Motility and Chemotaxis of *Erwinia herbicola* and Its Effect on *Erwinia amylovora*

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ABSTRACT

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Optimum conditions for motility and chemotaxis of *Erwinia herbicola* (112Y) were determined by capillary assay. Cells grown at 29 C and suspended in motility medium containing phosphate buffer, adjusted to pH 7, were very motile. The optimum assay temperature tested was 37 C for motility and 29 C for chemotaxis. *E. herbicola* was attracted chemotactically to Jonathan apple nectar extract and to all fractions (organic acid, amino acid, and neutral and basic) of this extract. All amino acids tested, especially asparagine, histidine, and serine, were excellent chemoattractants. *E. herbicola* exhibited strong taxis to all sugars tested, but only to a few organic acids (malate and tartrate). High concentrations of cells of *E. herbicola* ($> 2 \times 10^8$ cfu/ml) in both the pond and the capillary of a capillary assay inhibited motility and chemotaxis of *E. amylovora*. Culture fluids of *E. herbicola* cells suspended in chemotaxis medium reduced the chemotactic response of *E. amylovora* toward malate. However, culture fluids of *E. herbicola* enhanced motility of *E. amylovora*.

Additional key words: biological control, fire blight.

Erwinia amylovora (Burr.) Winslow et al., the causal agent of fire blight of apples and pears, enters host plants through wounds, stomata, lenticels, and blossom nectaries (31). Raymundo and Ries (25) proposed that the pathogen, *E. amylovora*, might locate blossom nectaries and swim to openings via flagellar motility and chemotaxis. They suggested that this ability to migrate toward attractants and away from repellents (21) may play an important role in infection. Raymundo and Ries also determined that *E. amylovora* is chemotactically attracted to apple nectar extract and reported the optimal conditions for motility and chemotaxis (25,26). Bayot and Ries (5,6) provided evidence that motility of *E. amylovora* enhances infectivity and demonstrated the presence of sufficient concentrations of attractant compounds in host plant flower nectars to account for attraction.

Beer et al (7) reduced the occurrence of fire blight blossom infection by spraying apple blossoms with two different strains of a common leaf saprophyte, *E. herbicola* Lohnis (Dye). One strain (112Y) produces a bacteriocin that inhibits *E. amylovora* in vitro (14). However, reduction in blossom infection does not appear to be bacteriocin-related because a nonbacteriocinogenic strain of *E. herbicola* gave comparable results. The ability of these strains of *E. herbicola* to prevent infection by *E. amylovora* may be due to the successful competition for specific nutrients or ecological niches within the apple blossom. Therefore, the interaction of motility and chemotaxis between this saprophyte and the pathogen may play an important role in the infection process of *E. amylovora*.

The objectives of this study were to determine: 1) the optimum conditions for motility and chemotaxis of *E. herbicola*; 2) whether *E. herbicola* is attracted chemotactically to apple nectar extract and to which fractions of the extract; 3) what compounds serve as attractants of *E. herbicola*; and 4) whether or not *E. herbicola* has a direct and measurable effect on *E. amylovora* in a capillary assay. Preliminary results have been reported previously (15,16).

MATERIALS AND METHODS

Bacterial strains. *E. herbicola*, strain 112Y, originally isolated from apple (*Malus* spp.) (courtesy of S. V. Beer, Department of Plant Pathology, Cornell University, Ithaca, NY) was used in these studies. It was shown to have biological control capabilities for fire blight (7,14). Another strain of *E. herbicola*, Tr_2-3 (from E. J. Klos, Department of Plant Pathology, Michigan State University, East Lansing), was compared with strain 112Y. This strain (Tr_2-3) has a slight antibiotic effect on *E. amylovora* (E. J. Klos, *personal communication*). *E. amylovora* 110, a rifampin-resistant strain, obtained from E. J. Klos was used in interaction studies. All strains were stored by lyophilization in sterile 10% skim milk or in frozen broth at −80 C (13).

Media and growth conditions. Cultures of *E. herbicola* and *E. amylovora* were grown in modified Emerson's medium (MEM) (27) or in modified Miller and Schroth's medium (MMS). Miller and Schroth's medium (18) was modified by omitting sodium taurocholate, Tergitol 7, nitriloacetic acid, bromothymol blue, neutral red, cycloheximide, thallium nitrate, and agar. Cells of *E. herbicola* grown in broth cultures were shaken in a water bath at 120 oscillations per minute to mid-log phase (approximately 12 hr) at 29 C (*E. amylovora*, 23 C), unless otherwise stated. Cells grown in MEM plates were incubated for 24–36 hr at 30 C.

Motility and chemotaxis were measured using the capillary technique developed by Adler (2) and modified by Bayot (5). All glassware used was rinsed in double distilled water and autoclaved before each assay. All assays were conducted at room temperature (23–25 C) unless otherwise stated. Each assay consisted of four replications per treatment and was repeated at least twice.

The number of colony-forming units (cfu) per capillary was determined by direct pour-plate counts. These counts were obtained by placing the entire capillary contents into 5 ml of sterile 1% peptone water and pouring into a sterile petri dish. Ten milliliters of melted MEM agar, cooled to 50 C, was poured onto the 1% peptone suspension swirled for even distribution, allowed to harden, and incubated at 30 C. After 24 hr, three circular areas (D = 2.5 cm) were randomly marked on the surface of the agar and the total number of colony-forming units in these areas counted. The mean colony-forming units of these three samples was used to calculate the total colony-forming units in the capillary, assuming even colony distribution on the plate.

Fresh bacterial cells were prepared before each assay by transferring cells from the −80 C frozen broth and placing them in 25 ml of sterile MEM or MMS broth. These cultures were shaken for 24 hr, after which 0.25 ml of the cell suspension was transferred

aseptically to fresh 25 ml of MEM or MMS broth. Cultures were grown to mid-log phase (approximately 12 hr) and a 4-ml aliquot was removed and centrifuged in an SS-34 rotor (Sorvall) at 4,000 rpm for 5 min. The growth medium was removed, and to avoid flagellar breakage, the pellet was very gently resuspended in motility or chemotaxis medium. This washing procedure was repeated twice and the final suspension diluted to 2×10^8 cfu/ml (motility assay) or 5×10^7 cfu/ml (chemotaxis assay).

Motility medium for *E. herbicola* consisted of 10^{-2} M K_2HPO_4-KH_2PO_4 buffer and 10^{-3} M ethylenediamine tetraacetic acid (EDTA). Chemotaxis medium was the motility medium plus 10^{-2} M mannitol. Chemotaxis medium for *E. amylovora* was similar to that described previously (25) except that 10^{-3} M mannitol was used instead of 10^{-2} M mannitol and 10^{-2} M $MgCl_2$ was added. Motility and chemotaxis media were adjusted to pH 7.0 with 0.1 N HCl or 0.1 N KOH and filter-sterilized.

Factors affecting motility of *E. herbicola*. Motile isolates of *E. herbicola* were selected from the periphery of soft agar (0.25%) MEM plates minus the ingredient nutrient broth. The effects of growth temperature, assay temperature, pH, and EDTA (chelating agent) concentration on motility were studied.

The effect of growth temperature in MEM broth on the motility of *E. herbicola* was determined by varying the temperatures of the shaker baths from 18 to 33 C. Shaker baths were placed in a cold room (4 C) when temperatures less than ambient (18 and 21 C) were required. Cells were harvested at mid-log phase of growth at the appropriate temperature, washed and resuspended in motility medium, and checked for motility by the capillary assay.

Assay temperatures, ranging from 18 to 37 C, were screened for optimum motility of *E. herbicola*. Slide warmers were used for temperatures greater than ambient (25, 29, 33, and 37 C) and slide warmers placed in the cold room (4 C) were used for temperatures less than ambient (18 and 31 C). The effect of pH on motility was determined by adjusting the pH of the motility medium with HCl or NaOH from pH 5 to 9 at 1-unit intervals. Concentrations of EDTA (10^{-1} to 10^{-4} M) also were adjusted in the motility medium to check for any effects on motility.

Factors affecting chemotaxis of *E. herbicola*. The effect of assay temperature and length of assay time on chemotaxis was determined by the capillary assay. Cells were prepared and suspended to a concentration of 4×10^7 cfu/ml under optimum conditions required for good motility as described above. The Jonathan apple nectar extract (10^{-1} dilution) used as the attractant in the capillary for these experiments was obtained by the procedure of Bayot and Ries (6).

Chemotaxis of *E. herbicola* toward various compounds. Chemotaxis of *E. herbicola* to various compounds (amino acids, organic acids, sugars, and Jonathan apple nectar extract fractions) was determined by capillary assay. All sugars tested were D-form, whereas the amino acids were L-form. Arbutin, phloridzin, and the amino acids were obtained from Sigma Chemical Company (St. Louis, MO). The sugars and organic acids were of reagent grade from various commercial sources. Jonathan apple nectar extract was fractionated by ion exchange chromatography according to the procedure of Bayot (5). All compounds were dissolved in chemotaxis medium to a concentration of 10^{-2} M, unless otherwise indicated, adjusted to pH 7.0 with 0.1 N KOH or 0.1 N HCl, and then filter-sterilized. The attractiveness of these compounds was compared using relative response values (ratio of colony-forming units per capillary containing the compound tested to that of a capillary containing chemotaxis medium only).

Effect of *E. herbicola* on motility and chemotaxis of *E. amylovora*. The effect of varying cell concentrations and culture fluids of *E. herbicola* 112Y on the motility and chemotaxis of *E. amylovora* 110 was investigated by using the capillary assay.

Cell concentration. Four different concentrations of *E. herbicola* cells were suspended in *E. amylovora* chemotaxis medium at A_{590nm} = 0.02, 0.1, 0.2, and 0.28. A suspension of *E. amylovora* in chemotaxis medium was also adjusted to A_{590nm} = 0.09. To determine whether or not different concentrations of *E. herbicola* cells had an effect on either motility or chemotaxis of *E. amylovora*, the different *E. herbicola* suspensions were combined in a 1:1 ratio with the *E. amylovora* suspension. This 1:1 dilution ratio yielded suspensions of $A_{590nm} = 0.045$ (4×10^7 cfu/ml) for *E. amylovora* and 0.01 (2×10^7 cfu/ml), 0.05 (1×10^8 cfu/ml), 0.1 (2×10^8 cfu/ml), and 0.15 (3×10^8 cfu/ml) for *E. herbicola*, respectively. These suspensions were placed in the pond for the capillary assay. Other *E. herbicola* cell suspensions were prepared at the absorbances listed above (0.01, 0.05, 0.1, and 0.15) in either chemotaxis medium or chemotaxis medium plus 10^{-2} M malate, the latter a strong attractant of *E. amylovora* (25). These suspensions were then drawn into the 1-μl capillary tubes for the capillary assay.

Motility and chemotaxis assays for *E. amylovora* were incubated for 30 min (25), after which the capillaries were removed, the exteriors washed, and the contents spread over the surface of modified Emerson's medium plates containing 500 μg/ml of rifampin (Sigma). The number of colony-forming units per capillary of *E. amylovora* was counted after 36 hr.

Culture fluid. Culture fluid (CF) of *E. herbicola* cells was prepared by suspending approximately 1×10^{10} cells per milliliter in *E. amylovora* chemotaxis medium alone or chemotaxis medium plus 10^{-2} M malate for 1 hr. The cells were removed by centrifugation and the supernatant filter-sterilized and called *E. herbicola* CF.

E. amylovora cells were prepared for capillary assay as described previously, suspended in *E. herbicola* CF to an $A_{590nm} = 0.045$, and placed into the pond. The capillaries contained either CF for the motility assay or CF plus 10^{-2} M malate for the chemotaxis assay. Controls consisted of *E. amylovora* cells suspended in regular chemotaxis medium and the capillaries containing chemotaxis medium alone or chemotaxis medium plus 10^{-2} M malate. Assays ran for 30 min, and the capillary contents were plated to determine the number of cells of *E. amylovora* (cfu) that swam into the capillary tube.

Utilization of selected organic acids. The organic acids that were assayed to determine whether or not *E. herbicola* 112Y or *E. amylovora* 110 could use them as energy sources (10) included fumarate, galacturonate, malate, malonate, succinate, and tartrate, all as sodium salts.

Data analysis. Means were compared using the Waller-Duncan (Bayesian least significant diffference–BLSD) multiple comparison test (K = 100). The number of *E. amylovora* cells accumulated in a capillary tube was regressed on the concentration of *E. herbicola* cells in the pond and the capillary. Significance of the model and of polynomial terms was tested by F-values.

RESULTS

Factors affecting motility. *Effect of growth temperature.* Actively motile cells of *E. herbicola* were detected at growth temperatures of 29 and 31 C (Fig. 1) using the capillary assay. Growth at temperatures ranging from 18 to 27 C and at 33 C yielded fewer motile cells than growth at 29–31 C. A growth temperature of 29 C was used in subsequent experiments because it produced the most motile cells.

Effect of assay temperature. The motility of cells increased linearly as the temperature of incubation in the capillary assay increased (Fig. 2). Significantly more cells accumulated per capillary at 37 C than at any other assay temperature tested including 29 C, the optimum growth temperature tested for motility.

Effect of pH and EDTA. Cells of *E. herbicola* suspended in motility medium at pH 7 were more motile than those at pH 6 or 8 (Fig. 3). Motility was greatly reduced in cells suspended in motility medium of pH 5 or 9. The presence of EDTA, a chelating agent, in the motility medium slightly enhanced the motility of cells suspended in all concentrations tested (Fig. 3). Therefore, a 10^{-3} M concentration was used in all experiments to simulate conditions previously reported optimal for *E. amylovora* (26).

Factors affecting chemotaxis. *Effect of assay temperature.* The optimum assay temperature for chemotaxis of *E. herbicola* toward Jonathan nectar extract (10^{-1} dilution) was 29 C (Fig. 2). The

number of cells per capillary increased as the temperature increased from 18 to 29 C and decreased markedly at 33 C.

Effect of assay time. Chemotaxis toward nectar extract as measured by the number of cells accumulating per capillary increased as a function of assay time. The number of cells per capillary increased sharply from 250 cells per capillary at 0 min to 39,800 cells per capillary at 60 min. Motility, as measured by the number of cells randomly swimming into the capillary in the absence of an attractant, increased gradually from 200 cells per capillary at 0 min to 1,585 cells per capillary at 60 min. An assay time of 40 min was chosen for all experiments concerning chemotaxis of *E. herbicola*.

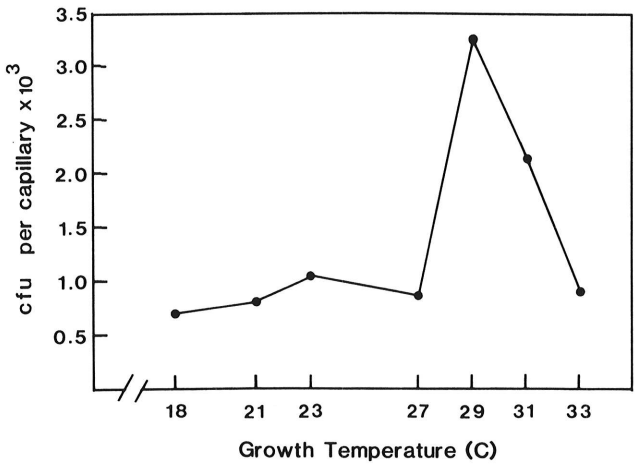

Fig. 1. Effect of growth temperature on motility of *Erwinia herbicola* 112Y. Cells were grown in modified Emerson's medium broth, harvested at mid-log phase, and suspended in 10^{-2} M potassium phosphate buffer and 10^{-3} M EDTA to a concentration of 2×10^{8} cfu/ml. Capillary assay run for 40 min.

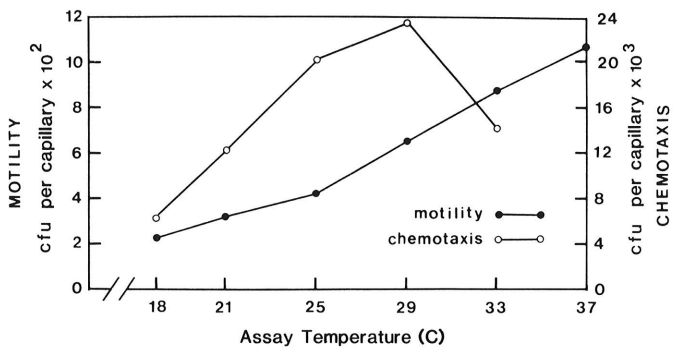

Fig. 2. Effect of capillary assay temperature on the motility and chemotaxis of *Erwinia herbicola* 112Y. Motility assays performed using 2×10^{8} cells per milliliter suspended in 10^{-2} M potassium phosphate buffer and 10^{-2} M EDTA adjusted to pH 7. Chemotaxis assays performed using 4×10^{7} cells per milliliter suspended in above buffer with 10^{-2} M mannitol added and Jonathan apple nectar extract (10^{-1} dilution) as the attractant. Assays were performed for 40 min.

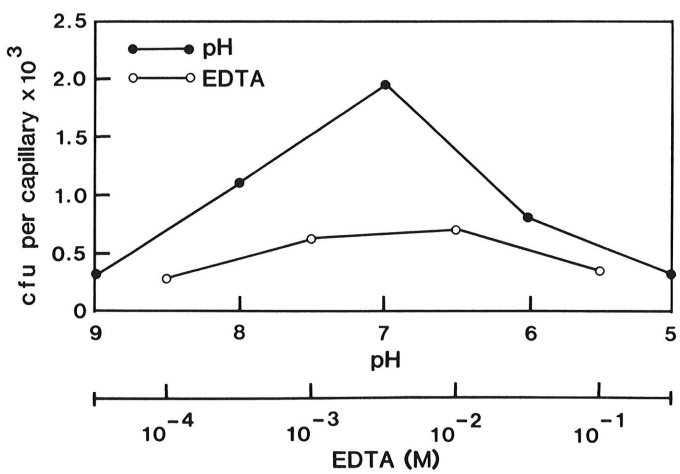

Fig. 3. Effect of ethylenediamine tetraacetic acid (EDTA) and pH on motility of *Erwinia herbicola* 112Y. Assays were performed for 40 min at 23 C using 2×10^{-8} cells per milliliter suspended in 10^{-2} M potassium phosphate buffer plus 10^{-3} M EDTA for pH experiments.

Chemotaxis to various compounds. *Nectar extract fractions. E. herbicola* showed positive taxis toward all fractions of Jonathan nectar extract (Table 1). The neutral and basic fraction of nectar extract and unfractionated nectar extract were significantly better attractants than the organic acid or amino acids fractions.

Chemotaxis to amino acids. *E. herbicola* exhibited positive chemotaxis to all amino acids tested (Table 2). Relative response

TABLE 1. Chemotactic response of *Erwinia herbicola* 112Y to different fractions of Jonathan apple nectar extract

Fraction[w]	cfu/capillary[x]
Neutral and basic	9,416 a[y]
Nectar extract[z]	8,670 a
Organic acid	4,639 b
Amino acid	4,282 b
Control	278 c

[w] Fractionated by ion-exchange chromatography and resuspended as a 10^{-1} dilution in chemotaxis medium.

[x] Each value is the mean of two trials with four replicates per trial, and the number of colony-forming units per capillary was determined by direct pour-plate counts. Assay was run with 5×10^{7} cfu/ml outside the capillary for 40 min at 23 C. Control capillaries were filled with chemotaxis medium alone.

[y] Means with same letter are not significantly different according to Bayesian least significant difference test ($K = 100$).

[z] Nectar extract at 10^{-1} dilution in chemotaxis medium.

TABLE 2. Chemotactic response of *Erwinia herbicola* 112Y to selected amino acids[w]

Amino acid	cfu/capillary[x]	Relative response[y]
Asparagine	165,307	256.7
Serine	96,863	150.4
Tryptophan	59,919	93.0
Histidine	57,896	89.9
Tyrosine	48,725	75.6
Lysine	46,816	72.7
Cysteine	42,769	66.4
Arginine	25,870	40.2
Threonine	24,633	38.2
Proline	21,925	34.0
Glycine	17,542	27.2
Leucine	16,565	25.7
Alanine	16,410	25.5
Glutamate	15,081	23.4
Phenylalanine	12,783	19.8
Methionine	12,220	18.9
Aspartate	9,975	15.5
Glutamine	8,246	12.8
Isoleucine	8,097	12.6
Valine	8,063	12.5
BLSD[z]	48,095	

[w] All amino acids were L-form and at 10^{-2} M concentration, except tyrosine (10^{-3} M) in chemotaxis medium.

[x] Each value is the mean of two trials with four replicates per trial, and the number of colony-forming units per capillary was determined by direct pour-plate counts. Assay was run with 5×10^{7} cfu/ml outside the capillary for 40 min at 23 C.

[y] Ratio of colony-forming units per capillary to that of control (644 cfu/capillary).

[z] Bayesian least significant difference test ($K = 100$). Data were log-transformed before analysis and calculation of BLSD. For purposes of presentation, values in the table are antilogs of means and BLSD value. To separate means with the BLSD, the reader should convert values back to $\log_{10}$.

values ranged from 12.5 to 256.7. Asparagine was significantly better as an attractant than all other compounds tested, but histidine, serine, and tryptophan also had high relative response values. All other amino acids tested were fair attractants with the exception of glutamine, isoleucine, and valine, which were weak attractants. Weak attractants were arbitrarily defined as having relative response values less than 15.

Chemotaxis to sugars. *E. herbicola* exhibited positive taxis to all sugars tested (Table 3). The six-carbon sugars, glucose and fructose, were significantly better attractants than the other sugars, having relative response values of 59.2 and 43.1, respectively. Strong attractants were arbitrarily defined as having relative response values greater than 40. Therefore, sucrose was considered a weak attractant since its relative response was 11.

Chemotaxis to organic acids. *E. herbicola* exhibited strong taxis toward only malate and tartrate (Table 4). The relative response values for malate and tartrate were 89.1 and 63.5, respectively. Relative response values of the other organic acids tested were less than 15, indicating that most were very weak or nonattractants.

Chemotaxis to other compounds. Arbutin, the principal glucoside of pear trees (9), and phloridzin (8), the principal glucoside of apple trees, were assayed as possible attractants of *E. herbicola* (Table 4). Arbutin (relative response = 1.0) and phloridzin (relative response = 4.0) were nonattractants of *E. herbicola*.

Combined analysis of all compounds as chemoattractants. A combined analysis of the relative response values for all compounds tested was conducted to determine which compounds served as the best overall attractants of *E. herbicola*. Asparagine and serine were the best attractants of *E. herbicola*. Seven of the best 10 attractants were amino acids. The organic acids, malate and tartrate, and the sugar, glucose, ranked 5, 9, and 10, respectively. Overall, *E. herbicola* showed strong taxis toward most amino acids and weak taxis to the majority of organic acids tested. Sugars generally served as strong attractants of *E. herbicola*, but not as strong as amino acids.

Inhibition of chemotaxis by other compounds. When asparagine, a strong attractant, was present in the capillary and the pond at 10^{-2} M, chemotaxis of *E. herbicola* toward serine was inhibited by 99%. Taxis toward glucose and malate was not affected. When malate, a strong organic acid attractant, was present in both the capillary and pond at 10^{-2} M, taxis of *E. herbicola* toward asparagine was inhibited by 46%, taxis toward glucose was by 30%, and toward Jonathan nectar extract by 52%.

Comparison of chemotaxis between two strains of *E. herbicola*. A second strain of *E. herbicola* (Tr_2-3) was used to compare its response to various attractants with those of strain 112Y. *E. herbicola* Tr_2-3 was chemotactic toward all compounds tested (Table 5). The neutral and basic fraction of Jonathan apple nectar extract showed the highest relative response, 107.7. Asparagine, glucose, malate, serine, and Jonathan nectar extract were all very strong attractants of Tr_2-3.

Effect of *E. herbicola* on motility and chemotaxis of *E. amylovora*. *Cell concentrations.* As the cell concentration of *E. herbicola* increased in the capillary and pond from $A_{590nm} = 0.01$ to $A_{590nm} = 0.15$, the motility and chemotaxis of *E. amylovora* decreased (Fig. 4). Regression analysis indicated a linear effect with the number of *E. amylovora* cells accumulating per capillary decreasing as the concentration of *E. herbicola* increased. This effect occurred in Trial A for both motility (random swimming) and chemotaxis toward malate for *E. amylovora*. Regression analysis of Trial B showed a quadratic relation between the number of bacteria per capillary and the concentration of *E. herbicola*.

Culture fluids enhanced the motility of *E. amylovora* when *E. amylovora* cells were resuspended in CF (Fig. 5). However, chemotaxis of *E. amylovora* toward malate (10^{-2} M) was reduced

TABLE 3. Response of *Erwinia herbicola* 112Y to selected sugars[w]

Sugar	cfu/capillary[x]	Relative response[y]
Glucose	38,138	59.2
Fructose	27,758	43.1
Lactose	20,428	31.7
Ribose	19,247	29.8
Raffinose	15,587	24.2
Sorbitol	14,325	22.2
Galactose	13,858	21.5
Sucrose	7,095	11.0
Glycerol	3,685	5.7
BLSD[z]	13,991	

[w] All sugars were D-form and at 10^{-2} M concentration in chemotaxis medium.
[x] Each value is the mean of two trials with four replicates per trial, and the number of colony-forming units per capillary was determined by direct pour-plate counts. Assay was run with 5×10^7 cfu/ml outside the capillary for 40 min at 23 C.
[y] Ratio of colony-forming units per capillary to that of control (644 cfu per capillary).
[z] Bayesian least significant difference test (K = 100). Data were log-transformed before analysis and calculation of BLSD. For purposes of presentation, values in the table are antilogs of means and BLSD value. To separate means with the BLSD, the reader should convert values back to $\log_{10}$.

TABLE 4. Chemotactic response of *Erwinia herbicola* 112Y to selected organic acids and other compounds[w]

Organic acid	cfu/capillary[x]	Relative response[y]
Malate	57,367	89.1
Tartrate	40,894	63.5
Galacturonate	9,542	14.8
Fumarate	5,216	8.1
Phloridzin	2,553	4.0
Oxalate	2,543	3.9
Succinate	2,105	3.3
α-Ketoglutarate	2,066	3.2
Citrate	1,751	2.7
Malonate	867	1.3
cis-Aconitic acid	751	1.2
Arbutin	654	1.0
Maleate	647	1.0
Control	644	1.0
Oxaloacetate	497	0.8
Pyruvate	473	0.7
Lactate	323	0.5
BLSD[z]	11,510	17.9

[w] All organic acids are at 10^{-2} M concentration in chemotaxis medium, except for phloridzin (10^{-3} M).
[x] Each value is the mean of two trials with four replicates per trial, and the number of colony-forming units per capillary was determined by direct pour-plate counts.
[y] Ratio of colony-forming units per capillary to that of control (644 cfu per capillary).
[z] Bayesian least significant difference test (K = 100). Data were log-transformed before analysis and calculation of BLSD. For purposes of presentation, values in the table are antilogs of means and BLSD value. To separate means with BLSD, the reader should convert values back to $\log_{10}$.

TABLE 5. Response of two strains of *Erwinia herbicola* to various attractants[x]

	Relative response[z]	
Attractant[y]	Tr_2-3	112Y
Neutral and basic fraction	107.7	33.9
Glucose	88.6	59.2
Serine	87.5	150.4
Malate	81.0	89.1
Asparagine	78.9	256.7
Jonathan nectar extract	69.5	31.2

[x] Attractants showing significant responses for strain 112Y in Tables 1, 2, 3, and 4.
[y] Ratio of number of colony-forming units per capillary to that of control value. Strain Tr_2-3 control = 1,368 cfu per capillary; strain 112Y control = 644 cfu per capillary.
[z] All at 10^{-2} M concentration in chemotaxis medium, except Jonathan nectar extract and neutral and basic fraction of nectar extract (10^{-1} dilution).

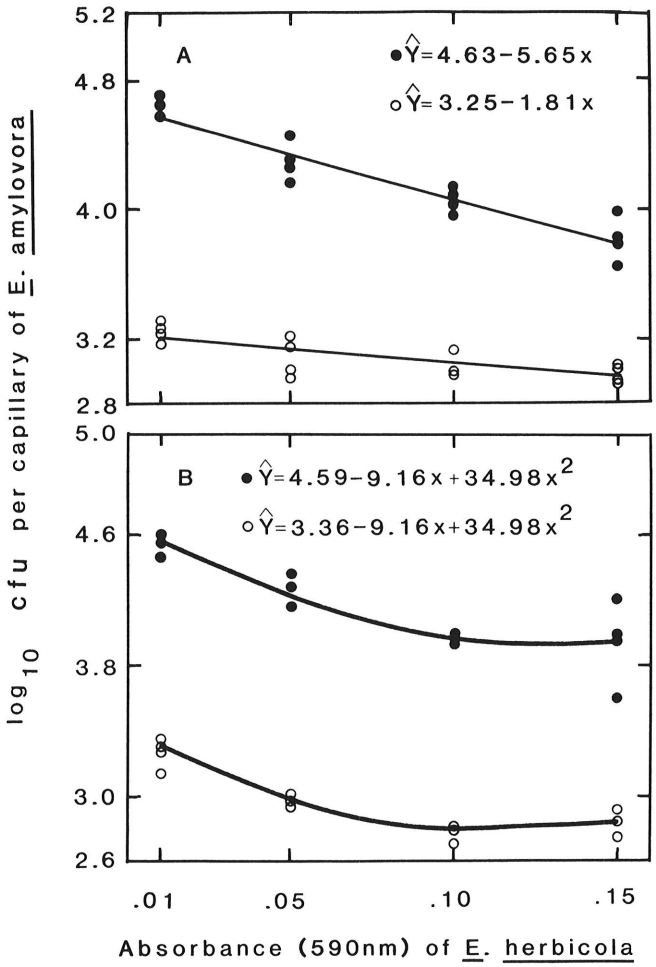

Fig. 4. Effect of different cell concentrations of *Erwinia herbicola* 112Y in the pond and capillary on the motility (open circle) and chemotaxis (bullet) of *Erwinia amylovora* 110 to malate. Two assays (**A** and **B**) were run for 30 min at 23 C. Concentrations of *E. herbicola* 112Y were 2×10^7 cfu/ml (0.01); 1×10^8 cfu/ml (0.05); 2×10^8 cfu/ml (0.1); 3×10^8 cfu/ml (0.15). Chemotaxis and motility assays performed using 4×10^7 cfu/ml of *E. amylovora* 110 using malate (10^{-2} M) as the attractant. Two assays (**A** and **B**) were run for 30 min at 23 C.

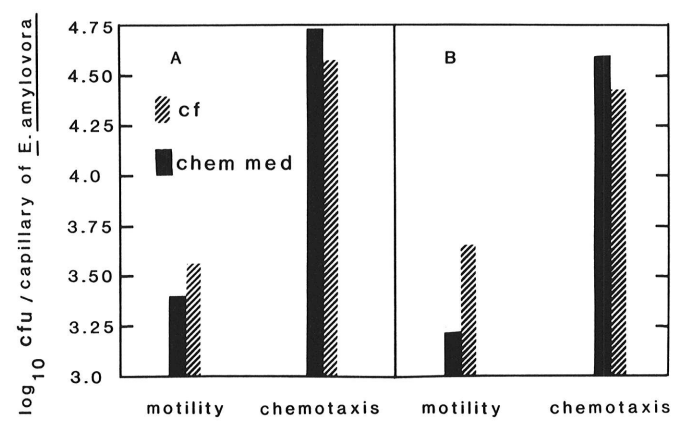

Fig. 5. Effect of *Erwinia herbicola* 112Y culture filtrate (CF) on motility and chemotaxis of *Erwinia amylovora* to malate (10^{-2} M). Cells of *E. amylovora* were resuspended to a concentration of 4×10^7 cfu/ml in chemotaxis medium alone (solid bar) or in CF (hatched bar). Assays (Trial **A** and **B**) were run for 30 min at 23 C. Bayesian least significant difference: Trial **A** = 0.0815 $\log_{10}$ colony-forming units per capillary; Trial **B** = 0.0678 $\log_{10}$ colony-forming units per capillary ($K = 100$).

in the presence of CF.

Utilization of selected organic acids. *E. herbicola* Tr_2-3 used all organic acids tested, whereas *E. herbicola* 112Y used all except galacturonate. *E. amylovora* 110 used all organic acids tested except for galacturonate and tartrate.

DISCUSSION

Warm growth temperatures (29–31 C) are required for the production of motile cells in *E. herbicola* 112Y. This contrasts sharply with the much cooler growth temperatures (23 C) required for the production of motile cells of *E. amylovora* (26). Erskine and Lopatecki (12) suggested that a change in environmental conditions later in the growing season, specifically an increase in temperature, favored predominance of the saprophyte over the pathogen. As temperatures warm throughout the growing season, more motile cells of *E. herbicola* may be produced, and, therefore, the saprophyte would be better able to swim and locate protected niches on the plant normally colonized by the pathogen.

The minimum temperature for the occurrence of blossom blight is from 18 to 20 C (24). *E. amylovora* is most motile at these temperatures (26), whereas *E. herbicola* is only slightly motile. *E. herbicola* requires much warmer temperatures (31–37 C) for active motility. Normal to warm temperatures (21 C) at flowering may favor *E. amylovora* motility and infection, whereas warm to hot temperatures (31 C) may favor motility and colonization of the blossom by *E. herbicola*.

Various cultural factors including pH and the addition of a chelating agent affect motility with many other bacteria, including *E. coli* (4). The optimum pH for motility of *E. herbicola* was pH 7. Motility was significantly reduced at pH values above or below neutrality. Interestingly, *E. amylovora* exhibits a broader pH range of 6–9 for optimal motility (26). Riggle and Klos (28) suggested that *E. herbicola* inhibits blossom blight infection by *E. amylovora* by reducing the pH within the apple blossom to a level inhibitory to *E. amylovora*. Our results suggest that lower pH values would probably not affect the motility of *E. amylovora* as significantly as *E. herbicola*.

E. herbicola 112Y is chemotactic toward Jonathan apple nectar extract and all fractions of this extract. Chemotaxis toward nectar extract also occurred with *E. amylovora* (25). However, the pathogen was attracted only to the organic acid fraction of this extract and probably only to fumarate or malate (5). The nonspecific attraction of *E. herbicola* to all components of the nectar extract may give *E. herbicola* an advantage over other microorganisms including *E. amylovora* in locating suitable niches for colonization of the blossom.

Temperature requirements for chemotaxis of *E. herbicola* and *E. amylovora* are similar, indicating that temperature may not be the limiting factor in the detection of attractants by these bacteria in the apple blossom. Temperatures above 29 C or below 20 C reduced chemotaxis in both *E. amylovora* (25) and *E. herbicola*. However, the optimum chemotaxis assay temperature appears independent of the motility assay temperature because *E. herbicola* is still motile at 37 C.

Many compounds, including amino acids, sugars, and a few organic acids, gave high relative response values as attractants of *E. herbicola*. This response is not unusual in that many other bacteria exhibit similar taxis (1,3,22,29,30). To verify whether some of the compounds are weak attractants of *E. herbicola*, purification of these compounds would be required. A small amount of a strong attractant as a contaminant in a nonattractant compound may result in a weak response.

E. herbicola exhibits positive taxis to many sugars, but *E. amylovora* is not known to be attracted to any sugar (25). Riggle and Klos (28) suggest that pear nectar contains high concentrations of fructose, glucose, and sucrose. They developed a medium containing these sugars and suggested that *E. herbicola* produces large amounts of acidic by-products on this high sugar medium, which reduces the pH and inhibits growth of *E. amylovora*. Because *E. herbicola* is attracted to these sugars it is conceivable that the saprophyte might metabolize these sugars, lower the pH in

the blossom, and thereby inhibit growth of *E. amylovora*. Previously reported research suggests that the motility and chemotaxis in *E. amylovora* is not affected over the range of pH 6–9 (25,26). Therefore, the control mechanism suggested by Riggle and Klos (28) may not be functioning.

E. herbicola was attracted toward most amino acids tested, including amino acids with acidic, basic, aromatic, and aliphatic side chains. Chemotaxis toward many different amino acids also occurs in other bacteria (17,19,20,22,30). Interestingly, *E. amylovora* is attracted to a single amino acid, aspartate, a four-carbon dicarboxylic acid (25).

Malate and tartrate are the only good organic acid attractants of *E. herbicola*. The strongest organic acid attractant of *E. amylovora* also is malate (25). Malate is found in nectar extract at concentrations detectable by *E. amylovora* (5). The similarity in taxis of *E. herbicola* and *E. amylovora* toward these organic acids, specifically malate, may be a clue to the mechanism by which the saprophyte inhibits infection by the pathogen.

The attractants of *E. amylovora* are detected by only one chemoreceptor (25). This receptor is highly specific for certain four-carbon dicarboxylic acids. The chemotactic response of *E. herbicola* 112Y to a wide variety of compounds is not unique. A different strain, Tr_2-3, which also has an inhibitory effect on *E. amylovora*, responded similarly to 112Y. Both strains were attracted to identical compounds. Because *E. herbicola* is attracted to many different compounds, it probably has many different chemoreceptors similar to the many receptors identified in other bacteria (1,23). Numerous receptors may be advantageous to the saprophyte for locating nonspecific nutritional sources on the plant surface. On the contrary, a single receptor site detecting specific compounds for a pathogen appears to be unique to *E. amylovora* and may represent a significant difference between a plant pathogen and a plant saprophyte.

It is conceivable that the presence of *E. herbicola* in the apple blossom may affect motility and chemotaxis in *E. amylovora* and thus explain its biological control capabilities. Our results, using one strain of *E. herbicola*, indicate that motility of *E. amylovora* is reduced in the presence of cells of *E. herbicola* 112Y but enhanced in the presence of *E. herbicola* 112Y CF. Possibly, *E. herbicola* may metabolize compound(s) in the chemotaxis medium, which inhibit motility of *E. amylovora,* or may secrete compound(s), which enhance motility in *E. amylovora*. Interestingly, chemotaxis of *E. amylovora* toward malate was reduced in both the presence of *E. herbicola* cells or CF. The presence of viable *E. herbicola* suspended in the chemotaxis medium containing malate may reduce the concentration of malate (the attractant) in the medium. *E. herbicola* 112Y and Tr_2-3 and *E. amylovora* 110 can use malate and other organic acids as sole carbon and energy sources. Dye (10,11) also reported similar results. This utilization of four-carbon dicarboxylic acids, especially malate, by *E. herbicola* could decrease the effective concentration of malate in the chemotaxis medium and thereby reduce the chemotactic response of *E. amylovora*.

The ability to detect many different compounds may enhance the effectiveness of *E. herbicola* as a biological control agent in apple blossoms. Large populations of this saprophyte, actively swimming and locating nutritional sources exuding from the host, may occupy many of the niches available to the pathogen. In addition, the saprophyte may use chemoattractants of the pathogen, thus confusing its sensory system and affecting invasion and subsequent infection.

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