotovora* recovered from stems and rhizospheres increased dramatically from low and sporadic levels in late May to over 10^5 cfu/g (fresh weight) by early July. Average *E. carotovora* populations for the Russet Burbank planting at field F were about 10-fold higher in the rhizosphere at midseason than those recovered from the middle section of stems (Fig. 3). Total bacterial populations ranged from 10^8 to 10^9 cfu/g (fresh weight) in the rhizosphere and from 10^6 to 10^7 cfu/g (fresh weight) in the middle section of stems at midseason.

Strains of *E. c. atroseptica* were recovered predominantly from Norgold Russet and Russet Burbank potato seed pieces. All strains of *E. c. atroseptica* from Norgold Russet seed tubers were typed to phage group EJ (85% of total *E. carotovora*); the strains of

BACTERIOPHAGE GROUP

SEROGROUP	AE	AEJ	E	EJ	EH	EF	F	FGI	G	GI	CF	CDGIM	D	DL	None	Total
I	8	1	12	93												114
IV			1		1										15	17
V			1				3								5	9
VII															1	1
XIV									5						1	6
XVIII												6				6
XXIX			3			4										7
XXXV															7	7
XXXVII													11	7		18
None			4		6			1		2	1				190	204
Total	8	1	21	93	7	4	3	1	5	2	1	6	11	7	219	389

Fig. 1. Diversity of 389 strains of *Erwinia carotovora* from Columbia Basin potato fields as related to bacteriophage group versus serogroup. Total number of strains that exhibit a particular combination of bacteriophage group and serogroup is identified within the square. Bacteria were isolated from seed tubers and from the stems and rhizospheres of potato plants collected at midseason in 1981 (fields A, B, and C) and at approximately 3-wk intervals during the 1982 (fields D, E, and F) growing season. All 114 strains identified as *E. c. atroseptica* were placed in serogroup I; the remaining 275 strains were identified as *E. c. carotovora*. Bacteria were isolated only from potato plants that did not show soft rot symptoms.

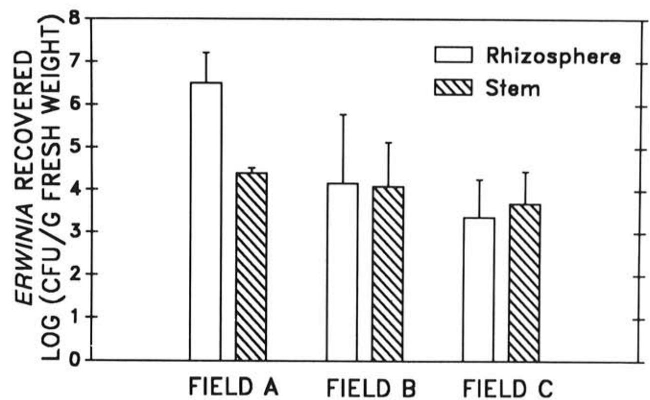

Fig. 2. Rhizosphere and stem (middle stem sections) populations of *Erwinia carotovora* recovered from Russet Burbank potatoes sampled at midseason from commercial fields located in the Columbia Basin of Washington (fields A, B, and C). Potatoes were sampled 23 July 1981. Bacterial populations are averages from four plants collected in different quadrants of each potato field. Vertical bars indicate standard error.

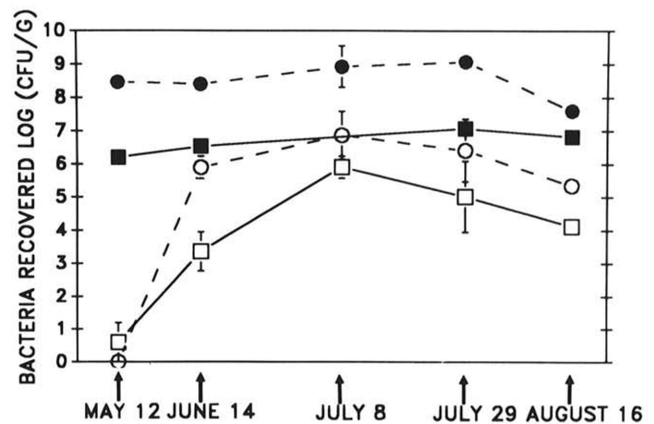

Fig. 3. Seasonal populations of *Erwinia carotovora* (open symbols) and total bacteria (closed symbols) recovered from the rhizosphere (○, ●) and stems (□, ■) (middle stem sections) of Russet Burbank potatoes grown in the Columbia Basin in 1982 (field F). Bacterial populations are averages of each collection. All collections consisted of three plants from each of three sites in the field. Vertical bars indicate standard error.

E. c. atroseptica from Russet Burbank seed tubers were typed to phage group EJ (78%) and E (11%). None of the strains of *E. c. carotovora* from seed tubers of both cultivars were susceptible to phages.

Of the 58 strains of *E. carotovora* recovered from the Norgold Russet planting at field F, 25 (43%) were identified as *E. c. atroseptica* in phage group EJ (Fig. 4A). All of the remaining strains were identified as *E. c. carotovora* and were not susceptible to any phage. In comparison, 164 strains were recovered from the Russet Burbank planting at field F and typed to phage groups AEJ (0.6%), E (3%), and EJ (34.8%) for *E. c. atroseptica* and phage groups E (2.4%), EF (0.6%), CF (0.6%), CDGIM (1.8%), and DL (2.4%) for *E. c. carotovora*; 88 (53.7%) strains of *E. c. carotovora* were not susceptible to phages (Fig. 4B). The phage groups appeared to be associated with neither sampling times early, midway, or late in the growing season nor samples collected from stems or rhizospheres. For example, members of phage group EJ were isolated in almost equal proportions from the stems and rhizospheres of Russet Burbank potatoes, and they were isolated from early June to late August. Moreover, no associations of phage group with isolation from either the top, middle, or bottom section of potato stems were observed.

Similar observations were made for strains of *E. carotovora* from fields D and E. *E. c. carotovora* was predominant, and over half of the strains were insensitive to all phages. None of the phage groups (summarized in Fig. 1) were associated clearly with a specific part of the plant or with a particular date of isolation. The only possible exceptions were phage groups D and DL, where 16 of the 18 strains originated from stem samples collected in Russet Burbank fields D, E, and F, and phage group EH, where all seven strains came from stem samples from fields D and E.

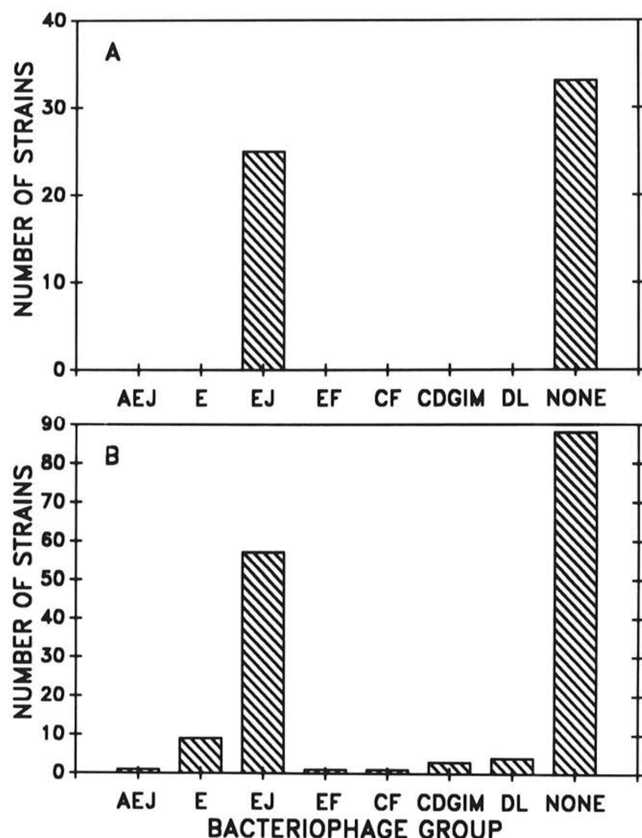

Fig. 4. Bacteriophage groups identified for strains of *Erwinia carotovora* from Norgold Russet (**A**) and Russet Burbank (**B**) potatoes grown in field F in 1982. Total numbers of strains analyzed were 58 from Norgold Russet and 164 from Russet Burbank. Bacteria were isolated from specimens collected at approximately 3-wk intervals during the growing season. Average seasonal populations of *E. carotovora* recovered from Russet Burbank potato rhizospheres and stems are shown in Fig. 3.

Serogroups and phage types of strains of *E. carotovora*. Results of serotyping and phage typing 389 strains of *E. carotovora* from potatoes grown in the Columbia Basin are summarized in Fig. 1. Strains susceptible to phages were arranged into 14 phage groups, accounting for 44% of the total strains of *E. carotovora*. In contrast, 48% of the strains were arranged into one of nine serogroups. Of the 14 unique phages tested (Table 2), only phages B (104-41) and K (SK68-1) and the *E. chrysanthemi* phage N (Ech-3) failed to lyse any of the field strains of *E. carotovora*.

All 114 field strains of *E. c. atroseptica* were members of serogroup I and were restricted to phage groups AE, AEJ, E, and EJ (Fig. 1). The predominant phage group was EJ, which contained 82% of the strains of *E. c. atroseptica*. However, only phages A (I-4) and J (465-2-3-6) were restricted in host range to some strains of *E. c. atroseptica*. Although phage E (101-1) infected all strains of *E. c. atroseptica*, 20 strains of *E. c. carotovora* were also susceptible to infection by this phage. A notable exception to the above observations is strain SK6 of *E. c. atroseptica*, which was placed in serogroup XVIII and was lysed solely by phage C (304-32) (Table 1). Of the 114 strains of *E. c. atroseptica*, 37% were isolated from stem samples.

The most common serogroups of *E. c. carotovora* were IV and XXXVII, containing 17 and 18 strains, respectively (Fig. 1). Several of the serogroups closely corresponded with specific phage groups: all six serogroup XVIII strains were also in phage group CDGIM; all seven serogroup XXIX strains were restricted to phage groups E and EF; all 18 serogroup XXXVII strains were restricted to phage groups D and DL; and serogroup XIV contained all five strains in phage group G. None of the strains identified as members of serogroups VII and XXXV were sensitive to any of the phages.

DISCUSSION

Although high populations and a diversity of strains of *E. carotovora* were recovered from potatoes grown in the Columbia Basin, virulent phages were isolated from only a few host-sample combinations. The sporadic occurrence of *E. carotovora* phages in nature was further illustrated by the fact that they infected only 13 of the 25 strains representing distinct serogroups. Moreover, only 44% of the field strains of *E. carotovora* were sensitive to one or more of the 14 phages displaying unique host ranges. Because 21 known serogroups of *E. carotovora* were reported to occur in Oregon (4), using strains from several serogroups as hosts to isolate phages by the enrichment method appeared to be an appropriate strategy for improving the rate of success. One possible limitation of this approach is that some strain types may not occur or occur only rarely in Columbia Basin potato fields. It is unclear why phages active toward *E. carotovora* are relatively rare, whereas phages against *E. amylovora* and *E. herbicola* are easily obtained from field specimens (2,5).

Interestingly, five of the 15 fields sampled yielded phages that solely infected a potato strain of *E. chrysanthemi*. Although none of the soft rot strains were identified as *E. chrysanthemi* in this study, Cappaert et al (4) showed that it composes less than 1% of the soft rot erwinias isolated as epiphytes or from diseased plants in Oregon. The frequent isolation of phages specific to *E. chrysanthemi* further suggests that this species is found throughout the Columbia Basin and may be a significant potato pathogen when suitable environmental conditions occur (4,26).

Despite the use of phage-typing systems for differentiating strains of several phytobacterial species (2,6,7,20), our results indicate that phages are of limited use for surveying strain diversity in field populations of *E. carotovora*. All strains of *E. c. atroseptica* and 75% of the strains of *E. c. carotovora* that were phage typed also were identified to serogroup. Moreover, 44% of the field strains of *E. carotovora* were identified to phage group versus 48% to serogroup. One advantage phage typing offers is the use of simple methods for preparing phages and screening for sensitivity. Phage typing appears to be potentially more useful for *E. c. atroseptica*, because all field strains tested were identified to phage group, as opposed to only 20% of the strains of *E.*

c. carotovora. Field strains of *E. c. atroseptica* were assembled into four phage groups based on reactions to phages A (I-4), E (101-1), and J (465-2-3-6). Although phage isolate E (101-1) infected all serogroup I strains and strain 465-2-3 of serogroup XVIII, a few strains of *E. c. carotovora* were also sensitive, limiting its use as a presumptive test for identifying subspecies. The reported separation of strain SK6 of *E. c. atroseptica* of serogroup XVIII from strains of serogroup I (12) was confirmed by its sensitivity to phage isolate C (304-32). The restricted host range of phage isolate N (Ech-3) to a potato strain of *E. chrysanthemi* may be indicative of a potential use in identifying other potato strains from the Columbia Basin, although this needs to be verified by testing a broad range of strains of *E. chrysanthemi.*

The serogroups of *E. carotovora* were found by De Boer et al (10,11) to be based on the specificity of the LPS O-antigen. In addition to identical reactions of antisera with purified LPS and whole cells, strains within a specific serogroup have the same LPS sugar composition. The correspondence of some phage groups, such as D and DL, with specific serogroups, such as XXXVII, indicates that the LPS serves as the receptor for their attachment to cells. It was also observed that phage isolate E (101-1) infected all strains in serogroups III and XXIX. De Boer et al (11) noted that strains in either of these two serogroups cross-react, possibly because of similar LPS sugar compositions. Because the specificity of phages resides in their requirement for one or more structural features of the cell surface to serve as a receptor (21), phages that use a receptor other than LPS might not correspond with any of the reported serogroups. Although phage typing was originally proposed as a potentially better method than serotyping for differentiating strains of *E. carotovora* because specificity would not necessarily be based on LPS receptors, few serologically unreactive strains proved to be sensitive to one or more of the phage isolates.

Regardless of field, populations of *E. carotovora* recovered from the rhizospheres and stems ranged from 10^3 to nearly 10^7 cfu/g (fresh weight) at midseason. Typically, populations of *E. carotovora* rise dramatically in June as mean temperatures increase and a lush plant canopy is established. The rhizosphere populations of *E. carotovora* detected coincide with earlier estimates from potatoes grown in the Columbia Basin (17,35) and elsewhere (13); however, stem populations from field-grown potatoes have not been previously reported. Nevertheless, Maher et al (22) noted dramatic increases in the percentage of Russet Burbank and Norgold Russet stems yielding *E. carotovora* over a period extending from early July to mid-August in Wisconsin. The strains of *E carotovora* we recovered from stems were apparently associated with the vascular tissue and may reflect an incipient infection that foreseeably could lead to potato early dying (29,32).

It was not surprising to find that all field strains of *E. c. atroseptica* were serogroup I, because other serogroups of this subspecies are isolated infrequently or are prevalent only in restricted geographic regions (10). For example, all strains of *E. c. atroseptica* isolated by Cappaert et al (4) in Oregon and by Peltzer and Sivasithamparam (26) in Western Australia were identified as members of serogroup I. Although our serogroup I strains of *E. c. atroseptica* could be further divided into four phage groups, 82% of the strains were members of phage group EJ. This indicates that populations of *E. c. atroseptica* in the Columbia Basin are highly homogeneous as compared with those of *E. c. carotovora.* Moreover, strains of *E. c. atroseptica* from some fields were identified to one of three phage groups with no clear association with either rhizosphere or stem samples. This suggests that strains of the four phage types of *E. c. atroseptica* are distributed within the same plant habitat, and that phage typing is of little value in tracing the spread of strains of *E. c. atroseptica* in potato fields.

Although several field strains of *E. c. carotovora* were typed to one of 11 phage groups, only 20% of the total number of strains were sensitive to one or more of the phages, which limits the usefulness of phage typing in making epidemiological observations. Of the noteworthy phage groups, D and DL contained a cluster of strains primarily from stems that were also members of serogroup XXXVII. Serogroup XXXVII also was frequently isolated from water, foliage, and diseased stems in Oregon (4). A total of 26% of the strains of *E. c. carotovora* were identified to serogroup, which closely corresponds with the percentage of strains serologically identified from various sources in Oregon (4). Serogroup XXIX occurred infrequently, despite reports (4,9,22,26,30) of its prevalence in soil, water, tuber, and stem samples collected in other potato-growing regions. The six strains identified as serogroup XVIII were nonfoliar in origin, although strains of this serogroup were reported (4,9) to be isolated most commonly from potato foliage. The diversity of strains of *E. c. carotovora* reflects the occurrence of several possible sources of inoculum in addition to contaminated seed tubers (4,19).

Control of potato diseases caused by *E. carotovora* is complicated by the diversity of strains that can occur in a field and by the continual spread of strains in irrigation water and contaminated seed pieces. Although black leg and bacterial soft rot of tubers and stems commonly occur in Columbia Basin potato fields and are easily recognized, potato early dying resulting from vascular infections by *E. carotovora* alone or in concert with other soilborne pathogens develops more slowly and is more difficult to diagnose (32). Potato early dying is generally considered the primary factor responsible for reductions in overall potato yields in the Columbia Basin (29). Only recently has the role of *E. carotovora* in potato early dying been demonstrated and its implications for potato production acknowledged (29,32). Prior attempts to develop a system of biological control of potato early dying using root-colonizing fluorescent pseudomonads antagonistic to *E. carotovora* have shown little success in the field despite the development of high rhizosphere populations for pseudomonad strains effective in suppressing seed piece decay in greenhouse trials (34,35). The lack of substantial suppression of rhizosphere populations of *E. carotovora* was partly attributed to the presence of a complex mixture of strains of *E. carotovora* in which individual strains differed in sensitivity to antagonistic pseudomonad strains (17,34,35). The complexity of populations of *E. carotovora* was verified in this study by both phage typing and serotyping. These methods may prove useful in identifying pseudomonads antagonistic to a broad spectrum of strains of *E. carotovora* that occur in Columbia Basin fields.

In conclusion, phage typing yielded no substantial improvement over serological methods in differentiating strains of *E. carotovora.* This is in sharp contrast to phage-typing schemes for other phytobacterial species where the method has proved to be a simple and reliable means of differentiating strains arising from different inoculum sources or geographic locations (2,20). Instead, phages may be of value in studies of cell surface components that may contribute critically to the establishment of the plant-bacterial interaction, as has been demonstrated for phages of *E. amylovora* (2,5). Because of the difficulty in isolating phages of *E. carotovora* from natural sources, serological methods of strain identification need to be improved to encompass a larger proportion of the strains of *E. c. carotovora* occurring in potato fields.

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