Mathematical Analysis of Growth and Interaction Dynamics of Streptomyces and a Bacteriophage in Soil

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We observed the infection cycle of the temperate actinophage KC301 in relation to the growth of its host Streptomyces lividans TK24 in sterile soil microcosms. Despite a large increase in phage population following germination of host spores, there was no observable impact on host population numbers as measured by direct plate counts. The only change in the host population following infection was the establishment of a small subpopulation of KC301 lysogens. The interaction of S. lividans and KC301 in soil was analyzed with a population-dynamic mathematical model to determine the underlying mechanisms of this low susceptibility to phage attack relative to aquatic environments. This analysis suggests that the soil environment is a highly significant component of the phage-host interaction, an idea consistent with earlier observations on the importance of the environment in determining host growth and phage-host dynamics. Our results demonstrate that the accepted phage-host interaction and host life cycle, as determined from agar plate studies and liquid culture, is sufficient for quantitative agreement with observations in soil, using soil-determined rates. There are four significant effects of the soil environment: (i) newly germinated spores are more susceptible to phage lysis than are hyphae of developed mycelia, (ii) substrate mycelia in mature colonies adsorb about 98% of the total phage protecting susceptible young hyphae from infection, (iii) the burst size of KC301 is large in soil (>150, 90% confidence) relative to that observed in liquid culture (120, standard error of the mean [SEM], 6), and (iv) there is no measurable impact on the host in terms of reduced growth by the phage. We hypothesize that spatial heterogeneity is the principal cause of these effects and is the primary determinant in bacterial escape of phage lysis in soil.

The filamentous nature of streptomycetes causes two problems for their quantification in soil and interaction with phage. First, the identification of a lysable unit during phage infection as a proportion of a hypha is unclear; second, the rate of phage adsorption to hyphae is dependent upon the age of the hypha (11, 22, 30). Phage interaction, therefore, changes over time as a function of colony heterogeneity. The effects of this heterogeneity are greatest in undisturbed colonies where interactions are dependent on diffusion. Growth on agar is limited to the boundary of the colony by the diffusion of nutrients and staling compounds (33). In contrast, colonies grown in liquid culture have little spatial heterogeneity since diffusion is rapid, and the uniform exposure of host to phage can result in efficient phage lysis (4). The effects of diffusion in soil are expected to be informed by, but distinct from, both of these cases and possibly underlies the environmental dependence observed in a number of systems (15, 17, 28).

Our objective in this study was to use population-dynamic modeling of the phage-streptomycete interaction to quantify and characterize the growth of streptomycetes and the efficiency of phage infection in soil. We examined whether specific growth and interaction characteristics were attributable to the soil environment.

Theoretical

The streptomycete life cycle includes germination, colony formation, and development (vegetative growth), and sporulation (7, 18). In brief, spores germinate via a primary germ tube that undergoes branching after a period of linear extension and nucleoid replication. In Streptomyces coelicolor, mature colonies grow exponentially (as measured by DNA content) through linear (apical) extension of individual hyphae with an exponential increase in the number of branches (2). Exponential growth continues until limited through depletion of an essential nutrient, occupancy of all spatial niches, or the accumulation of staling compounds (3). The nature of growth limitation in soil is unclear. Sporulation occurs following the formation of aerial mycelia.

Phage can be extracted from the soil environment, with viability decaying at the rate of 0.1 day -1 (10). Phage survival therefore requires either continuous production (virulence) or latency (lysogeny). Phage adsorb to mycelia in a two-step process (1): a process specific to the phage receptor and a non-specific process caused by hydrophobic and electrostatic forces. Specific adsorption may lead to infection and either lysis or lysogeny. The susceptibility of streptomycetes to phage infection varies with the age of the mycelia, with older mycelia being more resistant than young tips (11, 22, 30). This variation may be due to higher rates of DNA synthesis in hyphal-tip proximal regions (14) or to differences in the density of, or area covered by, surface receptor molecules (6, 12). The replication rate of phage therefore depends on the rate of adsorption to, and infection of, susceptible hosts, transportation processes such as rates of diffusion of phage, and the burst size of the virus. The burst size may be affected by the environment (15), presumably through the nutritional status of the host. We constructed a model of the lifecycle of the host and phage (Fig. 1) based on mass action adsorption effects. Colony heterogeneity was modeled by separate compartments for spores, germlings, and substrate mycelia. A germling is an intermediate between the spore and the exponentially growing colony and is physically identifiable as the germ tube and initial branchings (when growth is linear) prior to the exponential
growth in the number of branches. The relatively slow growth of the germling (compared to the exponential growth of the colony) means that the germling can be treated as a nongrowing state. Viable propagules can be lost when germination fails or when germlings fail to differentiate into viable substrate mycelia. We defined the probabilities $P_s$ and $P_p$ for successful germination and differentiation, respectively (Fig. 1). After differentiation, exponential growth of the substrate mycelia commences and ends with saturation of an unknown resource at the “vegetative capacity,” denoted by $K$, intrinsic to the soil and conditions. $K$ is less than the final soil capacity because mycelial growth ceases at saturation, while sporulation continues. The growth rate was modeled with a standard logistic form $\mu(1 - [M/K])$, i.e., the growth rate decreases linearly from a maximum value $\mu$ to zero as the total mycelial mass $M$ approaches the vegetative capacity $K$. The germlings and substrate mycelia are divided into susceptible and resistant compartments, designated $G^s$, $G^r$, $T^s$, and $T^r$, respectively. Germlings and young tips are initially susceptible and acquire resistance to phage lysis as they age (Fig. 1).

The heterogeneity of the colony structure was also reflected in the phage dynamics. Phage occurred in six states: a free state $V_f$, replicating phage within an infected host propagule $I$, or adsorbed on the surface of the available hyphae, i.e., adsorbed to susceptible and resistant substrate mycelium ($V_{Gs}$, $V_{Gr}$) and susceptible and resistant germlings ($V_{Cs}$, $V_{Cr}$) (Fig. 1). We excluded lysogeny because its low frequency in our experiments implies lysogeny does not have a significant affect on the growth cycle dynamics.

Experimentally, we measured the number of viable spores, total propagules ($T$), and free phage ($V_f$). Spores, germlings, and substrate mycelia were assumed to contribute equally to the propagule count, and phage lysis was assumed to remove a propagule. A model with differential contributions of spores and mycelia to the total propagule assay also was examined to see if this assumption affected the fit to the data.

A simulation of the mathematical model is shown in Fig. 2 that demonstrates four key features of the model. (i) Phage growth depends on susceptible propagule numbers. Phage growth is initially rapid since susceptible germling density is high after spore germination, but it declines as resistance is acquired. A second phase of phage growth can occur if the host produces a high density of susceptible tips, $T^s$, on day 3 of the stimulation, as indicated by the rise in phage adsorbed to susceptible hosts. (ii) Phage adsorption to substrate mycelia protects young tips from infection. Nonspecific adsorption of phage during the vegetative phase produces a decline in the free phage density (Fig. 2, day 3) even though the total phage level increases through the lysis of substrate mycelia. Free-phage density reduction protects young hyphae from infection since phage adsorbed to resistant mycelia are not infectious unless they reenter the free-phage pool by desorption (Fig. 1). This explains why although susceptible hosts (young tips) are in a 10-fold-higher density at day 4 than at day 0 they do not induce significant phage growth. (iii) Temporary cessation of growth occurs when the vegetative capacity is achieved. The increase in total propagule numbers temporarily ceases (Fig. 2, days 5 and 6) prior to the appearance of spores. Growth cessation has been observed experimentally (13, 26). (iv) Differentiation of germlings into substrate mycelium does not correlate with resistance acquisition. In this simulation, differentiation of germlings is slow, whereas the acquisition of resistance by germlings is rapid. Our data suggest that these time scales are in fact similar, a result that is not due to the linking of these effects in the model.

**MATERIALS AND METHODS**

Sterile, unamended Warwick soil (32) was inoculated with *Streptomyces lividans* spores (TK24) and phage (KC301) in two protocols. In experiment 1, 1.6 \times 10^9 CFU of TK24 g\(^{-1}\) and various amounts of KC301 were used as follows: microcosm 1A, 1.3 \times 10^3 PFU g\(^{-1}\); microcosm 1B, 1.3 \times 10^4 PFU g\(^{-1}\); microcosm 1C, 1.3 \times 10^5 PFU g\(^{-1}\); microcosm 1D, 1.3 \times 10^6 PFU g\(^{-1}\); microcosm 1E, 1.3 \times 10^7 PFU g\(^{-1}\); and microcosm 1F without KC301. In experiment 2, 2.0 \times 10^9 PFU of KC301 g\(^{-1}\) and various amounts of TK24 were used as follows: microcosm 2A, 2.0 CFU g\(^{-1}\); microcosm 2B, 2.0 \times 10^3 CFU g\(^{-1}\); microcosm 2C, 2.0 \times 10^4 CFU g\(^{-1}\); microcosm 2D, 2.0 \times 10^5 CFU g\(^{-1}\); microcosm 2E, 2.0 \times 10^6 CFU g\(^{-1}\); microcosm 2F, 2.0 \times 10^7 CFU g\(^{-1}\); microcosm 2G, 2.0 \times 10^8 CFU g\(^{-1}\); microcosm 2H, 2.0 \times 10^9 CFU g\(^{-1}\); and microcosm 2I without TK24.
significant growth in the next 24 h (P), following inoculation at days 1 and 2 (P) in the absence of phage (microcosm 1F).

Serum was added to neutralize the phage, and the suspension was diluted by a factor of 1,000 and assayed for PFU at specified times. 

Statistical significance is based on analysis-of-variance and Student t tests.

RESULTS

Streptomyces growth dynamics. We monitored a single streptomyces growth cycle (Fig. 3). Germination was complete by day 1, vegetative (exponential) growth occurred during days 2 to 5. Sporulation began during days 2 to 5, and by day 15 the growth cycle was complete. The final total propagule counts of microcosms 2B to 2F were \(3 \times 10^7\) to \(6 \times 10^7\) CFU g\(^{-1}\); microcosms 2D, 2E, and 2F were not significantly different (P > 0.05%), as were microcosms 2B and 2C (P > 10%). The total propagule count decreased significantly at day 1 (P < 1%) to approximately 10% of the initial value, with significant growth in the next 24 h (P < 1%). This fall occurred in the absence of phage (microcosm 1F).

All microcosms (Fig. 4) showed identical relative growth following inoculation at days 1 and 2 (P > 5%), even though the phage count varied by 3 logs (Fig. 4). Thus, S. lividans growth is independent of the phage at these inoculation densities, and the decrease in the total propagule count over the first 24 h cannot be attributed to either phage lysis or to a crowding effect (31). At day 5, the relative growth was significantly different between microcosms (P < 10^{-14}); microcosms 2D, 2E, and 2F, although the total propagule counts were not identical either (P = 3 \times 10^{-10}). The relative differences were significantly reduced by day 5 from an initial scaling of 10 between microcosms 2C, 2D, 2E, and 2F to 1.3, standard deviation (SD), 0.02 (best fit), indicating that exponential growth had ceased by this time. Thus, there is a weak dependence on the initial inoculation density in the total propagule count at day 5. However, microcosms 2D, 2E, and 2F were in an identical state of sporulation by day 5 (P > 20%). We attribute differences in growth between microcosms over the first 48 h solely to different inoculum levels, but during the subsequent 3 days of vegetative growth the microcosms all reached the same density and stage in the growth cycle to a fair approximation.

The total propagule count increased over days 5 to 15 by three- to eightfold (Table 1). This increase is caused by the increase in the spore count. In experiment 2, spores contributed 11% of the total propagule count at day 5 and 93% on day 15 (Table 1). In experiment 1, the spore contribution to the total propagule count at day 15 was low (12%), perhaps due to a crowding effect suggested by the poor gain in spores during the growth cycle (Table 1).

Since the microcosms all reached the same state of growth at day 5, the exponential growth phase ended by reaching a saturation capacity prior to day 5. This saturation capacity is the vegetative capacity (K) of the mathematical model.

Phage growth dynamics. Phage growth was dependent on the state of S. lividans. During the first 24 h, a large increase in phage density occurred for the higher streptomyces inoculum microcosms (Fig. 3C), was constant for 24 h, and then decreased dramatically in all microcosms between days 2 and 5. Day 5 and 15 counts were equal, indicating that the interaction was complete and that spores were not interacting with phage. Phage growth during the first 24 h in experiment 1 was poor compared to that expected from the trends in experiment 2 (Fig. 3C). In the absence of host, the phage count gradually
declined as phage particles were inactivated or became unavailable for detection by the assay (not shown). These data suggest that phage may be adsorbed by the mycelia, similar to the adsorption observed in liquid culture (22, 30). The phage assay detects only unadsorbed phage because of the gentle extraction process (23). Therefore, phage adsorption caused the dramatic decrease in phage count over days 2 to 5 correlating with the increase in mycelial density.

Since the increase in phage numbers occurs within 24 h of inoculation the number of germinated spores lost through lysis can be estimated as \( \frac{V_i - V_0}{b} \) for a burst size \( b \), where \( V_i \) denotes free phage density at day \( i \). The loss of host propagules can be compared directly to the number of available host propagules at day 0, \( S_{inoc} \), the inoculation density. For a burst size of 120 (see below), effectively all propagules would be lysed for microcosms 2E and 2F \( \left( \frac{V_i - V_0}{bS_{inoc}} \right) \) values estimated as 71%, SD 21% and 110%, SD 12%, respectively). Such high losses through phage lysis would dramatically affect host growth and should produce effects similar to those seen in liquid culture, where the bacteria can be eliminated (4). However, the growth of the host was unaffected by the presence of phage (Fig. 4), suggesting that phage growth resulted from lysis of only a small fraction of the host propagules.

**Lysogeny.** Throughout these experiments the lysogen counts were low, indicating that lysogens were a minority (<1% of host population) and that lysis was the more common outcome of infection.

**Liquid culture.** The burst size for KC301 on TK24 hosts was 120, standard error of the mean (SEM) 6. This is comparable to \( \phi \)C31 and \( \phi \)A7 with burst sizes of 10 to 80 (22, 23) and 70 to 100 (11), respectively. The rise time (i.e., the time from addition of antiserum to the end of phage increase) was 60 min.

FIG. 3. Growth curves (log_{10}) for experiment 1 (solid lines) and experiment 2 (dashed lines). (A) Spore counts. (B) Total propagules. (C) Phage counts (microcosms 2A, 2B, and 2C have less growth than microcosm 2D and are omitted for clarity). (D) Lysogen counts. For experiment 1, an average over microcosms is shown for spore and total propagules since streptomycete growth is identical across microcosms \( (P > 1\%) \) for microcosms 1A, 1B, and 1D). Typical error bars (95%) are shown as indicated, displaced to the right for clarity. Their skewed appearance is due to the logarithmic scale; the confidence intervals were computed based on the absolute values. Symbols (A and B): 2A, $\otimes$; 2B, +; 2C, □; 2D, ×; 2E, $\odot$; 2F, * with a dashed line; experiment 1 (average over microcosms), solid line. Symbols (C and D): 1A, $\otimes$; 1B, + (experiment 1, solid line); 2D, □; 2E, ×; 2F, $\triangle$ (experiment 2, dashed line). Data are reprinted from *Gene Transfers and Environment* (25) with permission from the publisher.

FIG. 4. Plot of total propagule growth relative to the inoculum against time on a log_{10} scale. Microcosms 2C to 2F and an average over microcosms for experiment 1 (adjusted for the difference in extraction efficiency) are shown. Symbols: 2C, $\otimes$; 2D, +; 2E, □; 2F, ×; experiment 1, $\odot$.
We estimated the growth and interaction parameters for the model schematically described in Fig. 1. Sporulation was excluded because we could not estimate the rate of this process from the data set. This restricts analysis to days 0 to 5, which are independent of the sporulation cycle. We initially restricted modeling to microcosms 2B to 2F. Microcosm 2A was removed because of its very low inoculum level. The number of datum points per microcosm is low (measurements were only made at days 0, 1, 2, and 5), but these data are sufficient since the doubling time of the streptomycetes is expected to be 6 to 16 h, although rapid changes in the first 24 h may have been missed. The availability of multiple microcosms with various inoculation conditions ensures model validity over a wide range of initial conditions.

We first developed a “basic model” that (i) ignored lysogeny, (ii) assumed that substrate mycelia were resistant to phage lysis (11), and (iii) assumed that the decrease in the propagule count at 24 h resulted from a failure of germlings to differentiate successfully. Modifications and extensions to this model were then examined.

**Determination of parameters.** Extraction efficiencies were estimated from day 0 measurements (Table 1). Three parameters—the germination rate ($g = 6.0 \text{ day}^{-1}$), the lysis rate of infected host propagules ($\gamma = 1.0 \text{ h}^{-1}$), and the decay rate of free phage in soil ($b = 0.12 \text{ day}^{-1}$)—were estimated directly because they determine an effect that is either very rapid or very slow and therefore they are not significantly correlated with the other parameters (verified by alteration of these values over reasonable ranges [data not shown]). The germination rate was estimated by comparing the spore counts on day 1 with those on day 0 by the formula $g = -\log (S_0/S_1)$ (Table 1), which models germination as a probabilistic event with an exponential distribution. The decay of free phage was estimated from microcosm 2G (data not shown), and the lytic time period was estimated from the liquid culture.

The nine remaining parameters were estimated by optimizing the fit of the model to the data using a sum-of-squares statistic denoted “SS” (see Appendix and reference 29). This method uses the 29 datum points (day 1, 2, and 5 measurements) to simultaneously estimate all nine parameters. The SS was minimized in the nine-dimensional parameter space, i.e., the total error between observed and model predictions of the total propagule and free phage counts summed over days 1 to 5 was minimized. The incline of SS away from that minimum determines the parameter confidence intervals. The burst size $b$ was treated separately from the other parameters (see below). The best fit of the basic model to experiment 2 is shown in Fig. 5 and 6, with the parameter estimates in Table 2. The only strong correlations in the parameters (correlations with $b$ were not determined; see below) were between $\mu$ and $P_d$ ($r = -0.869$) (Fig. 7), between $\omega_{c_2}$ with $\beta_+$ ($r = 0.633$), and between $\omega_{c_2}$ and $l$ ($r = 0.581$). All other parameters were weakly correlated ($r < 0.35$). The parameters are discussed in turn below.

**Burst size ($b$).** A good upper bound for the burst size could not be determined from this data set because streptomycete

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results (SD) from:</th>
<th>Expt 1</th>
<th>Expt 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction efficiency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total propagules</td>
<td>1.3 (0.03)</td>
<td>1.2 (0.11)</td>
<td></td>
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<tr>
<td>Spores</td>
<td>0.20 (0.01)</td>
<td>0.079 (0.008)</td>
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<tr>
<td>Phage</td>
<td>0.88 (0.07)</td>
<td>1.1 (0.07)</td>
<td></td>
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<tr>
<td>Soil capacity, CFU/g (total propagules)</td>
<td>$5.7 \times 10^7 (2.9 \times 10^6)$</td>
<td>$4.6 \times 10^7 (2.3 \times 10^6)$</td>
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<tr>
<td>Spore gain at:</td>
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<tr>
<td>Day 1 ($S_1/S_0$)</td>
<td>0.0035 (0.0004)</td>
<td>0.0020 (0.0003)</td>
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<tr>
<td>Day 15 ($S_{15}/S_0$)</td>
<td>3.4 (0.3)</td>
<td>&gt;70</td>
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<tr>
<td>Spores as fraction of soil capacity* at:</td>
<td></td>
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<tr>
<td>Day 5</td>
<td>0.014 (0.002)</td>
<td>0.11 (0.02)</td>
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<tr>
<td>Day 15</td>
<td>0.12 (0.01)</td>
<td>0.93 (0.16)</td>
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<tr>
<td>Total propagule count relative to soil capacity at day 5 ($T_5/T_0$)</td>
<td>0.37 (0.03)</td>
<td>0.12 (0.014)</td>
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* $S_k$ and $T_k$ refer to spore and total propagule measurements on day $k$. Corrected for extraction efficiencies of spores and total propagules, i.e., $S_k/T_0 \times$ ratio of the extraction efficiencies.

**FIG. 5.** Fit of basic model to total propagule data of experiment 2, microcosms 2B to 2F. The data are time displaced for clarity. Error bars are 95%. The parameters are given in Table 3. Note the uniformity in the growth over the first 3 days, which reproduces the scaling behavior of Fig. 4. Symbols: 2B, (———); 2C, (— — — —); 2D, (— — — —); 2E, × (· · · · ·); 2F, (· · · · ·).

**FIG. 6.** Fit of model to phage data for microcosms 2B to 2F. Symbols: 2B, ○ (———); 2C, (— — — —); 2D, □ (— — — —); 2E, × (· · · · ·); 2F, △ (— — — —).
growth was independent of phage density. Thus, although SS decreases as $b$ increases from 100 to 300, it plateaus over the range $b = 300$ to 1,000, where SS would normally have a valley. This prevents traditional estimation of $b$. We fixed the burst size at $b = 300$, a value equal to that in the first minimum, and derived estimates and confidence intervals of the other parameters subject to this constraint. A lower confidence bound for the burst size was then determined: $b > 150$ (90% confidence; see Appendix for details). Liquid culture measurements suggest that the burst size is 120; however, a burst size this low is inconsistent with our data set (Table 2).

**Colony formation ($\mu$, $K$, $P_g$, $k$, and $\omega_{c2}$).** The vegetative capacity estimate agrees with the day 5 total propagule count for microcosms 2D, 2E, and 2F. We predict that at this time these microcosms had ceased growth. The growth rate $\mu$ corresponds to a doubling rate of 5.5 h. The probability $P_g$ of a germling forming a colony was less than 1%, i.e., most of the germlings failed to produce successful colonies. Resistance to phage lysis was attained at the same rate as differentiation, $\omega_{c2} \sim k$, suggesting that acquisition of resistance might be associated with exponential growth and changes in the early colony structure. This similarity of time scale means that most germlings were available for lysis, and only later did a proportion (of those that escape lysis) successfully form colonies. The ability of the model to satisfy the lack of an impact of the phage on the host population is particularly apparent in microcosm 2F, where ca. 50% of the inoculum was lysed, and 1% of those remaining developed into colonies. In the absence of phage, the total propagule counts would have doubled at days 1 and 2, a change that could not be detected given the measurement errors.

**Phage infection ($\beta_1$ and $l$).** The rate-limiting step for phage infection (two-step process [1]) changed with the host density from adsorption to phage entry. Adsorption to mycelia was rate limiting for total propagule densities of $<10^6$ CFU g$^{-1}$. 3% (ratio $\beta_1 / \beta_2$) of the vegetative capacity $K$ (microcosms 2A to 2E). In microcosm 2F the rate of phage infection was primarily limited by phage entry after adsorption to the surface (Fig. 1). Liquid culture estimates of the adsorption constant $\beta_1$ are $2 \times 10^{-10}$ to $8 \times 10^{-10}$ ml min$^{-1}$ (1, 11, 22). By converting to an effective volume of water (equating $-67$ kPa matrix potential with 15% volume to weight), we calculated $\beta_1$ to be $2 \times 10^{-9}$ ml min$^{-1}$ from our data. The similarity of these values is consistent with our interpretation of the phage-host interaction.

The SS for this basic model has a probability of about 0.01%. This low value was caused by high contributions to SS from two points: microcosm 2B (phage on day 1) and microcosm 2E (phage on day 2), suggesting that these data are outliers. Excluding microcosm 2B or 2E reduced the SS to 30 ($P = 1.2\%$) and 31 ($P = 0.9\%$), respectively; removal of both gave an SS of 13 ($P > 16\%$).

**Variations on the basic model.** We analyzed four extensions of the basic model in which some of our original assumptions are relaxed.

(i) **Modification of saturation dynamics.** In the basic model, growth rate saturation was modeled as logistic, $\mu[1 - (M/K)]$, which can be interpreted as inhibition of growth by mass action effects, e.g., secretion of an inhibitor with high diffusivity and inhibition proportional to the local concentration of the inhibitor. However, growth is probably correlated over a colony, suggesting inhibition of a more general form, $\mu[1 - (M/K)^\alpha]$. As discussed in Results, the vegetative capacity also may depend on inoculum through a scaling behavior $K \sim AS_{in}^\alpha$ (Fig. 3B). These modifications lead to a (nonsignificant) reduction of SS to 46 (with a best fit for $\eta = 3, v = 0.05$). Thus, the exact form of the host growth dynamics is not critical in modeling these data.

We analyzed data from both experiments together with these modifications, attributing differences in phage dynamics

| Table 2. Fitted parameters for basic model (data sets 2B to 2F) |
|-------------------|--------------------|-------------------|
| Symbol            | Parameter          | Estimate          | 90% CI |
| SS                | Sum of squares statistic (20 degrees of freedom) | 51 | <65 |
| $\mu$             | Substrate mycelial basic growth rate | 3.0 day$^{-1}$ | 2.8–3.3 |
| $K$               | Vegetative capacity | $4.5 \times 10^9$ CFU g$^{-1}$ | 2.8–6.8 ($\times 10^9$) |
| $k$               | Rate of differentiation of germlings to substrate mycelia | 1.1 day$^{-1}$ | 0.65–1.6 |
| $P_g$             | Success probability of germling differentiation | 0.0083 | 0.0025–0.019 |
| $b$               | Burst size         | 300 | >150 |
| $\beta_1$         | Adsorption rate of phage to mycelia | $\beta_1,K = 99$ day$^{-1}$ | 23–440 |
| $\beta_2$         | Rate of release of phage from mycelia | $\beta_2 / \beta_1,K = 0.033$ | 0.019–0.052 |
| $l$               | Infection rate (rate of adsorbed phage entering host) | 1.9 day$^{-1}$ | 0.66–16 |
| $\omega_{c1}$     | Rate germlings become resistant to phage attack | 1.3 day$^{-1}$ | 0.6–6.2 |
| $\omega_{c2}$     | Rate mature mycelia become resistant to phage attack | >29 day$^{-1}$ |

* CI, confidence interval.
  * The rate $\beta_1,K$ is estimated normalizing adsorption to a mycelial mat at density $K$.
  * The ratio $\beta_2 / \beta_1,K$ is estimated since this determines the equilibrium state.
between experiments to a difference in burst size and differences in vegetative capacities to a weak dependence on the initial inoculum. The fit was very poor ($P = 10^{-8}$; SS = 115 with a contribution of 80 from experiment 2, 38 degrees of freedom), suggesting that the model can adequately describe either data set alone but cannot explain both data sets with all parameters identical except for burst size. We hypothesize that differences between these experiments are due to differences in phage adsorption rates and infection cycle dynamics.

(ii) Substrate mycelium susceptibility. In the basic model, germings could age but substrate mycelia were assumed to be resistant to phage lysis even though hyphal tips are probably susceptible to some extent (11, 22, 30). We added a parameter, $\omega_T$, for the rate of resistance acquisition by substrate mycelia and estimated the 10 parameters of Table 2 as before. Germings became resistant at rate 1.3 day$^{-1}$, independent of the value of $\omega_T$. For a $\omega_T$ value as low as 50 day$^{-1}$ there was no appreciable change in the goodness of fit (SS = 54). Below this value, lysis of substrate mycelium and total phage in the system increase as the $\omega_T$ decreases. For a $\omega_T$ value of >50 day$^{-1}$, the phage lysis of germings dominated. Lysis of substrate mycelia became important as the $\omega_T$ decreased to 30 to 50 day$^{-1}$ (Fig. 8). For a $\omega_T$ value of >80 day$^{-1}$, the free phage at day 5 constituted 2.5% of the total phage, whereas at $\omega_T = 25$ this fraction was 1%. Thus, phage derived from the lysis of substrate mycelia were adsorbed, while the quantity of phage produced by germing lysis remained relatively constant. A lower confidence limit on $\omega_T$ (90%) was 29 day$^{-1}$, corresponding to a susceptible half-life of 35 min. The important parameter for measuring susceptibility of hyphae to phage lysis is the ratio of half-lives $\omega_T/\omega_G$, estimated to be >30 at a 90% confidence level. This result suggests that the phage-hypha interaction depends on the state of the colony. Identical results were obtained by varying the rate constant $l$, i.e., substrate hypha exposure $\omega_T l_T$ was at least 30 times less than the germing exposure $\omega_G l_G$ (90% confidence).

For a $\omega_T$ value of 50 day$^{-1}$, a hyphal tip is susceptible for only about 20 min compared to 18 h for germings. During exponential growth, the fraction of the colony that was susceptible is $\mu/\omega_T$, with a value of ca. 6%.

(iii) Lysogeny. We assumed a constant probability of lysogeny per infection, a spontaneous reversal rate of zero, and that the extraction efficiency for the lysogens was the same as that of the total propagules. Under these assumptions the probability of lysogeny was $2 \times 10^{-8}$, with a 90% upper confidence limit of $5 \times 10^{-8}$ compared to a frequency of lysogeny of $\delta C31$ observed in liquid culture of 30 to 40% (22). The low frequency of multiple infection of hyphae predicted by the model for these soil experiments probably explains this difference and is consistent with an environmental dependence of the lysogeny switch (17).

(iv) Models for propagule loss after germination. In the basic model, the decrease in total propagule count during the first 24 h was attributed to germings failing to form colonies. Two alternative hypotheses are that not all spores form viable germings ($P_r < 1$; Fig. 1) or that spores, germings, and substrate mycelia contribute unequally to the total propagule count. We quantified the second alternative as:

$$T = e_T [S + \sigma_G (G' + G^*) + \sigma_T (T' + T')]$$

where $e_T$ is the assay efficiency (as measured on spores at day 0) and $\sigma_G$ and $\sigma_T$ are the relative assay efficiencies for germings and total propagules, respectively.

The statistic SS indicates that these two models are not significantly better at describing the data (SS = 51 and 49, respectively). Again significant contributions to SS came from two or three outliers. The SS plateaued in burst size at 400 and 200 for the two models, respectively. Parameters that could be suitably compared lay within the confidence intervals of Table 2. Therefore, our estimates are robust against the as-yet-unknown process causing total propagule loss during the first 24 h. Possible explanations are a 99% failure of germings to differentiate into viable mycelia, failure of 55% of spores to establish a colony on germination, or a 5:2 efficiency ratio for detection by the assay for spores and mycelia (or germings).

**DISCUSSION**

Our three main observations were a large burst size (Table 2), a lack of impact of the phage on host growth (Fig. 4), and a high susceptibility to infection of germings relative to young substrate mycelium (Table 2 and Fig. 8). The difference in the burst size estimates relative to liquid culture can be explained by environmental dependence (28), and molecular explanations for differing susceptibilities and adsorption have been proposed (6, 12). However, these factors also can be explained by spatial effects: spatial colony heterogeneity, localized modification of the environment by hyphae (19), and the spatial distribution-correlation of phage and hosts throughout the microcosm. Liquid culture destroys these spatial effects because of high diffusion and mixing, while growth on agar reduces these effects by restricting growth to the colony boundary. At the molecular level, phage adsorption may be affected by spore and hyphal modification of their local environment. For example, water absorption by the spore could localize phage to germings, and slow diffusion of phage relative to colony growth will produce high local concentrations of phage after lysis and reduce the effect of phage relative to the total amount of phage present. Local adsorption of phage by substrate mycelia protects substrate hyphal tips more than it does germings and could partially explain the increased susceptibility of germings to phage lysis relative to the substrate mycelium.

There are two biological uncertainties in this system: the identity of the adsorbing material and the quantification of
mycelial propagules. Because of the physical separation of the aerial mycelium from the substrate mycelium, the substrate mycelium is likely to be the adsorbing material (32), as assumed in the model. However, since adsorption is only important as the mycelial mat matures, the dynamics would be mathematically similar and the exact identity of the adsorbing material is not crucial for parameter estimation. The mycelial nature of streptomycetes is potentially problematic, principally because 1 CFU need not be a lytic unit. However, this problem does not affect parameter estimation since phage growth is restricted to the first 24 h and the streptomycetes are in discrete lysable units during this time because of the absence of septa (20). Further, differential contributions of spores and mycelia to the total propagule assay cannot explain the data any better than can alternative models.

Our analysis implies that the qualitative form of the streptomycete growth cycle and phage-streptomycete interactions observed in liquid (11) and agar plates (7, 18) can explain the dynamics in soil but that both the soil environment and colony heterogeneity are important variables. We note, specifically, the following points. (i) Vegetative exponential growth stops after reaching a vegetative soil capacity, at approximately 10% of the final soil capacity, prior to the sporulation cycle. (ii) Phage do not significantly affect streptomycete growth. (iii) Phage propagation on germlings is more successful than on developing colonies. Thus, mycelial susceptibility to phage lysis varies with age (11, 22, 30). (iv) Adsorption of phage to mycelia significantly reduces the density of free phage. Adsorption dominates the phage dynamics during the vegetative phase of the host, reducing free-phage density by a factor of 50 relative to the total phage in the system. (v) There is a phage-independent growth pause of the host during the first 24 h and a significant decrease in the total propagule count at 24 h. This decrease can be explained by either a failure of 55% of spores to establish a colony on germination, 99% of germlings failing to produce viable mycelia, or a 5:2 efficiency ratio for detection of spores and mycelia (and germlings), respectively, in the total propagule assay. (vi) The burst size is large, with a b of >150 (90% confidence) and a best estimate of b of 300 (germling differentiation failure model).

These results emphasize the role of the environment in the growth and interaction characteristics of streptomycetes and phage. However, in contrast to previous work highlighting the importance of the environment in affecting burst size (15, 28) and the lysis-lysogeny switch (17), which can be explained through the nutrient status of the host, our analysis suggests that physical processes within the environment (e.g., diffusion) significantly affect ecosystem dynamics. Further experimental work is required to clarify the contribution of the environment to the dynamics with regard to physical processes and nutrient status. Experiments incorporating in situ monitoring of DNA for phage (9) and streptomycetes would also enhance the analysis and contribute to model development. In particular, these methods could be used to test model predictions, for instance, the ratio of free phage to total phage in the microcosm at day 15 (estimated to be 1 to 3%) and the rate of decline of free phage over the vegetative phase, predicted by our models to be equal to the growth rate of the adsorbing medium.

**APPENDIX**

**Mathematical model.** The basic model schematically represented in Fig. 1 consists of 14 coupled ordinary differential equations. The model parameters are listed in Table 2. Susceptible G′ and resistant G′° germlings have dynamics described by:

\[
\frac{dG'}{dt} = \beta G^* S_{\text{max}} e^{-r} - kG' - IV_{G} - \omega_0 G' \tag{A1}
\]

and

\[
\frac{dG'}{dt} = \omega_0 G' - kG'. \tag{A2}
\]

In equation A1 the terms are the creation of susceptible germlings by germination (rate g), differentiation into substrate mycelia at rate k, infection by adsorbed phage V_{G}, and acquisition of resistance at rate \omega_0, corresponding to the first term of equation A2. Resistant germlings differentiate at rate k, the second term in equation A2. Germinnation reduces spore numbers as e^{-r}. Germination is successful with probability P_e.

Substrate mycelia occur in four states: susceptible mycelia (T^s), resistant mycelia (T^r), an intermediate state (T^i; not shown in Fig. 1), and nonviable mycelia (T^n). The last two states are introduced to ensure that germling differentiation and aging are independent processes (i.e., to prevent parameter correlations) and to prevent adsorbed phage release to the environment on differentiation failure, respectively. The intermediate state T^i is differentiated (giving rise to growing tips) but acquires resistance on a germling time scale \omega_0. T^n models the fact that failed germlings present an adsorbing surface for a period of time. Decay of this adsorbing surface is not included since the surface area is insignificant once exponential growth is underway, i.e., within 24 h. The substrate mycelium dynamics are as follows:

\[
\frac{dT^s}{dt} = \mu(M) (T^s + T^i + T^r) - IV_{T^s} - \omega_0 T^s, \tag{A3}
\]

\[
\frac{dT^i}{dt} = P_j kG^* + \omega_0 T^s + \omega_0 T^r, \tag{A4}
\]

\[
\frac{dT^r}{dt} = kP_j G^* - IV_{T^r} - \omega_0 T^r, \tag{A5}
\]

and

\[
\frac{dT^n}{dt} = k(1 - P_j)(G^* + G'). \tag{A6}
\]

Here M = T^s + T^i + T^r is the total growing mycelia with a growth rate \mu(M) = \mu(1 - M/K). The terms in equation A3 are the production of susceptible mycelia by growth, the loss by infection by adsorbed phage V_{T^s}, and the acquisition of resistance at rate \omega_0. For resistant mycelia, equation A4, the terms are differentiation from resistant germlings and the acquisition of resistance by the intermediate state and susceptible mycelia. Similarly, equations A5 and A6 record the production and loss of T^r and T^n.

The phage compartments consist of free phage V_p replicating phage in an infected host I, and phage adsorbed to the host compartments above, denoted V_{G^*}, V_{G^*}, V_{T^s}, V_{T^r}, and V_{T^n}. The phage dynamics are as follows:

\[
\frac{dV_p}{dt} = b_p I - \delta V_p - \beta I V_{G^*} M + G^* + G^* + T^n \frac{V_p}{K} + \beta I V_{T^s} + V_{T^r} + V_{T^n}
\]

\[
+ V_{G^*} + V_{G^*} - k V_{G^*} - (1 + s_G) V_{G^*} - \omega_0 V_{G^*}, \tag{A7}
\]

\[
\frac{dV_{G^*}}{dt} = \beta G^* V_p - \beta V_{G^*} - \omega_0 V_{G^*}, \tag{A8}
\]

\[
\frac{dV_{G^*}}{dt} = \beta G^* \frac{V_p}{K} - \beta V_{G^*} - k V_{G^*} - \omega_0 V_{G^*}, \tag{A9}
\]

\[
\frac{dV_{T^s}}{dt} = \beta T^s \frac{V_p}{K} - \beta V_{T^s} - (1 + s_G) V_{T^s} - \omega_0 V_{T^s}, \tag{A10}
\]

\[
\frac{dV_{T^r}}{dt} = \beta T^r \frac{V_p}{K} - \beta V_{T^r} + kP_p V_{G^*} - \omega_0 V_{T^r}, \tag{A11}
\]
Phage adsorption kinetics are based on mass action. The first two terms in equations A8 to A13 represent adsorption and desorption and correspond to the third and fourth terms of equation A7. Adsorbed phage compartments mirror aging, differentiation, and infection of the underlying host equation, except for an extra term at infection that models the additional phage adsorbed to the surface of the hypha at infection. The number of additional particles adsorbed per propagule under our experimental conditions this refinement is not important since infected propagules tend to have very few adsorbed infection. Because of destructive sampling. The measurement errors are estimated across the confidence intervals. The 90% level for the SS statistic is consistent with the approximation that the χ² distribution underestimation that may derive from variation between the parameters (Fig. 7). Multiparameter confidence intervals will be narrower than the single-parameter estimates. For parameters that are only constrained by the data in one direction, e.g., the burst size, we fix these parameters at a suitable minimum and calculate the SS statistic. Probability levels under these constraints. To obtain the appropriate SS statistic for the full parameter space, we use the χ² distribution to adjust for a change in the degrees of freedom, i.e., we normalize the SS to 93.1% on eight variables for 90% confidence on nine. These probability levels are reasonable approximations for the full unconstrained probability distribution since χ²(m) was a reasonable approximation to the SS. This is equivalent to imposing a weak prior on b.

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