Temperature Effects on the Relationships Between Xanthomonas pruni and its Virulent Phages

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ABSTRACT

The effects of temp on phage-*Xanthomonas pruni* interactions are described. The plating efficiencies of eight virulent *X. pruni* phage variants, isolated from morphologically distinct plaques, are about the same on four *X. pruni* isolates from 10 to 27 C. Growth of an apricot isolate of *X. pruni* is inhibited in the presence of phage isolate Xp3-A/ApB at 35 C and may be due to a type of abortive infection. Irreversible adsorption of phage Xp3-A/ApB to cells of the apricot isolate of *X. pruni* grown at 35 C is markedly reduced, presumably due to the inaccessibility or absence of phage-specific receptor sites. In addition, replication of phage Xp3-A/ApB and cell lysis at 35 C are inhibited in cells of this *X. pruni* isolate previously infected at 27 C. The same numbers of lesions develop at inoculation sites on seedling leaves of peach cultivar Sunhigh following inoculation with cells of the apricot isolate of *X. pruni* grown at 27 or 35 C. Thus, structural alterations, presumably of the cell wall, which occur on cells grown at 35 C, and that result in the reduced capacity of cells to irreversibly adsorb virulent pruniphages are apparently not related to virulence of this *X. pruni* isolate.

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Because of their ubiquitous distribution in nature, microbial viruses may be important ecological agents in the environmental biology of microorganisms (7, 21, 26, 27, 33, 38), including bacteriophages of phytopathogenic bacteria (2, 4, 5, 6, 16, 17, 20, 22, 31, 34, 36, 37, 41, 42, 43). Evaluation of the ecological significance of viruses of phytopathogenic bacteria as components of microbial communities with respect to plant diseases (4, 16, 17, 20, 34, 42, 43) necessarily depends on understanding the effects of various factors, including chemical and physical effects of the environment, on phage-bacteria interactions in natural environments.

Although the effects of temp on phages can be differentiated from temp effects on host bacteria, the influence of temp on phage-bacterial host interactions may be ecologically significant (36, 41). Plaque formation by virulent phages on lawns of susceptible *X. pruni* cells are qualitatively and quantitatively affected by the temp (24, 29, 32). In previous studies on the influence of various in vitro factors on phage-*X. pruni* interactions, infection occurred only in a narrow temp range from about 22 to 30 C (13, 24, 29, 32, 39, 40), well below the inactivation temp of virulent phages capable of infecting *X. pruni* (3, 9, 32). However, *X. pruni* can grow over a wide temp range from 7 C minimum to 38 C maximum (23).

Infection of susceptible stone fruit hosts in nature by *X. pruni* is probably initiated by relatively low numbers of cells. However, subsequent development of bacterial spot disease symptoms may be a function of bacterial multiplication in vivo, symptom production occurring only after certain population thresholds are attained (15). Spread of *X. pruni* and development of bacterial spot disease in nature occurs most rapidly and extensively at temp of about 20 to 30 C when other environmental conditions are favorable (11, 28). However, growth of *X. pruni* cells either in overwintering cankers or as resident bacteria, as well as primary infections early in the season, may not necessarily be limited to these temp. If phages are

important factors in the ecology of *X. pruni,* interaction between these viruses and their host bacteria would probably occur in the early spring even when temp may not be as high as 22-30 C. The purpose of this paper is to describe some effects of temp on the interaction between virulent phages and *X. pruni* to aid in evaluating the ecological significance of these bacterial viruses in their natural environment.

MATERIALS AND METHODS.—*Bacteria and bacteriophages.*—Isolates of *X. pruni* were obtained from the following naturally infected trees: peach seedling (Pe), apricot cultivar Blenril (ApB), plum cultivar Burmosa (PlB), and sweet cherry seedling (SC). Single-colony selections were maintained at 27 C on nutrient agar containing 2% glucose (3, 6). The apricot isolate was used for the cell lysis, irreversible adsorption, one-step growth, and intracellular growth experiments. The virulent phage variant, Xp3-A/ApB, which forms large clear plaques, was propagated in *X. pruni* (ApB) grown at 27 C in media containing 0.8% nutrient broth, 0.2% glucose and 0.5% NaCl (NGSB). Additional virulent *X. pruni* phage variants ϕ1, ϕ2A, ϕ2B, ϕ2C, ϕ3A, ϕ3B, ϕ4 and ϕ5 were isolated from morphologically distinct plaques (12, 13, 29, 31) produced on bacterial lawns of *X. pruni* (PlB). Each variant was purified by three to five successive single plaque transfers on the original *X. pruni* host and finally stored at 4 C in NGSB over $CHCl_3$. Only one morphological type of virus particle was present in preparations of each *X. pruni* phage variant examined in the electron microscope. Structurally, this type of virion has a hexagonally shaped head to which is attached a tail consisting of an outer, contractile sheath surrounding an internal core and terminating with a base plate with attached tail spikes (18; *unpublished*). The attachment sites for this morphological type of phage on *X. pruni* host cells are only known to occur on the cell wall (24).

Plaque count assays were made by a single-agar-layer method (3). Generally, 0.5 ml of *X. pruni* indicator containing approximately 2-5 $\times$ 10^8 colony-forming units/ml (c.f.u./ml) was added to 5 ml melted 0.7% nutrient agar containing 0.2% glucose and 0.5% NaCl at 45 C (NGSA) followed by addition of 0.1 ml of an appropriate dilution of phage in NGSB.

Effect of temperature on the relative efficiency of plating (EOP).—In quantitative tests, 0.1 ml of the appropriate phage dilution containing 40 to 200 plaque-forming units (p.f.u.) in NGSB was plated with indicator isolates as above. Phage-bacteria mixtures were incubated at 10, 15, 20, 27, and 35 C. The temp at which the highest number of plaques were formed was taken as the standard. Plates incubated at 20, 27, and 35 C were read after 24 h. Plates incubated at 15 C were read after 48 to 72 h. Plates incubated at 10 C were read after 4 to 5 days.

Temperature-shift experiments.—Bacteria were grown for 16 to 20 h in 10 or 100 ml NGSB at 27 or 35 C. For cell-lysis experiments, cells in log phase of growth from NGSB shake cultures were suspended in 5 ml of fresh, prewarmed NGSB. Shifts to higher or lower temp for irreversible adsorption, one-step growth, and intracellular growth experiments were made by transferring the desired tube containing 1 or 2 ml of phage-bacteria mixture to a second water bath shaker at the appropriate temp.

Cell lysis.—Lysis of *X. pruni* (ApB) by phage Xp3-A/ApB was followed by monitoring changes in optical density of inoculated cultures at 620 nm. Cells in log phase of growth from NGSB shake cultures grown at 27 C were suspended in fresh NGSB to contain approximately 1-3 $\times$ 10^8 c.f.u./ml. Cultures were inoculated at p.f.u./c.f.u. input ratios of 5 to 25 so that the majority of cells were infected. Cultures were incubated with shaking at 27 and 35 C. A similar volume of NGSB was added to noninoculated control cultures.

Irreversible adsorption, one-step growth, and intracellular growth experiments.—Irreversible adsorption was measured by the chloroform technique as previously described (3). Phage was added to bacteria, previously grown at 27 or 35 C, at p.f.u./c.f.u. input ratios of 0.01 to 0.2 in NGSB containing 10^{-3}M $MgSO_4 \cdot 7H_2O$. One-step growth experiments were performed as before (3). Phage was added to bacteria at p.f.u./c.f.u. input ratios of 0.02 to 0.2 in NGSB with 10^{-3}M $MgSO_4 \cdot 7H_2O$. After incubation for 20 min. at 27 C (more than 95% adsorption of input p.f.u.), the phage-bacteria mixtures were diluted into growth tubes containing fresh NGSB, incubated with shaking at the appropriate temperature, and assayed for infective centers. Intracellular phage growth was determined in the same cultures used for the one-step growth experiment by prematurely lysing infected cells with 1/20 vol. $CHCl_3$(3).

Pathogenicity tests.—An evaluation of the relative virulence of *X. pruni* was based on the number of lesions formed at inoculation sites on leaves of peach cv. Sunhigh seedlings. Log phase *X. pruni* (ApB) cells, grown in NGSB shake cultures at 27 or 35 C, were collected by centrifugation at approximately 5,000 *g* for 15 min. Cells were resuspended in sterile demineralized water and adjusted turbidimetrically to contain approximately 1 $\times$ 10^8 c.f.u./ml. Cell suspensions were then diluted to contain approximately 5 $\times$ 10^4 c.f.u./ml.

The standard method of leaf inoculation was based on a technique described previously (35). Each inoculation site was a selected circular area, 5 mm in diam., on the lower leaf surface. Inoculum was applied at each inoculation site exposed through a hole in an aluminum foil shield by spraying at 1.76×10^{-2} kg-force/cm^2 (25 psi) until the underlying tissue was thoroughly water-soaked. Approx. 10 μl of inoculum were introduced into the intercellular spaces at each inoculation site. Excess inoculum was rinsed off the leaves after the water-soaking disappeared. Inoculated plants were maintained in a controlled environment room at 27 $\pm$ 2 C and 98-100% RH for 5 days, and then returned to normal greenhouse conditions. Lesions at each inoculation site were counted 14 days after inoculation. In each test there were four inoculation sites on each of three young terminal leaves on each of three or six plants.

RESULTS.—*Effect of temperature on plaque formation by X. pruni phage isolates.*—No plaques were observed at 35 C, although bacterial indicator lawns were well developed within 24 h. In other tests, droplets of concd phage (10^8-10^{10} p.f.u./ml) were placed on the surface of *X. pruni*-seeded NGSA. After incubation of 35 C for 24 h, growth of the *X. pruni* indicator was slightly

inhibited at the droplet sites. This apparent growth inhibition became more evident after continued incubation of the plates at room temp. However, no distinct lytic reaction, as indicated by clearing of the indicator lawn at the droplet sites, occurred in plates previously incubated at 35 C.

The effect of temp on the relative EOP of several phage variants on various *X. pruni* isolates is shown in Table 1. In general, the EOP of these eight virulent *X. pruni* phage variants was about the same on the four *X. pruni* isolates between 10 and 27 C. There was no apparent difference in the final appearance of specific plaque types formed between 10 and 27 C although plaques formed at 27 C may be larger initially than those formed at 10, 15, and 20 C.

Effect of temperature on lysis of X. pruni by Xp3-A/ApB.—The effect of a temp shift from 27 to 35 C on growth of *X. pruni* and lysis of this isolate by phage Xp3-A/ApB was followed by monitoring the change in absorbance of noninoculated and phage-inoculated cultures at 620 nm (Fig. 1-A to F). Following inoculation at 27 C of *X. pruni* with phage Xp3-A/ApB at p.f.u./c.f.u. input ratios of 5-25, the turbidity of the culture decreased rapidly and extensively for at least 500 min under these conditions (Fig. 1-B). This decrease in turbidity only indicated cell lysis and did not necessarily indicate that phage replication had occurred.

When an aliquot of a 16- to 20-h NGSB shake culture of *X. pruni* in the log phase of growth at 27 C was diluted into fresh NGSB and transferred immediately to 35 C, the turbidity of the culture initially increased very rapidly for about 75 to 100 min (Fig. 1-C). As in cultures grown at 27 C (Fig. 1-A), the turbidity of cultures may continue to increase at least up to 500 min after transfer to 35 C, but at a lower rate. Although the rate of increase and extent of turbidity of cultures grown at 35 C may be greater than that at 27 C under certain conditions, this is not invariably the case (5).

At p.f.u./c.f.u. input ratios of 5-25 phage Xp3-A/ApB nearly completely lysed *X. pruni* at 27 C, while no lysis occurred when this phage variant and *X. pruni* are incubated at 35 C (Figs. 1-D, E, F). Nevertheless, growth of *X. pruni* was markedly inhibited in the presence of phage X-3-A/ApB at 35 C (Figs. 1-D, E, F) at least when the initial p.f.u./c.f.u. ratio was much greater than 1.0. Both the rate of increase and the maximum level of turbidity were significantly lower in the cultures containing phage Xp3-A/ApB than in noninoculated control cultures to which NGSB was added. Growth inhibition of *X. pruni* apparently occurred at 35 C even when phage-inoculated cultures were previously incubated at 27 C for 30 to 60 min (Figs. 1-E, F).

Irreversible adsorption of Xp3-A/ApB to X. pruni.—At 27 or 35 C, more than 97-99% of the input p.f.u. were irreversibly adsorbed within 20 min to cells previously grown at 27 C, while only 20-30% of the input p.f.u. were irreversibly adsorbed at 35 C within 20 min to cells previously grown at 35 C (Fig. 2). About 60% of the input p.f.u. were irreversibly adsorbed within 20 min at 27 C to log phase cells previously grown at 35 C for 16-20 h (Fig. 2).

Effect of temperature on replication of phage Xp3-A/ApB.—Although Xp3-A/ApB-specific receptor sites are either not present or are not accessible on *X. pruni* cells grown at 35 C, it was not possible to determine from the above experiments how temp affects replication of phage in *X. pruni*. The effect of transfer from 27 C to 35 C on Xp3-A/ApB replication in *X. pruni* is presented in Table 2.

At p.f.u./c.f.u. input ratios greater than one, the average titer after 1.0 h at 35 C of the samples transferred at 0 min after the adsorption period was the same as the

TABLE 1. Average relative efficiency of plating (EOP) of eight virulent *Xanthomonas pruni* bacteriophage variants from morphologically distinct plaques at different temp on four *Xanthomonas pruni* isolates from peach, apricot, plum, and sweet cherry

Incubation temp (C)	*X. pruni* isolate indicator	Relative efficiency of plating of phage variant:[a]							
		ϕ1	ϕ2A	ϕ2B	ϕ2C	ϕ3A	ϕ3B	ϕ4	ϕ5
10	peach	0.78	1.00	0.79	1.00	1.00	0.60	0.80	0.70
	apricot	1.00	1.00	0.94	0.83	1.00	0.87	0.75	0.74
	plum	0.83	1.00	0.79	0.82	1.00	0.73	1.00	1.00
	sweet cherry	1.00	0.94	0.79	0.92	0.91	0.71	0.97	0.91
15	peach	0.73	0.97	0.94	0.76	1.00	0.99	0.85	0.89
	apricot	0.77	0.94	1.00	0.89	0.95	0.87	0.66	0.84
	plum	0.84	0.94	0.77	1.00	0.76	0.93	0.86	0.88
	sweet cherry	0.91	0.79	0.96	0.81	0.74	0.70	0.67	1.00
20	peach	0.76	0.96	1.00	0.86	0.98	1.00	1.00	1.00
	apricot	0.89	0.86	0.94	1.00	0.73	1.00	1.00	1.00
	plum	0.65	0.92	1.00	0.83	0.85	1.00	0.82	0.84
	sweet cherry	0.92	0.98	1.00	1.00	1.00	1.00	1.00	0.92
27	peach	1.00	0.93	0.89	0.83	0.89	0.99	0.89	0.87
	apricot	0.79	0.81	0.86	0.93	0.83	0.83	0.98	0.84
	plum	1.00	0.93	0.83	0.83	0.84	0.96	0.70	0.85
	sweet cherry	0.92	1.00	0.78	0.96	0.90	0.78	0.84	0.85

[a]Triplicate plates of each phage-bacterium combination were incubated at each temp. The relative EOP at the temp at which the largest number of plaques produced for each phage-bacterium combination was taken as one.

original culture at 27 C at the time of transfer. Therefore, no phage replication occurred in samples transferred to 35 C. However, there was approximately an 8 to 10-fold increase in the average titer after 1 h at 35 C in samples transferred 15 and 30 min after the adsorption period. Thus, limited phage replication may occur at 35 C or virus replication may be initiated at 27 C and completed at 35 C with no virus synthesis being initiated at 35 C. There were only slight increases (0 to 10-fold) in titers after 1 h at 35 C in samples incubated at 27 C for 15 to 45 min before being transferred to the higher temp. In contrast, the titer of the original culture increased 1,000-fold in 60 min at 27 C. Therefore, replication of Xp3-A/ApB phage in *X. pruni* was apparently shut off immediately after transfer to 35 C.

Similar events occurred when the p.f.u./c.f.u. input ratio of the original culture was less than one. A notable difference, however, was the apparent 10-fold increase in phage titer after 1.0 h at 35 C in samples transferred from 27 to 35 C immediately after the end of the adsorption period.

The greatest increase in phage titer occurred when the p.f.u./c.f.u. ratio of the original culture was less than 1.0. Furthermore, the relative phage titer, after 1 h at 35 C compared to that of the original culture at 27 C at the time of the temp shift, increased from about 10 in the samples transferred at 0 and 15 min after the adsorption period, to a maximum of 20 in samples transferred 30 min after the adsorption period. When the p.f.u./c.f.u. ratio of the original culture was greater than 1.0, the relative phage titer, after 1 h at 35 C compared to that of the original culture at 27 C at the time of transfer, increased from less than 1.0 in samples transferred at 0 min after the end of the adsorption period to 9 and 8 in the samples transferred at 15 and 30 min, respectively, after the end of the adsorption period.

One-step and intracellular growth of Xp3-A/ApB in X. pruni at 35 C.—The kinetics of Xp3-A/ApB replication in *X. pruni* at 35 C is shown in Fig. 3. At 35 C there was slight, but continual, decrease in infective centers for at least 60 min after the adsorption period. It is unlikely that this continual decrease in phage titer was due to additional irreversible adsorption of input phage at 35 C, since the titer of total infective centers in control growth tubes at 27 C increased from an average value of 1.47×10^5 p.f.u./ml through a 30-min latent period to a titer of 2.73×10^6 p.f.u./ml. On the other hand, it is possible that there is a temp-related inactivation of replicating phage, or an inhibition of cell lysis, at 35 C.

At 35 C, there was a rapid initial decrease in the yield of intracellular phage in the first 10 min after the adsorption

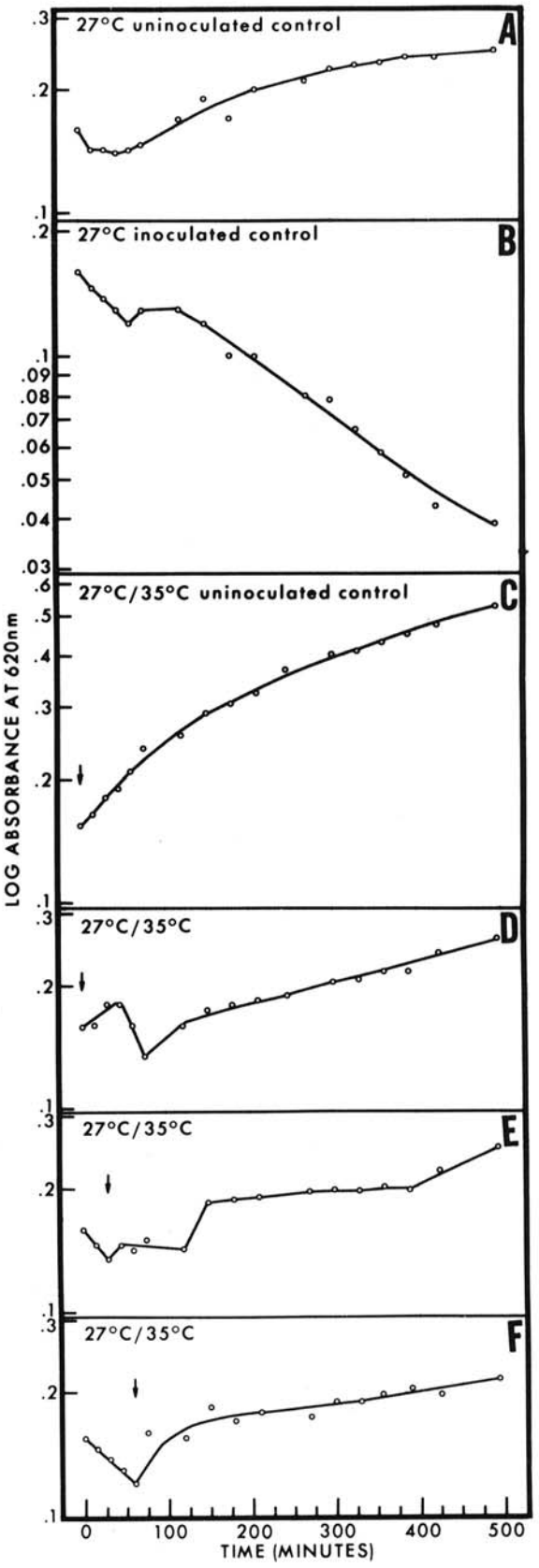

Fig. 1-(A to F). Effect of temp on the lysis type of interaction between *Xanthomonas pruni*/ApB and phage Xp3-A/ApB in NGSB shake cultures. **A)** Growth of *X. pruni* (ApB) at 27 C in a noninoculated control culture; **B)** Lysis of *X. pruni* (ApB) by Xp3-A/ApB at 27 C; **C)** Growth of *X. pruni* (ApB) at 35 C in a noninoculated control culture initiated by cells previously grown at 27 C; **D, E, F)** Growth of *X. pruni* (ApB) at 35 C following addition of Xp3-A/ApB at 0 (D), 30 (E), and 60 (F) min after transfer to 35 C. Ratio of phage (plaque-forming units) bacteria (colony-forming units) approx. 5.0.

TABLE 2. Effect of transfer from 27 to 35 C on replication of Xp3-A/ApB in *Xanthomonas pruni* (ApB)

Input ratio[a] (p.f.u./c.f.u.)	Time of transfer to 35 C (min.)	Phage titer (p.f.u./ml)		Ratio (B)/(A)[b]
		Original culture at 27 C at time of transfer to 35 C (A)	1 ml sample after 1 h at 35 C (B)	
6-17	0	7.34×10^6	5.88×10^6	0.80
	15	4.56×10^6	4.20×10^7	9.21
	30	3.67×10^6	2.90×10^7	7.90
	45	3.08×10^8	8.84×10^8	2.87
	60	2.66×10^9	4.18×10^9	1.57
0.2 - 0.5	0	5.61×10^4	5.32×10^5	9.48
	15	4.51×10^4	4.85×10^5	10.75
	30	4.11×10^4	7.89×10^5	19.20
	45	6.55×10^5	4.83×10^6	7.37
	60	6.77×10^7	7.57×10^7	1.12

[a]Xp3-A/ApB (plaque-forming units, p.f.u.) was added to *X. pruni* (ApB) (colony-forming units, c.f.u.) in NGSB with 10^{-3}M $MgSO_4 \cdot 7H_2O$ and incubated at 27 C for 20 min to allow adsorption of more than 95% of the input p.f.u. At regular intervals after the adsorption period, 1-ml samples were transferred to 35 C for 1 h. Phage titers after 1 h at 35 C were compared to those in the original culture at 27 C at the time of transfer to 35 C. Each value is the average from three experiments.

[b]Change in ratio of phage titer after 1 h at 35 C (B) to the titer of the original culture at the time of transfer from 27 to 35 C (A). Each value is the average from three experiments.

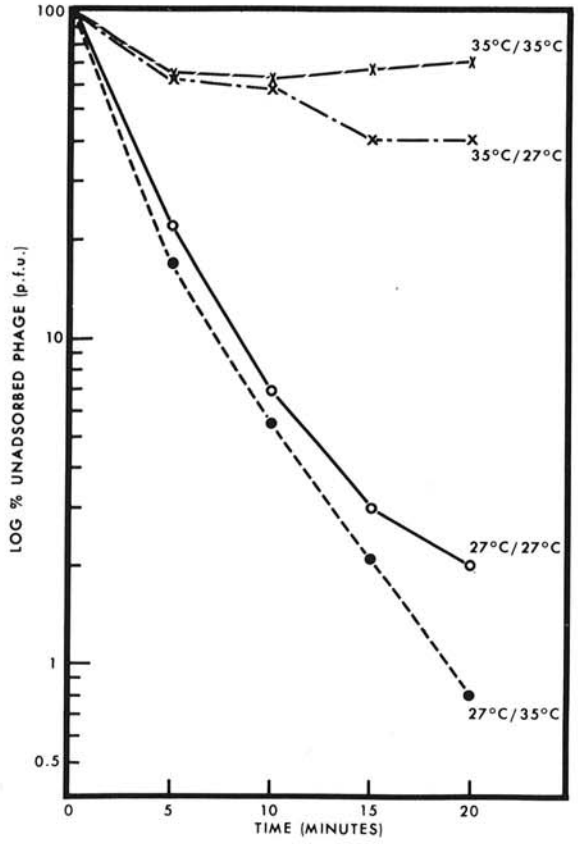

Fig. 2. Irreversible adsorption at 27 and 35 C of Xp3-A/ApB to *X. pruni* (ApB) cells previously grown at 27 and 35 C in NGSB. Xp3-A/ApB was added to log phase *X. pruni* (ApB) cells in NGSB with 10^{-3}M $MgSO_4 \cdot 7H_2O$ (plaque-forming units/colony-forming units input ratio = 0.01 - 0.2). Unadsorbed phage was measured by diluting aliquots removed at 5 min intervals into fresh NGSB with 1/20 vol. $CHCl_3$. Temperature at which the cells were grown is given first, followed by temp at which irreversible adsorption was measured.

period. This was followed by an increase in intracellular phage titer for 60 min after the adsorption period. However, the final yield of intracellular phage was less than a 2-fold increase above the initial level of intracellular phage. In contrast, the titer of intracellular phage in control growth tubes at 27 C increased steadily from an average value of 6.00×10^2 p.f.u./ml during a 20 min eclipse period to 4.16×10^6 p.f.u./ml 60 min after the adsorption period. Therefore, replication of phage Xp3-A/ApB in *X. pruni* at 35 C is markedly inhibited.

Effect of growth at 35 C on X. pruni.—No gross morphological changes were detectable by light microscopy in *X. pruni* cells grown at 35 C, compared to cells grown at 27 C. Cells from cultures grown and inoculated with Xp3-A/ApB at 35 C appeared to be less motile than cells from noninoculated cultures grown at 35 C. Although it was not apparent in these studies, bursting of at least some cells could occur in the presence of phage at 35 C.

Pathogenicity.—There was no significant difference between the average number of lesions that developed at each inoculation site by *X. pruni* cells grown at 27 C or at 35 C (Table 3). Thus, the relative virulence of *X. pruni* cells grown at 35 C may be similar to that of cells grown at 27 C. It was not possible to evaluate the effect of temp on the *X. pruni*-plant host interaction resulting in development of bacterial spot disease. Nevertheless, the ability of *X. pruni* cells grown at 35 C to attach to presumed multiplication sites (14) within susceptible peach leaf tissue, and to initiate infection, apparently was not directly related to the ability of the cells to irreversibly adsorb or support replication of the virulent pruniphage variant Xp3-A/ApB.

DISCUSSION.—At least eight virulent phage variants are capable of infecting and lysing susceptible cells of four *X. pruni* isolates [Table 1, (24, 29, 31, 41)]. However, the ecological balance between phages and their hosts may be affected by temp (5, 36, 41). Although other factors also affect disease development, any possible role of phage-*X. pruni* interactions in development of bacterial spot

disease may not be necessarily limited by temp. Indeed, from the relative EOP results in vitro at 10, 15, and 20 C, it might be concluded that any effect of virulent pruniphages on infection by *X. pruni* and bacterial spot disease development can occur early in the growing season when generally low temp prevail as well as later in the season. Infection, lesion development, and bacterial oozing are not prevalent below 20 C (11, 28).

At 35 C, a differential growth rate between infected and uninfected cells, and not cell lysis, could account for the apparent growth inhibition in bacterial cell lawns at sites of application of concd phage suspensions. Inhibition of *X. pruni* growth in the presence of phage Xp3-A/ApB at 35 C [Fig. 1, (5)] is similar to the action of EN phages on cells of *Erwinia rubrifaciens* (43). Unlike *E. rubrifaciens* cells which become spherical in the presence of EN phages, rod-shaped *X. pruni* cells did not appear to undergo any gross morphological changes in the presence of phage Xp3-A/ApB at 35 C. Cells from phage-inoculated cultures at 35 C appeared to be less motile than cells from noninoculated cultures.

Alternatively, lysis from without (10) might explain the apparent growth inhibition by the high concn of phage at 35 C. Although no bursting of cells was apparent in these studies as reported in the case of "lysis from without" of phage-infected *Escherichia coli* under certain conditions (8, 10), bursting of some *X. pruni* cells in the presence of an excess of phage Xp3-A/ApB at 35 C cannot be precluded. Although irreversible adsorption of phage Xp3-A/ApB to *X. pruni* cells at 35 C is greatly reduced compared to that at 27 C, it is possible that the apparent inhibition of *X. pruni* growth in the presence of phage Xp3-A/ApB represents a type of abortive infection (1, 43). This inhibition of *X. pruni* growth may or may not be virus-specific.

Phage particles morphologically similar to those described herein are only known to adsorb to *X. pruni* cell walls (24). There is no evidence for the occurrence of *X. pruni* phage receptors on other cell structures such as flagella and pili. The markedly reduced capacity of *X. pruni* cells grown at 35 C to irreversibly adsorb phage Xp3-A/ApB at 35 C and 27 C (Fig. 2) may be due to the inaccessibility or absence of phage receptor sites on the cell walls. Data about the specific location, and the chemical and structural nature of phage receptor sites on *X. pruni* cells are not available.

Nevertheless, cell alterations at 35 C that prevent or block irreversible adsorption of the virulent phage Xp3-A/ApB apparently do not affect virulence of *X. pruni*.

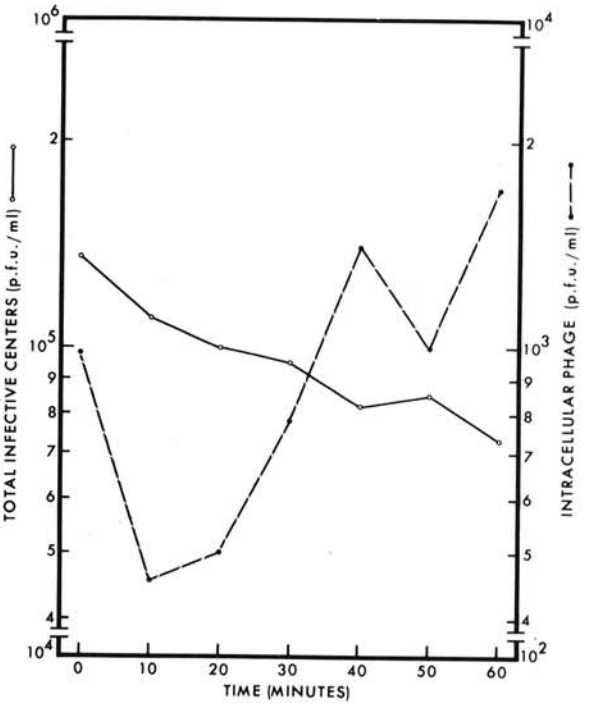

Fig. 3. One-step growth (_____) and intracellular growth (- - -) of Xp3-A/ApB in *X. pruni* (ApB) at 35 C in NGSB. Cells were grown and infected with Xp3-A/ApB at 27 C, added to growth tubes with fresh NGSB, and incubated at 35 C.

These results do not necessarily preclude the possibility that phage receptor materials and/or sites on the cell surface or wall may be related to *X. pruni* virulence determinants (16, 17, 20, 42). In preliminary pathogenicity tests, an *E. amylovora* mutant sensitive to coliphage P1 (19) was as virulent as a nonsensitive, wild type isolate.

It cannot be determined from the irreversible adsorption data whether phage nucleic acid is injected into a small proportion of *X. pruni* cells grown at 35 C that apparently adsorb phage Xp3-A/ApB irreversibly at 27 and 35 C. However, in addition to the reduced capacity of *X. pruni* cells grown at 35 C to irreversibly adsorb phage Xp3-A/ApB, replication of this phage and cell lysis at 35 C also appear to be inhibited. Thus, when phage Xp3-A/ApB is allowed to adsorb to cells at 27 C for 20

TABLE 3. Lesions at 5mm diameter inoculation sites on seedling leaves of peach cultivar Sunhigh inoculated with *Xanthomonas pruni* (ApB) cells grown at 27 and 35 C

Expt. no.	Temp at which *X. pruni* (ApB) grown[a] (C)	Number of inoculation sites with lesions per total number of inoculation sites	Avg. number of lesions/inoculation site
1	27	36/36	36
	35	36/36	34
2	27	68/72	45
	35	72/72	46

[a]Inocula contained ca. 5×10^4 colony-forming units per ml in sterile distilled, demineralized water.

min and the infected cells are shifted to 35 C up to 45 min after the end of the adsorption period when at least the early stages of virus replication have occurred (3), there is a marked inhibition of mature phage production (Table 2; Fig. 3). It is unlikely that a temp-sensitive restriction mechanism is involved, since virus replication apparently is inhibited at 35 C even if infected cells are preincubated at 27 C for 45 min (Table 2). The reduced yield of phage from cells incubated at 35 C for 1 h, compared to that from cells incubated at 27 C, could be due to inactivation of phage-specific intermediate replicative enzymes at 35 C (30).

X. pruni cells grown at 35 C are apparently as virulent as those grown at 27 C (Table 3), as evaluated by the ability of cells grown at 35 C to initiate infections resulting in the same number of lesions on peach cultivar Sunhigh seedling leaves as cells grown at 27 C. However, other factors related to bacterial disease development, such as toxin production, may be affected by temp (25, 41). Nevertheless, inhibition of *X. pruni* growth by phage at 35 C, as well as lysis of cells at lower temp, may be factors affecting infection of susceptible leaf tissue and subsequent development of bacterial spot disease.

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