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DENSITY-DEPENDENT HOST–PATHOGEN DYNAMICS IN SOIL MICROCOSMS[1]

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Abstract. Temporal density-dependent parasitism and a host threshold density are important features of disease induced by infectious parasites in populations of aboveground, macroscopic organisms. We determined whether these features also occur in soil microcosms containing a microscopic host (the nematode *Heterodera schachtii*) and its parasite (the nematophagous fungus *Hirsutella rhossiliensis*). Soil microcosms are especially interesting because (1) the environment and scale are quite different from conventional host–parasite systems and may result in considerably different disease dynamics, (2) the small size of the soil microcosms, although biologically appropriate, facilitates experimentation and parameter estimation, and (3) some soil-borne, microscopic organisms (such as *H. schachtii*) are important agricultural pests.

Temporal density-dependent parasitism was directly assessed with laboratory experiments in which host density and environment were controlled. A theory, which complements and extends the experiments, was developed to enable direct comparison of observed and predicted dynamics and to provide a stringent test of our understanding of processes underlying the dynamics. The theory was simple, yet explicitly described the essential biology. Parameters for the theory were measured with short-term experiments.

We found that the disease dynamics in soil microcosms exhibited both temporal density-dependent parasitism and a host threshold density. However, epidemics were slow to develop. Observed and predicted dynamics were quite similar, indicating that our understanding of the underlying biology was correct.

Key words: biological control; epidemiology; epizootiology; fungus; host threshold density; microcosm; nematode; soil; temporal density-dependent parasitism.

INTRODUCTION

Ecological questions associated with the dynamics of disease are important from both applied and basic perspectives. These questions include: How does disease contribute to the regulation of population size? Under what conditions do epidemics start or end? How can humans intervene to prevent undesirable epidemics or to start epidemics in pest populations?

Enormous progress has occurred since the original quantitative formulation of the dynamics of disease by Kermack and McKendrick in the 1920s (see Anderson 1991 for review and discussion). Two of the most important qualitative insights from the study of disease in human and vertebrate populations are the following. First, a host threshold density is common. This density is the level of hosts in the population required to insure that the pathogen does not become locally extinct. Second, transmission rates depend upon host and pathogen population densities, and this leads to temporal density-dependent parasitism. Recent work has shown that these concepts also apply to diseases in insect populations (e.g., Anderson and May 1981, Brown 1987, Onstad and Carruthers 1990).

The density-dependent nature of disease is intuitively understood in that we expect an increased probability of epidemics as host population density increases to high levels. Temporal density dependence occurs primarily because transmission of parasites among hosts is a function of parasite density, which in turn is a function of host density. In addition, susceptibility of hosts may increase as host density, and associated physiological stresses, increase (Anderson and May 1981).

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Using differential equation models, Anderson and May (1981) described direct, horizontal transmission of microparasites in invertebrate populations in two ways. In model A, the net rate of transmission is βXY, where X is the density of susceptible hosts, Y is the density of infected hosts, and β is a transmission parameter. This expression of transmission is termed the mass action principle and has been used for disease models in human (Anderson 1991) and insect (Brown 1987) populations. In model G, Anderson and May assumed that transmission occurs when susceptible hosts contact propagules of the parasite, rather than when susceptible hosts contact infected hosts. In this model, the net rate of transmission is νWX, where W is the density of parasite propagules, X is the density of susceptible hosts, and ν is a proportionality constant measuring transmission efficiency. Note that W is a function of past density of susceptible hosts. In this paper, we offer a slightly more complicated model for the transmission function. Our model, however, is based on empirical data from which parameters can be estimated. In addition, we provide a biologically mechanistic understanding of the parameter ν.

Transmission, parasite reproduction in infected hosts, and parasite longevity determine the host threshold density. The host threshold density tends to be low if the propagules of the parasite are highly transmissible, are produced in high numbers per infected host, and are long-lived. In other words, the host threshold density is directly related to the parasite's rate of mortality and inversely related to the parasite's rate of reproduction and transmissibility. In some host–parasite systems, parasite mortality is directly related to virulence because the parasite dies when the host dies.

Although epidemics among populations of vertebrates and insects have been studied for a number of years (Onstad and Carruthers 1990), epidemics among populations of soil-borne microscopic organisms have seldom been considered but are worth studying for several reasons. First, the soil environment differs greatly from the aboveground environment and may result in considerably different dynamics. Second, the small scale of disease in a population of microscopic organisms facilitates experimentation and control of environment and other factors such as host density. Finally, some soil-borne microscopic organisms, such as plant-parasitic nematodes, are destructive pests of crop plants and are subject to epidemics (Kerry 1987, Stirling 1988). Epidemiology may help us maximize disease in order to achieve biological control of these pests and thus reduce the use of toxic chemical controls.

The work described in this paper has three main objectives:

1) To determine whether density-dependent parasitism and a host threshold density are features of disease in a soil environment containing a microscopic host and its parasite.

2) To test our understanding of the biological processes underlying density-dependent parasitism in the soil environment. To accomplish this objective, we developed a theory for the disease dynamics and compared experimental and predicted dynamics. The theory was designed to facilitate direct comparison of these dynamics and thus to provide a stringent test of our understanding of fundamental biological mechanisms. Parameters were estimated independently of the model tests.

3) To determine how density-dependent disease dynamics in the soil are affected by changes in transmissibility and changes in rates of parasite mortality and reproduction. This objective required use of the theory developed in Objective 2.

The Model System

An appropriate model system for addressing the three objectives is a soil-borne nematode and its obligate fungal parasite. In this section, we describe the nematode host, its obligate fungal parasite, and the soil microcosms used for experimentation.

The plant-parasitic nematode Heterodera schachtii

The nematode *H. schachtii* is an obligate parasite of plant roots. Juveniles (second stage) are ≈450 μm long and 20 μm wide. They hatch from eggs in cysts in soil, move through soil pores, penetrate host roots, and develop into adult males or females in ≈1 mo at 20°C. The females are nonmotile, but their swollen bodies rupture the root surface where fertilization by motile males occurs. The females become egg-filled (≈200–500 eggs per female), die, and are passively moved into the soil when the root or soil is disturbed. The dead female's body is called a cyst and protects the eggs until they hatch.

The nematophagous fungus Hirsutella rhossiliensis

The fungus *H. rhossiliensis* is a soilborne hyphomycete that produces nonmotile spores on the tip of flask-shaped phialides (one spore per phialide) in soil pores (Sturhan and Schneider 1980). The phialide is ≈30 μm long, and the spore is 4 × 8 μm. The spore adheres to passing nematodes and detaches from the phialide. A germ tube penetrates the cuticle of the nematode (immunity has not been observed) directly beneath the spore and forms an infection bulb within the body cavity. Assimilative hyphae extend from the infection bulb and, at 20°C, the nematode is dead and colonized (filled with fungal hyphae) ≈4 d after spore acquisition. Fungal hyphae then radiate from the cadaver into the soil where phialides and spores are produced (Jaffee and Zehr 1983, Jaffee and Muldoon 1989).

The external network of hyphae and phialides that surrounds each cadaver is important because (a) it provides for local distribution of spores, and (b) it is required for spore acquisition (which we also call transmission in this paper). When the network is destroyed by soil disturbance, the current crop of spores loses the

TABLE 1. Summary of laboratory experimental procedures.

Experiment	Number of colonized nematodes added at week 0	Weeks when microcosms were inoculated with host nematodes	Number of host nematodes added per microcosm at each inoculation	Weeks when microcosms were assayed	Number of replicates
1	39 ± 5	2, 5, 8, 11, 14	0, 25, or 94	2, 5, 8, 11, 14, 17	4
2	51 ± 3	2, 5, 8, 11, 14, 17, 20	0, 25, or 270	2, 8, 14, 20, 23	3 or 5
3	49 ± 10	3, 6, 9	0, 16, 32, 64, 117, 234, or 469	12	4
4	51 ± 3	2, 5, 8, 11	0, 9, 17, 33, 65, 133, or 266	14	4

ability to adhere to nematodes, apparently because the spores are detached from phialides and detached spores die unless attached to a host (McInnis and Jaffee 1989). Thus, the fungus cannot be successfully introduced into soil in the form of spores. For experiments, we add the fungus to soil in the form of colonized nematodes from which the fungus sporulates (Jaffee et al. 1990).

H. rhossiliensis can grow, albeit slowly, on standard laboratory media in the absence of other organisms, but has no saprophytic activity in soil and appears to be an obligate parasite (Jaffee and Zehr 1985); the growth of *H. rhossiliensis* on sterilized media is probably a laboratory artifact.

High numbers and percentages of nematodes are parasitized by *H. rhossiliensis* in some agricultural fields (Jaffee et al. 1989). In a recent study, Jaffee and McInnis (1991) found that the percentage parasitism was related to host density based on spatial sampling, but the constancy of parasitism and nematode density over time precluded inferences on the temporal relationship between these variables.

Soil microcosms

Because epidemics of soilborne, microscopic hosts and parasites are difficult to study in the field (Jaffee and McInnis 1991), we used soil microcosms (25 mL vials containing soil) to control the environment and to control and measure the critical factors governing host–parasite dynamics. The significant features of these microcosms are (a) the scale is appropriate for studying the population dynamics of slow-moving, microscopic organisms (Wiens 1989); (b) the small size enables us to establish many replicate microcosms (by periodically removing and assaying some of the replicates, we can sample the "same" interacting populations repeatedly through time); (c) the environment is controlled (loamy sand, bulk density 1.54, temperature 20°C, moderate water potential); and (d) the small volume minimizes spatial heterogeneity and allows us to focus on temporal dynamics.

An additional and important feature of the microcosm is that no host plant is present and thus nematodes are unable to reproduce. The absence of a host plant enables experimental control of host recruitment: new hosts (healthy juveniles) enter each microcosm only when they are added by the experimenter rather than by birth or immigration. This experimental decoupling of host reproduction and parasitism facilitates quantification of density-dependent parasitism and the host threshold density.

EXPERIMENTS

The main thrust of our experimental work was to study the effect of host recruitment (numbers of juveniles added) on rates of parasitism and the dynamics of parasitism. A synoptic view of the experiments is given in Table 1.

General procedures

For all laboratory experiments, we used a loamy sand that had been heated to 60°C for 2 h to kill native nematodes and *H. rhossiliensis* (Jaffee et al. 1990). The soil was dried, wetted to 10.3% of soil dry mass with 4.6 mmol/L KCl), and packed into vials (17 cm^3 soil, bulk density = 1.54). Because spores of this fungus are not viable unless produced in situ (McInnis and Jaffee 1989), the fungus was mixed into the soil (as the soil was wetted before packing) in the form of *H. rhossiliensis*-colonized juveniles of *H. schachtii.* The fungus then produces spores in situ. Other details on the soil and procedures are provided by Jaffee et al. (1990).

Effect of host recruitment on parasitism

In four separate experiments, we examined the effect of controlled host recruitment on the percentage of assay nematodes acquiring spores. Selection of host recruitment levels was based on a preliminary estimation of the host threshold density using published parameter values for transmission, sporulation, and spore mortality (Jaffee et al. 1990). In the first experiment, microcosms containing 39 ± 5 colonized *H. schachtii* (mean ± 1 SE, unless stated otherwise) were inoculated with 0, 25, or 94 hosts (healthy juveniles of *H. schachtii* for all experiments) at 3-wk intervals (Table 1). At each inoculation of host nematodes and at week 17, four microcosms per treatment were inoculated with assay nematodes (*Heterorhabditis bacteriophora*) instead of host nematodes. *Heterorhabditis*

bacteriophora is an entomogenous nematode that is morphologically distinct from *H. schachtii* and facilitates enumeration of assay nematodes with and without spores (McInnis and Jaffee 1989); when *H. schachtii* is used as both the host and assay nematode (see experiment 2, next paragraph), hosts surviving from previous inoculations may be confused with newly added assay nematodes. The assay nematodes were extracted 42 h later and the percentage with at least one *H. rhossiliensis* spore determined.

In the second experiment, *H. schachtii* juveniles were used as both the host and the assay nematode. Microcosms containing 51 ± 3 colonized *H. schachtii* in loamy sand were inoculated with 0, 25, or 270 hosts at 3-wk intervals (Table 1). At 3- or 6-wk intervals, three microcosms receiving 0 hosts per interval and five microcosms receiving 25 or 270 hosts per interval were assayed (Table 1). The microcosms selected for assay were inoculated with 250 healthy *H. schachtii.* These were extracted 66 h later, and the number n_w of nematodes with spores and the number n_{wo} of nematodes without spores were determined. A possible problem arising from using the same species as both host and assay nematode was that n_w and n_{wo} might include host nematodes (added 3 wk before) in addition to assay nematodes. Although we expected the potential for carry-over to be minimal (see *Theory* section), we quantified carry-over by extracting and counting host nematodes with and without spores from five control microcosms per assay time. Inoculation of these microcosms stopped 66 h before extraction. The percentage of assay nematodes with spores was calculated after n_w and n_{wo} were corrected for the carry-over of hosts. In general, $<1\%$ of the nematodes with spores were carry-over nematodes.

In the third experiment, microcosms containing colonized *H. schachtii* were inoculated with one of seven levels of hosts at week 3, 6, and 9 (Table 1). On week 12, 250 assay nematodes (*H. bacteriophora*) were added to each microcosm and extracted after 42 h. The percentage of assay nematodes with at least one *H. rhossiliensis* spore was determined. There were four replicate microcosms per level of host recruitment.

In the fourth experiment, microcosms containing colonized *H. schachtii* were inoculated with one of seven levels of hosts at week 2, 5, 8, and 11 (Table 1). Assay nematodes (*H. bacteriophora*) were added, extracted, and examined at week 14. There were four replicate microcosms per level of host recruitment.

Parameter values for spore acquisition

Although Jaffee et al. (1990) determined spore acquisition parameters for *H. schachtii,* we reassessed these parameters for three reasons. First, Phillips (1990) demonstrated that contact could be interpreted mechanistically through the contact function (see *Theory* section and the Appendix). Second, we used a different species of nematode (*H. bacteriophora*) to assay for spores in three of the laboratory experiments, and parameters for spore acquisition may vary with species of nematode. Third, the water regimes of the present and previous studies differed, and soil water affects spore acquisition (E. Tedford, *unpublished data*). The initial water content and the volume added per inoculation were less in the present study than in Jaffee et al. (1990) to prevent saturation of the soil with repeated addition of hosts.

To assess the effect of spore density on spore acquisition by *H. schachtii* and *H. bacteriophora,* we packed microcosms with soil containing 0–460 colonized juveniles of *H. schachtii.* At 20°C, the fungus produces 112 ± 7 spores from each colonized nematode after 14 d; no spores are produced after this time because the nematode substrate is depleted (Jaffee et al. 1990). Thus, we estimated the number of spores added to microcosms and present at day 14 by multiplying the number of colonized nematodes added per microcosm times 112. After 14 d, we added 250 *H. bacteriophora* or *H. schachtii* in 0.2 mL of 4.6 mmol/L KCl to the surface of each microcosm and extracted them after 42 or 66 h, respectively. The percentage of nematodes with at least one *H. rhossiliensis* spore adhering to the cuticle was determined. We performed this experiment a second time with minor variations in numbers of colonized nematodes. Data from the two experiments were combined for nonlinear regression analysis (SAS 1988). We assessed spore acquisition by *H. schachtii* after 66 h because most (84%) *H. schachtii* that acquire spores do so within 66 h (Jaffee et al. 1990). We assessed spore acquisition by *H. bacteriophora* after 42 h to spread the laboratory work load.

Theory

In order to test our understanding of density-dependent dynamics, we developed a dynamic model of the interaction between *H. schachtii* and *H. rhossiliensis.* The experimental results represent the interaction of a number of different biological processes, and a successful theory can help tease these apart and show how they produce the final results. In addition, experimental determination of the host threshold density is difficult; one would need many levels of host recruitment and experiments that ran for long periods (at least 200 d) due to the longevity of *H. rhossiliensis* spores. We now develop a theory from which the dynamics of spores and the host threshold density can be predicted using experimental parameters that are readily measured on short time scales.

We will develop two variants of a theory. In the first, we concentrate on the experiments in which hosts were added at 3-wk intervals. In the second, we increase the temporal resolution by considering 1-wk intervals.

To begin, let S_t denote the number of spores present in the microcosm at the start of week t. To model the experiments, we want to relate S_t and S_{t+3}, i.e., the number of spores present at a given week and the num-

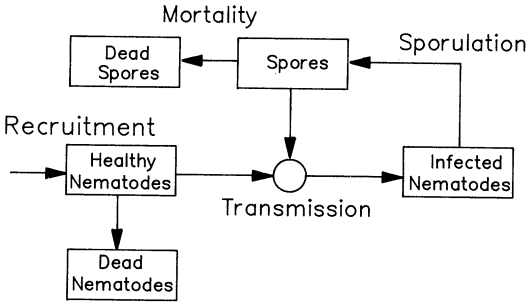

FIG. 1. Flow diagram of parasitism of nematodes by the nematophagous fungus *Hirsutella rhossiliensis* with controlled recruitment of hosts. In each time period, a fraction of the healthy nematodes contacts spores and becomes infected. This fraction depends upon spore density. Nematodes that do not acquire spores within 3 d are assumed to die before the start of the next period. Infected nematodes are killed, assimilated, and converted into spores. Spores either initiate a new infection or die.

ber of spores present 3 wk later. This relation depends (Fig. 1) upon (1) survival of existing spores, (2) removal of spores that contact hosts and (3) production of new spores from infected hosts.

First consider the survival of existing spores. Jaffee et al. (1990) found that, at 20°C, spore mortality can be described by exponential decay with a constant rate of 0.072 per week, which is equivalent to 0.201 per 3-wk interval. That is, the number of spores surviving from week t to week $t + 3$, given S_t spores in week t, is $(1 - \mu)S_t$ where the parameter $\mu = 0.201$.

Next consider the removal of spores due to contact with hosts. Suppose that the number of hosts (juveniles) added at the start of week t is H_t. If f_t is the fraction of hosts added at week t that acquires spores, and each contact removes exactly one spore (see below), then the removal of spores between weeks t and $t + 3$ is f_tH_t. We will describe forms for the contact term f_t after we describe spore production.

Spore production at 20°C can be described as follows (Jaffee et al. 1990): Sporulation occurs 2 wk after spore transmission; i.e., if a juvenile is added on week t and contacts a spore, it contributes 112 spores at the start of week $t + 2$. Assuming that those spores survive to week $t + 3$, the number of new spores contributed to the population at week $t + 3$, due to infections in week t, is ϵf_tH_t, where $\epsilon = 112$.

Combining these three processes leads to the equation for spore dynamics,

$$S_{t+3} = (1 - \mu)S_t - f_tH_t + \epsilon f_tH_t. \quad (1)$$

Note that if we choose 3-wk intervals, corresponding to the experiments, then $H_t = R$, the number of hosts added at 3-wk intervals. Also note that the last two terms on the right-hand side of Eq. 1 can be combined as $f_t(\epsilon - 1)H_t$.

Eq. 1 is not a complete description of the dynamics without a description of the contact term f_t, which is the fraction of hosts acquiring spores. Based on empirical evidence (Jaffee et al. 1990), juveniles either contact spores within 3 d of recruitment or cease movement and, for the purpose of the model, are assumed to be dead (Fig. 1). Jaffee et al. (1990) found that the fraction of juveniles that acquired spores in the 3-d interval following recruitment increases, but at a decreasing rate, as the number of spores increases. In addition, >90% of the juveniles that acquire spores become infected. These data suggest that the infection probability is a nonlinear function of spore numbers and that it approaches 1 as the number of spores becomes large. Here we consider three models of spore transmission:

$$f_t(s) = 1 - e^{-bs} \quad (2)$$

$$f_t(s) = \frac{s}{s + c} \quad (3)$$

$$f_t(s) = \frac{as^2}{s^2 + c^2}. \quad (4)$$

The contact term (Eq. 2) corresponds to Nicholson-Bailey dynamics (Hassell 1978, Perry 1978); in the Appendix we provide a mechanistic interpretation of the parameter b. The contact term (Eq. 3) was used by Jaffee et al. (1990). We have not been able to find mechanistic interpretations of Eqs. 3 or 4. In the *Results* section, we fit the observed data to these models. We will see that Eqs. 2 and 3 give virtually identical results but prefer Eq. 2 because of the mechanistic interpretation. We also found that Eq. 4 provides a better fit to some of the data than does Eq. 2.

Our basic model (Eq. 1) changes only slightly when we consider 1-wk rather than 3-wk intervals. In particular, the vast majority of new spores in any week are produced by nematodes that were infected 2 wk previously. Hence, if we choose to model spore dynamics over the finer time scale of 1 wk, Eq. 1 must be modified to

$$S_{t+1} = (1 - \mu_1)S_t - f_tH_t + \epsilon f_{t-1}H_{t-1}, \quad (5)$$

where μ_1 is the weekly spore mortality rate (Jaffee et al. 1990).

Host threshold density

A main analytical prediction of the theory is the determination of the number of hosts required to insure the persistence of the fungus. To find this level we consider the case, corresponding to the experiments, of 3-wk intervals in which $H_t = R$, a constant. Using the contact term (Eq. 2), the equation for spore dynamics becomes

$$S_{t+3} = (1 - \mu)S_t + (\epsilon - 1)R(1 - e^{-bS_t}). \quad (6)$$

At equilibrium, $S_t = S_{t+3} = S_{eq}$, so that Eq. 6 becomes

$$S_{eq} = (1 - \mu)S_{eq} + (\epsilon - 1)\,R(1 - e^{-bS_{eq}}). \quad (7)$$

Eq. 7 is a nonlinear equation that determines the equilibrium spore population in terms of the fundamental biological parameters of spore mortality (μ), spore production (ϵ), spore transmission (b), and host recruitment (R). This equation can be rewritten as

$$S_{eq} = \frac{(\epsilon - 1)R}{\mu}(1 - e^{-bS_{eq}}). \quad (8)$$

Because Eq. 8 is a nonlinear equation, it is difficult to interpret the dependence of S_{eq} on the parameters ϵ, R, μ, and b. We can, however, describe the condition that insures that S_{eq} is positive (i.e., that the spores persist). A positive value of S_{eq} exists if, near $S_{eq} = 0$, the curve $y = \frac{(\epsilon - 1)R}{\mu}(1 - e^{-bS_{eq}})$ rises faster than the line $y = S_{eq}$ (Fig. 2). To determine when this is true, we differentiate the right-hand side of Eq. 8 with respect to S_{eq}. The condition that $S_{eq} > 0$ then becomes

$$1 < \frac{(\epsilon - 1)R}{\mu} be^{-bS_{eq}}. \quad (9)$$

Since $e^{-bS_{eq}} < 1$ if the equilibrium number of spores is positive, the condition described by Inequality 9 is equivalent to

$$1 < \frac{(\epsilon - 1)R}{\mu} b. \quad (10)$$

Rearranging Inequality 10 gives the level of host recruitment, R, that insures that the equilibrium spore population exceeds 0,

$$R > \frac{\mu}{(\epsilon - 1)b}. \quad (11)$$

This condition defines a host threshold density which is the smallest number of hosts that must be present to insure that the parasite does not go extinct (see Onstad et al. 1990 for a discussion of the definition of host threshold density). From Inequality 11 we see that the host threshold density increases linearly with spore death rate μ, and decreases with both spore production ϵ and contact rate b.

Similar results are obtained if other contact terms are used. For example, when the contact term (Eq. 3) is used, we find that the threshold condition is $R > \frac{\mu c}{\epsilon - 1}$. This condition has the same dependence on μ and ϵ as before, but now depends linearly on the saturation constant c, which is analogous to the parameter b in Eq. 2. Computation of the host threshold density for the contact term (Eq. 4) is more complicated and we will not present it here.

Dynamics of spores and parasitism

Although the host threshold density, which is an equilibrium or steady state quantity, can be determined analytically as just described, the dynamics of spores and hosts cannot be determined analytically. That is, we must numerically solve the equation for spore dynamics to describe the temporal behavior of nematode and fungus populations.

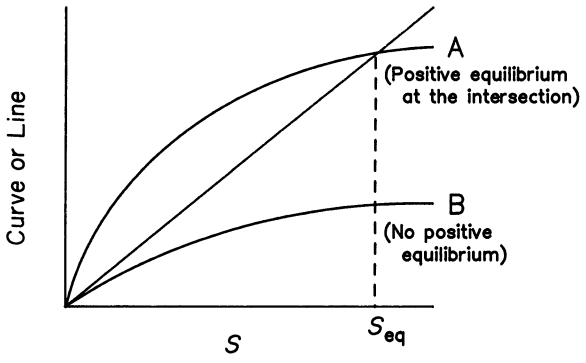

FIG. 2. Illustration of the numerical solution of Eq. 8. We wish to determine the condition that the equation

$$s - \frac{(\epsilon - 1)R}{\mu}(1 - e^{-bs}) = 0$$

has a positive solution. To do this, we plot the line $y_L = s$ and the curve

$$y_C = \frac{(\epsilon - 1)R}{\mu}(1 - e^{-bs})$$

as a function of s on the same graph. These always intersect at $s = 0$, corresponding to extinction of spores. In addition, if they intersect at a positive value of s, then this value will be the equilibrium population level. Thus, the curve marked A has a positive spore equilibrium but the curve marked B does not.

In order to capture the dynamics of spores more closely, we used Eq. 5, in which the time interval is 1 wk. In addition, to increase biological realism, we allowed each juvenile to acquire more than one spore. Based on our observations of infected nematodes (Jaffee et al. 1990), we assumed that each juvenile acquiring spores removes $1 + 3f_t$ spores. For the purposes of predicting host threshold density and spore dynamics, this modification had virtually no impact: the dynamics of spores were essentially the same whether we used the simpler or the more complicated formulation. Parameter values (Table 2) for spore acquisition (transmission) were determined in the present study and values for spore mortality and removal were determined by Jaffee et al. (1990).

We used the numerical solution of Eq. 5 to determine the dynamics of spores and parasitism, and to explore how the dynamics and rates of parasitism depended upon parameter values.

RESULTS: COMPARISON OF THEORY AND EXPERIMENT AND FURTHER THEORETICAL PREDICTIONS

In this section, we (1) describe spore acquisition and parameter estimation, (2) compare predicted and observed dynamics of parasitism, (3) compute the host

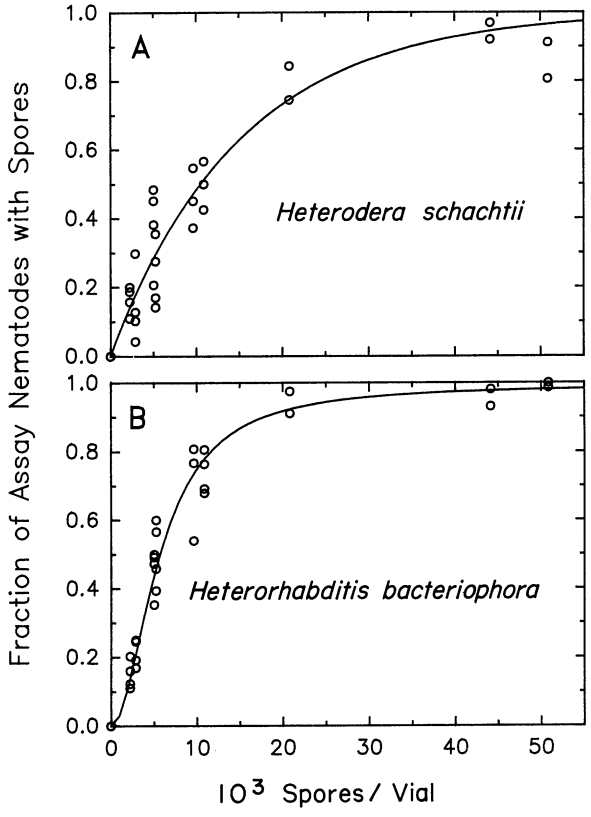

FIG. 3. Acquisition of spores of *Hirsutella rhossiliensis* as affected by spore density. Symbols represent observed data. When *Heterodera schachtii* was the assay nematode (A), the relationship was $Y = 1 - e^{-0.066X}$, $r^2 = 0.90$. When *Heterorhabditis bacteriophora* was the assay nematode (B), the relationship was $Y = \frac{0.993X^2}{(5.7)^2 + X^2}$, $r^2 = 0.96$.

threshold density, and (4) provide other theoretical predictions.

Spore acquisition

For *H. schachtii,* the relationship between spore acquisition and spore density was described ($r^2 = 0.90$) by the random contact term (Eq. 2) with $b = 0.066 \pm 0.005$ (parameter estimate ± 1 asymptotic standard error) (Fig. 3A). The contact term (Eq. 3) also fits the data well ($c = 11.0 \pm 2.1$, $r^2 = 0.89$). The values of b or c correspond to spores measured in increments of 1000 per microcosm. For *H. bacteriophora,* the contact term (Eq. 2) overestimated spore acquisition at low spore densities, although the r^2 was high (0.93) with $b = 0.117 \pm 0.006$. The contact term (Eq. 4), $P = (as^2)/(c^2 + s^2)$, with $a = 0.993$ and $c = 5.7$, fits ($r^2 = 0.96$) the observed data better than did the contact term (Eq. 2), especially at low spore densities (Fig. 3B). This better fit is not unexpected, because Eq. 4 has two rather than one adjustable parameter. However, because neither Eq. 3 nor Eq. 4 is mechanistic, we choose the contact term that best fits the data. Thus, the contact term (Eq. 2) was used for *H. schachtii* and the contact term (Eq. 4) was used for *H. bacteriophora* (Table 2).

Density-dependent parasitism

Density-dependent parasitism was predicted by the theory and was observed in the laboratory experiments (Figs. 4 and 5). The predicted and observed values of parasitism were similar in three experiments in which *H. bacteriophora* was the assay nematode (Fig. 4A, and 5). In experiment 2, *H. schachtii* was the assay nematode and observed values were higher than predicted values when hosts were added at high or moderate levels (Fig. 4B). In all four experiments, the predicted decline in parasitism in the absence of host recruitment was very similar to the observed decline.

Empirical and theoretical values of the host threshold density

Using Inequality 11 and the data in Table 2 and converting b to a per spore value (6.6×10^{-5}), we computed the host threshold density to be 27 juveniles per microcosm. In other words, the theory predicts that *H. rhossiliensis* will go extinct if R is <27 juveniles of *H. schachtii* per microcosm per 3 wk. Extinction, however, may require a long time. For example, in our experiments with $R = 25$ hosts per 3 wk, there was no substantial decline of spores (Fig. 4A) in experiment 1 and a slight increase in spores in experiment 2 (Fig. 4B). Using Eq. 1, we predict a 20% decline of spores over the 20-wk experimental period when $R = 25$ (Fig. 6), with extinction requiring >5 yr. Thus, the experi-

TABLE 2. Parameter values used to predict nematode–fungus dynamics.

Parameter	Symbol	Value ($\bar{X}$ ± 1 SE)
Spore mortality per 3 wk	μ	0.201 ± 0.007
Spore mortality per wk	μ_1	0.072 ± 0.003
Spores produced per infected *Heterodera schachtii*	ϵ	112 ± 7
Spore transmission parameter for *Heterodera schachtii**	b (search rate)	0.066 ± 0.005
Spore transmission parameters for *Heterorhabditis bacteriophora*†	a (maximum probability)	0.993 ± 0.006
	c (saturation constant)	5.7 ± 0.9

* The function for *H. schachtii* was $f_i(s) = 1 - e^{-bs}$, where s is measured in increments of 1000 spores per microcosm.

† The function for *H. bacteriophora* was $f_i(s) = \frac{as^2}{s^2 + c^2}$, where s is measured in increments of 1000 spores per microcosm.

ments and theory are consistent with a rough value of 20–30 juveniles per 3 wk as the host threshold density.

Further theoretical predictions

We also examined the dependence of parasitism on ranges of parameter values derived from published or unpublished data. With the given range and combination of parameter values, dynamics of parasitism appeared to be more sensitive to spore production per host (ϵ) than to spore transmission (b) or mortality (μ) (Fig. 7). These predictions also indicate that the variances in parameter estimations (Table 2) are relatively unimportant.

Discussion

The disease described in the present study is unusual because it occurs in the soil and because the host is microscopic. However, the disease is conventional in two key features; namely, parasitism is temporally dependent on host density, and the parasite requires some minimum number of hosts to persist in the population. These features of general epidemiological theory appear to be robust and useful for understanding this and similar systems.

Our evidence for density dependence was strong because we directly addressed this process with experiments in which host recruitment was controlled. Controlled host recruitment also has been employed in studies of disease in populations of laboratory fish (Scott and Anderson 1984) and mice (see Anderson and May 1979). In addition to providing a direct test for density dependence, controlled recruitment facilitates quantification of the density-dependent processes. The experimental evidence for a host threshold density was

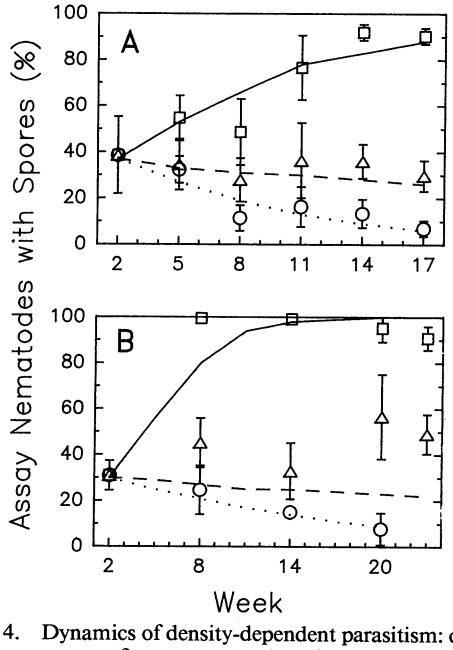

FIG. 4. Dynamics of density-dependent parasitism: change in the percentage of assay nematodes with *Hirsutella rhossiliensis* spores as affected by numbers (R) of healthy host nematodes (*Heterodera schachtii*) added to soil microcosms. Observed values are mean ± 1 SD. (A) Experiment 1. Microcosms containing 39 *H. rhossiliensis*-colonized nematodes at week 0 were inoculated with R hosts at week 2, 5, 8, 11, and 14. At each 3-wk interval, four replicates were assayed with *Heterorhabditis bacteriophora*. R = 94: □ Observed; ——Predicted. R = 25: △ Observed; – – – Predicted. R = 0: ○ Observed; ····· Predicted. (B) Experiment 2. Microcosms containing 51 *H. rhossiliensis*-colonized nematodes at week 0 were inoculated with R hosts at week 2, 5, 8, 11, 14, 17, and 20. At week 2, 8, 14, 20, and 23, five replicates previously receiving 25 or 270 hosts and three replicates receiving no hosts were assayed with *H. schachtii*. R = 270: □ Observed; ——Predicted. R = 25: △ Observed; – – – Predicted. R = 0: ○ Observed; ····· Predicted.

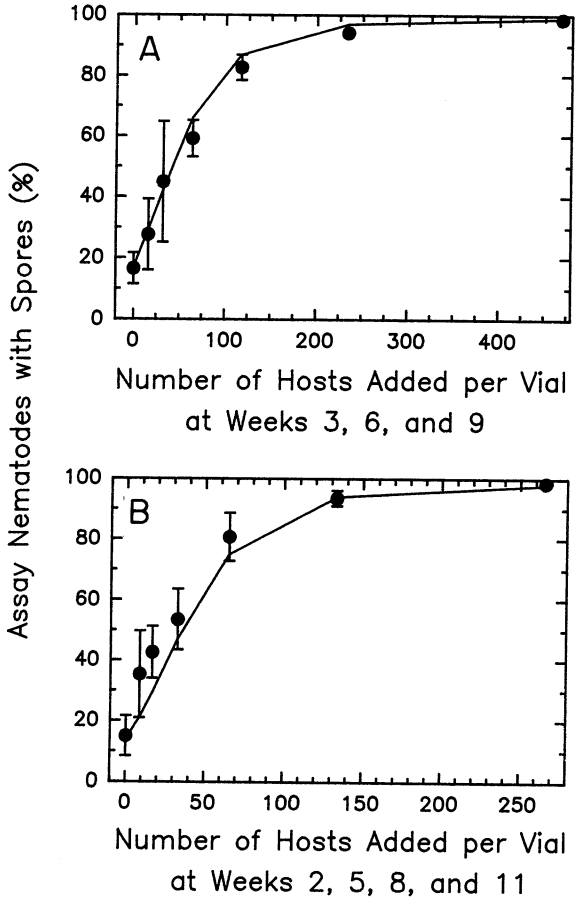

FIG. 5. Density-dependent parasitism: percentage of assay nematodes (*Heterorhabditis bacteriophora*) with *Hirsutella rhossiliensis* spores as affected by numbers (R) of healthy host nematodes (*Heterodera schachtii*) added to soil microcosms. Observed values (mean ± 1 SD) are represented by ● and predicted values are represented by ——. (A) Experiment 3. Microcosms containing 49 *H. rhossiliensis*-colonized nematodes at week 0 were inoculated with 0, 16, 32, 64, 117, 234, or 469 hosts at week 3, 6, and 9, and were assayed at week 12; n = 4. (B) Microcosms containing 51 *H. rhossiliensis*-colonized nematodes at week 0 were inoculated with 0, 9, 17, 33, 65, 133, or 266 hosts at week 2, 5, 8, and 11, and were assayed at week 14; n = 4.

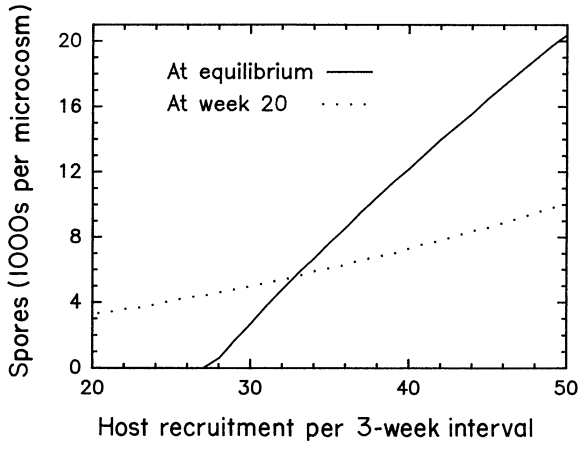

FIG. 6. Predicted effect of host recruitment on number of spores per microcosm at equilibrium and at week 20. The microcosm initially contained 5600 spores and parameter values were $\mu = 0.201$ spore deaths per 3 wk, $\epsilon = 112$ spores produced per infected host, and a spore transmission rate of $b = 0.066$. Note that extinction occurs if <27 hosts are recruited per 3-wk interval. However, the decline in spore number is very slow when 25 hosts are recruited per interval.

not as strong because the experiments did not run long enough to allow the parasite population to go extinct, and because we used few low levels of host recruitment. However, the observed data suggest that ≈25 hosts were needed every 3 wk to maintain the parasite.

Our concentration on density-dependent parasitism is justified by observations that the process is important in the field. For example, Kerry and co-workers (see Kerry 1987) found that continuous cropping of cereals, which are attacked by the cereal cyst nematode, caused soils to become biologically suppressive to that pest. Suppression followed an "inductive phase" during which large densities of pest nematodes supported an increase in parasites of those nematodes. Similar observations have been made with a bacterial parasite of nematodes, *Pasteuria penetrans* (Stirling and White 1982, Oostendorp et al. 1991), and the parasite used in the present study, *H. rhossiliensis* (B. Jaffee, *unpublished manuscript*). Density-dependent parasitism also may be operative in the suppression of nematodes in soils amended with organic matter (Linford et al. 1938). We therefore believe that understanding density-dependent parasitism is central to understanding how obligate parasites suppress nematodes.

In soil systems such as those described in the preceding paragraph, levels of parasitism change relatively slowly, i.e., high levels of parasitism may occur but explosive epidemics do not. This may be due to the low transmissibility of pathogens in soil. For both *H. rhossiliensis* (Fig. 3) and *P. penetrans* (Stirling et al. 1990), high probabilities of transmission require very high densities of spores. It follows that natural infestations of pathogens may not result in rapid (within-season) regulation and limitation of pest nematodes.

Although the experimental data demonstrate density-dependent parasitism and suggest the existence of a host threshold density, the underlying mechanisms remain qualitatively and quantitatively unclear without reference to a theoretical foundation. The theory was designed to describe the biology as explicitly as

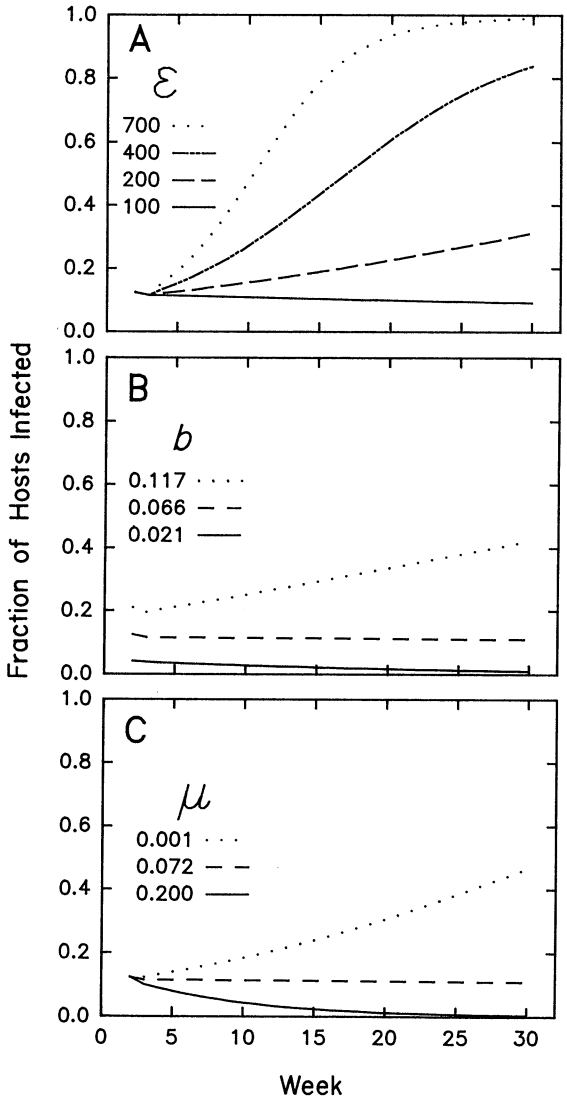

FIG. 7. Predicted temporal change in parasitism as affected by (A) spore production per nematode ϵ, (B) spore transmission parameter b, and (C) spore mortality per week μ. Host recruitment was 10 juveniles of *Heterodera schachtii* per week starting at week 2, when 2000 spores were present. Except as indicated in the figure, $\epsilon = 112$, $b = 0.066/1000$ spores, and $\mu = 0.072$ per week. The range of values of ϵ was based on data for sporulation of *Hirsutella rhossiliensis* from *Criconemella xenoplax* (Jaffee and Zehr 1983) and from *H. schachtii* (Jaffee et al. 1990). The range in values of b was based on a reanalysis of data for *H. schachtii* and *H. rhossiliensis* (Jaffee et al. 1990) and Fig. 3 of the present study. The range in values of μ was based on presumed low values of μ for resting spores of certain fungi (Perry 1978) and a subjectively selected upper value (0.200).

possible. For example, the mathematical description of transmission differs from those provided by Anderson and May (1981) in that our function was not linear with respect to propagule density. A nonlinear function both fits the data and describes the biology of transmission. As explained in detail in the Appendix, the per capita efficiency of spores is reduced as spore density increases. This occurs because the probability of a host contacting more than one spore (and thus "wasting" spores) also increases with spore density. Although transmission is linear at low spore densities, we do find high densities of *H. rhossiliensis* in the field (Jaffee and Muldoon 1989, Jaffee et al. 1989, McInnis and Jaffee 1989).

Our theoretical formulation enables direct comparison of observed and predicted dynamics. The agreement between these dynamics demonstrates that our understanding of the density-dependent mechanism is correct. The theory also allows us to estimate the host threshold density without performing time-consuming and extensive experiments, allows us to explore the effect of parameter values on disease dynamics, will serve as a foundation for more elaborate theories, and may be applicable to other host–parasite systems. Finally, the agreement between predicted and observed dynamics shows that, under appropriately controlled but biologically relevant conditions, accurate parameter estimation is possible in this host–parasite system.

The interaction of the nematode and fungus will be more complex in the field than in the laboratory microcosms. In the field, the environment will be variable, other organisms may directly or indirectly affect the epidemic, spatial heterogeneity may be important, plant roots may provide refuge for the host nematodes, and host reproduction and parasitism will not be uncoupled. The philosophy of our approach was to simplify the existing system to insure that basic components were fully understood. Once they are, we can add complexity to the theory and experiments (Black and Singer 1987, Onstad and Carruthers 1990). The alternative is to begin with complex theoretical descriptions. Both approaches have value, but ours facilitates the estimation of parameters and comparison of theory and experiment.

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APPENDIX

A Mechanistic Understanding of the Spore Transmission Term

In this appendix, we derive the contact term (Eq. 2) from mechanistic considerations of a nematode moving in the soil and contacting spores. Nematodes of mean radius r move through soil that contains nematodes already colonized by the fungus. Each cadaver supports a network of external hyphae, phialides, and spores. Suppose that r' denotes the radius of an individual spore. The nematode will contact a spore if the distance between the center of the nematode and the center of the spore is $<r + r'$.

Here we concentrate on very short time periods (at most 3–4 d) and use the variable u to measure time. As nematodes move through the soil, they "sweep out" a possible contact volume. That is, if a nematode moves with speed v, then in a short interval of time du, it has moved a distance $v\,du$ and swept a contact volume $\pi(r + r')^2\, v\, du$. Suppose that the total volume of the microcosm is V and that it contains S spores, so that the density of spores is $s = S/V$. Given a density of spores s, the number of contacts in du is $s\pi(r + r')^2 du = S\pi(r + r')^2\, v du/V$.

We thus conclude that a single nematode contacts $bSdu$ spores in a short time du, where b is the fraction of the soil pore volume covered per unit time per nematode, i.e., $b = \pi(r + r')^2\, v/V$. When N nematodes are present, the total number of encounters per unit time is bSN.

Over short time intervals, we use a continuous time model for nematode and spore dynamics. Viable spores are lost by natural death (at rate μS) or by attaching to nematodes (at rate bSN). Nematodes disappear only by infection (also at rate bSN). Over short time intervals, there will be no production of spores (which requires 2 wk). If $\frac{dS}{du}$ and $\frac{dN}{du}$ are the rates of disappearance of spores and nematodes, we have

$$\frac{dS}{du} = -\mu S - bSN \tag{A.1}$$

$$\frac{dN}{du} = -bSN. \tag{A.2}$$

Suppose that initial numbers of spores and nematodes are $S(0) = s_0$ and $N(0) = n_0$. Instead of measuring absolute numbers of spores and nematodes, we can measure spores and nematodes relative to these initial values. That is, we scale the equations relative to the initial densities by defining

$$x = \frac{S}{s_0} \qquad y = \frac{N}{n_0}. \tag{A.3}$$

Thus, x and y measure spore and nematode population sizes as fractions of the original numbers; because both populations decline, x and y will always be <1.

In terms of x and y, the spore and nematode numbers are $S = xs_0$ and $N = yn_0$. If we substitute these into (A.1) and (A.2), we obtain

$$s_0\frac{dx}{du} = -\mu s_0 x - bs_0 n_0 xy \tag{A.4}$$

$$n_0\frac{dy}{du} = -bs_0 n_0 xy. \tag{A.5}$$

Note that bs_0 has the dimensions of a rate. Thus $bs_0 u = \tau$ is a nondimensional measure of time.

First we consider the dynamics of the nematode population, given by (A.5). Setting $u = \tau/bs_0$ in Eq. A.5 and simplifying, we obtain

$$\frac{dy}{d\tau} = -xy. \tag{A.6}$$

This equation provides a description of the dynamics of nematodes that involves quantities that are all dimensionless but related to the detailed biology of the system.

Now let us consider the dynamics of spores. Dividing both sides of Eq. A.4 by s_0, we obtain

$$\frac{dx}{du} = -\mu x - bn_0 xy \tag{A.7}$$

We can convert to the dimensionless time τ by dividing the entire equation once again by bs_0:

$$\frac{dx}{d\tau} = -\frac{\mu x}{bs_0} - \frac{n_0 xy}{s_0}. \tag{A.8}$$

Note that a new parameter, $\frac{\mu}{bs_0}$, which we will call ω, appears in Eq. A.8. This parameter compares the rate at which spores disappear by natural mortality to the rate at which they disappear by contact with hosts. Using the values of μ and b typical to the experiments, we find that ω is approximately $2.3/s_0$, so that if $s_0 \gg 1$, then $\omega \ll 1$. That is, for the experimental regime we studied, ω is a "small parameter" (Murray 1990 gives a thorough treatment of the kinds of methods that we are using here) and:

$$\frac{dx}{d\tau} = -\omega x - \frac{n_0}{s_0}xy. \tag{A.9}$$

In the experiments, s_0 is typically much greater than n_0, so that $n_0/s_0 \ll 1$ as well. To summarize this, we write $\frac{n_0}{s_0} = \kappa\omega$, which defines the value of κ.

In terms of the new dimensionless parameters κ and ω, the dynamics of the nematodes are now

$$\frac{dx}{d\tau} = -\omega[x + \kappa xy]. \quad \text{(A.10)}$$

We now consider the solutions of Eqs. A.10 and A.6. Since ω and κ are both $\ll 1$ and x and y are both < 1, the right-hand side of (A.10) is very small. Hence, as a first approximation, we can assume that x is a constant over the small intervals of time. Since the initial value of x is 1, we conclude that over a short interval of time $x = 1$.

If $x = 1$, then the dynamics of nematodes (Eq. A.6) become

$$\frac{dy}{d\tau} = -y. \quad \text{(A.11)}$$

Since $y(0) = 1$ as well, we conclude that $y(\tau) = \exp(-\tau)$.

In terms of the original variables we find that

$$N(u) = N(0)e^{-bSu} \quad \text{(A.12)}$$

so that the number of nematodes infected during a unit interval of time is

$$N(0) - N(1) = N(0)(1 - e^{-bS}). \quad \text{(A.13)}$$

Recall that in a Poisson process with rate constant λ, the probability of more than one occurrence in a time interval u is given by $1 - e^{-\lambda u}$. Comparing our results, we have a rate constant $\lambda = bS$ with $u = 1$, and the probability that a nematode encounters at least one spore and becomes infected during the time it is motile is

$$f(s) = 1 - e^{-bs}. \quad \text{(A.14)}$$

This analysis provides intuition about the functional form (A.14). In particular (A.10) shows that when ω is small, the spore death rate is negligible on the time scale of nematode infection. This causes a decreasing per capita efficiency of spores as spore population density increases.