Interaction of *Xanthomonas campestris* pv. *pruni* with Pruniphage and Epiphytic Bacteria on Detached Peach Leaves

P. S. Randhawa and E. L. Civerolo

Fruit laboratory, U.S. Department of Agriculture, Beltsville Agricultural Research Center, Beltsville, MD 20705.
Accepted for publication 7 November 1985.

ABSTRACT

Randhawa, P. S., and Civerolo, E. L. 1986. Interaction of *Xanthomonas campestris* pv. *pruni* with pruniphage and epiphytic bacteria on detached peach leaves. Phytopathology 76:549-553.

A bioassay for measuring the interaction of *Xanthomonas campestris* pv. *pruni* with pruniphage and epiphytic bacteria is described. Detached young peach leaves were surface-sterilized with 70% ethanol and placed on 0.5% water agar with their adaxial side up. Inoculum mixtures of *X. c.* pv. *pruni* and epiphytic bacteria (47 strains from stone fruit trees and two nonepiphytic strains from crucifer seeds and roots) or pruniphage were placed as 20 drops of 5 μl each on a leaf and incubated for 120 hr at 25 C under 16-hr photoperiods. Populations of *X. c.* pv. *pruni* were significantly reduced on the surface and within leaves by seven and 17 strains of epiphytic bacteria, respectively. Colonization of leaves depended upon the specific strain of epiphytic bacterium. Strains that had a higher degree of colonization caused stronger inhibition of *X. c.* pv. *pruni*. Inhibition of *X. c.* pv. *pruni* by epiphytic strains on nutrient glucose agar and leaves was not related. Coinoculation of pruniphage and *X. c.* pv. *pruni* resulted in reduction of *X. c.* pv. *pruni* on and within leaves. However, surviving *X. c.* pv. *pruni* developed pruniphage resistance especially at high ratios of plaque-forming units to colony-forming units. Approximately 50% of the pruniphage-resistant population was significantly less virulent than the parent strain.

Additional key words: antagonism, biocontrol, Prunus bacterial spot disease.

Detached plant organs have often been used to determine pathogenicity of plant-pathogenic bacteria (9). *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye and *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, and Wilkie, which are present in low numbers among saprophytic bacteria, are selectively increased within detached leaves of pepper and tomato, respectively (16). However, a reduction or elimination of the pathogenic bacteria also may occur if competitive and antagonistic microorganisms or specific bacteriophages are present (15,19,20). Selection of antagonistic bacteria based upon inhibition of pathogen on plant tissue is superior (8,18,22) to the commonly used approach of inhibition on agar media (3). Detached peach (*Prunus persica* (L.) Batsch) leaves may be suitable for such a selection procedure because of their relatively long keeping quality and the specific response to *X. c.* pv. *pruni* (Doidge) Dye in bacterial spot disease development (14). Our objective, therefore, was to examine the interaction of *X. c.* pv. *pruni* with epiphytic bacteria associated with *Prunus* spp. and pruniphage on detached peach leaves to identify beneficial microbial interactions that can be exploited for biocontrol of Prunus bacterial spot disease. A preliminary report has been published (13).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Bacteria and pruniphage. Epiphytic bacteria were periodically isolated from the leaves of field-grown peaches and apricots during the 1983 and 1984 growing seasons. Leaves (100 g) were shaken for 10 min in a plastic bag with 100 ml of sterile water containing one drop of Tween 20. An aliquot (5 ml) of the suspension was transferred to a sterile vial. Bacteria also were isolated from rainwater on shoots of apricots. Immediately after rain, a shoot was enclosed in a plastic bag and shaken to collect the adhering rainwater. Rainwater was pooled from at least 10 shoots and an aliquot was transferred to a sterile vial. The suspensions collected by these two methods were serially diluted and plated on nutrient agar containing 0.25% glucose (NGA). After 3–5 days at 27 C, bacterial colonies that appeared relatively often were isolated. *X. c.*

pv. *pruni* strain XP-1 and pruniphage strain PP-1 were isolated previously in this laboratory (4,5). A spontaneous mutant resistant to 100 μg of streptomycin per milliliter (strain XP-1 Str^r) was derived from XP-1. Strain NC-1 of *Bacillus* sp. from crucifer seeds and an unidentified strain SO-9-4 from crucifer roots were obtained from N. W. Schaad, Moscow, ID. These two strains produced wide zones of inhibition against *X. c.* pv. *pruni* on NGA.

To prepare inoculum, bacterial strains were grown on NGA for 24–48 hr at 27 C. A loopful of bacterial culture was suspended in sterile distilled water and the suspension was adjusted to $A_{535\,nm} = 0.1$ by using a Beckman spectrophotometer. The inoculum was diluted, as needed, for inoculations. Pruniphage was propagated on strain XP-1 in 1 L of nutrient glucose sodium chloride broth for 24 hr on a rotary shaker at 27 C (4,5). To kill any surviving bacterial cells in the cleared lysate, 100 ml of chloroform was added and incubation was continued for an additional 30 min. Sodium chloride was added to the lysate to a final concentration of 0.5 M and bacterial debris was removed by centrifugation at 12,100 *g* for 10 min. Polyethylene glycol was added at 7.5% and the lysate was stored overnight at 2–4 C. The precipitated phage was collected by centrifugation at 12,100 *g* for 10 min. This partially purified phage (23) was suspended in 0.01% $MgSO_4 \cdot 7H_2O$ and passed through a 0.22-μm Millipore filter. The titer of this stock suspension was 2×10^9 plaque-forming units (pfu) per milliliter.

Leaf inoculation. Leaves of greenhouse-grown peach (cultivar Sunhigh) seedlings were used. Young leaves (third to sixth from the shoot terminal) were detached and adjusted to an approximate length of 60 mm by cutting the proximal and distal ends with scissors. The leaves were briefly washed under tap water and surface-disinfested. Based on the comparative effectiveness of 70% ethanol (treatment of leaves for 60 sec followed by one rinse with sterile water), 0.2% sodium dodecyl sulfate (SDS) and 1% sodium hypochlorite (treatment of leaves for 60 sec followed by five rinses with sterile water), surface disinfestation was routinely done with ethanol. The leaves were lightly blotted with sterile paper towel and placed, adaxial side up, on 0.5% water agar in 100-mm-diameter plastic petri dishes. Two or three randomly chosen leaves were placed in each dish. To investigate multiplication of *X. c.* pv. *pruni* on leaves over a period of time, XP-1 inoculum was diluted 10-fold with sterile water and 0.1 ml was placed (one 5-μl drop at each of 20 scattered sites) on the surface of each leaf. Bacterial population on leaves was assayed at 24-hr intervals up to 168 hr postinoculation. To determine interaction of *X. c.* pv. *pruni* with epiphytic bacteria, XP-1 Str^r inoculum was mixed with inoculum of each epiphytic strain (1:1, v/v) and inoculation was performed as described above. Likewise, pruniphage stock suspension was serially diluted to 10^{-4} with sterile 0.01% solution of $MgSO_4 \cdot 7H_2O$. One milliliter from each dilution was mixed with 1 ml of suspension of XP-1 (1×10^8 colony-forming units [cfu]/ml) and 0.1 ml of the mixture was immediately inoculated as described. For controls, water was substituted for epiphytic bacteria or pruniphage. Each treatment was replicated five times with one leaf in each replication. Inoculated leaves were incubated for 120 hr at 25 C under 16-hr photoperiods provided by warm fluorescent lights at a photon flux density of approximately 60 $\mu Ein \cdot m^{-2} \cdot sec^{-1}$.

Population assays. Bacterial or pruniphage population on each of five inoculated leaves was assayed at 0 hr and after 120 hr of incubation. Each leaf was aseptically cut with a flame-sterilized scalpel into three portions (to facilitate dispersion) and shaken at 200 rpm (Fermentation Design, Inc., model OS31 rotary shaker) in 10 ml of sterile water containing 0.01 ml of Tween 20 in a 50-ml beaker for 10 min at 27 C. Bacteria harvested from leaf surfaces by this method were concentrated by centrifugation at 12,100 *g* for 10 min and resuspended in 1 ml of sterile distilled water. To determine bacterial population within leaves, each leaf was surface-sterilized with 1% sodium hypochlorite containing 0.2% SDS for 2–3 min and rinsed five times with sterile water. The homogenate obtained by grinding the leaf in 10 ml of sterile water in a mortar with a pestle was centrifuged at 484 *g* for 5 min. The bacteria in the supernatant were concentrated by centrifugation at 12,100 *g* for 10 min and resuspended in 1 ml of sterile distilled water. These bacterial suspensions obtained from surfaces and interior of the leaf (= external and internal populations, respectively) were serially diluted and 0.1 ml from each dilution spread onto NGA with or without streptomycin. Bacterial colonies from NGA plates containing 20–200 colonies were counted after 3–5 days. Populations of epiphytic bacteria were estimated only from suspensions prepared from leaf surfaces. This procedure allowed a minimum detection limit of 10 bacterial cfu per leaf. Degree of leaf colonization was calculated as: (population at 120 hr) / (population at 0 hr).

To determine if surface disinfestation with a mixture of sodium hypochlorite and SDS was toxic to bacteria within leaves, three circular areas (10 mm in diameter) on each of two leaves were exposed through a hole cut in a piece of aluminum foil placed on the leaves. XP-1 inoculum (10^6 cfu/ml) was sprayed at 1.25×10^{-1} kg/cm until the exposed areas were water-soaked. Leaves were then air-dried until the water-soaking disappeared. One leaf was washed several times with tap water to remove surface bacteria while the other leaf was treated with 1% sodium hypochlorite containing 0.2% SDS for 3 min followed by five successive rinses with water. Each leaf was ground in 10 ml of water and the bacterial population in the homogenate was determined as above. A recovery of $2.5 \pm 0.8 \times 10^4$ cfu from extensively washed leaves and $1.8 \pm 0.7 \times 10^4$ cfu from disinfested leaves indicated that this disinfestation treatment was not toxic to bacteria present within the leaf.

Pruniphage pfu were determined from aliquots of suspensions passed through a 0.22-μm Millipore filter (4). Minimum detection limit was 100 pfu per leaf. To determine XP-1 population on pruniphage-treated leaves, the suspensions were treated with a 32-fold dilution of anti-phage-serum in 1:1 (v/v) ratio for 2 min and dilution plated on NGA as described. This anti-phage-serum treatment was shown to completely inactivate 2×10^8 pruniphage pfu in 0.1 ml of water. Several XP-1 colonies that developed from assays conducted at 120 hr were tested for resistance to pruniphage and virulence to peach leaves. To determine pruniphage resistance, bacterial lawns were produced by mixing 10^8 cfu with 5 ml of phage assay medium (per liter—nutrient agar, 7 g; sodium chloride, 5 g; and glucose, 2.5 g) in petri dishes. Ten microliters from a routine test dilution (10^5 pfu/ml) of strain PP-1 were spotted on the surface of the bacterial lawn and lytic zones were observed after 24 hr at 27 C.

Virulence of phage-resistant clones, relative to the parent strain XP-1, was determined by infiltrating inoculum (1×10^6 cfu/ml) with a needleless syringe to produce 2- to 4-mm-diameter water-soaked zones at a selected site on a young leaf on a susceptible peach (cultivar Sunhigh) tree in the field. Ten sites on each of four leaves on the same tree were inoculated. Disease incidence (number of inoculated sites showing lesions) and severity (extent of symptom expression) were recorded after 14 days. Disease severity for each leaf was rated on a 0–3 scale: 0 = no symptoms or slight chlorosis, 1 = blackening of veins or grayish white lesions, 2 = water-soaked, discrete lesions, and 3 = confluent necrosis.

For statistical analysis, bacterial and/or phage populations were transformed to $\log_{10}$ values and undetected populations were given a value of 1.0.

RESULTS

Assays of surface washings of 10 uninoculated leaves resulted in 0–185 cfu per leaf representing minimal natural bacterial flora on the leaves used in this study. This population was independent of the leaf size. When these leaves were detached and incubated without surface sterilization on 0.5% water agar for 5 days, a visible bacterial growth sometimes developed along the leaf-agar interface. In addition, 1–26 fungal colonies per leaf developed beneath leaves on water agar. The leaves, however, remained intact and apparently healthy. This natural bacterial and fungal flora were reduced by treatment of leaves with 0.2% SDS for 60 sec and eliminated by treatment with 70% ethanol for 60 sec without causing any toxic effect on the leaves. Treatment with 1% sodium hypochlorite for 60 sec reduced flora but caused white flecks on leaves after incubation for 4 days. Addition of 0.2% SDS increased the effectiveness of sodium hypochlorite to remove bacteria from the leaf surface without being toxic to bacteria within leaves.

Multiplication of *X. c.* pv. *pruni* on individual leaves was variable (Fig. 1). When assayed for total population, only two of 10 untreated leaves supported multiplication of *X. c.* pv. *pruni* at 120 hr. Further assays on another set of 10 leaves indicated multiplication of *X. c.* pv. *pruni* within only one leaf. This suppression of *X. c.* pv. *pruni* was associated with an increase in saprophytic bacteria that were naturally present on untreated leaves. These saprophytic bacteria were recovered from the surface of all 10 untreated leaves (7×10 to 3×10^4 cfu per leaf). In contrast, only two of 10 SDS-treated leaves (80–200 cfu per leaf) and none of 10 ethanol-treated leaves supported growth of saprophytic bacteria. Likewise, leaves treated with SDS or ethanol supported multiplication of *X. c.* pv. *pruni* with increased uniformity and mean populations were significantly greater than those that developed on untreated leaves (Fig. 1).

X. c. pv. *pruni* increased on the surface of ethanol-treated leaves from 4×10^5 to 1×10^8 cfu per leaf in 168 hr. Based on regression analyses, there was a significant quadratic relationship between length of incubation time and population size (Fig. 2). On the other hand, there was a significant linear relationship between population size within leaves and length of incubation time. However, no disease lesions developed on these leaves even after extended incubation up to 3 wk. Multiplication of XP-1 and XP-1 Str^r on leaves was similar.

Of the 49 bacterial strains tested, seven and 17 strains significantly inhibited external and internal populations of *X. c.* pv. *pruni* on leaves, respectively (Fig. 3). Six of these seven strains did not produce any inhibition to *X. c.* pv. *pruni* on NGA but had a relatively higher degree of leaf colonization. Similarly, 12 of 17 strains capable of inhibiting the pathogen within the leaf were not inhibitory on NGA. Epiphytic bacteria, depending upon the strain, varied in their capacity to colonize leaves. While the populations of

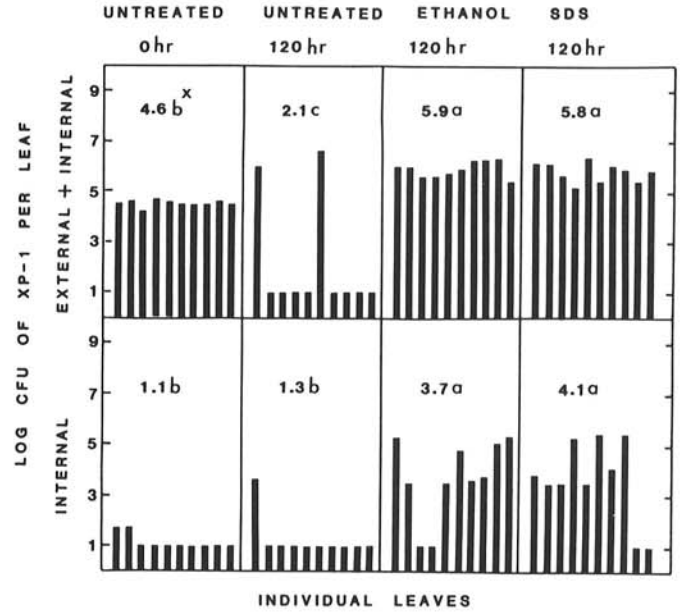

Fig. 1. Multiplication of streptomycin-resistant strain XP-1 Str^r of *Xanthomonas campestris* pv. *pruni* on individual detached peach leaves nondisinfested or treated with 70% ethanol or 0.2% sodium dodecyl sulfate (SDS). Inoculum (1×10^5 cfu/ml) of strain XP-1 was placed as a 5-μl drop at each of 20 sites scattered over the surface of each leaf and the bacterial population was determined at 0 and 120 hr of incubation. A value of 1.0 (based upon minimum detection limit) was assigned for undetected populations. Within rows, means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

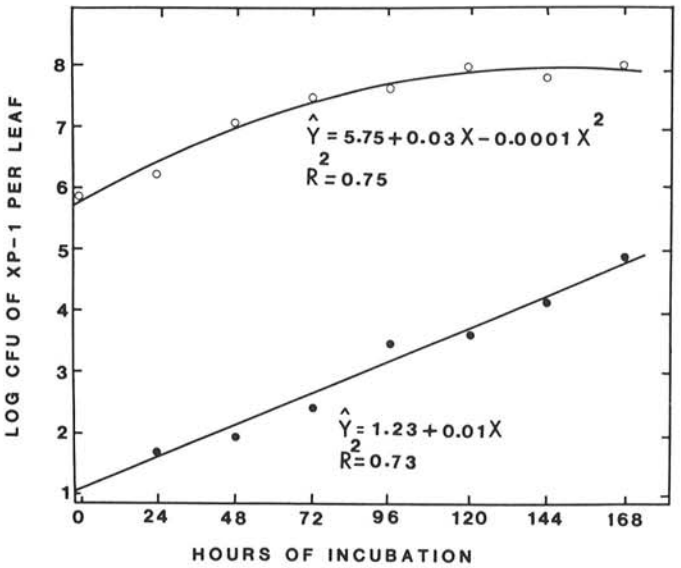

Fig. 2. Relationship of multiplication of strain XP-1 of *Xanthomonas campestris* pv. *pruni* on the surface (○) and within (●) detached peach leaves with incubation period. Inoculum (1×10^7 cfu/ml) of strain XP-1 was placed as a 5-μl drop at each of 20 sites scattered over the surface of an ethanol-treated leaf and bacterial populations determined at 24-hr intervals. Means of five replications are plotted.

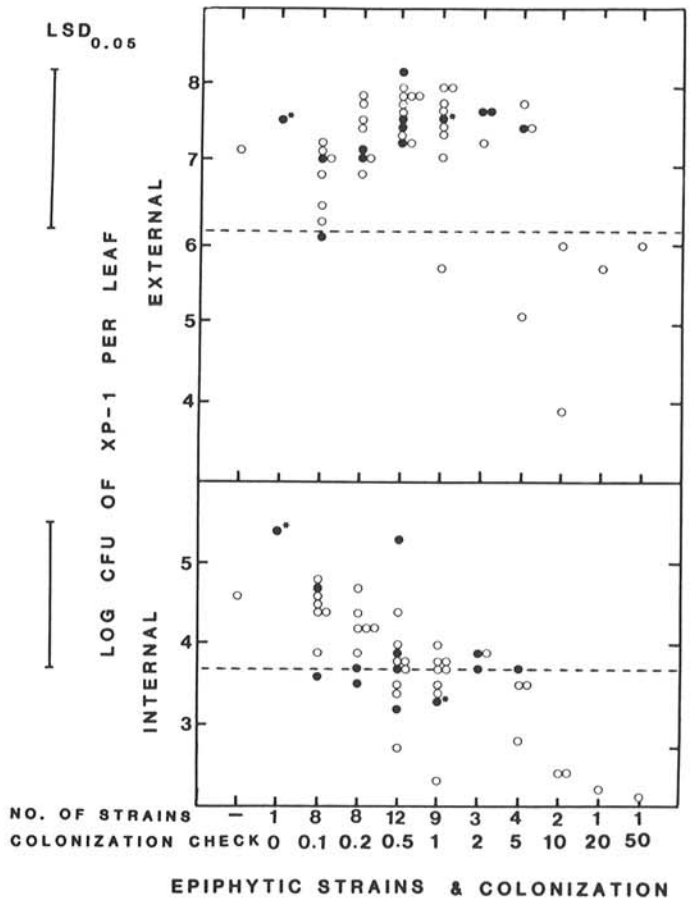

Fig. 3. Changes in populations of streptomycin-resistant strain XP-1 Str^r of *Xanthomonas campestris* pv. *pruni* on the surface of (external) and within (internal) detached peach leaves by epiphytic bacteria (two nonepiphytic strains are shown by asterisks). Inoculum of strain XP-1 Str$_r$ containing 1×10^8 cfu/ml and each epiphytic strain ($A_{535\ nm} = 0.1$) was mixed in 1:1 ratio and placed as a 5-μl drop at each of 20 sites scattered over a leaf. Population of XP-1 Str^r was determined at 120 hr and epiphytes at 0 and 120 hr of incubation. Means of five replications are plotted. Epiphytic strains were categorized according to their abilities to colonize leaves. Colonization refers to population at 120 hr per population at 0 hr. Solid dots represent strains inhibitory to XP-1 Str^r on nutrient glucose agar. Strains under the dotted horizontal line significantly reduced populations of XP-1 Str^r.

29 strains were decreased, nine remained unchanged and 11 increased in number in 120 hr. Strain NC-1 of *Bacillus* sp. was not recovered but another crucifer strain SO-9-4 remained unchanged. Inhibition of *X. c.* pv. *pruni* on leaves and on nutrient glucose agar was not related.

The population of pruniphage strain PP-1 recovered after 120 hr varied from 2 to 4×10^7 pfu per leaf on leaf surfaces and 4×10^2 to 6×10^4 pfu per leaf within leaves at various pfu/cfu ratios. Increasing pfu/cfu ratios altered the populations of *X. c.* pv. *pruni* on leaf surface and there was a significant cubic relationship (Fig. 4). However, there was a significant quadratic relationship between pfu/cfu ratio and population of *X. c.* pv. *pruni* within leaves. The inhibition was greatest at pfu/cfu ratios of 0.2 to 2.0. With an increase of pfu/cfu ratio from 2.0 to 20.0, *X. c.* pv. *pruni* populations also increased.

Although at pfu/cfu ratios of 2 to 20, only one or two colonies of *X. c.* pv. *pruni* were recovered immediately after inoculation, a large population developed in 120 hr. This population comprised a mixture of phage-resistant and phage-susceptible cells of *X. c.* pv. *pruni*. High pfu/cfu ratios yielded high proportions of phage-resistant clones (Table 1). Ten of 19 phage-resistant clones were significantly less pathogenic compared to the parent strain XP-1 (Fig. 5). Six phage-susceptible clones tested were as pathogenic as the parent strain.

DISCUSSION

Interaction of *X. c.* pv. *pruni* with pruniphage or epiphytic bacteria resulted in significant reduction in its population on and within detached peach leaves. Considerable variation among leaves in the final population size of *X. c.* pv. *pruni* was apparent. Since experiments were done under controlled microbial flora and environmental conditions, this variation suggests an active role of leaves in determining microbial interactions. Differential leaf response to other bacterial plant pathogens has been observed before (7,10,12). Leaf detachment did not contribute to variation since attached and detached leaves responded similarly (14). As observed before (18), ethanol treatment reduced variation among leaves and increased multiplication of *X. c.* pv. *pruni* by removing the microbial flora on the leaves.

Investigation of microbial interactions on leaves may lead to development of a biocontrol strategy for foliar pathogens. Bacterial pathogens in their epiphytic phase may be especially vulnerable to specific antagonists (2,12,19). Selection of suitable microbial antagonists should be made by biologically relevant methods because techniques based on inhibition on agar (3) are inaccurate predictors of in planta antagonism (2,8,11,15,19,22).

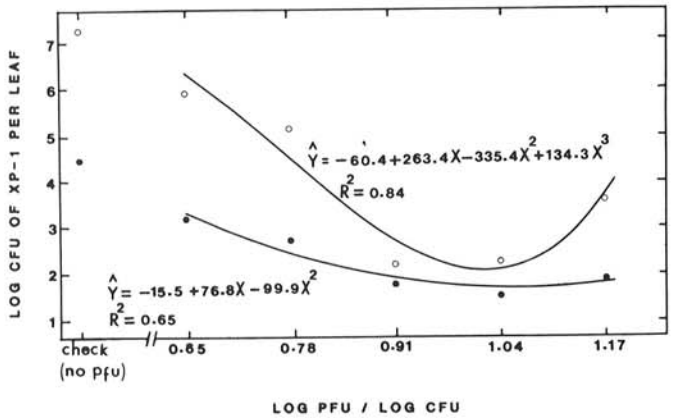

Fig. 4. Reduction of strain XP-1 of *Xanthomonas campestris* pv. *pruni* by pruniphage on the surface (o) and within (•) detached peach leaves. Serial 10-fold dilutions of pruniphage varying in number of pfu/ml from 2×10^5 to 2×10^9 were prepared. One milliliter from each phage dilution was mixed with 1 ml of strain XP-1 containing 1×10^8 cfu and the mixture was immediately inoculated as a 5-μl drop placed at each of 20 sites scattered over a leaf. Bacterial populations were determined after 120 hr of incubation. Means of five replications are plotted.

The use of detached leaves (16,18) and the microbial interactions reported herein may be useful in selecting strong antagonists for foliar pathogens. Our results suggest that chances of obtaining antagonistic strains are greater if selection is made on the basis of their ability to colonize host tissue (Fig. 3). A practical advantage would be fewer applications for disease control in the field. Host-colonizing strains also may be isolated from nonhost sources (15,19). Host-colonizing strains may further be selected for antibiotic production on the host plant but not on the artificial agar media. The reduction of *X. c.* pv. *pruni* by inhibitory bacteria was often high on detached leaves. Nevertheless, an elimination of the pathogen under these conditions is desirable to identify a potential antagonist since a small amount of inoculum of bacterial pathogens can initiate an epidemic.

Bacteriophages reduce the incidence of Prunus bacterial spot disease (4,6) but have not been recommended (21) for disease control because of development of phage resistance by the bacterium and possible transduction. In our studies, populations of *X. c.* pv. *pruni* were reduced by pruniphage; however, at high phage:bacterium ratios, a considerable proportion of the bacterial population became resistant to pruniphage. Resistance development was partially associated with reduced virulence as observed before (5). Phage-resistant clones of *Rhizobium trifolii*

TABLE 1. Development of phage-resistance in strain XP-1 of *Xanthomonas campestris* pv. *pruni* on detached peach leaves[a]

Pfu/cfu[b] ratio	Clones tested (no.)	Phage-resistant clones (no.)
0.0	10	0
0.2	22	0
2.0	8	4
20.0	41	40

[a]Serial 10-fold dilutions of strain PP-1 of pruniphage were mixed 1:1 (v/v) with XP-1 suspension (1×10^8 cfu/ml of distilled water) to generate various pfu/cfu ratios and 0.1 ml of the mixture inoculated as 5-μl drops at 20 sites on each of five leaves. After 120 hr of incubation, pruniphage resistance of recovered XP-1 was determined by spotting 10 μl (10^3 pfu) of PP-1 over bacterial lawns (4).

[b]Pfu = plaque-forming units, cfu = colony-forming units.

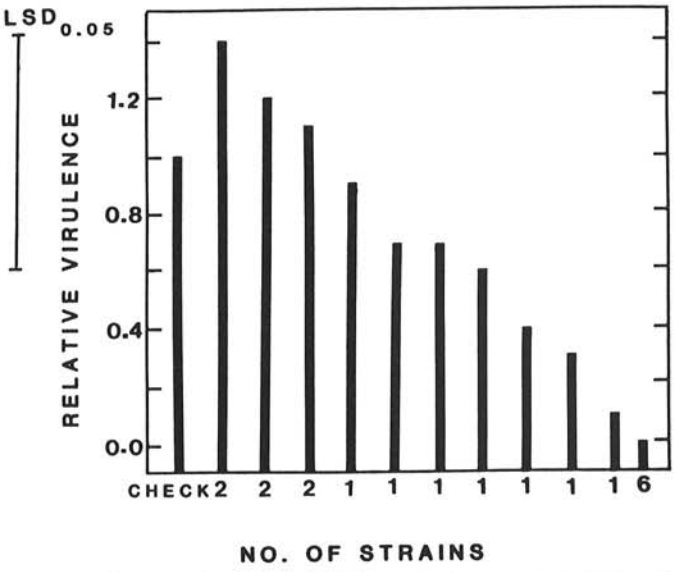

Fig. 5. Relative virulence of pruniphage-resistant mutant strains of *Xanthomonas campestris* pv. *pruni*. Inoculum (1×10^6 cfu/ml) of each strain was infiltrated with a needleless syringe to produce a 2–4 mm water-soaked area at a selected site on a young leaf on a peach tree in the field. Ten sites on one leaf, replicated four times, were inoculated. After 14 days, number of infected sites and disease severity on infected sites were recorded. Phage-sensitive parent strain XP-1 was included as a check. Relative virulence is calculated as: (no. of infected sites × disease severity caused by a test strain)/(no. of infected sites × disease severity caused by XP-1).

Dangeard also lose the ability to nodulate clover roots (1). Use of a mixture of two or more phages may minimize the development of resistance and increase attenuation of a phage-resistant population (17). A direct in vivo laboratory test, based on disease reduction on detached leaves by a bacterial or phage strain, is needed to identify useful biocontrol agents for disease management.

LITERATURE CITED

1. Barnet, Y. M. 1979. Properties of *Rhizobium trifolii* isolates surviving exposure to specific bacteriophage. Can. J. Microbiol. 25:979-986.
2. Blakeman, J. P. 1982. Phylloplane interactions. Pages 307-333 in: Phytopathogenic Prokaryotes. Vol. 1. M. S. Mount and G. H. Lacy, eds. Academic Press, New York. 541 pp.
3. Chakravarti, B. P., Leben, C., and Daft, G. C. 1972. Numbers and antagonistic properties of bacteria from buds of field-grown soybean plants. Can. J. Microbiol. 18:696-698.
4. Civerolo, E. L. 1973. Relationships of *Xanthomonas pruni* bacteriophages to bacterial spot disease in *Prunus*. Phytopathology 63:1279-1284.
5. Civerolo, E. L. 1976. Phage sensitivity and virulence relationships in *Xanthomonas pruni*. Physiol. Plant Pathol. 9:67-75.
6. Civerolo, E. L., and Keil, H. L. 1969. Inhibition of bacterial spot of peach foliage by *Xanthomonas pruni* bacteriophage. Phytopathology 59:1966-1967.
7. Crosse, J. E. 1959. Bacterial canker of stone fruits. IV. Investigation of a method for measuring the inoculum potential of cherry trees. Ann. Appl. Biol. 47:306-317.
8. Cullen, D., and Andrews, J. H. 1984. Epiphytic microbes as biocontrol agents. Pages 381-399 in: Plant-Microbe Interactions: Molecular and Genetic Perspectives. Vol. 1. T. Kosuge and E. W. Nester, eds., Macmillan, New York. 444 pp.
9. Fahy, P. C., and Hayward, A. C. 1983. Media and methods, isolation and diagnostic tests. Pages 337-379 in: Plant Bacterial Diseases, a Diagnostic Guide. P. C. Fahy and G. J. Parsley, eds. Academic Press, Sydney, Australia. 393 pp.
10. Hirano, S. S., Nordheim, E. V., Arny, D. C., and Upper, C. D. 1982. Log-normal distribution of epiphytic bacterial populations on leaf surfaces. Appl. Environ. Microbiol. 44:695-700.
11. Leben, C., Daft, G. C., Wilson, J. D., and Winter, H. F. 1965. Field tests for disease control by an epiphytic bacterium. Phytopathology 55:1375-1376.
12. Leben, C., Rusch, V., and Schmitthenner, A. F. 1968. The colonization of soybean buds by *Pseudomonas glycinea* and other bacteria. Phytopathology 58:1677-1681.
13. Randhawa, P. S., and Civerolo, E. L. 1984. Inhibition of *Xanthomonas campestris* pv. *pruni* by bacteria and pruniphage. (Abstr.) Phytopathology 74:864.
14. Randhawa, P. S., and Civerolo, E. L. 1985. A detached-leaf bioassay for *Xanthomonas campestris* pv. *pruni*. Phytopathology 75:1060-1063.
15. Randhawa, P. S., and Schaad, N. W. 1984. A seedling bioassay chamber for determining bacterial colonization and antagonism on plant roots. Phytopathology 75:254-259.
16. Sharon, E., Okon, Y., Bashan, Y., and Henis, Y. 1982. Detached leaf enrichment: A method for detecting small numbers of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in seeds and symptomless leaves of tomato and pepper. J. Appl. Bacteriol. 53:371-377.
17. Smith, H. W., and Huggins, M. B. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. J. Gen. Microbiol. 129:2659-2675.
18. Spurr, H. W., Jr. 1979. Ethanol treatment—A valuable technique for foliar biocontrol studies of plant diseases. Phytopathology 69:773-776.
19. Spurr, H. W., Jr. 1981. Introduction of microbial antagonists for the control of foliar plant pathogens. Beltsville Symposia in Agricultural Research: Biological Control in Crop Production 5:323-332.
20. Thomson, S. V., Schroth, M. N., Moller, W. J., and Reid, N. O. 1976. Efficacy of bactericides and saprophytic bacteria in reducing colonization and infection of pear flowers by *Erwinia amylovora*. Phytopathology 66:1457-1459.
21. Vidaver, A. K. 1976. Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. Annu. Rev. Phytopathol. 14:451-465.
22. Vidaver, A. K. 1982. Biological control of plant pathogens with prokaryotes. Pages 387-397 in: Phytopathogenic Prokaryotes. Vol. 2. M. S. Mount and G. H. Lacy, eds. Academic Press, New York. 506 pp.
23. Yamamoto, K. R., and Alberts, B. M. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large scale virus purification. Virology 40:734-744.