Field Evaluations of the Interactions Among Fluorescent Pseudomonads, *Erwinia carotovora*, and Potato Yields

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ABSTRACT

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Fluorescent pseudomonad strains that inhibited *Erwinia carotovora* in vitro and which reduced potato seed piece decay in the greenhouse were tested in the field for ability to colonize potato roots and increase yield. Some strains colonized roots with populations exceeding 10^5 colony-forming units per gram (fresh weight) and were detected throughout the growing season. In 1982, the population of *E. carotovora* on roots of pseudomonad-treated potatoes (cultivar Russet Burbank) was only 1–8% of that on the untreated check; in 1983, it was less than 25% of that on the check. Strain W4P63 of *Pseudomonas putida* increased the yield of Russet Burbank by 11.7% in 1982 and by 10.2% in 1983; in both years it suppressed the soft rot potential of the tubers.

Additional key words: bacterial soft rot, blackleg, siderophore.

Erwinia carotovora (Jones) Bergey et al is an important pathogen of potatoes in the Columbia Basin in the state of Washington where it causes blackleg and stem soft rot (30,31). The pathogen develops high populations on roots, stems, and tubers even in the absence of typical soft rot symptoms. De Boer et al (7) reported a population of *E. carotovora* of up to 3×10^8 colony-forming units (cfu) per gram (dry weight) of potato roots in the absence of disease symptoms and by harvest almost all daughter tubers were infested with *E. carotovora*. In the Columbia Basin, plants often die 1–2 mo before harvest because of systemic infection by *E. carotovora* (13,32). Early death occurs at midseason when potatoes are heavily irrigated and soil temperatures are highest; at that time, the population of *E. carotovora* is at its highest level. The primary inocula of *E. carotovora* can originate from several sources including contaminated potato seed, infested soil, and irrigation water (1,27,31). Therefore, control procedures besides crop rotation and the planting of clean seed are needed to effectively protect plants from colonization and infection by *E. carotovora*.

Application of fluorescent pseudomonads to potatoes may be a method of suppressing *E. carotovora* in the field (14). Fluorescent pseudomonads are normal inhabitants of the potato rhizosphere (2,3) and produce a diverse array of siderophores and antibiotics (21) that are important in disease suppression (19). Populations of indigenous fluorescent pseudomonads frequently exceed 10^8 cfu per gram of potato roots grown in the Columbia Basin (37). Kloepper and Schroth (18) detected a shift in the populations of various microorganisms on roots by applying fluorescent pseudomonads referred to as plant growth-promoting rhizobacteria (PGPR). These beneficial bacteria produced siderophores that suppressed growth of deleterious root-inhabiting microorganisms by means of iron deprivation (15,16). Application of PGPR to potato increased yields up to 17% in field trials in California (20). Kloepper (14) also reported that some PGPR applied to seed pieces significantly suppressed infestation of daughter tubers with *E. carotovora*. For example, plant growth-promoting strain B10, which produces the fluorescent siderophore called pseudobactin, suppressed the population of *E. carotovora* on roots by 96–100% in California and 53% fewer daughter tubers were infested with the pathogen (14).

Several other laboratories also have selected and evaluated fluorescent pseudomonads for beneficial effects on potato growth and yield. Howie and Echandi (11) selected fluorescent pseudomonads for production of siderophores inhibitory to *E. carotovora* and then tested representative strains in the field for ability to improve plant growth. Yields were increased significantly ranging from 17 to 37% over those of untreated potatoes at two locations in North Carolina. Severe yield depressions observed by Geels and Schippers (8) in fields frequently cropped to potatoes in Holland were offset, in part, by treatment of seed pieces with fluorescent pseudomonads selected for ability to inhibit *E. carotovora* and several other potato pathogens; yields and numbers of tubers from treated plants were increased in greenhouse experiments up to 70 and 93%, respectively. Vrany and Fiker (35) selected fluorescent pseudomonads on the basis of antagonism to *E. carotovora* on King's medium B (KB) agar and some strains improved plant growth and tuber yield by 4–30% in field trials. However, the effects of the pseudomonads on populations of *E. carotovora* in the field were not evaluated in these studies.

Colyer and Mount (4) isolated a strain (M17) of *Pseudomonas putida* (Trevisan) Migula that inhibited *E. carotovora* in vitro. Seedpieces treated with the pseudomonad had significantly less postharvest soft rot potentials (up to 50%) of daughter tubers. Strain M74, a noninhibitory mutant of strain M17, colonized the rhizosphere of potatoes equal to strain M17, but yielded intermediate effects on overall soft rot potentials of daughter tubers. However, the size of the populations of *E. carotovora* on pseudomonad-treated tubers were not directly measured and tuber yields were not reported.

A screening procedure was devised for selection of fluorescent pseudomonads that suppress *E. carotovora* subsp. *atroseptica* (Van Hall) Dye (37). Selection was based on four criteria: production of inhibitory siderophores with high iron affinities, production of antibiotics, colonization of potato root surfaces in relatively high numbers, and greenhouse suppression of preemergence seed piece decay as measured by plant emergence. The most effective strains improved potato emergence up to 63% and increased plant growth six- to seven-fold 2 wk after planting compared to that of plants produced from seed pieces inoculated with only *E. c.* subsp. *atroseptica*.

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This study was conducted to quantitatively evaluate fluorescent pseudomonads for ability to colonize the root systems of potatoes, suppress populations of *E. carotovora* on roots and daughter tubers, and to improve yields of potatoes grown in the Columbia Basin. Selected strains from the Columbia Basin were compared to plant growth-promoting strain B10 (noted for its antagonism to *E. carotovora* both in vitro and on field-grown potatoes) and strain Pf-5 of *Pseudomonas fluorescens* (Trevisan) Migula (9,10) (an isolate [from cotton] that produces two potent antibiotics [pyrrolnitrin and pyoluteorin] which have been implicated in the control of cotton seedling diseases).

MATERIALS AND METHODS

Pseudomonad strains. Mutants of strains W4F35, W4F49, W4F68, W4F151, and W4F156 of *P. fluorescens* and strains W4P5, W4P59, W4P63, and W4P144 of *P. putida* were selected for resistance to rifampicin (100 μg/ml) and nalidixic acid (100 μg/ml) as described by Weller and Cook (36). Antibiotic-resistant strains were checked for in vitro inhibition of *E. carotovora* on KB agar (12) and potato-dextrose agar (PDA). Strain Pf-5 of *P. fluorescens* and plant growth-promoting strain B10 of *P. fluorescens-P. putida*, obtained from G. D. Easton (Irrigated Agricultural Research and Extension Center, Prosser, WA), were isolated and described by Howell and Stipanovic (9,10) and Kloepper et al (15), respectively. All strains were preserved as previously described (37).

Preparation of pseudomonads for potato seed piece treatments. Fluorescent pseudomonads were prepared in talc-methylcellulose essentially as described by Kloepper and Schroth (17). Bacteria from 24-hr cultures grown on KB agar were suspended in magnesium sulfate (0.1 M) (10^{10} colony-forming units [cfu] per milliliter) mixed with a equal volume of a 2.5% methylcellulose-water suspension; then, after 20 min, talc was added fourfold (v/w) and thoroughly mixed. The resultant paste was dried at room temperature for 3 days and stored at 4 C prior to seed piece treatment. Small portions of each preparation were stored at 4 C and bacterial populations were determined monthly over a 6-mo period. Populations of strains W4P63, W4F35, W4F151, W4F156, and B10 were determined by shaking 1 g of the dried inocula in 10 ml of magnesium sulfate (0.1 M) for 30 min and plating appropriate dilutions onto KB agar containing rifampicin (100 μg/ml) and nalidixic acid (100 μg/ml).

Characteristics of the field plots. The fields located at Plymouth, WA, had been bare-fallowed (not planted to potatoes) for 4 yr (1982) or 5 yr (1983). The soil was a Quincy loamy sand (mixed, xeric, Torripsamments; pH 6.5) that lacked a detectable indigenous population of *E. carotovora* as determined by an enrichment procedure specific for the pathogen (28). Fields were fertilized with 336 kg of N per hectare prior to planting.

Planting, maintaining, and harvesting potatoes. Potatoes (*Solanum tuberosum* L.) were planted on 30 March 1982 (cultivars Norgold Russet and Russet Burbank) and on 14 April 1983 (Russet Burbank). Potato seed pieces (approximately 60 g per seed piece) were cut just prior to planting and treated with the pseudomonads at a ratio of 60 g of dried bacterial preparation per 90 seed pieces by shaking 30 to 60 sec in paper bags. Clean bags and gloves were used between treatments to avoid cross contamination. Treated seed pieces were planted by hand every 30 cm, covered with soil and hilled. Treatments were arranged in a randomized block and replicated five times in 1982 and six times in 1983. Treatments in 1982 were strains W4P63, W4P5, W4F49, W4P59, W4F68, B10, Pf-5, and a check (talc-methylcellulose only); in 1983, the treatments were strains W4P63, W4F35, W4P144, W4F151, W4F156, B10, and a check (talc-methylcellulose only). Treated rows consisting of two 12-m rows were separated by two uninoculated buffer rows and treatments within rows were separated by a 1.5-m-wide strip of fallow ground.

Plants were irrigated by overhead sprinklers from May to September for 2 hr each day and received 13.2 mm of water per application.

In 1982, Norgold Russet and Russet Burbank potatoes were harvested, respectively, on 16 August and on 7 September; in 1983, Russet Burbank potatoes were harvested on 14 September. Two 6-m rows per replication were dug by a single-row mechanical digger from an area not previously sampled. Potatoes were bagged, and subsequently graded and weighed. The means of yields from each treatment were compared by calculating the protected least significant difference (LSD).

Monitoring populations of the antagonistic pseudomonads and *E. carotovora*. Populations of the antagonistic pseudomonads and *E. carotovora* were determined at 3-wk intervals from plant emergence to harvest. Treatments were sampled six times in 1982 (18 May, 14 June, 8 and 29 July, 16 August, and 7 September) and five times in 1983 (3 and 24 June, 15 July, and 4 and 30 August). The exception was Norgold Russet on 7 September 1982. Samples consisted of three bulked plants from each of three replications of each treatment. Tubers were counted and weighed. Roots and tubers were bagged, chilled for transport back to the laboratory, and stored at 4 C for 12–18 hr prior to analysis for bacterial populations.

Roots (2 g) from each of three plants were shaken for 30 min in 20 ml of 0.1 M magnesium sulfate at 250 rpm. Nine medium-size daughter tubers were shaken for 30 min at 250 rpm with approximately the same volume of sterile water. Serial dilutions were plated in duplicate on KB agar containing rifampicin (100 μg/ml) and nalidixic acid (100 μg/ml) (to detect introduced pseudomonads), crystal violet-pectate agar (to detect *E. carotovora*) (5), and tryptic soy agar (TSA) (to detect total bacteria) (26). Plates were incubated for 48 hr at 22–24 C. The surface area of tubers was determined by a standard procedure (25). Mean bacterial populations were compared by calculating the protected LSD.

Determination of tuber soft rot potential. Ten medium-sized tubers were randomly sampled by hand-harvesting outside the area used for yield determination; samples were collected from each of three replications per treatment. Tubers were stored at 4 C for 2–4 wk and then assayed for bacterial soft rot potential according to the method of De Boer and Kelman (6). Tubers were rinsed under tap water to remove loosely adhering soil and ten lenticels per tuber were wounded with sterile toothpicks. Individual tubers were wrapped in a moist paper towel, covered with Saran Wrap (Dow Chemical Company, Indianapolis, IN), placed in a covered plastic box, incubated for 5 days at 20 C, and examined for soft rot. Means of the percentage of decayed lenticels were compared by using the protected LSD.

Characterization of *E. carotovora*. At each sampling, three to five colonies of *E. carotovora* per replication were transferred to fresh crystal violet-pectate agar and subsequently purified to colony homogeneity. Isolates of *E. c.* subsp. *atroseptica* were distinguished from *E. c.* subsp. *carotovora* by using the determinative tests described by De Boer et al (7), including production of acid from α-methyl D-glucoside, production of reducing substances from sucrose, and growth at 36 C.

RESULTS

Viability of dried pseudomonad preparations. The number of pseudomonads in the talc-methycellulose mixtures ranged between 10^8 and 10^9 cfu per gram after 3 days of drying at room temperature (approximately 24 C). Although all strains survived following initial desiccation, their viability decreased either gradually or precipitously with time at 4 C depending on the strain. For example, the population of strain W4P63 declined from 10^9 cfu per gram at day 3 to 10^8 cfu per gram at day 5 and then remained stable for at least 14 days. Between days 14 and 117 the populations of strains W4P63, W4F151, and W4F156 dropped only one log unit. After 6 mo of storage at 4 C, populations of strains W4P63, W4F151, W4F156, and B10 ranged from 10^4 to over 10^6 cfu per gram. However, the population of strain W4F35 declined to less than 10^3 cfu per gram 2 mo after preparation and was undetected in the dried talc-methylcellulose after 6 mo.

Populations of *E. carotovora* on seed tubers and in soil. Seed tubers of cultivars Russet Burbank and Norgold Russet used in field trials in 1982 were naturally contaminated with over 10^6 cfu of *E. carotovora* per seed piece. However, in 1983 less than 10 cfu of *E. carotovora* per seed piece were detected on the Russet Burbank potatoes planted at Plymouth.

In both 1982 and 1983, *E. carotovora* was not detectable in soil collected from the Plymouth plot even when the soil enrichment technique of Meneley and Stanghellini (28) was used.

Effects of fluorescent pseudomonads on colonization and infection of potatoes by *E. carotovora*. Prior to June, the populations of *E. carotovora* on root surfaces were low and sporadic (Fig. 1). With an increase in mean soil temperature to between 16 and 18 C (measured at seed piece level) the population rapidly increased on roots of untreated potatoes reaching a maximum of 2×10^7 cfu per gram on 8 July and remaining high (10^6 to 10^7 cfu per gram) throughout the season. *E. carotovora* comprised nearly 1% of the total bacteria on root surfaces during the period when the mean soil temperature exceeded 16 C.

Following treatment of seed pieces with approximately 10^9 cfu per gram of either strains W4P63 or B10 in dried talc-methycellulose, about 10^4 cfu of each strain per gram of root was recovered on 18 May (49 days after planting) (Fig. 1A and B). Strain W4P63 spread to newly developing roots and the population increased to nearly 10^5 cfu per gram by 16 August (Fig. 1A). The population declined 10-fold in September just prior to harvest, corresponding with the senescence and death of the plants. On potatoes treated with strain W4P63, the population dynamics of *E. carotovora* was similar to that of the check, however, on treated potatoes the population of *E. carotovora* was less than 10% that of the check throughout the growing season. In contrast, plant growth-promoting strain B10 colonized roots poorly and the population never increased beyond the initial root populations measured in May (Fig. 1B). By 8 July, the population of *E. carotovora* on roots was approximately 100-fold > that of strain B10. Despite the fact that the population of *E. carotovora* was high and generally exceeded that of strain B10, total populations of *E. carotovora* were only about one-tenth that of the untreated check throughout the growing season.

All of the fluorescent pseudomonad strains colonized potato roots and were detected throughout the growing season (Table 1). In 1983, the average root populations for all strains over the whole season ranged from 10^2 cfu per gram for strain W4F35 to 10^5 cfu per gram for strain W4F151. Colonization was relatively consistent for individual strains regardless of cultivar or year. For example, there was only a 0.32 difference in the average log populations for strain W4P63 (a good colonizer) and a 0.60 difference for strain B10 (a weak colonizer). The most aggressive colonizers were strains W4P63, W4P5, and W4F68 in 1982 and W4P63, W4P144, and W4F151 in 1983 with average populations over 10^4 cfu per gram of root. Strain W4F35 had the lowest population (1.1×10^2 cfu/g) of all strains due to its poor survival following planting. Strains B10 and Pf-5 did not colonize potato roots as well as most of the pseudomonads isolated from potatoes in the state of Washington. Antibiotic-resistant pseudomonads were not isolated from any of the check plants in 1982 and 1983.

Pseudomonads also colonized daughter tubers; the average populations during the 1983 season ranged between 26 cfu/cm^2 for strain B10 and 4.5×10^2 cfu/cm^2 for strain W4P63.

The average population of *E. carotovora* on roots of untreated (check) potatoes for the entire season ranged from 1.9×10^3 cfu per gram on Norgold Russet in 1982 to $2\text{–}5\times 10^5$ cfu per gram for Russet Burbank in 1982 and 1983 (Table 1). Despite colonization of potato roots by the applied pseudomonads, high populations of *E. carotovora* developed. Furthermore, there was no direct relationship between the extent of colonization by pseudomonads and suppression of *E. carotovora*. Populations of *E. carotovora* were, however, lower than the check for all but the strain B10 treatment in 1983. The average populations of *E. carotovora* on most pseudomonad-treated Russet Burbank and Norgold Russet plants were only about one-tenth that of the respective untreated check.

The fluorescent pseudomonads did not affect the incidence of disease caused by *E. carotovora*. For example, the incidence of blackleg in all treatments averaged 2% for Norgold Russet potatoes, and 1.3 and 0.7% in 1982 and 1983, respectively, for Russet Burbank potatoes. Stem soft rot and severe early dying, caused by *E. carotovora*, occurred in both cultivars in July 1982, which corresponded to peak seasonal root populations of *E. carotovora* (Fig. 1). Potatoes in all treatments developed disease to the same extent and senesced at the same rate. Similar results were observed in 1983; however, the incidences of stem soft rot and early dying were less.

Effects of fluorescent pseudomonads on potato yield. The weights of plants harvested at each sampling and the corresponding number and weights of tubers were not significantly different from those of the check, nor was plant growth visibly stimulated in any of the treatments. Nevertheless, several fluorescent pseudomonads significantly increased the yield of Russet Burbank (Table 2). The best strain was W4P63, which increased yield by 11.7% (significant at $P = 0.05$) in 1982 and by 10.2% in 1983. Furthermore, treatment with strain W4P63 significantly ($P = 0.05$) increased the yield of U.S. No. 1 Russet Burbank by 25.2% and 10.2% in 1982 and 1983, respectively. Strains W4F68 and W4P5 improved Russet Burbank yields by 7.5% (significant at $P = 0.05$) and 4.5% (significant at $P = 0.10$), respectively. Plant growth-promoting strain B10 also increased yield in 1982 by 9.8% (significant at $P = 0.05$) and in 1983 by 7.2%). Treatment with the remainder of the strains, possible exceptions being W4P144 and W4P156, did not consistently increase Russet Burbank yields.

None of the pseudomonads improved total yield or yield of U.S. No. 1 tubers of Norgold Russet (Table 2). Moreover, potatoes

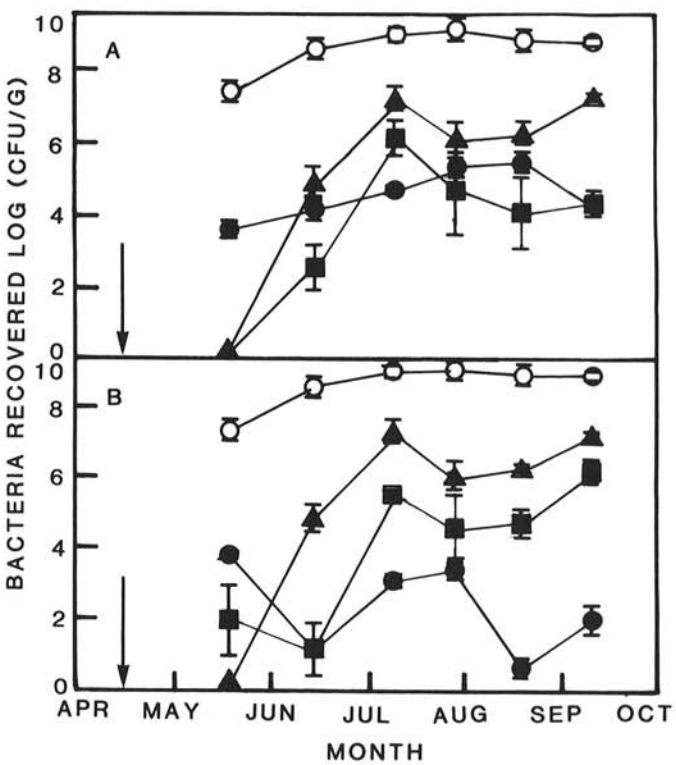

Fig. 1. Population dynamics of applied fluorescent pseudomonad strains W4P63 (●, **A**) and B10 (●, **B**) and *Erwinia carotovora* (from check plants, ▲) on roots of Russet Burbank potatoes grown at Plymouth, WA, in 1982. The effect of applied fluorescent pseudomonads on the populations of *E. carotovora* (■) are indicated in **A**, (seed pieces treated with strain W4P63) and **B**, (seed pieces treated with strain B10). Total bacterial populations on roots (o), populations of *E. carotovora*, and applied pseudomonads were averages of each collection. Each collection consisted of three plants from each of three replications. Seedpieces were treated with pseudomonads at planting (denoted by arrows) to give approximately 10^8 cfu per seed piece. Vertical bars indicate standard error.

treated with four of the strains (W4F49, W4F59, W4F68, and B10) yielded up to 17% less than the untreated check.

Effect of pseudomonads on soft rot potential of tubers. Despite the presence of populations of *E. carotovora* in excess of 10^3 cfu/cm^2 at harvest, daughter tubers from Russet Burbank check treatments showed low susceptibilities to decay in 1982 (18.3%) and 1983 (8.3%)(Table 3). Nevertheless, the percentage of decayed lenticels was significantly less ($P = 0.05$) on tubers developing from plants treated with pseudomonads. Nearly all strain treatments had soft rot potentials less than 10% that of the checks, including strain W4P63 in both 1982 and 1983. These results, however, were not reflected in the comparative bacterial populations at harvest. The pseudomonad populations on tubers were low relative to those of *E. carotovora* and the size of the populations of *E. carotovora* for the pseudomonad-treated potatoes were largely indistinguishable from those of the checks.

Identification of *E. carotovora* subspecies. *E. c.* subsp. *carotovora* was detected more often than *E. c.* subsp. *atroseptica* at Plymouth in both 1982 and 1983 (Fig. 2). Of 262 and 508 isolates of *Erwinia* in 1982 and 1983, respectively, 72 and 83% were identified as *E. c.* subsp. *carotovora*. The percentages of *E. c.* subsp. *atroseptica* were highest early in each season and were progressively less as soil temperatures increased. For example, on 3 June 1983, when overall populations were very low, isolations of *E. c.* subsp. *atroseptica* were 35% as great as those of *E. carotovora*, but by 4 August, when the total root population of *E. carotovora* was highest (1.1×10^6 cfu per gram), the isolations of *E. c.* subsp. *atroseptica* were only 4% as great as those of *E. carotovora*. Although the proportion of *E. c.* subsp. *atroseptica* may have risen slightly by harvest, populations of *E. c.* subsp. *carotovora* continued to be higher on the surfaces of roots and daughter tubers.

DISCUSSION

The ultimate test of a biological control agent is field demonstration of its growth and survival on plant surfaces and its effectiveness in suppressing disease. The latter can be measured as lower pathogen numbers, suppression of pathogen growth, or enhancement of plant growth and/or yield. In general, the fluorescent pseudomonads, selected from potato root systems and shown to be inhibitory to *E. carotovora* in vitro and in the greenhouse, aggressively colonized emerging roots following seed piece treatment. More importantly, treatment with some strains (e.g., W4P63) resulted in populations of *E. carotovora* only one-tenth those on the roots of the untreated check throughout the growing season, increased total potato yield up to approximately 12% and increased tuber quality (e.g., up to 25% more U.S. No. 1 tubers). Perhaps the most important effect of the pseudomonads was a 10-fold reduction in the soft rot potential of daughter tubers. However, further improvements in the biological control system are needed before the antagonistic bacteria can be used commercially to control *E. carotovora*. Populations of *E. carotovora* ranging up to 10^6 cfu per gram (fresh weight) developed on root systems with previously established populations of some of the best biocontrol strains, including strain W4P63. Accordingly, soft rot incidences on treated and untreated plants were indistinguishable. Although complete eradication of *E. carotovora* appears unnecessary for commercial control of *E. carotovora*, and is unlikely to be achieved by biocontrol, more effective suppression of growth of *E. carotovora* is needed for substantial disease control and yield gains.

The establishment of high populations of pseudomonads on roots was dependent on the initial dose of the bacterial preparation

TABLE 1. Average populations of introduced fluorescent pseudomonads on potato roots in field trials at Plymouth, WA, and their effects on the average size of populations of *Erwinia carotovora*

Year and cultivar	Fluorescent pseudomonad strain	Log cfu/g[a]		*E. carotovora* (% of check)
		Applied pseudomonads	*E. carotovora*	
1982				
Norgold Russet	W4P63	4.73	2.01 †	5.7
	W4P5	4.46	2.77	31.0
	W4F49	3.22	2.54	18.6
	W4P59	3.77	1.94 †	4.7
	W4F68	4.07	2.42	14.1
	B10	3.17	2.51	17.4
	Pf-5	2.96	2.09 †	6.6
	Check[b]	...	3.27	100.0
	LSD 0.10	0.46	1.10	
	LSD 0.05	0.60	1.42	
Russet Burbank	W4P63	4.61	3.40 *	1.2
	W4P5	4.78	4.04 *	5.1
	W4F49	3.51	3.40 *	1.2
	W4P59	3.10	4.07 *	5.5
	W4F68	4.43	2.66 *	0.2
	B10	2.57	4.19 †	7.8
	Pf-5	3.52	3.23 *	0.8
	Check[b]	...	5.33	100.0
	LSD 0.10	0.39	0.96	
	LSD 0.05	0.50	1.24	
1983				
Russet Burbank	W4P63	4.41	5.13 †	28.2
	W4F35	2.06	5.22	34.7
	W4P144	4.05	4.74 *	11.5
	W4F151	5.00	4.83 *	14.1
	W4F156	3.78	4.78 *	12.6
	B10	2.61	5.70	104.0
	Check[b]	...	5.68	100.0
	LSD 0.10	0.53	0.50	
	LSD 0.05	0.69	0.67	

[a] Root populations are averages of five sampling dates for 1982 Norgold Russet and 1983 Russet Burbank potatoes, and six sampling dates for 1982 Russet Burbank potatoes. The protected least significant difference (LSD) between the means for the check and fluorescent pseudomonad treatments are shown at $P = 0.10$ (†) and $P = 0.05$ (*).

[b] Treatment with talc-methylcellulose only.

and ability of the strain to multiply and colonize the first roots. Initial populations of the pseudomonads were as high as 10^8 cfu per seed piece; these were applied as a dry coating that adhered well to cut seed pieces. Most pseudomonads survived drying in talc-methylcellulose in high numbers, thus confirming the usefulness of the procedure first reported by Kloepper and Schroth (17) for drying PGPR strains. The factors that influenced successful colonization by the bacteria following planting include soil pH, temperature, and moisture (33). The soil pH at Plymouth was 6.5, which is favorable to good bacterial growth. Soil temperatures at planting averaged 8 C, which is suitable for pseudomonad growth, albeit growth would be slow. Soil temperatures that were highly conducive for rapid growth did not occur until May; overall populations of strain W4P63 increased to roughly correspond with increasing soil temperatures. Initial soil temperatures in California would likely be higher than those in the Columbia Basin and would provide more suitable conditions for growth of PGPR pseudomonads as observed by Kloepper et al (20) on potato.

Loper et al (22) critically evaluated the influence of soil temperature on the potato rhizosphere population of PGPR strains B4 and B10 and noted that overall populations were generally higher at 12 or 18 C than at 24 C, where growth rates were more rapid. Cool soil temperatures apparently enhanced survival of the fluorescent pseudomonads in the rhizosphere or gave them a competitive edge over indigenous bacteria. In the Columbia Basin, soil temperatures between 12 and 18 C are characteristic for irrigated potatoes prior to and following development of a plant canopy. These soil temperatures are also highly suitable for growth of *E. carotovora* (29).

Soil moisture may be the most important factor influencing root colonization. Weller and Cook (36) observed that pseudomonads colonized wheat roots in field soils that contained higher moisture, and Suslow and Schroth (34) noted that inadequate moisture may have been responsible for the failure of PGPR strains to promote significant growth increases in some sugar beet field trials. Potatoes in this study were irrigated intermittently until plants emerged, and then irrigated daily to ensure adequate moisture for optimum potato growth in the sandy loam soil. These conditions were also conducive for growth, survival, and colonization of potato roots by most of the pseudomonad strains and should not have been an important factor in limiting their effect as biocontrol agents. Nevertheless, Loper et al (22) noted that the size of the rhizosphere population of various strains of PGPR on potatoes was correlated with greater osmotolerance in vitro. For instance, strain B10 has a relatively low osmotolerance; this may partly explain its relatively poor growth and survival on potatoes in the Columbia Basin (Table 1).

Populations of fluorescent pseudomonads on potato varied with the strain and ranged from 10^3 to 10^6 cfu per gram of roots (fresh weight) over the entire season. These populations are similar to those of the indigenous total fluorescent pseudomonads that occur on potato roots at midseason in the Columbia Basin and are consistent with those reported for PGPR strains on potato roots (11,20). Kloepper et al (20) found that the PGPR populations at several locations in a sandy loam soil ranged from 1.9×10^2 to 9.6×10^5 cfu/cm of roots 2 wk after plant emergence. One of the more effective strains, monitored at 2-wk intervals throughout the growing season, markedly decreased to a population of approximately 10^3 cfu/cm of root 4–6 wk after emergence and remained stabilized at this population level until irrigation was terminated. Populations of strain B10 (originally isolated from potato) 2 wk after emergence in a sandy loam soil ranged from 2.3×10^2 to 3.8×10^4 cfu/cm, depending on the location and/or year (20). Recently, Loper et al (24) showed that rhizosphere bacterial populations, including those of the PGPR, can vary by a factor of 100 to 1,000 and show a lognormal distribution on potato roots.

Our results show plant growth-promoting strain B10 was not as effective in developing and maintaining high populations on roots as several of the pseudomonads originally isolated from potatoes grown in the Columbia Basin; average populations of strain B10 were only one-tenth those of the more aggressive colonizers such as strain W4P63 (Table 2). The root population of strain B10 was shown by Loper et al (22) to markedly decrease with distance from the initial seed piece. In contrast, plant growth-promoting strain B4 was distributed over the whole root at a relatively uniform population. Consequently, patterns of root colonization will vary with the strain. Poor distribution of strain B10 along roots may explain its low populations relative to other strains tested in the Columbia Basin. Strain Pf-5 of *P. fluorescens*, which originated from a cotton rhizosphere (9,10), also was a relatively poor colonizer of potato roots and may not be adapted to potatoes and/or Columbia Basin conditions.

Despite the presence of pseudomonad populations on root systems, populations of *E. carotovora* sharply increased in June and eventually surpassed the populations of several pseudomonad strains in 1982 and all strains in 1983. For example, appreciable populations of *E. carotovora* did not develop on roots of untreated potatoes early in the season, when mean soil temperatures were less than 15 C (measured at seed piece level). They, however, approached 10^7 cfu per gram when soil temperatures reached approximately 18 C and were maintained between 10^6 to 10^7 cfu per gram until harvest. If plants were treated with pseudomonads, the populations of *E. carotovora* at mid-season were generally only one-tenth as high as on plants that were without introduced pseudomonads, but the magnitude of their populations reflected the lack of adequate control. Strain B10, which was reported (14) in California to reduce *E. carotovora* by 96–100% at harvest, did not reduce populations of *E. carotovora* to a similar degree in the Columbia Basin. This is despite our observations that strain B10 colonized roots and characteristically produced the fluorescent

TABLE 2. Influence of introduced fluorescent pseudomonads on potato yield at Plymouth, WA

Year and cultivar	Fluorescent pseudomonad strain	Yield (kg/plot)[a]	Increase (%)	U.S. no. 1 (kg/plot)[a]	Increase (%)
1982					
Norgold Russet	W4P63	42.4	0.0	34.7	3.5
	W4P5	42.7	0.7	35.0	4.2
	W4F49	35.2	−17.0	27.8	−17.6
	W4F59	41.9	−1.1	34.0	1.2
	W4F68	36.6	−13.0	29.1	−13.0
	B10	38.2	−10.2	31.3	−6.9
	Pf-5	45.8	8.0	37.5	11.8
	Check[b]	42.4	0.0	33.6	0.0
	LSD 0.10	6.8		5.7	
	LSD 0.05	8.1		6.8	
Russet Burbank	W4P63	56.4 *	11.7	34.1 *	25.2
	W4P5	52.8 †	4.5	28.8	6.0
	W4F49	52.1	3.1	27.3	0.0
	W4F59	52.5	3.9	29.0	6.7
	W4F68	54.3 *	7.5	31.5	15.8
	B10	55.5 *	9.8	31.5	15.8
	Pf-5	47.4	−6.3	24.6	−9.7
	Check[b]	50.5	0.0	27.2	0.0
	LSD 0.10	2.3		5.4	
	LSD 0.05	2.9		6.8	
1983					
Russet Burbank	W4P63	69.7	10.2	56.0 *	10.2
	W4F35	63.2	0.0	48.1	−5.3
	W4P144	69.0	9.3	53.7	5.7
	W4F151	65.1	3.4	50.6	0.0
	W4F156	68.0	7.6	51.5	1.4
	B10	67.7	7.2	51.3	1.0
	Check[b]	63.2	0.0	50.8	0.0
	LSD 0.10	8.2		4.1	
	LSD 0.05	9.9		4.9	

[a] Mean of five (1982) or six (1983) replications for all treatments, two 6-m rows per replication. The protected least significant difference (LSD) between the means for the check and fluorescent pseudomonad treatments are shown at $P = 0.10$ (†) and $P = 0.05$ (*).

[b] Treatment with talc-methylcellulose only.

pigment, pseudobactin. Thus, the pseudomonads at best resulted in a relatively small level of suppression of numbers of *E. carotovora* at midseason when *E. c.* subsp. *carotovora* predominated. This may reflect our earlier observation (37) that the antagonistic pseudomonads were generally less effective in controlling seed piece decay caused by *E. c.* subsp. *carotovora* than that caused by *E. c.* subsp. *atroseptica*.

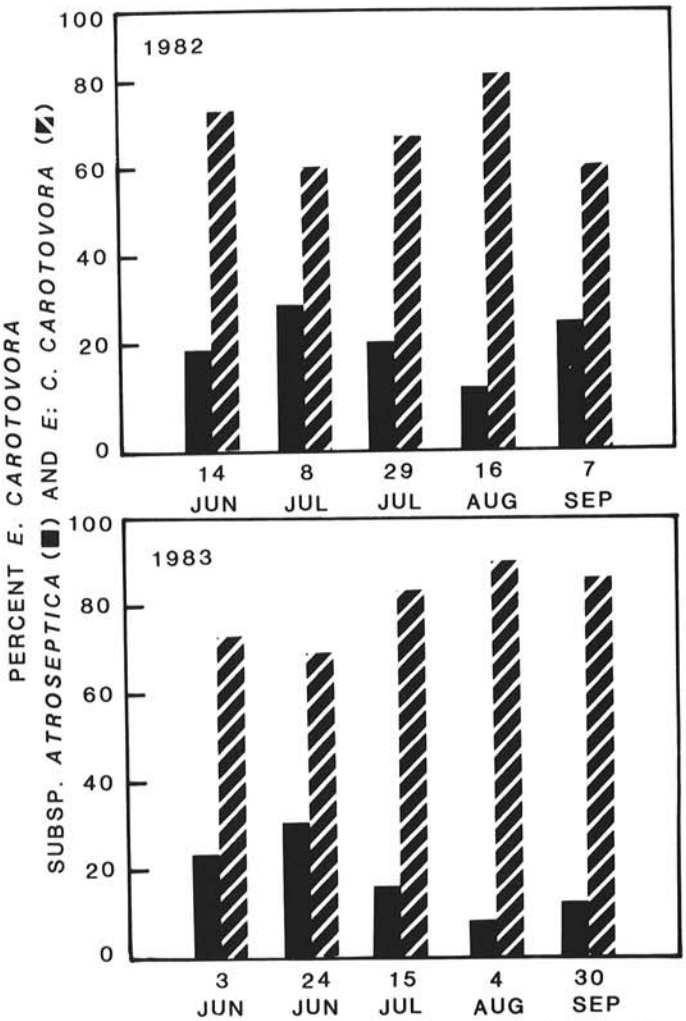

Fig. 2. Ratio of *Erwinia carotovora* subsp. *atroseptica* (solid bars) to *E. c.* subsp. *carotovora* (diagonally shaded bars) in Russet Burbank field plots on a seasonal basis at Plymouth, WA. Percentages were based on between 50 and 100 isolates of *E. carotovora* at each sample date in 1982 and 1983.

The soft rot potentials of tubers from pseudomonad-treated plants were generally only about 10% that of the respective check. It is cautioned, however, that the percentages of decayed lenticels were very low (Table 3), even for the check tubers which harbored 10^3–10^4 pectolytic *Erwinia* per square centimeter at harvest. Nevertheless, the extent of postharvest soft rot suppression is consistent with that reported by Colyer and Mount (4) for strain M17 of *P. putida*. Consequently, the greatest effect of the pseudomonads on *E. carotovora* may reside in a lowering of the incidence of postharvest tuber infection.

The pseudomonads tested in the field were originally isolated from potatoes in the Columbia Basin, and selected for in vitro antagonism of strain W3C37 of *E. c.* subsp. *atroseptica* and for ability to control seed piece decay in the greenhouse (37). All were highly effective in reducing seed piece decay with plant emergence ranging from 27 to 63% over that of the checks. Screenings for antagonism were based on production of bacteriostatic siderophores on KB agar and/or bactericidal antibiotics on PDA. All of the pseudomonads in this study produced fluorescent siderophores that were inhibitory to strain W3C37 of *E. c.* subsp. *atroseptica*. With the exceptions of strains B10, W4F49, W4P63, and W4F68, the pseudomonads produced siderophores with high affinities for iron, reversing iron starvation in the presence of 1,000 μg/ml ethylenediamine-di-(*o*-hydroxyphenyl acetic acid) (EDDA). The siderophores of the other four strains, including strain B10 that produces pseudobactin (15), were intermediate in iron affinity (>250 to <1,000 μg of EDDA per millliter). Despite the production of siderophores with relatively high iron affinities by all strains used in this study, the field populations of *E. carotovora* were suppressed no more than one log unit. This raises questions concerning the value of selecting antagonists to *E. carotovora* on the basis of production of inhibitory fluorescent siderophores. It should be noted, however, that environmental factors such as iron availability and temperature may have influenced siderophore production. Loper and Schroth (23) showed that PGPR pseudomonads varied greatly in the optimum temperature for siderophore production; at 30 C, strain B10 produced only one-tenth the amount of siderophore that it produced at 28 C. Nevertheless, soil temperatures of this magnitude are not achieved at midseason in irrigated fields under a plant canopy in the Columbia Basin. Repression of siderophore synthesis would furthermore occur in soils naturally high in available iron. These conditions, however, would likely be conducive to antibiotic production. Of the pseudomonads tested in the field, only strains B10, W4P59, and W4F68 did not produce an antibiotic on PDA. Nevertheless, these pseudomonad strains did not differ from the antibiotic-producing strains in their effect on *E. carotovora* in the

TABLE 3. Effect of applied fluorescent pseudomonads on the soft rot potential of potato tubers harvested from field trials at Plymouth, WA

Year and cultivar	Fluorescent pseudomonad strain	Log (cfu/cm² tuber surface) at harvest		Decayed lenticels[a] (%)	Decayed lenticels as % of check
		Applied pseudomonads	*Erwinia carotovora*		
1982					
Russet Burban	W4P63	1.67	4.30	1.7 *	9.3
	W4F68	0.70	0.60	2.0 *	10.9
	Check[b]	0.0	4.74	18.3 *	100.0
	LSD 0.05			10.5	
1983					
Russet Burbank	W4P63	2.54	3.36	0.3 *	4.0
	W4F35	0.84	4.05	0.3 *	4.0
	W4P144	1.80	3.67	2.3	27.0
	W4F151	2.01	3.96	0.0 *	0.0
	W4F156	1.20	4.00	0.0 *	0.0
	B10	1.40	4.05	0.0 *	0.0
	Check[b]	0.0	3.11	8.3	100.0
	LSD 0.05			6.9	

[a] Mean of three replications, each replication consisted of 10 tubers each with 10 injured lenticels per tuber. The protected least significant difference (LSD) between the check and fluorescent pseudomonad treatments are shown at $P = 0.05$ (*).

[b] Treatment with talc-methylcellulose only.

field. Regardless of the iron status of the soil, most of the strains tested in the field were capable of producing inhibitory siderophores or antibiotics under low or high iron conditions, respectively. The reasons for the relatively poor suppression of growth of *E. carotovora* by the antagonistic pseudomonads may be attributed to production of insufficient quantities or instability of these inhibitory factors in the root environment.

The observed increase in potato yields by treatment with some pseudomonad strains (e.g., W4P63) may have resulted from inhibition or displacement of minor pathogens responsible for plant growth suppression (18,33). Suslow and Schroth (34) found deleterious rhizobacteria to occur at relatively high frequencies and to be a normal component of the root microflora of sugar beets. Treatment of potato seed pieces with PGPR also resulted in up to a 64 and 93% lower population of fungi and Gram-positive bacteria, respectively, within the root zone (34). Apparently, pseudomonads with the ability to promote growth and yield of potato are common in nature and have shown promise in several experiments with natural field soils (4,8,11,20,35). For example, Howie and Echandi (11) observed significant yield increases of 17–37% over controls in field trials in North Carolina. In our study, the population of *E. carotovora* at midseason was only reduced from about 10^6 to 10^5 cfu per gram on Russet Burbank (Fig. 1), and, even though the incidence of blackleg was indistinguishable for plants treated or untreated with pseudomonads, yield was increased by 10–12% in response to treatment with strain W4P63. This yield increase may have resulted from suppression of major or minor root pathogens of potato. Although the identity of such pathogens is unknown, the negligible incidence of Verticillium wilt indicated that *Verticillium dahliae* Kleb. was not the target pathogen.

Field trials indicated that fluorescent pseudomonads are potentially useful in suppressing *E. carotovora* and/or promoting potato growth and yield in the Columbia Basin. Commercial use of the pseudomonads, however, must await further improvements in the effectiveness of strains to ensure consistent benefits to potato culture. Improvements in biocontrol of *E. carotovora* may be achieved by selecting strains of fluorescent pseudomonads more antagonistic to *E. c.* subsp. *carotovora*, the subspecies most often the cause of "early dying" (13). It would also be necessary to maintain higher populations of introduced pseudomonads on root surfaces. At least 10^6 cfu per seed piece were needed for significant suppression of preemergence seed piece decay in greenhouse trials (37). In subsequent field trials, a peak population of about 10^6 cfu per gram of root was obtained for the more aggressive strains. Consequently, a number of challenges remain in developing an effective biological control system for *E. carotovora*. This will require the elucidation of the environmental factors that influence pseudomonad growth and survival and the basic mechanism(s) underlying their antagonistic interaction with *E. carotovora*.

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