Ecology and Epidemiology

Role of Motility in Apple Blossom Infection by *Erwinia amylovora* and Studies of Fire Blight Control with Attractant and Repellent Compounds

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A portion of the senior author's Ph.D. dissertation submitted to the University of Illinois.

ABSTRACT

Bayot, R. G., and Ries, S. M. 1986. Role of motility in apple blossom infection by *Erwinia amylovora* and studies of fire blight control with attractant and repellent compounds. Phytopathology 76: 441-445.

A nonmotile strain (Nm22) and its motile revertant (Mr22) of *Erwinia amylovora* were obtained after treating cells of the wild type with 0.3 M ethyl methanesulfonate. Pathogenicity of both strains was similar to that of the wild type on young Jonathan apple (*Malus domestica*) seedlings. Significantly more infection was recorded in blossoms inoculated with Mr22 than with Nm22 at inoculum concentrations of 5×10^5 and 1×10^7 colony-forming units per milliliter. Neither the repellents, sodium benzoate or sodium salicylate, nor the attractants, sodium malate or sodium tartrate, applied to apple blossoms as 10^{-2} or 10^{-3} M solutions, provided consistent protection against infection by *E. amylovora*. Negative chemotaxis of *E. amylovora* was constitutive for benzoate and salicylate but inducible for L-isoleucine, L-leucine, and L-phenylalanine.

Additional key words: bacterial movement.

Flagellar motility is characteristic of many plant pathogenic bacteria. Most plant pathogenic bacteria that infect aboveground plant parts generally enter and invade tissues through natural openings or mechanical wounds in the presence of sufficient moisture (5,9). Motility and chemotaxis confer survival advantages to certain bacterial species (8,16) and may increase the infection potential of bacterial plant pathogens by allowing them to actively enter infection sites (11).

Erwinia amylovora is motile by means of peritrichous flagella (4,6). The factors affecting its motility (14) and chemotaxis (13) have been studied extensively under laboratory conditions. The pathogen was attracted chemotactically to nectar extracts of apple blossoms (13). Raymundo and Ries (13) identified several attractants for *E. amylovora* and showed that the pathogen was strongly attracted to malate and weakly attracted to a structurally

similar compound, tartrate. Repellent compounds for *E. amylovora* have not been reported. *Escherichia coli* and *Salmonella typhimurium* are reported to be repelled by several compounds (17,18). Spray applications of attractant and/or repellent chemicals to susceptible rosaceous plants might confuse the pathogen and provide a novel control strategy.

The objectives of this study were to determine whether motility aids *E. amylovora* in its invasion of apple blossoms and to determine whether chemical attractants or repellents could be used to reduce fire blight infection.

MATERIALS AND METHODS

Media and bacterial strains. Modified Emerson's medium (MEM) (15), modified Miller and Schroth medium (MMS), and a chemotaxis medium (13) were used. Semisolid tryptone medium (STM) contained 1.0% tryptone (Difco) and 0.4% agar (3). All media were adjusted to pH 7. The chemotaxis medium (13) was filter-sterilized.

A rifampin-resistant strain of *E. amylovora* (isolate 110) was obtained from E. J. Klos, Plant Pathology Department, Michigan

State University, East Lansing 48823. A nonmotile strain (Nm22) was obtained after mutagenesis of isolate 110 by using a modification of the procedure of Ordal and Adler (10). One milliliter of a 24-hr-old culture grown in MEM broth was transferred to 20 ml of MEM broth and was grown in a shaker bath for 12 hr at 23 C. Cells were harvested by centrifugation at 5,000 rpm in an SS-34 (Sorvall) rotor for 5 min, washed in 4 ml of 10 mM $MgSO_4$, and resuspended in 4 ml of MMS to a concentration of about 2.7×10^8 colony-forming units (cfu) per milliliter (Absorbance = 0.22 at 590 nm). Ethyl methanesulfonate was added to the suspension to a final concentration of 0.3 M, the suspension was mixed with a vortex mixer, incubated for 1 hr at room temperature (23–25 C), centrifuged, and washed twice with MMS. The suspension was diluted 1:1,000 in MEM broth, incubated in a shaker bath for 24 hr, diluted again in MEM (1:50) and single small drops (approximately 0.1 ml) of the suspension were placed at the center of STM plates. All plates were incubated overnight at room temperature. The center of each colony was touched with a transfer needle to obtain nonmotile cells. Nonmotile cells remain at the colony center while more motile cells swim toward the colony edge. These nonmotile cells were transferred to the center of another plate of STM and this process was repeated each day for 4 days. The final transfer was done by streaking onto MEM agar plates and a portion of a colony that developed was suspended in a drop of chemotaxis medium and observed under a phase-contrast microscope for motility. Colonies with nonmotile cells failing to spread rapidly on STM plates were cultured and used in subsequent tests.

Nonmotile strains were grown in STM for 72 hr. Colonies that produced revertants were evident by the appearance of "flares" of bacteria spreading away from the inoculation point. Cells were taken from the edges of such flares and were transferred to fresh STM plates daily. The final transfer was done by streaking on MEM plates. Colonies with highly motile cells as viewed under a phase-contrast microscope were cultured and used in subsequent tests.

Testing for rifampin resistance and pathogenicity. Nonmotile strain Nm22 and its motile revertant (Mr22) were streaked on MEM plates containing 500 μg of rifampin (Sigma Chemical Co., St. Louis, MO) per milliliter to determine if the rifampin-resistance trait was retained after mutagenesis. The pathogenicity of strains was tested and compared with the wild type on greenhouse-grown Jonathan apple seedlings. All strains were grown on MEM plates for 24 hr at 30 C, the cells were washed from the MEM plates with sterile distilled water, and the suspension was adjusted to approximately 1×10^8 cfu/ml (Absorbance = 0.075 at 590 nm). Young shoots were inoculated with a hypodermic needle.

Motility assay. *Measurement of colony diameter on STM.* Colonies (24 hr old) of Nm22 and Mr22 were touched separately with the point of a transfer needle and placed on separate STM plates by stab inoculation. Two stab inoculations were made per plate and each strain was replicated four times. The average growth in diameter of both colonies per plate was measured after 18 hr of incubation at room temperature.

Capillary assay. This assay was performed by using a modified Raymundo and Ries (14) technique. Sterile glass slides were used to prepare the assay chambers and 0.25 ml of a bacterial suspension $(4 \times 10^7$ cells per milliliter) in motility medium was placed in each chamber. Assay chambers were incubated for 10, 20, 30, and 45 min at room temperature. The number of bacteria in a capillary at the end of the incubation period is a function of their rate of movement. Each treatment was replicated four times and the number of bacteria per capillary was based on duplicate plate counts on MEM.

Response of isolates to apple flower nectar extract. Nectar extract was collected from Jonathan apple flowers at full bloom. Flowers were collected and kept in plastic bags at 4 C until processed. Glass-distilled water (10) was deposited on flower nectaries exposed by manually removing other flower parts and the nectar extract was removed by using a modification of the method of Raymundo and Ries (13). A collection flask attached to a vacuum source was used instead of an Eppendorf pipet. This was prepared by fitting a bent 1-ml glass pipet into a rubber stopper. The

pipet was heated and while molten pulled to make a long and slender tip. Extracts were immediately filter-sterilized, placed in vials, and stored at −20 C.

The tactic response of Mr22 and Nm22 to apple flower nectar was compared to that of the wild type (strain 110). The same procedure as in the capillary motility assay was followed except some capillaries contained a (filter-sterilized) 10^{-1} dilution of apple flower nectar in chemotaxis medium, while others contained chemotaxis medium alone. The assay chambers were incubated for 30–45 min at room temperature. Each treatment was replicated six times. The relative response values were obtained by dividing the number of colonies recovered from capillaries containing flower nectar by the number of colonies recovered from control capillaries.

Screening for repellent chemicals. L-isoleucine, L-leucine, L-phenylalanine, sodium benzoate, and sodium salicylate (all obtained from Sigma Chemical Co.) were tested for repellent action (10) against *E. amylovora*. Each compound was dissolved separately to 0.1 M in the chemotaxis medium, and the solution was adjusted to pH 7.0 with 0.1 N NaOH or 0.1 N HCl.

The response of *E. amylovora* to each compound was determined by the capillary assay described previously except that the cell concentrations were 5×10^7 per milliliter. Repellent compounds were identified by comparing the number of bacteria in capillaries with and without (control) the test chemicals. Some bacteria enter the control capillaries (background number) due to random motility. If the chemical is a repellent, fewer than the background number will accumulate inside the capillary. However, similar results would be obtained if flagellar motility is inhibited by the compound. The test-tube method of Tso and Adler (18) was used to determine whether the negative response to benzoate and salicylate was due to inhibition of flagellar motility. With this method, a clear band or zone results as motile bacteria move away from the agar containing the repellent compound.

The threshold concentration for negative taxis to benzoate and salicylate was determined by measuring the response of strain 110 of *E. amylovora* to a range of concentrations. The inducibility of negative taxis for L-isoleucine, L-leucine, and L-phenylalanine was determined by growing the bacteria in 10^{-3} M solutions of each compound in MMS broth. Response to each compound was measured by the capillary assay. The repellent action of benzoate and salicylate against *E. amylovora* in the presence of 10^{-1} and 10^{-2} dilutions of Jonathan apple flower nectar also was tested by using this assay.

Preparation of blossoms for inoculation. Flower clusters containing at least four flower buds were selected from 18-yr-old Jonathan apple trees growing at the Plant Pathology Research Center in Urbana. Each cluster was bagged in a $5 \times 10 \times 30$-cm plastic bag 2–3 days before inoculation.

Chemical preparation. The chemical attractants used were malic acid (a strong attractant) and tartaric acid (a weak attractant) (13). They were dissolved separately in distilled water and adjusted to pH 7. Only 10^{-3} M solutions of both attractants were used in 1982, but 10^{-2} and 10^{-3} M solutions were used in 1983. The chemical repellents used were sodium benzoate and sodium salicylate. They also were dissolved in distilled water and the solutions were adjusted to pH 7. The effect of these repellents was studied only in 1983. Due to a limited number of open flowers per tree, two different experiments were conducted. In the first experiment, 10^{-1} M and 10^{-2} M solutions of each repellent were used. In the second experiment, only 10^{-3} M solutions were applied.

All solutions were applied with a hand sprayer 1 day prior to inoculation when 50% of the flowers were open to simulate the application of a protectant chemical. Control blossom clusters were sprayed with distilled water. Treatments were arranged in a randomized complete block design with each tree a block or replicate. Ten flower clusters made up a replicate. Each treatment was replicated six times in all trials in all experiments.

Inoculum preparation and blossom inoculation. *Effect of motile and nonmotile isolates on blossom infection.* The Mr22 and Nm22 strains were grown separately in 25 ml of MMS in a shaker bath at 23 C. Cells were harvested after 20–24 hr and centrifuged in an

SS-34 rotor (Sorvall) at 4,000 rpm for 8 min. To maintain high motility, the cells were resuspended in a solution containing 10^{-3} M $MgSO_4$ and 10^{-4} M EDTA. Bacterial concentrations were adjusted to 1×10^7 cfu/ml during the spring 1981 trial and to 5×10^3, 5×10^5, and 3×10^7 cfu/ml during the 1982 trial to determine the optimum concentration for detection of differences in blossom infection between strains. Care was taken during suspension and handling of inocula to minimize flagellar breakage.

Inoculations were made by slitting the plastic bag to create an opening and inserting the nozzle of a plant mister (hand sprayer). Approximately 6 ml of inoculum was sprayed on each open blossom cluster to run-off, then the opening in the bag was stapled closed to prevent rapid drying. All bags were removed 2 days after inoculation. Treatments were arranged in a randomized complete block design with each tree considered a block or replicate. Each treatment was replicated six (1982) or 10 (1981) times with 10 flower clusters per replicate.

Effect of attractant and repellent chemicals on blossom infection. Strain 110, prepared in a similar manner as Mr22 and Nm22, was used as inoculum. Inoculum concentrations of 5×10^5 and 3×10^7 cells per milliliter were used in 1982 and 5×10^5 cells per milliliter in 1983. The inoculation procedure described previously was used.

Disease assessment. Disease was assessed 7–10 days after inoculation, when symptoms of blossom infection were clearly visible but had not progressed to the base of the peduncle. The total number of flowers infected was divided by the total number of flowers inoculated. Disease ratings were expressed as percentages and transformed for statistical analysis. Means were compared by using Fisher's least significant difference test at $P = 0.05$ unless otherwise indicated.

RESULTS

Isolation of nonmotile and motile revertant mutants. Many of the isolates tested were slightly motile as indicated by their colony diameters which were broader than those of Nm22 on STM. Nm22 did not spread on STM and produced an average colony diameter of 2.5 mm after 18 hr. Mr22 spread rapidly and produced a colony diameter of 31.5 mm in 18 hr. The wild type had a colony diameter of 28.5 mm. Therefore, Mr22 and Nm22 were selected for further tests. These results were confirmed by using the capillary assay wherein the number of Nm22 cells that entered the capillaries did not increase significantly with increasing incubation time whereas a significant increase in cell accumulation per capillary was observed with Mr22 with increases in incubation time (Fig. 1). The average number of Nm22 and Mr22 cells that accumulated per capillary

after 10 min was approximately 30 and 540, respectively. After 45 min, only 120 Nm22 cells were recorded per capillary compared to 1,650 cells for Mr22.

Pathogenicity tests. Mr22 and Nm22 were resistant to rifampin after mutagenesis. Both isolates were as pathogenic as the wild type. Symptoms were observed 4 days after inoculation and infection caused by all three strains progressed down the shoots at the same rate.

Response of isolates to apple flower nectar extract. Mr22 was as chemotactic towards apple flower nectar as the wild type. In the presence of 10^{-1} dilution of the apple flower nectar extract, 36,800 Mr22 cells entered the capillary while 4,900 cells entered in the absence of the apple flower nectar. For Strain 110, 19,300 and 2,450 cells entered the capillaries in the presence and absence, respectively, of the 10^{-1} dilution of flower nectar. Nm22 did not respond chemotactically to flower nectar extract, since 350 and 300 cells entered in capillaries without and with flower nectar, respectively.

Screening for chemical repellents. *E. amylovora* was repelled by 10^{-1} M solutions of benzoate and salicylate without induction (Table 1). The mean number of cells in capillaries containing a 10^{-1} M solution of either compound was about eight times less than in capillaries containing the chemotaxis medium alone; that difference was statistically significant ($P = 0.01$). The responses for L-isoleucine, L-leucine, and L-phenylalanine were not significantly different than the control. However, after growing the bacterial cells in MMS containing 10^{-3} M of each compound, cells were induced to respond negatively to 10^{-1} M solutions of L-isoleucine, L-leucine, and L-phenylalanine. The average number of bacteria that accumulated in the capillaries containing 10^{-1} M of any of the compounds was about one-third that in the control capillaries. This difference was significant ($P = 0.01$).

Benzoate and salicylate were selected for further tests because the negative chemotaxis of *E. amylovora* for these two compounds appeared to be constitutive. The lowest concentration that gave a discernable negative response was a 10^{-3} M solution of benzoate and a 10^{-2} M solution of salicylate (Table 2). Lower concentrations of either compound (10^{-4} M benzoate and 10^{-4} M to 10^{-5} M salicylate) resulted in significantly more cells recovered than in the controls. When a 10^{-1} dilution of flower nectar extract was combined with a repellent compound in the capillary, only a 10^{-1} M solution of either repellent compound elicited a negative response that was significantly lower than the chemotaxis medium control

TABLE 1. Response of *Erwinia amylovora* to repellent compounds before and after induction with the test compound

| Treatment[b] | Bacterial cells (no.) per capillary[a] | |
	Uninduced[c]	Induced[d]
Control	5,281 x	3,700 x
L-isoleucine	5,712 x	825 y
L-leucine	5,150 x	1,431 y
L-phenylalanine	4,605 x	1,244 y
Sodium benzoate	644 y	nd[e]
Sodium salicylate	631 y	nd

[a] Each figure is the mean of two trials with four replicates per trial and the number of bacteria per capillary was based on duplicate plate counts. Assay was run with 5×10^7 cells per milliliter outside the capillary for 30–45 min at 23–25 C. Figures with the same letter in each column are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

[b] Each chemical repellent was tested at 10^{-1} M in chemotaxis medium, pH 7, within capillaries. Control capillaries were filled with chemotaxis medium alone.

[c] Cells were grown in modified Miller and Schroth (MMS) broth.

[d] Cells were grown in individual cultures on MMS supplemented with 10^{-3} M of each compound.

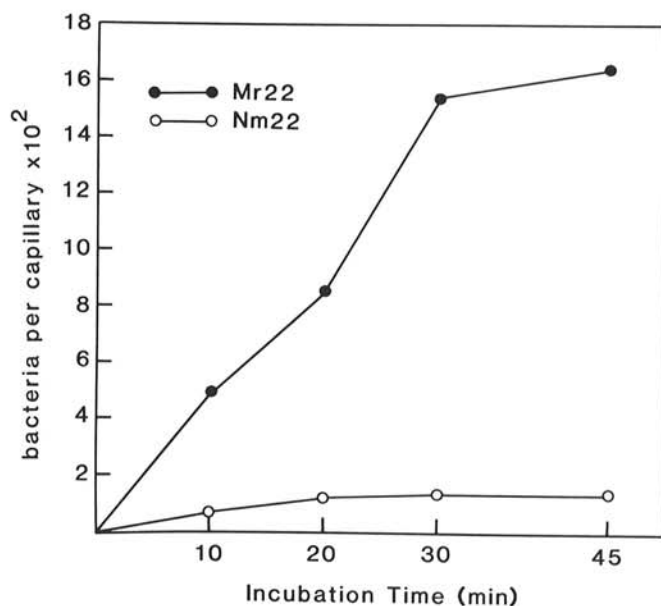[e] Abbreviation "nd" means "not determined."

Fig. 1. Capillary assay for motility of motile revertant isolate Mr22 and nonmotile isolate Nm22 of *Erwinia amylovora*. Each data point is the mean of two trials with four replicates per trial. Assay was run with 5×10^7 cells per milliliter outside the capillary at 23 C.

(Table 3). A 10^{-2} dilution of flower nectar extract reduced the repellent effect of 10^{-2} M solution of either benzoate or salicylate. In such experiments, solutions of repellent compounds masked the attractiveness of flower nectar extract.

In the test tube method of Tso and Adler (18), a clear band or zone was formed above the agar containing 1 M and 0.1 M benzoate or salicylate 30 min after introducing the bacterial suspension. The zone of inhibition was barely visible in tubes containing 10^{-2} M and 10^{-3} M solutions of the repellents. This zone was not observed with Nm22 at any concentration.

Blossom infection by Mr22 and Nm22. During the spring 1981 trial, blossom infection ranged from 48 to 86% (mean, 71%) in blossoms inoculated with Mr22 and from 29 to 67% (mean, 39%) in blossoms inoculated with Nm22 when a concentration of 1×10^7 cells per milliliter was used. These differences were statistically significant.

In the spring of 1982, when percent blossom infection was compared with the nonmotile strain, significantly higher infection occurred in blossoms inoculated with the motile revertant at 5×10^5 and 3×10^7 but not at 5×10^3 cfu/ml (Fig. 2).

Effect of chemical attractants on blossom infection. The average incidences of infection in malate-treated (72%) and tartrate-treated (58%) blossoms were not significantly different from the control (66%) at 5×10^5 cfu/ml. However, the difference in blossom infection was significant between the malate- and tartrate-treated blossoms. At 3×10^7 cfu/ml, no significant differences in the amount of blossom infection were observed among treatments. In

experiments conducted in 1983, significantly lower percentages of infection were observed in blossoms sprayed with 10^{-2} M and 10^{-3} M solutions of either compound than in the control.

Effect of chemical repellents on blossom infection. Benzoate and salicylate at 0.1 M were phytotoxic to apple blossoms. Open flowers looked scorched and brown 24 hr after application. Therefore, these flowers were not inoculated. Phytotoxicity did not occur with 10^{-2} M solutions of either compound. The average incidence of infection in salicylate- and benzoate-sprayed blossoms were 30 and 29%, respectively, and 41% for the control blossoms. The differences were not statistically significant. In another experiment, in which 10^{-3} M solutions of the same compounds were used, the average infection was 30, 22, and 24% in the control, salicylate-sprayed, and benzoate-sprayed blossoms, respectively. The differences were not significant.

Effect of temperature on blossom invasion. In 1982, the lowest, minimum, and highest maximum temperature from 7 May (inoculation date) to 16 May (disease assessment date) were 9 and 32 C, respectively, with an average mean temperature of 21 C. In 1983, the lowest minimum temperature was 1 C and the highest maximum temperature was 28 C during the 10 day period from 6 May (date of inoculation) to 15 May (date of disease assessment). The average mean temperature during this period was 15 C.

DISCUSSION

Apple blossom infection by a motile and a nonmotile strain of *Erwinia amylovora*. To test the hypothesis that motility enhances the infection potential of bacterial pathogens such as *E. amylovora*, it is necessary to have motile and nonmotile strains that are near isogenic. The nonmotile strain was as pathogenic as either the wild type or the motile revertant when injected into Jonathan apple seedling shoots. Infections by all strains progressed equally rapidly in succulent shoots. Nm22, reisolated from infected field and greenhouse-grown shoots, did not revert to a motile strain. This indicates that motility is not a requisite for pathogenicity in *E. amylovora* and supports earlier reports of experiments with *P. phaseolicola* (11).

TABLE 2. Taxis of *Erwinia amylovora* towards sodium benzoate and sodium salicylate

Concentration in capillary[b]	Bacterial cells per capillary[a]	
	Sodium benzoate	Sodium salicylate
0 (Control)	4,181 y	2,569 x
10^{-2} M	1,175 x	750 w
10^{-3} M	1,375 x	2,544 x
10^{-4} M	5,669 z	3,406 y
10^{-5} M	4,869 y	4,412 z

[a] Each number is the mean of two trials with four replicates per trial and the number of bacteria per capillary was based on duplicate plate count. All assays were done with 5×10^7 cells per milliliter outside the capillary for 30–45 min at 23–25 C. Figures with the same letter in each column are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.
[b] Each chemical was diluted in chemotaxis medium, pH 7. Control capillaries were filled with chemotaxis medium alone.

TABLE 3. Taxis of *Erwinia amylovora* towards sodium benzoate and sodium salicylate in the presence of apple (cultivar Jonathan) nectar extract

Treatment[b]	Nectar[a]	
	10^{-1} dilution[c]	10^{-2} dilution
Nectar alone	27,250 z[d]	8,262 z
Nectar + 10^{-1} M sodium salicylate	819 x	694 x
Nectar + 10^{-2} M sodium salicylate	5,681 y	1,312 xy
Nectar + 10^{-1} M sodium benzoate	750 x	681 x
Nectar + 10^{-2} M sodium benzoate	4,469 y	1,550 xy
Control[e]	3,862 y	2,169 y

[a] Nectar extracted from excised apple flowers.
[b] Nectar alone or combined with solutions of repellents dissolved in chemotaxis medium at pH 7 and placed in capillaries.
[c] The dilution of nectar contained in each capillary.
[d] Refers to the number of bacterial cells accumulating per capillary. Each number is the mean of two trials with four replicates per trial and the number of bacteria was based on duplicate plate counts. All assays were done with 5×10^7 cells per milliliter outside the capillary for 30–45 min at 23–25 C. Figures followed by the same letter in each column are not significantly different ($P = 0.05$) according to Fisher's least significant difference test.
[e] Control capillaries were filled with only chemotaxis medium. They contained neither attractant nor repellent.
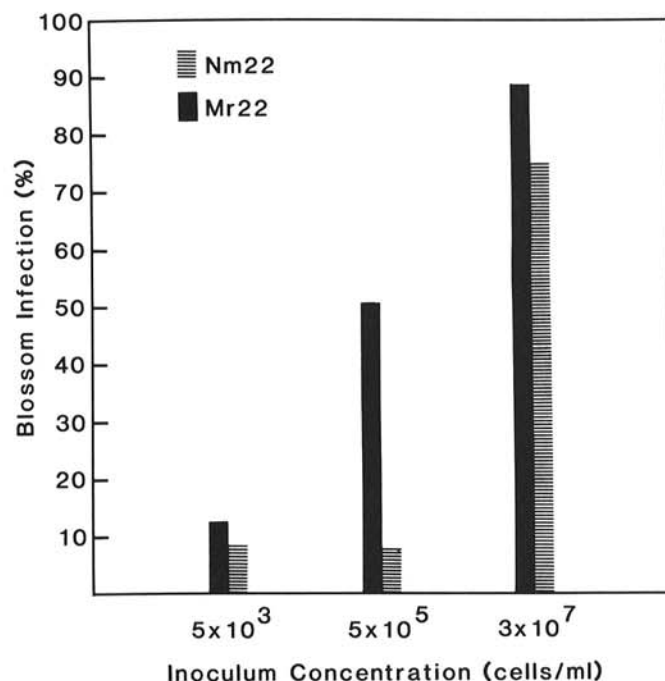

Fig. 2. Blossom infection in cultivar Jonathan (1982 trial) after inoculation with motile revertant isolate Mr22 and nonmotile isolate Nm22 of *Erwinia amylovora* at three inoculum concentrations. The percentage infection was on a per individual flower basis, determined by dividing the total number of flowers infected by the total number of flowers inoculated. Each number represents the mean of six replicates with 10 flower clusters per replicate.

The chemotactic response of Mr22 to Jonathan flower nectar extracts was comparable to that of the wild type, suggesting that the genes (*che*) involved in chemotaxis (12) were not affected during mutagenesis. Most probably, either the structural genes (*hag*) involved in flagellin synthesis and therefore in filament shape (2) or the genes (*fla*) that regulate flagellar formation (7) were affected in the Nm22 strain. Based on the results of blossom inoculations, motility enhances the infectivity of *E. amylovora* especially at inoculum concentrations of 5×10^5 and 1×10^7 cfu/ml.

Screening for chemical repellents. Negative chemotaxis of *E. amylovora* may be constitutive for both benzoate and salicylate and inducible for L-isoleucine, L-leucine, and L-phenylalanine. In the test-tube assay, a clear band or inhibition zone formed above the agar containing a high concentration of either compound. Therefore, the negative response observed in the capillary assay was not due to the inhibition of motility; most probably, the pathogen was repelled and moved away from benzoate and salicylate. It is not certain whether benzoate and salicylate act as weak attractants for *E. amylovora* at lower concentrations. If both compounds function as such, the mechanism involved and the biological significance to the pathogen are not understood. However, since neither compound was purified before use, the attraction observed at the lower concentration of both compounds may be due to contaminating attractant(s). This is a possibility since the threshold concentration for a repellent is usually 100- to 10,000-fold higher than for attractants (18).

The pathogen's response when presented simultaneously with a repellent and an attractant at varying concentrations suggests that apple flower nectar extract can minimize the repelling effect of benzoate and salicylate. Similar results with the same type of assay for *E. coli* were obtained by Adler and Tso (1) and by Tsang et al (17) who used a gradient-sensing apparatus. They suggest that bacteria respond to whichever compound is present in the more effective concentration. These results are consistent with the concept of algebraically additive stimuli, and the repellents, like attractants, operate through specific receptors and utilize a common memory mechanism for taxis.

Effect of attractant and repellent chemicals on blossom infection. There was a trend for reduced infection in blossoms sprayed with solutions of either attractant or repellent chemicals. One possible explanation of why attractants used in this study did not provide consistent protection of apple blossoms against the disease is that spring temperatures were higher in 1982 than in 1983. Perhaps the lower temperatures during the 1983 trial reduced the effective inoculum concentration of the pathogen, thereby confounding measurements of the efficacy of the attractant chemicals on *E. amylovora*. This is a possibility, considering that chemotaxis of *E. amylovora* toward apple flower nectar is reduced at temperatures lower than 18 C and higher than 30 C (13).

There was also a trend for reduced infection in blossoms sprayed with solutions of the chemical repellents. This observation is consistent with the result of the competition experiment wherein

the repelling effect of these compounds was observed at 10^{-1} M in the presence of a 10^{-1} dilution of flower nectar extract. Unfortunately, 10^{-1} M solutions of these repellents were highly phytotoxic to apple blossoms.

For a disease control strategy designed to disorient *E. amylovora* before it enters its host to work, compounds must be sought which repel the pathogen at very low concentrations and are not phytotoxic.

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