Seasonal Population Dynamics and Interactions of Competing Bacteriophages and Their Host in the Rhizosphere

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We describe two prolonged bacteriophage blooms within sugar beet rhizospheres ensuing from an artificial increase in numbers of an indigenous soil bacterium. Further, we provide evidence of in situ competition between these phages. This is the first in situ demonstration of such microbial interactions in soil. To achieve this, sugar beet seeds were inoculated with Serratia liquefaciens CP6RS or its lysogen, CP6RS-ly-Φ1. These were sown, along with uninoculated seeds, in 36 field plots arranged in a randomized Latin square. The plots were then sampled regularly over 194 days, and the plants were assayed for the released bacteria and any infectious phages. Both the lysogen and nonlysogen forms of CP6RS survived equally well in situ, contradicting earlier work suggesting lysogens have a competitive disadvantage in nature. A Podoviridae phage, identified as ΦCP6-4, flourished on the nonlysogen-uninoculated plants in contrast to those plants inoculated with the lysogen. Conversely, the Siphoviridae phage ΦCP6-1 (used to construct the released lysogen) was more abundant from the lysogen-treated plants but almost never on the nonlysogen-uninoculated plants. The uninoculated plants also harbored some ΦCP6-1 phage up to day 137, yet hardly any ΦCP6-4 phages were found, and this was consistent with previous years. We show that the different temporal and spatial distributions of these two physiologically distinct phages can be explained by application of optimal foraging theory to phage ecology. This is the first time that such in situ evidence has been provided in support of this theoretical model.

Bacteria are ubiquitous in the environment, with a global estimate of 4 \times 10^{30} to 6 \times 10^{30} cells (26). With this ubiquity comes an importance to the biosphere that is well recognized; thus, any process that substantially affects natural bacterial communities will also be significant. One such process may be predation by bacteriophages (phages). It is thought that predatory phages could control the numbers of bacteria and facilitate gene transfer between bacteria by transduction (5, 6, 14).

Certainly phages are as common as bacteria. In addition, estimates of phage abundance in aquatic habitats suggest their numbers are 10 times greater than those of bacteria (5). Extrapolating this estimate to the biosphere at large would make phages the most abundant organisms on earth.

Clearly then, phages have a potentially significant global impact. But is this potential realized? This is a difficult question to answer, as the natural population ecology of phages has been little studied. Most knowledge derives from investigations using chemostats, mainly because phage-bacterium interactions serve as a useful paradigm of predator-prey interactions generally, and chemostats afford the opportunity to test the validity of mathematical models (see reference 16 for a review). However, chemostat conditions are far removed from the complexity of nature. A few studies have attempted to follow long-term phage population changes in situ, but these concentrated on aquatic habitats, considering only gross over-

Our present study is the first to describe interactions between competing phages within a natural habitat over a prolonged (i.e., 6-month) time scale. The data we present provide compelling evidence of competition between two indigenous predatory phages for the same prey bacterium within a natural environment that is consistent with established interspecific-competition theory (19).

MATERIALS AND METHODS

Bacteria used in this study. The bacteria used in this study (Table 1) were derived from Serratia liquefaciens CP6, previously isolated from a sugar beet grown at our field site (3). For the present study, a spontaneous spectinomycin- and nalidixic acid-resistant mutant of S. liquefaciens CP6 was isolated and called CP6N. In addition, a lysogen of S. liquefaciens CP6RS was isolated (4) from a CP6RS culture inoculated with the temperate Siphoviridae phage ΦCP6-1, and this lysogen was named CP6RS-ly-Φ1. These isogenic forms of the wild-type CP6 grew equally well in soil in the laboratory. All bacteria were maintained on nutrient agar (CM3; Oxoid) at 4°C, with stocks kept at ~ 80°C in 50% glycerol.

Phages ΦCP6-1 and ΦCP6-4 are double-stranded-DNA-tailed phages belonging to the families Siphoviridae and Podoviridae, respectively (4). They both infect S. liquefaciens CP6 and were previously isolated from our field site (3).

First field experiment, 1997. On 16 May 1997 (day zero), five soil samples were randomly collected from a 2.25- by 5.1-m plot within the field site. Next, around 500 sugar beet seeds (EB3 pellets; Germaines UK Ltd., Kings Lynn, United Kingdom) were soaked in sterile quarter-strength Ringer’s solution (QSR) (BR52; Oxoid) for 5 min and sown at 15-cm intervals within the plot.

Homogenates were prepared from the soil samples by mixing 1 g of soil with 20 ml of QSR and thoroughly homogenizing the resulting suspension by adding sterile 5-mm-diameter glass beads, vortex mixing the suspension for 1 min, and then shaking it on an orbital shaker for 10 min. The homogenates were screened for phages antagonistic towards CP6 by the overlay agar technique (2). The base medium was nutrient agar, while the overlay agar was made from nutrient broth (CM1; Oxoid) (13 g liter⁻¹) and bacteriological agar (L11; Oxoid) (6.5 g liter⁻¹). The homogenates (1 ml) were centrifuged for 5 min at 14,000 \times g, and 100 µl of the resulting supernatant was used to inoculate the overlay agar (2.5 ml) with an equal volume of CP6 culture.

The plates were incubated overnight at a plaque size-optimizing temperature.
TABLE 1. *S. liquefaciens* strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype*</th>
<th>Comments</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP6</td>
<td>Wild type</td>
<td>Sugar beet rhizosphere isolate from farm</td>
<td>3</td>
</tr>
<tr>
<td>CP6-ly-Φ1</td>
<td>ΦCP6-1*</td>
<td>Phage ΦCP6-1 lysogen of CP6</td>
<td>4</td>
</tr>
<tr>
<td>CP6-ly-Φ2</td>
<td>ΦCP6-2*</td>
<td>Phage ΦCP6-2 lysogen of CP6</td>
<td>4</td>
</tr>
<tr>
<td>CP6-ly-Φ3</td>
<td>ΦCP6-3*</td>
<td>Phage ΦCP6-3 lysogen of CP6</td>
<td>4</td>
</tr>
<tr>
<td>CP6-ly-Φ5</td>
<td>ΦCP6-5*</td>
<td>Phage ΦCP6-5 lysogen of CP6</td>
<td>4</td>
</tr>
<tr>
<td>CP6RS</td>
<td>Rif* Strp*</td>
<td>Sp* Na*</td>
<td>This study</td>
</tr>
<tr>
<td>CP6RS-ly-Φ1</td>
<td>Rif* Strp* ΦCP6-1*</td>
<td>Phage ΦCP6-1 lysogen of CP6</td>
<td>4</td>
</tr>
<tr>
<td>CP6SpN</td>
<td>Sp* Nal*</td>
<td>Spontaneous antibiotic-resistant mutant of CP6</td>
<td>This study</td>
</tr>
</tbody>
</table>

* Phages ΦCP6-1 to ΦCP6-5 were isolated from sugar beet rhizosphere samples collected from our field site at Oxford University Farm (3). Rif, rifampin; Strp, streptomycin; Sp, spectinomycin; Nal, nalidixic acid.

of 15°C, and any resulting plaques were counted. Phages from these plaques were identified using the methodologies detailed in our previous studies (3, 4). That is, the plaques were initially classified according to appearance (the two most dominant phages at the field site have very distinct plaque morphologies [4]). The classifications were then tested by producing phage lysates from the plaques and assaying them for the ability to lyse the CP6 lysogens listed in Table 1 (i.e., superinnunity testing [4]). As a final confirmatory step, DNA was extracted from representative lysates (3) and cut with EcoRI (Promega) as described by the manufacturer. Digests were run on 0.7% agarose gels at 0.32 V cm⁻², along with HindIII-cut lambda DNA (D-9780; Sigma), and the resulting restriction profiles were compared with the expected banding patterns (4). When no phages were detected, the homogenates were enriched with nutrient broth, spiked with an overnight culture of CP6, and resuspended after overnight incubation at 25°C to optimize phage proliferation.

Soil bacteria were counted by plating the homogenates on tryptone soy broth agar (TSBA) (17), which, when enumerated, gave an estimate of total viable heterotrophic bacterial numbers, and on *Pseudomonas* selection isolation agar (PSIA) (15) to determine total pseudomonad counts. TSBA plates were incubated at 15°C for 7 days before enumeration, while PSIA plates were incubated at 30°C for 48 h.

On days 25, 52, 82, 123, 160, 209, and 286 after being sown, 10 sugar beet plants were collected at random from the field site and weighed. After as much loose soil as possible was dislodged, the rhizosphere from each plant was sampled by scraping the surface of each root with a sterile scalpel and collecting the resulting thin layer of soil in a sterile 50-ml centrifuge tube. Homogenates were then prepared and assayed for phages and bacteria, as described for the soil samples. Additionally, the homogenates were enumerated on a *Serratia* selective medium (SSM) that we had designed for estimating total CP6-like bacterial numbers (1). Prior tests with CP6-spiked soil samples had shown that, when incubated for 48 h at 30°C, this medium selected against most non-*Serratia* bacteria while CP6 appeared as small (~1-mm-diameter) orange colonies with yellow borders that were clearly discernible against the dark-blue background of the surrounding agar. The identities of randomly selected CP6-like isolates were confirmed by checking their sensitivities to our CP6 phenotype collection (3) and their API 20E strip (bioMérieux sa) profiles.

**Second field experiment, 1998.** On 22 May 1998 (day zero), in a separate region of the field site, 3.9 by 4.8 m was partitioned off and divided into 36 plots in a six-by-six matrix, with a 30-cm-wide border separating each plot from its neighbor. Soil was collected from each plot. The plots were then randomly assigned to one of five sugar beet seed treatments (Fig. 1). The seeds were treated as follows: for treatment 1, the seeds were un inoculated as in 1997; for treatment 2, the seeds were inoculated with CP6SpN; for treatment 3, the seeds were inoculated with CP6SpN and CP6RS; for treatment 4, the seeds were inoculated with CP6RS-ly-Φ1; for treatment 5, the seeds were inoculated with CP6SpN and CP6RS-ly-Φ1. The purpose of these inoculations was to release differently marked lysogen (CP6RS-ly-Φ1) and nonlysogen (CP6SpN) forms of *S. liquefaciens* to investigate cross infection of the temperate phage ΦCP6-1 in situ and to see whether transduction might occur. CP6RS was released as a nonlysogen control of CP6RS-ly-Φ1. The inoculations were achieved by soaking the seeds for 5 min in the appropriate bacterial suspensions prepared with QSR (3). Estimates of inoculum density were obtained by drop plate counting (12) on nutrient agar with the appropriate selective agents. Inocula were also checked for phages by similarly drop plating serial dilutions of samples filtered through 0.2-μm-pore-size membranes onto CP6-inoculated overlay plates.

Directly after inoculation, the seeds were sown within their designated plots in ascending order of treatment. Latex gloves and sterile forceps were used throughout for handling the seeds. Within each plot, the seeds were sown at 15-cm intervals in a 4-by-3 matrix. Three seeds were planted in each hole to maximize germination success, with multiple germinations being thinned to single seedlings as they emerged.

As in 1997, homogenates were prepared from the collected soil samples, except that this time 1 g of soil was suspended in 10 ml of QSR. The homogenates were screened for phages antagonistic towards CP6 as described for 1997. Bacterial numbers were estimated by plating the homogenates onto TSBA, SSM, SSM supplemented with streptomycin at 1,000 μg ml⁻¹ (to select for bacteria with the CP6RS phenotype), and SSM supplemented with spectinomycin at 100 μg ml⁻¹ (to select for the CP6SpN phenotype). Plates were incubated as described above.

On days 19, 47, 96, 194 after being sown, one plant from each of the 36 plots was randomly selected and weighed. The rhizosphere of each plant collected was then sampled and assayed for phages and bacteria as for the soil samples. Putative *S. liquefaciens* CP6RS or *S. liquefaciens* CP6SpN colonies were confirmed by growing them on nutrient agar supplemented with rifampin (100 μg ml⁻¹) or nalidixic acid (200 μg ml⁻¹), respectively, which selected for their second phenotypic markers. Additionally, selected isolates were assayed for sensitivity towards phages ΦCP6-1 to ΦCP6-6 (4). This confirmed whether CP6 bacterial strains (i.e., CP6RS and CP6SpN) had been reisolated and identified any CP6-like lysogens (i.e., CP6RS-ly-Φ1) by their sensitivities to all phages except ΦCP6-1. Lysogeny was confirmed by stabbing colonies onto CP6-inoculated overlay lawns to detect zones of lysis after incubation.

**Statistics.** Calculations were done using the MINITAB version 11 computer package (Minitab Inc., University Park, Pa.). Sugar beet weights and bacterial counts were compared statistically using analysis of variance, after log₂(x + 1) transformation, with group means compared by calculating the minimum significant difference at a P value of 0.05, according to the Tukey-Kramer method (8). Phage counts were compared using the mood-median test, and linear regression lines were compared by analysis of covariance (8). Contour plots showing in situ phage distributions were generated using MINITAB.

**RESULTS**

**The 1997 field experiment.** Prior to sowing, the mean total viable bacteria count per gram of soil was 2.5 × 10⁷ CFU, as

FIG. 1. Arrangement of plots during 1998 field experiment. Each square represents a separate plot. Plots were assigned to one of five treatments by following a randomized Latin square arrangement. The squares shaded light gray indicate the plots containing plants inoculated with CP6RS, squares shaded dark gray indicate the plots containing plants inoculated with CP6RS-ly-Φ1, and unshaded squares represent plots containing uninoculated plants, either by design (i.e., treatment 1), or through inoculation failure (i.e., treatment 2); see the text for descriptions of treatments.
estimated on TSBA medium. A mean of $2.6 \times 10^6$ CFU of pseudomonads g$^{-1}$ was also calculated from PSIA plates. No phages were detected from any of the five soil homogenates directly after preparation (limit of detection, 200 PFU per g of soil). Only after the homogenates had been enriched with host bacteria were phage detected in one of the soil samples, and these were all identified as CP6-1.

The sugar beets grew, reaching their maximum weight by around day 123 (mean, 1,107.8 g). We monitored the densities of indigenous Pseudomonas and CP6-like bacteria (Fig. 2A) and those phages antagonistic towards S. liquefaciens CP6 (Fig. 2B). Phage abundances determined through enrichment (Fig. 2B) illustrated the fact that phages were present throughout the experiment, but often at densities below our preenrichment limit of detection (mean, $2.3 \times 10^2$ PFU g$^{-1}$). Small significant (i.e., $P < 0.05$) variations in numbers of both CP6-like bacteria and pseudomonads were seen (Fig. 2A), but these did not coincide with significant changes in the populations of their phages.

As in 1996 (3), we also analyzed the isolated phages antagonistic to bacterium CP6 in depth. Plaque morphology, superimmunity tests, and restriction fragment length polymorphism analysis showed that this phage population consisted predominately of the Siphoviridae phage CP6-1 (Fig. 2C) and, to a lesser extent, CP6-4, a Podoviridae phage frequently isolated from our site in 1996 (3). A few CP6-3 (Myoviridae) phage (3) and several previously unisolated phages were also present.

The 1998 field experiment. Prior to sowing, there was no significant difference among the treatment plots in terms of total viable bacterial counts ($P = 0.897$) or total CP6-like bacteria ($P = 0.879$). The mean total viable count was $3.3 \times 10^7$ CFU g$^{-1}$ of soil, while for CP6-like bacteria it was $8.6 \times 10^6$ CFU g$^{-1}$. As in 1997, no CP6-antagonistic phages were isolated from freshly prepared soil homogenates (limit of detection, 100 PFU g$^{-1}$). Only after the samples had been enriched were 3 of the 36 samples shown to harbor phages for this bacterium. Specifically, soil from plot A6 was shown to contain an unidentified CP6-antagonistic phage and plot B4 soil carried CP6-4, while soil from plot E4 harbored phage CP6-3.

All inocula had bacterial counts of around $10^9$ CFU ml$^{-1}$ prior to seed inoculation. Cultures of the lysogen CP6RS-ly-Φ1 contained $10^3$ PFU ml$^{-1}$ of phage CP6-1 due to spontaneous lysis. No free phages were detected from either the CP6SpN or CP6RS cultures.

The sugar beets reached maximum weight by around day 137. The resulting mean weight, 275.4 g, was much less than in the previous year, probably due to the combined effects of later sowing and a particularly dry summer. In addition, analysis of variance showed that the overall mean total viable bacterial count recorded from the rhizosphere samples of these 1998 plants ($9.3 \times 10^7$ CFU g$^{-1}$) was significantly lower ($P < 0.05$) than that determined from the 1997 plants ($1.3 \times 10^8$ CFU g$^{-1}$).

Both S. liquefaciens CP6RS and S. liquefaciens CP6RS-ly-Φ1 inocula survived well after release (Fig. 3B and C). S. liquefaciens CP6SpN, however, was never detected on any plants sampled, showing that this bacterium had not survived in situ. Moreover, a comparison of sugar beet weights and bacterium and phage counts between treatments revealed that the S. liquefaciens CP6SpN inoculation had no detectable effect on the experiment (all $P > 0.05$). Consequently, for the purposes of subsequent analysis, treatments 1 and 2 were judged to be the same (i.e., “uninoculated” controls), and their results were combined (Fig. 3A, D, and G). Treatments 4 and 5 were also determined to be equivalent (i.e., a CP6RS-ly-Φ1 release), and their data sets were merged (Fig. 3C, F, and I). Treatment 3 was effectively a release of CP6RS alone (Fig. 3B, E, and H).

The nonlysogen and lysogen forms of S. liquefaciens CP6RS survived equally well in the sugar beet rhizosphere and established large populations within the rhizospheres that were not different ($P = 0.747$) from each other. These populations declined at the same rate, with no significant difference in regres-
neither release noticeably changed the population dynamics of the indigenous bacteria from that seen on the uninoculated sugar beets (total viable bacterial count, \(P = 0.882\); CP6-like bacterial count, \(P = 0.390\)).

On the first sampling occasion after sowing (day 19), no released bacteria were detected on any of the uninoculated control plants. However, by the next sampling occasion (day 47), 5 of the 18 untreated plants sampled harbored released bacteria (geometric mean, \(5.1 \times 10^2\) CFU g\(^{-1}\)). Subsequently, fewer control plants carried released bacteria, until by day 194, only one plant harbored these organisms. When bacteria were detected, the abundances were around \(10^2\) CFU g\(^{-1}\). In total, 14 out of the 108 control plants collected over the experiment had detectable quantities of released bacteria.

Overall, five different types of *S. liquefaciens*-infecting phages were identified from sugar beet samples, and these corresponded to phages \(\Phi\)CP6-1 to \(\Phi\)CP6-5 (3, 4). The vast majority isolated (84.4%) were either \(\Phi\)CP6-1 or \(\Phi\)CP6-4.

(i) Phage \(\Phi\)CP6-1. Relatively small numbers of \(\Phi\)CP6-1 phage were isolated from uninoculated plants (Fig. 3D and 4A), and they were only very apparent when the homogenates were enriched (Fig. 3D and 4B). No \(\Phi\)CP6-1 phage were isolated from plants inoculated with bacterium CP6RS (Fig. 3E and 4A) unless their homogenates were also enriched, whereupon plants from two plots (B3 and E4) were shown to harbor small amounts of \(\Phi\)CP6-1 on days 68 and 137 (Fig. 3E and 4B).

In contrast, all but one of the plots inoculated with lysogen CP6RS-ly-\(\Phi\)1 repeatedly produced plants harboring high densities of \(\Phi\)CP6-1 from fresh homogenates (Fig. 3F and 4A),
and high titers (up to $1.6 \times 10^4$ PFU g$^{-1}$) were regularly recorded. The only exception was plot A4, and even then, when enriched, homogenates from this plot occasionally elicited phages (Fig. 4B). The FCPh-1 titers decreased with time, and this decline mirrored the observed drop in CP6RS-ly-F1 numbers on the same plants (Fig. 3C). From days 19 to 96 inclusive, the mean phage and lysogen counts appeared to decrease at the same rate, and a comparison of the slopes confirmed this, with no significant difference in gradient detected ($P < 0.972$).

In all, FCPh-1 counts were significantly greater ($P < 0.001$) in lysogen-inoculated plots (mean, $2.7 \times 10^3$ PFU g$^{-1}$) than in both uninoculated (mean, $5.8 \times 10^2$ PFU g$^{-1}$) and nonlysogen-inoculated plots, where FCPh-1 were below the limit of detection unless enriched.

(ii) Phage FCPh-4. Phage FCPh-4 was rarely isolated from untreated plants (Fig. 3G and 4C) even after nutrient enrichment (Fig. 3G and 4D). However, for inoculated plants the patterns of significant difference between treatments were different for FCPh-4 and FCPh-1. That is, phage FCPh-4 titers were significantly higher in nonlysogen-treated plots (mean, $9.1 \times 10^2$ PFU g$^{-1}$) than in either the uninoculated (mean, $3.6 \times 10^2$ PFU g$^{-1}$; $P < 0.001$) or the lysogen-inoculated (mean, $3.5 \times 10^2$ PFU g$^{-1}$; $P = 0.009$) plots. There was no difference between uninoculated and lysogen-inoculated plots ($P = 0.110$). Phage FCPh-4 was not inoculated into the site, and so it was not as abundant or as widely distributed as FCPh-1 (Fig. 4). More of the nonlysogen-inoculated plants (44.1%) carried FCPh-4 than either the lysogen-inoculated plants (19.7%) or uninoculated plants (12.0%). Furthermore, 83.3% of all nonlysogen-inoculated plots, 66.7% of all lysogen-inoculated plots, and 55.5% of uninoculated plots harbored plants with detectable FCPh-4 at some point during the experiment (Fig. 4D).

**DISCUSSION**

This is the first in situ study to unambiguously show that an increase in numbers of an indigenous soil bacterium can lead
to an equally substantial rise in a naturally occurring bacterio-
phage (ΦCP6-4). This work is unique, because it took place in a 
completely natural environment with native bacteria and 
phages and occurred over a long, ecologically relevant time 
scale. In no other natural habitat has this been achieved. Pre-
vious equivalent terrestrial studies have all employed micro-
cosms, over far shorter time scales, and often with very sim-
plified microbial communities (7, 9, 21, 22). A few aquatic 
studies have been undertaken in situ. However, these followed 
gross changes in total bacterial and virus populations (6, 24).
Interactions between individual bacterial and phage species in 
water have only been reported from microcosms (10, 20).

What makes our results so remarkable is that another S.
liquefaciens phage, ΦCP6-1, failed to benefit from the release of 
S. liquefaciens. ΦCP6-1, composed of the almost complete ab-
sence of this phage within CP6RS-inoculated plots (Fig. 3E) 
with its repeated occurrence elsewhere (Fig. 3D and F). This 
statistically significant difference leads us to conclude that 
phages ΦCP6-1 and ΦCP6-4 competed with each other in situ 
and that the different state of health of the released CP6RS, 
relative to wild-type CP6 indigenous within the soil, predis-
posed it to successful predation by ΦCP6-4 in preference to 
ΦCP6-1.

We assert these two conclusions for the following reasons. 
We already have strong evidence of temporal succession, and 
hence competition, between ΦCP6-1 and ΦCP6-4 occurring in 
situ in 1996 (3). During that field experiment, we observed an 
explosion in ΦCP6-1 numbers between days 48 and 99. This 
situation continued until day 156, when a dramatic decline in 
abundance occurred; thereafter numbers remained low until 
the end of the experiment. Concurrent with this decline was an 
even more substantial increase in the numbers of phage 
ΦCP6-4, which until that point had been almost completely 
absent.

Our subsequent research (4) confirmed ΦCP6-1 and 
ΦCP6-4 to be very different. (i) We found no DNA homology 
between the two phages. (ii) ΦCP6-1 was shown to be a Sipho-
viridae phage, while ΦCP6-4 was a member of the family 
Podoviridae. (iii) ΦCP6-1 was temperate for CP6, while 
ΦCP6-4 was entirely virulent. (iv) The latent period for 
ΦCP6-4 was almost three times that of ΦCP6-4, while its burst 
size was over five times greater. The last attributes are partic-
ularly pertinent to this discussion, as they have been identified 
as possible phage survival strategies (1, 23, 25).

For example, Stewart and Levin (23) theorized that viru-
rence would be favored as a survival mechanism over lysogeny 
in those environments where there are high numbers of a 
physiologically “suitable” host available. According to their 
theory, ΦCP6-4 would predominate over ΦCP6-1 at our field 
site when such host cells became abundant. CP6RS may have 
been this physiologically suitable host. Besides being abundant 
as a consequence of our release, CP6RS would have been 
physiologically different from contemporaneous indigenous 
CP6.

The scenario we outline is also consistent with the work of 
Abedon (1) and Wang et al. (25), who applied optimal foraging 
theory to phage ecology. From their theoretical models, they 
concluded that phages with short latent periods and small burst 
sizes (like ΦCP6-4) would outcompete phages with longer la-
tent periods and larger burst sizes (like ΦCP6-1) when the 
numbers of physiologically suitable host bacteria are high (as 
for CP6RS). Taken together, all these factors provide strong 
evidence of competition occurring between phages in situ.

We did not add ΦCP6-4 phage to our site, so the bloom we 
triggered derived entirely from naturally present virions. Phage 
ΦCP6-1 was also native; however, in this experiment its num-
bers were only increased substantially by a lysogen release. 
This inoculation, coincident with the CP6RS release, gener-
ated large numbers of ΦCP6-1 phage in all but one of the 
lysogen-inoculated plots. In these plots, ΦCP6-1 was up to 
1,000-fold more numerous than in uninoculated plots. This and 
the pattern of significant differences in observed phage titers 
showed that these large titers came from the inoculated lys-
ogen.

Several points arise from this concurrent release of lysogen and 
nonlysogen. First, it is clear that the proximity of all the 
plots to one another led to some movement of phage and 
released bacteria between plots, with small numbers of re-
leased bacteria repeatedly occurring in untreated controls (Fig. 
3A) and the apparent spread of ΦCP6-1 from lysogen-inocu-
lated plots to neighboring plots (Fig. 4B). Yet, in spite of these 
factors favoring ΦCP6-1, it was ΦCP6-4 that entirely domi-
nated the nonlysogen plots, emphasizing its competitive advan-
tage over ΦCP6-1 for CP6RS.

Second, ΦCP6-4 did not do so well in the lysogen-inoculated 
plots. It is unclear why this should be, as in the laboratory the 
lysogen was ΦCP6-4 sensitive. Perhaps the lysogen had a level 
of resistance to ΦCP6-4 that was only discernible under the 
nonoptimal growth conditions experienced in situ.

Third, this is the first study to simultaneously release lysogen 
and nonlysogen forms of the same bacterium into a natural 
environment and, in doing so, to demonstrate that a bacterium 
“burdened” with prophage DNA can survive as well as its wild 
type. This contradicts earlier microcosm studies (11, 18) that 
found lysogens surviving less well than nonlysogens.

Fourth, our study also illustrates what can happen to an 
environment into which a lysogen is released artificially. Not 
only is the microbial community altered by the new bacterium, 
but phages released from that lysogen also have the potential 
to affect indigenous bacteria and facilitate gene transfer 
through transduction. ΦCP6-1, for example, is a transducing 
phage (4).

A final important point to be drawn from our study is the 
unique observation that the temporal dynamics of specific 
phage populations in soil are repeated over successive years. 
Specifically, our results show that, over three consecutive years, 
ΦCP6-1 predominated at the beginning of the growing season 
(3) (Fig. 2C and 3D). In contrast, ΦCP6-4 was never abundant 
at that time. If it did bloom, it did so some time after the sugar 
beets had fully matured and ΦCP6-1 numbers had begun to fall 
(3). Thus, we conclude that the seasonality described in this 
paper highlights the potential predictability of bacteriur-
phage interactions in soil.

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