Efficacy of Bactericides and Saprophytic Bacteria in Reducing Colonization and Infection of Pear Flowers by Erwinia amylovora

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ABSTRACT

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Colonization of healthy pear flowers by *Erwinia amylovora* was reduced as much as fourfold in experimental plots sprayed with bactericides. The bactericides that effectively reduced the number of healthy flowers colonized by *E. amylovora* also were the most efficacious in reducing the incidence of fire blight. Accordingly, an assay of the percentage of flowers colonized by *E. amylovora* is an alternative method of determining the efficacy of bactericides, especially when conditions are not conducive for disease development. Fire blight incidence was significantly reduced in a Bartlett pear orchard sprayed eight times during bloom with three saprophytic *Pseudomonas* spp. and an *Erwinia* sp. These bacteria, in laboratory studies, multiplied from 10^2 cells per flower to approximately 10^5 to 10^6 cells per flower in 24 hours. The *Erwinia* sp. produced a bacteriocin lethal to *E. amylovora* in vitro.

Additional key words: antibiotic, biological control.

The evaluation of chemicals for control of fire blight (caused by *Erwinia amylovora*) is based upon differences in disease incidence on trees treated with different materials. Frequently, insufficient disease prevents a reliable comparison and additional tests in succeeding years are required to obtain efficacy data. Although inoculation of plants with *E. amylovora* to increase disease is a possible alternative, doubts arise as to the significance of the method because simultaneous inoculation of all plant parts with high numbers of bacteria is not a natural occurrence. Furthermore, most of our tests are conducted in commercial orchards under natural conditions and spraying trees with fire blight inoculum is unacceptable to growers.

In searching for other means to evaluate the efficacy of chemicals for fire blight control, it appeared that the efficacy of a chemical might be related to the percentage of flowers colonized or the number of bacterial cells per flower. Preliminary experimentation indicated that populations of *E. amylovora* frequently were present in healthy blossoms of host plants without necessarily inciting disease and that the populations were affected by the bactericides applied (7). This phenomenon might enable the screening of bactericides regardless of disease development, which is sporadic in California.

In testing the efficacy of chemicals on flower colonization by *E. amylovora* it also was of interest to test whether selected saprophytic bacteria that multiply in flowers would reduce the capacity of *E. amylovora* to colonize them. Parker (4) attempted to control fire blight with antagonistic bacteria but obtained inconsistent results in the field. Riggle and Klos (5) obtained partial control of fire blight in the greenhouse and in the field by pre-inoculating apple blossoms with *Erwinia herbicola*.

This paper reports the effect of various chemicals and several saprophytic bacteria on epiphytic populations of *E. amylovora* in pear flowers and subsequent disease development.

MATERIALS AND METHODS

Selection of bacteria for biological control.—Saprophytic bacteria used in biological control studies were selected on the basis of their ability to multiply in pear flowers. These bacteria were obtained by spraying flowering pear trees with a 10^{-2} (w/v) aqueous dilution of soil collected from the orchard floor. Bacterial strains that multiplied in the flowers and which were isolated in the highest numbers on King's B medium (KB) (2) were selected for laboratory tests. Bacterial suspensions of selected isolates at 10^2 cells per flower were placed in 20 open flowers on forced excised pear twigs in the laboratory (7). The inoculated flower clusters were incubated at 29 C and 90% RH. Individual flowers were washed after 24-hour intervals to isolate the bacterial species that had multiplied. Three different *Pseudomonas* sp. isolates were selected for further tests as biological control agents. An *Erwinia* sp. that causes a newly described disease of sugar beet (6) also was included as a test bacterium.

In vitro tests for bacteriocin production were performed according to the techniques of Fredericq (1). The three *Pseudomonas* spp. and the *Erwinia* sp. were spotted on KB and modified Miller-Schroth selective medium (MSM) with mannitol as the carbon source (3, 7) and incubated for 24 hours at 29 C. The bacteria were

killed by exposing the plates to chloroform vapor for 30 minutes and then removed with a sterile glass slide. The plates were individually seeded with 62 isolates of *E. amylovora* and one isolate each of *Pseudomonas syringae, Erwinia carotovora* var. *atroseptica,* and *Erwinia carotovora* var. *carotovora* and incubated for 24 hours at 29 C.

Antibiosis was determined by spotting the three *Pseudomonas* spp. and the sugar beet *Erwinia* sp. on 2-hour-old lawns of *E. amylovora* on MSM and KB. A zone of inhibition after 24 hours at 29 C was an indication of antibiosis.

Inoculum of saprophytic bacteria for spraying pear flowers in the field was prepared on KB agar. After incubation for 24 hours at 29 C, the bacteria were washed from the plates, suspended in water, and applied to trees within 1 hour.

Application of bactericides and saprophytic bacteria.—A 15-year-old Bartlett pear orchard in Butte County (Gridley, California) was used to compare the efficacy of different chemicals and saprophytic bacteria to control fire blight. The three *Pseudomonas* spp. and the *Erwinia* sp. were included in the same application at concentrations of approximately 3.2×10^7 cells per milliliter for each isolate. The bactericides used in this orchard were Citcop (48% copper salts of fatty and rosin acids, 4% metallic copper), Kocide (83% cupric hydroxide, 54% metallic copper), streptomycin (17% streptomycin sulfate), Terramycin (17% oxytetracycline hydrochloride), and MBR 10995 (25% active, Minnesota Mining and Manufacturing, St. Paul, Minnesota). Citcop, streptomycin, and Terramycin each was applied to trees in six completely randomized blocks (20 trees per block with two buffer rows between each treatment). These trees were treated at the rates described in Table 1 using a concentrate sprayer (1.9 liters per tree) approximately every 5 days from 16 March through 18 April 1973 with a total of eight applications. MBR 10995, Kocide, and the biological control organisms also were applied (at the rates indicated in Table 1) to adjacent blocks of three trees, replicated six times. These applications were made with a single-nozzle sprayer (2.0 liters per tree) on the same dates that the concentrate sprays were applied.

Monitoring of flowers.—The population of bacteria in individual rattail flowers (3) was determined 7 days after the most recent bactericide application (18 April) by inserting a fully-opened flower into a sterile test tube and detaching it from the tree by sliding a plastic cap over the tube. Each flower was washed with 10 ml of sterile tap water in a rotary tube mixer for 10 seconds. The wash water was dispensed in 0.01 ml aliquots to three plates each of KB or modified Miller-Schroth selective medium (MSS) with sorbitol as the carbon source (3, 7). The number of *E. amylovora* colony-forming units was determined according to procedures outlined previously (7). Thirty individual flowers were taken from each replicated plot of the 10 different treatments.

RESULTS

Selected biological control bacteria.—The pseudomonads selected for the biological control tests were oxidase-positive and produced a green fluorescent pigment on KB; two were arginine dihydrolase-positive. Colony morphology of the three isolates was not substantially different on KB and upon subsequent reisolation we did not attempt to distinguish them.

None of the *Pseudomonas* sp. produced inhibition zones against *E. amylovora* or the other test organisms which were assayed on MSM and KB. However, the sugar beet *Erwinia* sp. produced inhibition zones against 60 of 62 isolates of *E. amylovora* on MSM but not on KB. No inhibition zones were produced against *P. syringae, E. carotovora* var. *atroseptica,* or *E. carotovora* var. *carotovora.* In vitro tests according to Fredericq (1) established that inhibition zones were caused by bacteriocins.

Multiplication of saprophytic bacteria.—The three saprophytic pseudomonads selected for field tests multiplied in all pear flowers in the laboratory from 10^2 cells per flower to approximately 2×10^6 cells/flower within 24 hours after inoculation. The *Erwinia* sp. multiplied from 10^2 cells per flower to approximately $5 \times$

TABLE 1. Effect of control measures on reducing the colonization of pear flowers by *Erwinia amylovora* and in reducing the incidence of fire blight in Bartlett pear trees in Gridley, California (Butte County)

Treatment	Amount applied (per hectare)	Flowers colonized with *E. amylovora*[a,b] (%)	No. of bacteria per colonized flower ($\times 10^6$)[b]	Flower infections (no. per tree)[b]
Control	. . .	52 wx	1.5 w	3.2 w
Citcop	9.28 liters	51 wx	1.3 w	1.7 xy
Citcop	18.56 liters	48 wx	1.2 w	2.2 wx
Saprophytic bacteria[c]	464.00 liters	46 wx	0.8 w	1.0 xyz
Terramycin (17%)	0.70 kg	40 wx	0.9 w	0.8 yz
Streptomycin (17%)	0.70 kg	37 wx	1.5 w	0.8 yz
Kocide	0.56 kg	32 wx	0.8 w	1.0 xyz
Kocide	2.24 kg	28 x	0.4 w	0.4 z
MBR 10995 (25%)	0.28 kg	19 y	0.4 w	0.4 z
MBR 10995	1.12 kg	12 y	0.2 w	0.4 z

[a]Correlation coefficient (r) between the percent of flowers colonized and number of infections per tree is 0.87, significant $P = 0.01$.
[b]Values followed by different letters are significantly different, $P = 0.05$, as determined by Duncan's multiple range test.
[c]Approximately 3.2×10^7 cells per milliliter of suspension of each of three saprophytic pseudomonads and a saprophytic *Erwinia* sp. were applied every 5 days from 16 March through 18 April for a total of eight applications.

10^5 cells per flower in the same time interval. Although 90% of the flowers inoculated with the saprophytic bacteria in the laboratory became necrotic within 5 days, symptoms were not detected when flowers were inoculated in the field.

Populations of Erwinia amylovora in flowers and incidence of disease.—The bactericides that effectively reduced the incidence of fire blight also reduced the percentage of healthy flowers colonized by *E. amylovora*. For example, 52% of the rattail flowers from the nontreated plots in the Gridley orchard were colonized with *E. amylovora,* corresponding with 3.2 flower infections per tree. In contrast, only 12-19% of the flowers from the trees treated with MBR 10995 were colonized and only 0.4 flower infections per tree were recorded. There was no significant difference in the incidence of colonized flowers and the population per colonized flower in plots treated with Kocide by means of the single-nozzle sprayer or the concentrate sprayer.

Although chemical applications significantly reduced the incidence of pear flowers colonized with *E. amylovora,* their effect on the population per flower was less dramatic. There appeared to be a reduction in the population of *E. amylovora* in infested flowers from chemical application but the large variability among populations per flower prevented the drawing of any conclusions (Table 1).

The incidence of fire blight in trees treated with saprophytic bacteria was significantly less than in control trees or trees treated with Citcop but did not significantly differ from trees treated with Kocide, streptomycin, Terramycin, or MBR 10995 (Table 1).

DISCUSSION

These studies indicate that the efficacy of a bactericide can be evaluated by determining the number of pear flowers that are colonized with *E. amylovora* after bactericide applications. Information concerning the incidence of flower colonization may provide the only means for making comparisons of chemicals in years when natural fire blight symptom development is negligible. Excellent correlation (r = .87) was obtained between the percentage of colonized flowers and disease incidence. These procedures have been repeated in subsequent years and similar results were obtained. The determination of the bacterial populations per flower, however, does not appear to be a good method to evaluate chemicals, since the pathogen might continue to multiply internally in flower tissue even after bactericides have been applied to the surface. Furthermore, the bactericides currently registered for use are not eradicants and only reduce surface populations. Also flowers which open after a bactericide application are not protected from colonization. There was a 7-fold decrease in the number of bacteria per infested flowers with those chemicals which were most effective in controlling disease (Table 1). However, due to the extreme variability of bacterial populations in flowers, no significant difference was detectable between treatments when the data were analyzed statistically.

Biological control of fire blight in the Gridley orchard by means of saprophytic pseudomonads and an *Erwinia* sp. was nearly as effective as the currently recommended bactericides. There was a 68% reduction in the amount of disease when compared with the control plot. However, the incidence of disease was relatively low in the Gridley orchard during this study and it would be desirable to test the effectiveness of the saprophytic bacteria in controlling the disease during years when conditions are highly conducive for disease development. Our results are comparable to those of Riggle and Klos (5) who obtained control ranging from 13-50%.

The results of this study prompt further exploration for biological control agents of fire blight. An ideal bacterium for biological control would appear to be one that would multiply and occupy the same host sites as *E. amylovora* during environmental conditions optimal for growth of the pathogen. The saprophytes also should be capable of spreading throughout the orchard and colonizing newly opened flowers. This would keep the number of applications to a minimum and reduce costs.

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