Spatial and Temporal Dynamics of Entomopathogenic Nematodes

Jonathan Paul Fairbairn

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ABSTRACT

The life-history and infection parameters of the entomopathogenic nematodes *Steinernema feltiae* (Filipjev)(Nematoda:Rhabditida) and *Heterorhabditis megidis* (Poinar, Jackson & Klein)(Nematoda:Rhabditida) were examined to provide specific details for the construction of mathematical SI models for biological control of soil insect pests. Laboratory experiments using the Greater Waxmoth, *Galleria mellonella* as the model host were undertaken to specifically examine the transmission behaviour of infective juvenile nematodes.

The proportion of infective juveniles of *S. feltiae* which infected hosts was dependent on time. Previous studies declared that the proportion of infective juveniles which can infect is static, however, over a period of 5 days most of the infective juveniles infected hosts, demonstrating that the proportion infecting is dynamic.

Infection of hosts by both species of nematode was compared using two mathematical representations of the transmission rate. Whereas the most parsimonious form of transmission for *H. megidis* was the linear Mass Action function, it was evident that, when measured at the individual nematode scale, *S. feltiae* transmission was non-linear. I postulated that this functional difference is due to the biology of the two species of nematodes. The subsequent effect of including the non-linear response on model predictions were investigated and it was demonstrated that the dynamics of the host:nematode interaction became less stable.

Spatial models of *S. feltiae* infection were parameterised from laboratory experiments, and control prediction of these models examined. The horizontal rate of dispersal through sand columns was determined in the presence and absence of hosts. Infective juveniles were found to disperse preferentially towards hosts. The predicted dynamics of pest control using the spatial model were highly dependent on the degree of nematode dispersal, host dispersal and the attraction of nematode infective juveniles towards hosts.

The overall findings of this thesis have been placed in the context of epidemiological models created elsewhere, and predict that entomopathogenic nematodes may be targeted to specific pest systems with a high degree of success. An understanding of the infection biology of these nematode species is crucial in determining how and when pests may be controlled, and equally importantly, which systems successful control is not predicted.

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Statement of Originality

The work in this thesis is original research carried out by myself and has not been submitted for consideration previously for a higher degree at this or any other university. Any references henceforth used have been appropriately acknowledged.

Jonathan P. Fairbairn

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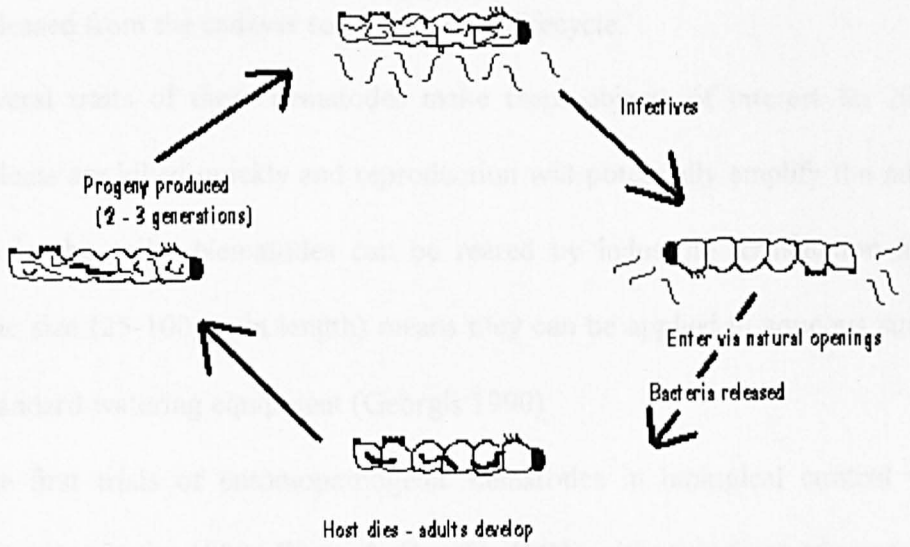
CONTENTS

Chapter 1

General Introduction

Entomopathogenic nematodes belonging to the families Steinernematidae and Heterorhabditidae are obligate lethal pathogens of soil insects. These families have an ubiquitous distribution, having been found in the soils of every continent with the exception of Antarctica (Downes & Griffin 1996). The lifecycle (Fig 1) comprises a free living infective juvenile stage which seeks out hosts by following chemical and physical cues (Grewal *et al.* 1993; Grewal *et al.* 1997; Lewis *et al.* 1992; Lewis *et al.* 1995; Lewis *et al.* 1996; Moyle & Kaya 1981). Infection then takes place through the spiracles, mouth or anus, or in the case of Heterorhabditid species by piercing the cuticle directly. Once inside the host the larvae migrate to the haemocoel where symbiotic bacteria (*Xenorrhabdus* spp in Steinernematidae and *Photorhabdus* spp in Heterorhabditidae) are released from the pharnyx of the nematode into the haemolymph. The bacteria produce toxins which counteract the hosts immune response (Woodring & Kaya 1988) whilst acting as antibiotics, preventing the establishment of other bacteria species within the host cadaver. The host tissues are lysed by the bacterial toxins and the host dies, normally within 48 hours. The juvenile nematodes feed on the host nutrients and develop to the adult stage. Heterorhabditid species develop into hermaphrodites at this stage and produce hundreds of new offspring within the host cadaver which develop into separate sexes and reproduce sexually. *Steinernema* species are gonochoristic and require the infection of one male and one female in order for reproduction to take place (Woodring & Kaya 1988) although there is a recently discovered *Steinernema* sp T87 which is self-fertile (Griffin *et al* 2001). Depending upon the size of host several generations may occur before the host resources become limiting. At this stage, usually within 12-16 days post infection, third stage juveniles develop as infective juveniles,

Figure 1. Lifecycle of entomopathogenic nematodes



retaining the juvenile 2 stage cuticle. This helps protect the nematode from environmental stresses outside the host, and may even prevent foreign bodies adhering to the nematode and initiating an immune response within the host, as this cuticle is shed prior to invasion (Downes, *personal communication*). Upwards of 10 thousand of these infective juveniles are then released from the cadaver to complete the lifecycle.

Several traits of these nematodes make them objects of interest for biological control. Hosts are killed quickly and reproduction will potentially amplify the number of nematodes in the soil. Nematodes can be reared by industrial fermenation and their microscopic size (25-100μm in length) means they can be applied in aqueous suspension through standard watering equipment (Georgis 1990)

The first trials of entomopathogenic nematodes in biological control were on *Popillia japonica* in the 1930s (Kaya & Gaugler 1993), although these attempts resulted in only a degree of success. To date they have been used successfully to control pests in citrus orchards, turf grass, glasshouse crops, strawberry crops and mushroom houses (Downes & Griffin 1996; Kaya & Gaugler 1993). However, economic success has mainly been limited to control of sciarid mushroom flies (Scheepmaker *et al.* 1997) following a decision by the Dutch Ministry of Agriculture legislating to reduce the use of chemical pesticides in mushroom production, which lead to a search for viable alternatives. More recently, Black Vine weevils in glasshouse flower crops and strawberry production has benefited from successful nematode control (Kakouli 1995).

Trial and error have predominated in experiments aiming to detect where, when and how many nematodes should be applied in order for successful control to be achieved.

However, the nature of these parasites lend themselves to a mathematical modelling approach utilising and adapting existing epidemiology models (Anderson & May 1981).

Using coupled differential equations describing the population changes of nematodes, susceptible hosts and cadavers it is possible to describe the temporal interactions of nematodes and their hosts(Fenton *et al.* 2000; Hudson & Norman 1995); See appendix for details). Some of the parameters can be gleaned from the literature, such as the rate of infective juvenile production within different hosts and the rate of nematode induced host death. Others must be calculated specifically for the system being studied. Spatial models may then be created, using cellular automata to include the interaction of host and nematode dispersal (Fenton *et al* 2000b). With a fully parameterised model it is possible to make broad predictions for the interactions between host and nematode through space and time. Whereas trial and error may yield specific solutions to specific questions, modelling can offer a quick and easy tool to investigate the outcomes of many different scenarios which can then be validated.

First of all there are many aspects of nematode biology which require investigation. The infective juveniles of *Steinernema* species do not all appear to be infective (Fan & Hominick 1991; Bohan & Hominick 1995; Bohan & Hominick 1996; Campbell *et al* 1999). Experiments have shown that at any one time between 60 and 80% of infective juveniles are not infective. This is a very large proportion and for a biological control to contain so many apparently redundant agents is an important aspect which needs to be more fully understood, especially as the cost of production is high (Scheepmaker *et al.* 1997). From a modelling point of view it is also important to include the correct

information describing this phenomenon. Questions such as: does this proportion change with time or exposure to hosts or is it constant? need to be answered.

There is evidence too, that nematodes will not invade a host which is already infected. Furthermore, they will not invade fresh hosts in the presence of infected ones (Glazer 1997). The implications to biological control seem obvious - where hosts are present at high densities a few hosts will be invaded and then confer resistance to the others by 'switching off' the nematodes in the surrounding environment. To what extent does this occur and how long after infection do the nematodes initiate this response? Experiments asking these questions can offer up information which, once included in models of the system, can inform us what the implications to control are.

One of the most difficult parameters to measure in epidemiological models is the transmission coefficient. The coefficient of transmission describes the probability that a nematode will encounter a host and infect it (Knell *et al.* 1998). The form that this transmission rate takes in models may also alter the predictions of the model (Briggs & Godfray 1995; Hochberg 1991). The models of Anderson & May (1981) include a representation of transmission where the rate of transmission is proportional to the number of susceptible hosts and parasites. The *per capita* transmission rate is therefore the same for individuals when they are part of a small population or a large one. This form of transmission is derived from physical gas laws which assumes that individuals are randomly mixed. The number of contacts an individual has with others in a population of 1 million is therefore ten times the number in a population of 100 thousand. For some parasites, such as sexually transmitted diseases this is clearly not the case, as the number of contacts will reach a maximum as the 'handling time' becomes limiting, following

which, transmission will remain constant. Here, transmission rate can be included in models as a type two functional response. As the numbers of susceptible hosts increases so does transmission rate. Above a certain number of susceptible hosts transmission rate levels off as the number of contacts becomes limited. Predator/prey models often assume a predation rate (equivalent to transmission rate) with a type three (sigmoidal) function. That is, initially transmission increases less than linearly with increasing prey numbers. This is equivalent to learning response as a new prey type is encountered more and more. The rate then increases linearly before levelling off as the numbers of prey exceed the number that a predator can handle in a given time period.

Different formulations of transmission can effect predictions of host/parasite dynamics (Briggs & Godfray 1995; Hochberg 1991), and several experimental studies have presented evidence that mass action, as used by Anderson & May (1981) is not always a good approximation (Dwyer 1991; Knell *et al.* 1998). Mathematical models of entomopathogenic nematodes designed to predict biological control outcomes must include the correct formulation of the transmission process if they are to accurately describe the system dynamics.

Finally, a key process that can affect model predictions is the mode of host seeking. Entomopathogenic nematodes have been shown to exhibit two distinct infection behaviour types, with some (*S. glaseri*) cruising through the soil in search of hosts and others (*S. carpocapsae*) using an ambushing strategy. Other species fall somewhere between these two behaviour types, spending some time cruising and some time ambushing. This clearly offers a description of a possible mechanism of dispersal that is essential to know for accurate model predictions. Where a host has a greater dispersal

rate than its pathogen it may escape infection at the extremes of its range. Where the pathogen has a dispersal rate equal to or greater than the host then all hosts will be spatially vulnerable to infection. How far nematodes travel may therefore be linked to their ability to infect (Westerman & Stapel 1992). If this is the case then we might expect a relationship between infectivity and dispersal from a point source, such as a cadaver. This thesis describes a number of experimental investigations into aspects of entomopathogenic nematode/host interactions that were deemed to be important in creating mathematical models for biological control. This work does not, therefore describe the creation of model themselves, as this was not the remit of my work. Instead, the work presented here is part of a two way process of experimentation and model investigations, where the experiments were driven by the need for such information in models created elsewhere. However, simulations of host/nematode dynamics were undertaken using the pre-existing models of Fenton *et al* (See appendix for details).

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Chapter 2

Materials and methods

NEMATODE REARING

Infective juveniles of *Steinernema feltiae* (Filipjev) site 76 strain were kindly received from Dr. B. Briscoe (CABI Biosciences, UK). This strain of nematodes had been reared through laboratory cultures for several years and have been utilised by biocontrol companies. The nematodes were reared through *Galleria mellonella* by adding a suspension of several hundred infective juveniles to moist sand in a petri dish. Several late instar *Galleria* were then placed onto the sand and the dishes were stored at 20°C for two days. All dead *Galleria* were placed onto dry filter paper in a new petri dish and stored at 20°C for ten days. Insects that die of infection by entomopathogenic nematodes have distinctive appearances and in particular, do not have a putrid odour. Insects dying from *S. feltiae* infection turn beige in colour and are soft to touch, but do not split when picked up. All *Galleria* which died of infection were then placed onto a Whitehead trap (Kaya & Stock 1997) to harvest fresh infective juveniles. This is a simple piece of apparatus consisting of a small upturned petri dish lid placed inside a large petri dish. Filter paper is then placed over the small lid so that it extends into water which has been placed into the larger petri dish. Five or six infected *Galleria* were then placed on the filter paper overhanging the small lid. Over the period of one week infective juveniles were released onto the moist filter paper and migrated into the water. These nematodes were removed each day and stored at 20°C to be used in experiments or rearing. Experiments used nematodes which were no more than six days post-emergence.

All *Heterorhabditis megidis* infective juveniles used in experiments were the commercial strain NemasysH received from Microbio. Nematodes were removed from the storage medium (vermiculite) by adding water.

INSECT REARING

The greater waxmoth, *Galleria mellonella* was reared on a diet according to (Woodring & Kaya 1988). This diet consisted of wheat middlings, honey, glycerol, dried yeast and beeswax. Approximately 150 eggs were placed onto the diet in plastic sandwich tubs (17.5 x 11.5 x 6 cm) and stored at 20°C. After ten days the larvae were divided between two tubs and more diet added. In all experiments, except where mentioned otherwise, late instar larvae were used. These were typically aged between three and four weeks and weighed 0.2 – 0.3 grams. The whole life-cycle takes around 4 weeks to complete under these conditions.

NEMATODE COUNTS

Where nematodes were used in experiments they were suspended in water to a known density of individuals. Nematodes were counted in 1ml volumes using a Gilson pipette and a 1ml counting slide. Stock flasks of nematodes were diluted or else nematodes were allowed to settle before removing excess water until the correct density was achieved. Six counts were taken per final dilution to give an accurate estimate of the density of nematodes. As an example, four suspensions of 200 nematodes ml^{-1} were each sampled six times. The estimates were not significantly different from 200 (t-test; mean=206.8, P=0.23; mean=207.3, P=0.069; mean=199.8, P=0.98; mean=205.8,

P=0.18). Prior to each pipette of suspension being counted or used in an experiment the flasks were agitated to bring all settling juveniles into suspension.

Where single nematode infective juveniles were used a dilute suspension of nematodes was placed into a petri dish under a dissecting microscope. Single nematodes were collected in a Hamilton 10µl syringe. Only live nematodes were used. Live nematodes can be distinguished from dead nematodes as dead nematodes are gently curved and rigid whilst live ones when not moving retain a definite 'j' shaped curve at the posterior end.

Nematodes were recovered from sand using sand washing techniques after (Bohan, 1995). This method involves adding 50ml water to 20ml sand in a large beaker. Swilling the sand and water around, the nematodes become suspended and can be removed in suspension. Repeating this washing three times removes around 80% of the infective juveniles from the sand (Bohan, 1995).

In experiments with a single nematode in 1ml of sand, the sand was washed in a petri dish under dissection microscope and the nematode was found by searching through the sand with a mounted pin.

Counts of nematode adults were made by dissecting hosts which had been dead for 72 hours. This 72 hour delay before dissection allows the infective juveniles which infected to develop into adult worms without allowing them time to produce progeny. Some caterpillars were dissected fresh, in which case they were dissected in Ringer's solution (H_2O (1 litre), NaCl (9g), KCl (0.4g), $CaCl_2$ (0.4g), NaH_2CO_3, (0.2g)) to prevent

the nematodes rupturing before they were counted. Where many dissections needed to be performed caterpillars were frozen first to prevent the nematodes developing further and adding a second generation to the cadaver. Freezing did not disrupt the nematode sheath and adults remained intact. Caterpillars were then dissected in water at a later date.

GALLERIA INFECTION

Where infected *Galleria* were used as part of the experimental protocol, these were infected using a Hamilton 10μl syringe. Nematodes were counted as above, but were suspended in sterilised Ringers solution. The caterpillars were then surface sterilised by wiping them with ethanol, the syringe sterilised in ethanol and washed out with sterilised Ringers. The nematodes were then inserted through the proto-leg of the caterpillar into the haemocoel, avoiding rupturing the gut. Caterpillars did not die wherever control injections were performed with only Ringers solution.

GALLERIA INFECTION EXPERIMENT

INTRODUCTION

The greater wax moth, *Galleria mellonella* has been used in many studies of entomopathogenic nematodes as a bioassay host. This is generally because *Galleria* are highly susceptible to nematode infection (Woodring & Kaya 1988). However, often the literature describes bioassays that use late instar larvae rather than defining the exact instar. It is well known that different life stages of insects are often differentially susceptible to infections (Dwyer, 1991; Knell *et al*, 1996). Here I present an experiment

aimed at determining whether different instars of *G. mellonella* are differentially susceptible to nematode infection.

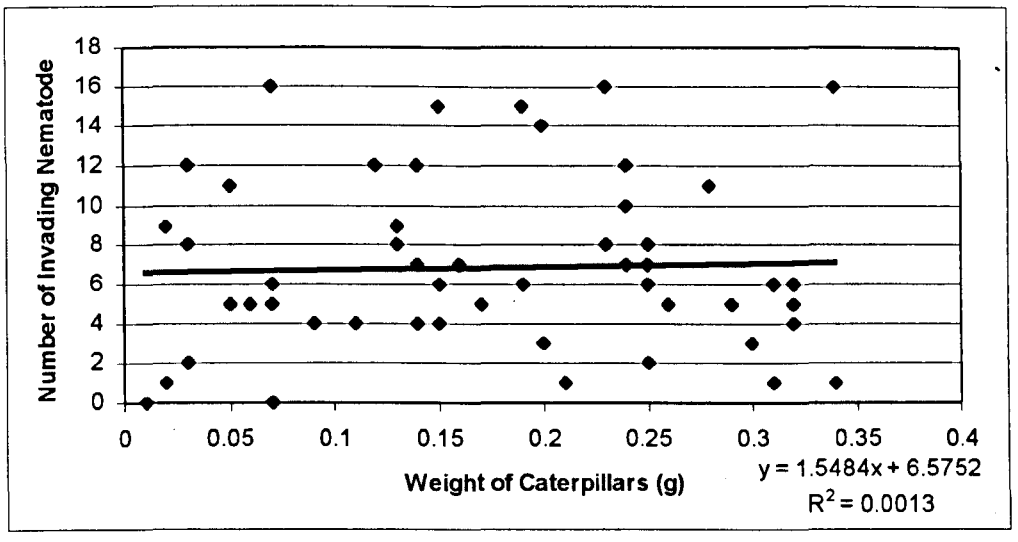
METHODS

Galleria were divided into six size classes according to their mass. The size increments between classes were 5mg and host mass ranged from 0.00g to 0.35g with eight individuals in each group. For the purposes of the experiment infection was assured by placing 1ml sterilised silver sand into 2ml plastic Eppendorf tubes followed by a single *Galleria* larva. In most cases 1 ml of sand was enough to assure contact with the caterpillar, but for smaller sized hosts more sand was added until the caterpillar and nematodes were in contact. A 250µl solution containing 50 nematodes was then poured into the tube and the lid closed. Tubes were ventilated by creating a hole in the lid with a mounted pin. The tubes were then inverted and left at 20°C for 6 hours. Following this period the caterpillars were washed to remove any nematodes that had adhered to the cuticle. The caterpillars were finally placed into 50mm petri dishes containing filter paper for 72 hours. The cadavers were then dissected and the number of invading nematodes counted.

RESULTS

A regression showed that there was no significant effect of host size on the number of nematodes successfully infecting (Fig 1. P=0.79, F=0.07). This indicates that *Galleria mellonella* larvae are highly susceptible to infection by *S. feltiae* at all larval stages. The use of different aged caterpillars as experimental bioassays can therefore be justified.

Figure 1. Effect of size of *G. mellonella* on the mean number of *S. feltiae* individuals which infected. 50 infective juveniles were placed on to moist sand in Eppendorf tubes containing one *G. mellonella* larvae for 6 hours before being removed and dissected. Regression (number invading = 6.5752 + 1.5484 x weight) showing no significant effect of host weight on the number of nematodes invading (n=56, df= 1, F=0.07, *P*=0.79, $R^2 = 0.0013$).



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Chapter 3

Re-assessing the infection strategies of the entomopathogenic nematode *Steinernema feltiae* (Rhabditidae; Steinernematidae)

Fairbairn, J.P., Fenton, A., Norman, R.A. & Hudson, P.J. (2000)

Parasitology 121, 211-216

INTRODUCTION

Entomopathogenic nematodes are used as biological control agents of insect soil pests such as sciarid mushroom flies (*Lycoriella* spp.) and the black vine weevil (*Otiorhynchus sulcatus*). Nematodes are typically applied in high numbers for example 3 million infective juveniles per square metre to control the mushroom sciarid fly (MicroBio, UK). Although such a large number of nematodes is used to protect crops, laboratory studies have shown that the majority of the free living larvae are not infective (Bednarek & Nowicki, 1986; Fan & Hominick, 1991a; Bohan & Hominick, 1995a, b, 1996, 1997; Glazer, 1997). The aim of this study is to understand the process that causes this phenomenon.

Following the work of Bednarek & Nowicki (1986) two hypotheses have arisen in the literature to describe the invasion strategies of these nematodes. The first states that infective juveniles can be rigidly divided into two sub-populations where one is infective and the other is not. To demonstrate this Bohan & Hominick (1995 a, b; 1996) showed that the overall proportion of *Steinernema feltiae* infecting in a given time period did not change, and never reached above 40% even when the initial number of nematodes applied was varied. Further support for a sub-population comes from a series of experiments by Fan & Hominick (1991 a), Mannion & Jansson (1993) and Hay & Fenlon (1995). However, these experiments did not take into account the mortality of nematodes during the time of the experiment, a factor which is known to be very high (Smits, 1996). By ignoring the mortality of nematodes over time it is impossible to evaluate the true proportion of infectious nematodes in the free living population as many of the nematodes will have died before they could infect.

The second hypothesis stems from work by Glazer (1997) and states that some part of the nematode/infected-host complex produces a chemical cue that results in infective juveniles in the surrounding environment not infecting hosts. He demonstrated that the number of infective juveniles infecting a fresh host, after exposure to an infected host, was reduced.

It should be noted that the experimental work carried out on the invasion tactics of entomopathogenic nematodes has largely focused on testing only one of these hypotheses (Fan & Hominick, 1991a; Mannion & Jansson, 1993; Bohan & Hominick, 1995a, b). For their subpopulation infection-model, Bohan & Hominick (1995a, b) explicitly stated an assumption that mortality was unimportant over the short time-scale of infection and implicitly assumed that no host derived cues operated to reduce nematode infection. However, given that mortality may be significant (Smits, 1996) and the findings of Glazer (1997), the conclusions of Bohan & Hominick (1995a, b) and others need re-evaluating.

These two hypotheses may not be mutually exclusive and in this paper we undertake a series of experiments to answer the following questions. (1) Can a subpopulation be identified by investigating groups of invading nematodes? (2) Does the infection rate of individual nematodes vary temporally? (3) Do infected hosts release a chemical cue that prevents infection? (4) If so, is the rate of cue production dependent on the initial density of infecting nematodes?

By answering these questions our objective is to determine the major factors limiting the infection rates of entomopathogenic nematodes.

MATERIALS AND METHODS

All experiments were carried out at 20°C, a temperature similar to that where *S. feltiae* is used in biological control.

Experiment1: Can a subpopulation be identified by investigating groups of invading nematodes?

Previous studies (Fan & Hominick, 1991a, Bohan & Hominick, 1995a, b) reported two subpopulations for entomopathogenic nematodes following experiments involving large groups of infective juveniles. The simplest way of determining the presence of two subpopulations where one is infective and the other is not, is to perform replicated challenges of the host with one nematode. To validate the work of Bohan & Hominick (1995a, b) where they showed no change in the measured infectious proportion when groups of between 100 and 800 nematodes were challenged against *Galleria*, we undertook two extreme tests. The infectivity of single nematodes exposed to a single host was compared with that of 1000 nematodes exposed to a single host.

Nematodes were placed either singly or as a group of 1000 into 1.5ml Eppendorf tubes filled with moist sand. Singletons were collected under dissection microscope using a Hamilton 100µl syringe whilst groups of 1000 were obtained from suspensions of 4000 nematodes per ml with a 250µl pipette. Following the addition of these nematodes, the Eppendorf tubes were incubated at 20°C for 6 hours to allow the nematodes time to disperse around the sand, reducing the activity levels of the nematodes that may have been enhanced during handling. A total of 388 replicates of single nematodes versus *Galleria* and 20 each of 1000 nematodes versus *Galleria* and contol tubes were performed. A

single *Galleria* was then placed into each tube and incubated for 24h at 20°C. We used such a small arena size to limit the effect of space in an attempt to ensure that the nematodes had the maximum opportunity to infect. A ventilation hole was provided in the lid of the tubes and they were placed into a plastic bag with moist cotton wool to maintain humidity. Host mortality was assessed after a further 48 hours and the number of nematodes that became infective was determined by dissection of the dead *Galleria*. Nematode mortality was controlled for by placing populations of 1000 nematodes into tubes without hosts for 24h and assessing the number of surviving nematode under the microscope following sand washing (Bohan & Hominick, 1995). The results were analysed using a χ^2 test, to distinguish whether or not the same proportions of nematodes infected when in a large numbers or as singletons.

Experiment 2: Does the infection rate of nematodes vary temporally?

Five hundred Eppendorf tubes were set up in the same manner as Exp. 1 with one nematode and one *Galleria*, incubated at 20°C for up to 5 days. Every 12 hours, 50 tubes were removed from the experiment, the *Galleria* extracted, washed and incubated for a further 48 hours and mortality recorded. Controls for nematode mortality consisted of 50 tubes containing single nematodes but no host. At each 12 hour interval, 10 tubes were washed to recover the nematodes and mortality of the nematodes determined under a dissection microscope. The number of new infections over the 5-day period was corrected for this mortality rate.

Experiment 3: Do infected **Galleria** *release a chemical cue which prevents infection?*

Galleria were injected with 250 nematodes in 10µl sterile Ringers solution and placed on moist sand in similar arenas to those in experiments 1 and 2. Injecting *Galleria* has no effect on the subsequent infection rates of nematodes (Fairbairn, unpublished data). Injected *Galleria* were assigned to one of 2 treatments: incubated on the sand for either 24 hours or 48 hours. Controls for each treatment consisted of *Galleria* injected with sterile Ringers with all treatments replicated 20 times. Following exposure on the sand, the infected *Galleria* were removed and 1000 nematodes pipetted onto the sand. These nematodes were left for 6 hours to acclimatise after which a single fresh *Galleria* was added to the arena. These fresh *Galleria* were incubated in the tubes for 24 hours and then removed, washed and further incubated for 48 hours. Infected *Galleria* were then dissected to establish the number of nematodes that had infected. The results were analysed using an ANOVA test where each treatment was tested against its corresponding control.

Experiment 4: Is the production of a cue dependent upon the number of invading nematodes?

Galleria were injected with 10, 50, 100 and 250 nematodes and placed into sand-filled Eppendorf tubes in a similar manner to that described above. The tubes were incubated at 20°C for 48 hours after which time the *Galleria* were removed and 1000 nematodes placed onto the sand for 6h, as in experiment 3. Fresh *Galleria* hosts were then added and incubated at 20°C for a further 24 hours. *Galleria* were then removed, washed and

dissected after 48 hours to determine the number of nematodes infecting. Again, each condition was replicated 20 times. The results were analysed by GLM.

RESULTS

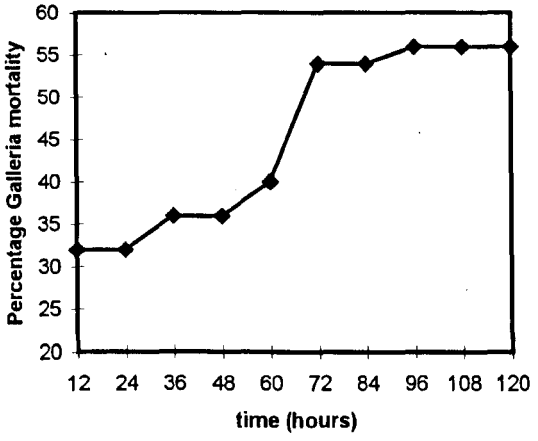
Experiment 1: Can a subpopulation be identified by investigating groups of invading nematodes?

There was no significant difference (χ^2=0.56, P=0.44, df=1) in the number of nematodes infecting as singletons (17.5%) or as a group (mean 14.3% s.d.=8.3). This demonstrated that the infective behaviour of nematodes is independent of factors arising from the presence of many other nematodes.

Experiment 2: Does the infection rate of nematodes vary temporally?

After adjusting for nematode mortality, which reached 50 % during the experiment, 32% of the infective juveniles infected after the first 12 hours. After this initial infection, the rate at which nematodes infected the hosts increased in a sigmoidal manner (logistic regression $P < 0.001$) over 4 days until, at the end of the experiment, 56% of the population had infected (Figure 1). Following the initial infection, the subsequent time to infect followed a continuous distribution with a mean of 64.9 hours (s.d.=16.2, n=10) showing that the proportion of non-infectious nematodes is not static.

Figure 1. The cumulative number of nematodes becoming infective over a 5-day period. *Galleria* mortality as a percentage is plotted against time.



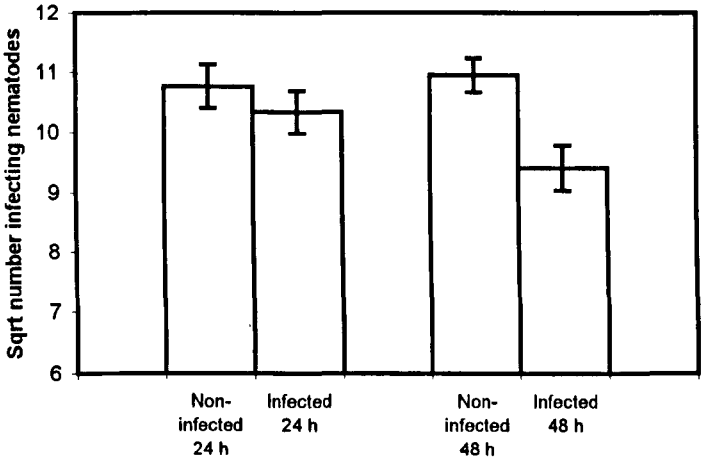
Experiment 3: Do infected* Galleria *release a chemical cue which prevents infection.

There was no significant effect on the degree of secondary infection at 24h or 48h (GLM, time $F_{1,77}$=0.23, P=0.63, treatment $F_{1,77}$=1.66, P=0.20). There was no significant interaction with time or treatment ($F_{1,76}$=0.76, P=0.39). From this, we conclude that infected *Galleria* did not emit a cue onto the sand that could inhibit the infection behaviour of nematodes following 48 hours post infection.

Experiment 4: Is the production of a cue dependent upon the number of invading nematodes?

The number of nematodes infecting a fresh *Galleria* following the presentation of infected *Galleria* was not significantly different between treatment groups ($F_{1,78}$=0.26, p=0.608) where the mean number of nematode infecting fresh *Galleria* following injection with 10, 50, 100 and 250 nematodes was 37.8 (se± 1.48), 38.75 (se± 1.76), 38.35 (se±1.74) and 33.75 (se±1.40) respectively. Therefore the number of infecting nematodes was found not to be dependent on the number of nematodes which had infected the primary hosts.

Figure 2. Square root mean number of nematodes infecting *Galleria* which had been placed on sand previously exposed to a non-infected *Galleria* and an infected *Galleria* for 24 or 48 hours with standard error bars shown.



DISCUSSION

The objective of this work was to determine the major factors limiting infection rates of entomopathogenic nematodes. Two hypotheses examined were: (1) an intrinsic mechanism, acting within each individual infective juvenile results in a proportion being infective at any one time, (2) an extrinsic chemical cue, arising from an infected host, prevents infective juveniles becoming infective.

Experiments demonstrated that the infective juveniles of *S. feltiae* exhibit considerable variation in the time until they become infective. The initial dynamics of the system were not observed as nematodes used in experiments were from a population that was up to six days old. After this time it appeared that an initial group of the infective juvenile population was seen to be immediately infectious with the remainder subsequently joining the infectious class following a continuous distribution with time. This behaviour was shown not to be the result of interactions between individual nematodes. However, as the nematode infection behaviour was not investigated for the first six days post emergence it was only possible to determine the mean time until infection from the start of the experiment.

There are four possible explanations exist to explain our findings. Firstly, the arena size may have been too big to allow more than the observed percentage of nematodes to encounter the host within the time-scale of the experiment. This is unlikely, since in a similar experiment Bohan & Hominick (1996) found that hosts and nematodes occupied the same space when placed into 30ml universal tubes and the 2ml Eppendorf tubes used in this study were considerably smaller than those used by Bohan. Also, nematodes have potential to move up to 16cm through sand within 24 hours (Koch-

Osborne unpublished data). The second explanation for the observed proportion infecting may be that hosts exhibit differential susceptibility to infection from nematodes. *Galleria* were chosen for this experiment because they have been demonstrated to lack immunity to *S. feltiae* (Bohan, personal communication) although this may not be true for all systems and nematode species (Hay & Fenlon, 1995; Campbell *et al.* 1999). The third possibility is that hosts may be differentially attractive to nematodes but this would create a non-random distribution of nematodes between hosts as would have been the case for immunity. Bohan & Hominick (1995a, b) explicitly used a Poisson model to describe the distribution of nematodes, thereby demonstrating that nematodes were distributed randomly amongst hosts. Finally, the infective behaviour of nematodes may be dependent on intrinsic mechanisms which act within each individual. As the null hypothesis was that no more than 20 to 30 percent of any population of infective juveniles ever infect hosts (Bohan & Hominick, 1995a,b) our findings best support this latter hypothesis and we suggest that it is this intrinsic mechanism that drives the infection process of the entomopathogenic nematode *S. feltiae*.

A further caveat as a result of the *Galleria* being present in the tubes for up to five days is that we cannot distinguish between host susceptibility and infectivity.

Further experiments (3 and 4) demonstrated that the infectious behaviour of infective juveniles was not altered by extrinsic factors. Previous studies have shown that infective juveniles react positively to cues derived from a host in a fashion likely to enhance infection (Lewis, Gaugler & Harrison, 1992, 1993; Grewal, Gaugler & Lewis, 1993). Here we have shown that infective juveniles do not react to cues derived from the infected host-nematode complex to decrease the likelihood of infection up to 48 hours

following exposure to an infected host. This finding is not in agreement with the results of Glazer (1997) who first proposed that a chemical cue was produced from an infected cadaver inhibiting subsequent infectivity of nematodes.

The implications of these findings for biological control are promising. We have shown that the proportion of nematodes that are infectious is not fixed but dynamic. Therefore nematodes which do not infect immediately following application will become infective over time. In mushroom houses populations have been shown to survive for periods extending 1 week with low rates of decline (R.L.Gwynn, unpublished data) so, in these cases the total number of nematodes which will become infective will be upwards of 50%.

Finally, entomopathogenic nematodes appear to exhibit mixed strategies of host infection. It remains to be shown whether these strategies affect the efficiency of these nematodes as biological control agents and a combination of modelling and experiments designed to incorporate both of these behaviours need to be adopted to investigate this.

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Chapter 4

Testing non-linear transmission in the entomopathogenic nematode *Heterorhabditis megidis*

INTRODUCTION

Mathematical models of parasite-host interactions have played a very important role in our understanding of ecological disease patterns and have provided an insight into effective biological control measures. In particular the work of Anderson and May (1979, 1991) has provided a sound framework that has allowed us to explore the dynamics of microparasitic infections in detail. In a number of instances these models have been tailored to specific human disease systems such as measles (Keeling, Rohani & Grenfell, 2001) or to specific infections of pest species such as infections of invertebrates (Briggs & Godfray, 1995). However, a major limitation with our understanding of any disease system is the accurate estimate of the important parameters. In disease models, the most important and yet one of the most elusive parameters to estimate accurately is the transmission coefficient, β (Anderson & May, 1981, Dobson and Hudson 1992, McCallum 2001). This transmission coefficient has been defined as the probability that contact between an infectious unit (infected individual or free living infective stage) and a susceptible host occurs and results in the susceptible host becoming infected (Knell *et al*, 1998). The formulation of the transmission process can have major influences on the predicted dynamics of the system under investigation (Briggs & Godfray, 1995), although the typical form of the rate of transmission is the mass action term:

$$\text{Number of new infections} = \beta SI$$

where β is the transmission coefficient, S is the density of susceptible hosts and I is the density of infectious units (Anderson & May 1979). This term is derived from physical gas laws and in this case constrains the interactions between host and pathogen by assuming linear increases in the transmission rate with increasing host and pathogen

density (Fig 1). When measured *per capita*, this translates as a constant value of transmission at increasing host and pathogen density (Fig 2). However, it is important to realise that mass action is only one of several possible formulations of the transmission process, with others allowing for non-linear transmission rates with changing host and pathogen densities (Hochberg, 1991, Briggs & Godfrey, 1995). Here, *per capita* transmission becomes a function of the density of host and/or pathogen density, rather than the constant value assumed with mass action (Fig 1, Fig 2).

Figure 1. Representations of three different forms of transmission rate. Linear Mass Action where the transmission rate is directly proportional to density of hosts. Accelerating response where the transmission rate increases in a non-linear function with increasing host density. Decelerating response where the transmission rate decreases with increasing host density.

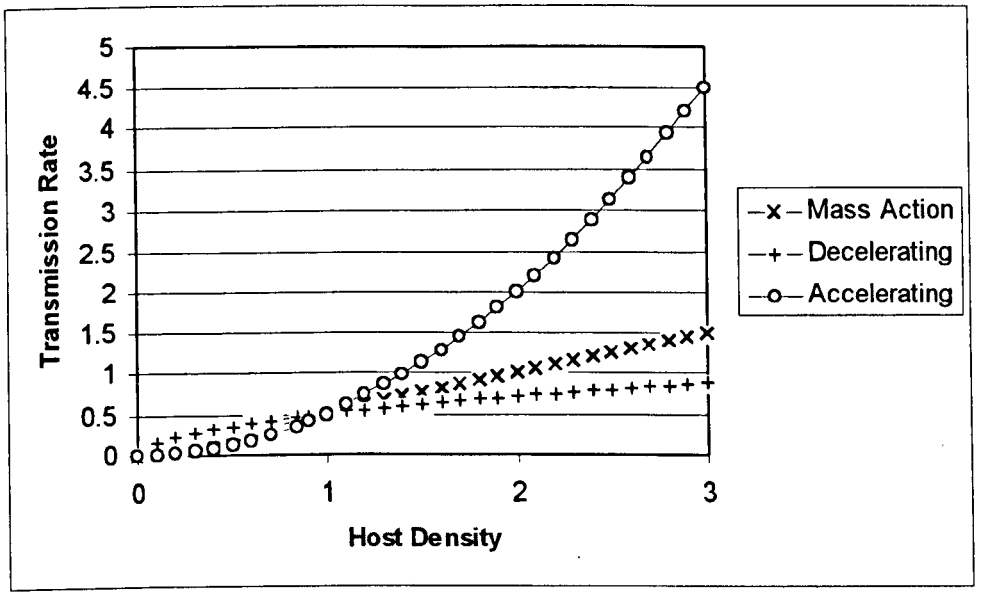
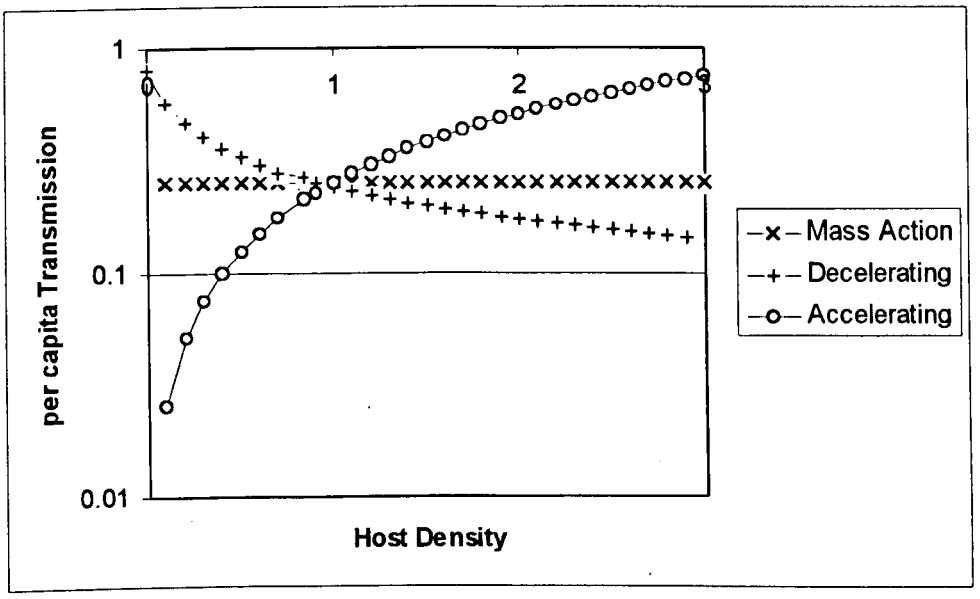


Figure 2. *Per capita* transmission (β) with three different forms of the transmission rate. Mass Action where *per capita* transmission remains the same at different host densities. Accelerating where *per capita* transmission increases with increasing host density. Decelerating response where *per capita* transmission declines with increasing host density.



Hochberg (1991) cited several instances that may give rise to non-linear transmission rates and suggested a formula which incorporated these dynamics by adapting models of directly transmitted microparasites, replacing the *per capita* transmission term, β with:

$$\beta=\beta' S^p I^q \qquad \text{eqn 1}$$

where β' is the rescaled transmission parameter and p and q are constants which represent the independent effects of the densities of susceptible (S) and infected hosts (I) on the *per capita* transmission rate. Such non-linearities arising from changing densities are therefore a product of the combined effects of both susceptible and infected densities. For the case where p=0 and q=0, *per capita* transmission is constant and the linear case of mass action is recovered. Where p or $q > 0$ the transmission response accelerates as the densities increase and where p or $q < 0$ a decelerating response is observed (Fig 1 & 2). Hochberg (1991) went on to demonstrate that increases in per capita transmission with host density ($p>0$) tends to lead to stable regulation of the host. Conversely, a reduction in the per capita transmission with density ($p<0$), similar to a type two functional response from predator-prey theory, led to non-stable behaviour.

Such non-linear transmission rates have been observed with *Plodia interpunctella* and its granulosis virus (Knell *et al*, 1998). In this case transmission primarily took place through cannibalism, a behaviour which is likely to increase at high host densities as resources become limiting. Other instances where this may occur are when hosts become stressed at high densities and their susceptibility to infection increases. Several workers

have recently started to test the linear transmission assumptions of mass action with microparasites (Dwyer, 1991; Goulson *et al.*, 1995; D'Amico *et al.*, 1996; Knell, Begon & Thompson, 1996; Knell, Begon & Thompson, 1998; Begon *et al*, 1998; Begon *et al*, 1999). In addition, for some macroparasites, similar investigations have shown that *per capita* transmission may not be a simple constant value with changing parasite and host densities (Keymer & Anderson, 1979; Carter, Anderson & Wilson, 1982). Even so, as McCallum *et al* (2001) points out, there is a need for more experimental data on the dynamics of transmission and the subsequent integration of these data with the original models.

A series of models exploring the use of entomopathogenic nematodes as biological control agents have shown that the success of these nematodes is greatly influenced by the precise value of the transmission coefficient β (Fenton *et al.*, 2000, 2001; Appendix A). However, at present only model fitted estimates are available for the value of β (Fenton *et al.*, 2001). Following from Hochberg (1991) and McCallum *et al* (2001) we recognised the importance of understanding the effects of changing densities on the form of transmission and evaluating how transmission rates are influenced by processes happening both at the level of the individual parasite and at the level of the host population. Our ultimate aim is to create a model that describes these effects in detail; only then can we use modelling to make accurate predictions of control success.

Dwyer (1991) presented an experimental method of estimating the transmission coefficient directly using a simple formula that estimated the number of healthy larvae that became infected. Assumptions included: first, a constant number of infectious units and second, the non-virus induced host death must be negligible. Such assumptions can be

met if the duration of the experiment is relatively short, although we assume one infective juvenile is lost from the nematode population per infection. This is as a result of including an equation which explicitly represents the free living infective stages which can each only infect one susceptible host, rather than the case for other microparasites where infected hosts may re-infect several susceptible individuals.

A Susceptible Infected model similar to that applied by Dwyer (1991) and by Fenton *et al* (2000; Appendix A) was used:

$$\frac{dX}{dt} = r X - \beta L X \qquad \text{eqn 2}$$

$$\frac{dY}{dt} = \beta L X - \alpha Y \qquad \text{eqn 3}$$

$$\frac{dL}{dt} = \Lambda\alpha Y - \beta L X - \mu L \qquad \text{eqn 4}$$

where L is the density of infectious free-living nematodes and *r* represents the rate of increase of the susceptible population, X. Y is the density of infected cadavers, which are lost at rate α, each producing Λ infective juveniles per unit time. By running the experiment for a short enough period of time, we can discount host reproduction (*r*), nematode mortality (μ) and nematode recycling (Λ and α) and the system can be described by:

$$\frac{dX}{dt} = - \beta L X \qquad \text{eqn 5}$$

$$\frac{dY}{dt} = \beta L X \qquad \text{eqn 6}$$

$$\frac{dL}{dt} = - \beta L X \qquad \text{eqn 7}$$

We can therefore determine the transmission coefficient β using the following formula:

$$\beta = -\frac{1}{t(L_o - X_o)} \ln\left[\frac{L_o \cdot X_t}{X_o(L_o + X_t - X_o)}\right] \qquad \text{eqn 8}$$

where L_o is the initial density of nematodes, t is the time over which the hosts are exposed to nematodes, X_t is the density of susceptible hosts alive at the end of the exposure period and X_o is the initial density of susceptible hosts. This is different to the formula used by Dwyer (1991) where he assumed there was no change in infectious particle density with each infection ($dL/dX = 0$).

METHODS

All assays used late instar larvae of the wax moth *Galleria mellonella* which had been reared on a diet of wheat middlings, honey and beeswax. Infective juveniles of the nematode *Heterorhabditis megidis* were the commercial strain, NemasysH (©MicroBio, UK).

Densities 25, 50, 75 and 100 thousand infective juveniles m^{-2} were point injected onto the surface of pots 3.8cm in radius, filled with moist sand (8%v/v). Host densities used were $3528m^{-2}$, $1764m^{-2}$, $882m^{-2}$ and $441m^{-2}$, translating as 16, 8, 4 and 2 hosts per

pot respectively. Each different host density was run with each density of nematodes in a factorial design.

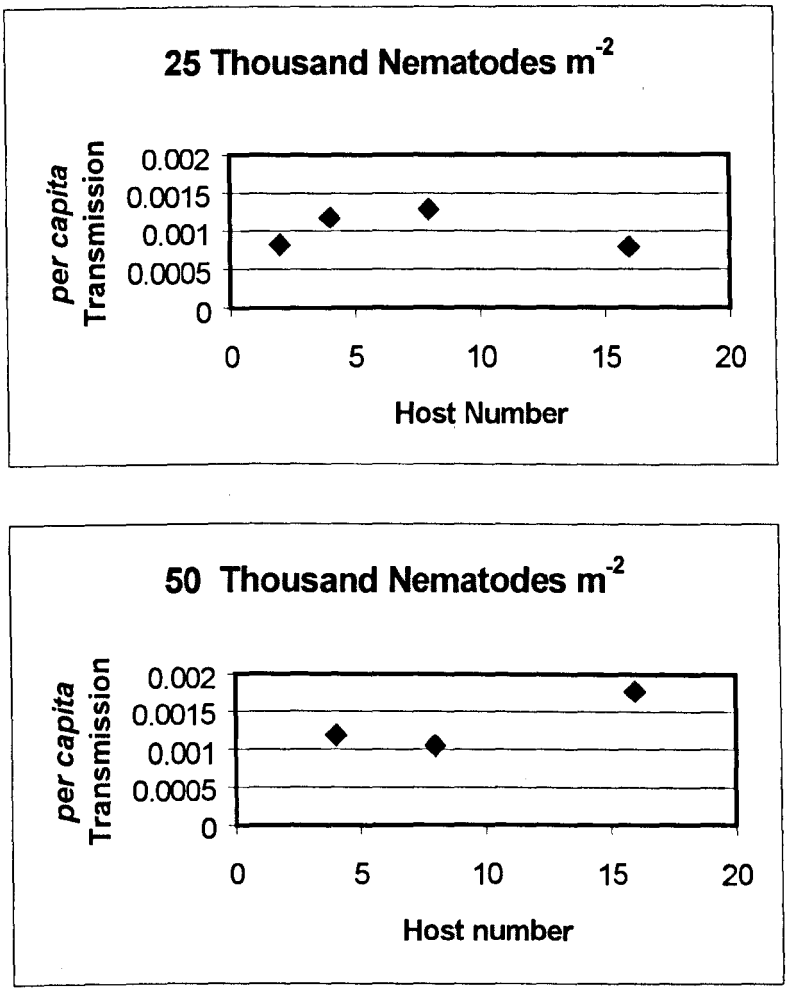
The hosts were left on the sand for 12 hours at 20°C, following which they were removed, washed to remove any nematodes adhering to the cuticle, and left for a further 48 hours to determine the number which had been infected. Death from *H. megidis* gives the host a distinctive red colouration. No hosts died of non-nematode induced causes. Each treatment combination was replicated six times, resulting in a total of 96 (4x4x6) experimental arenas.

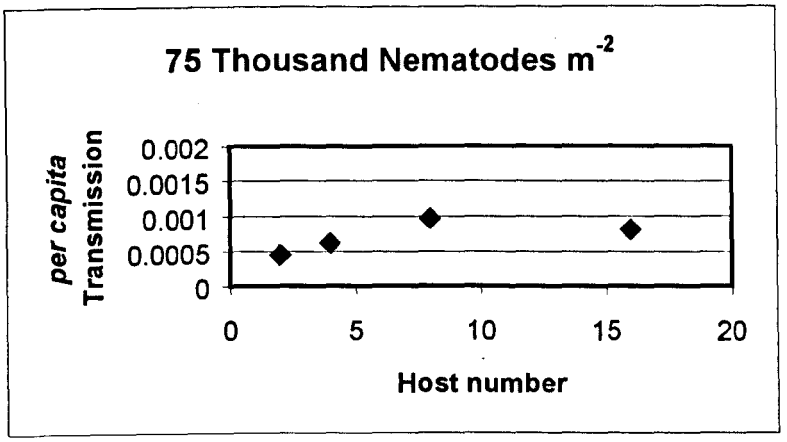
When all hosts died in a replicate it was not possible to calculate the transmission coefficient in which case a 'best estimate' of β was achieved by averaging the number of hosts that died between replicates. Where all but one host was killed in one set of replicates for one nematode and host density the value for surviving hosts was divided amongst all six replicates before calculating β. Although this approach removes some of the variance in the system it encapsulates the high mortality achieved, and therefore gives a more realistic estimate of the transmission coefficient than if such data were ignored.

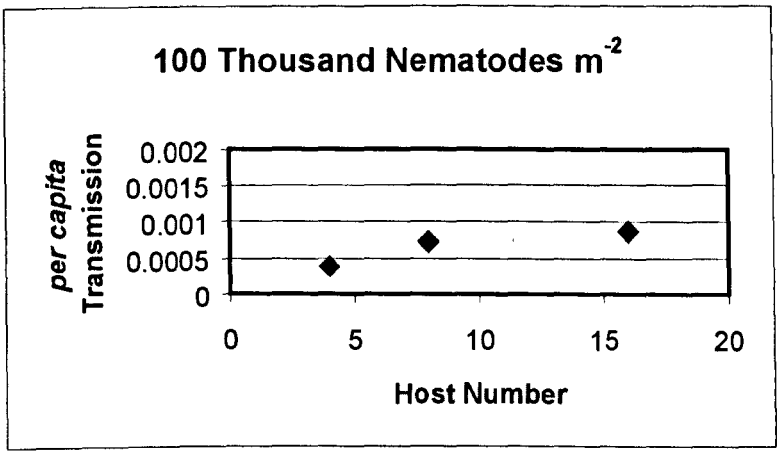
RESULTS

A factorial GLM of the estimated values of β across all host and nematode densities showed a significant effect of nematode density ($df=1$, $F=5.6$, $P=0.037$) but not host density ($df=1$, $F=2.97$, $P=0.11$). Therefore, at changing host density, *per capita* transmission remains constant, obeying the laws of mass action although changing nematode density does not.

Figure 3. Effects of changing host number on *per capita* transmission of *H. megidis* applied in four different densities (m^{-2}) to hosts in sand pots.




75 Thousand Nematodes m-2
per capita Transmission
0.002
0.0015
0.001
0.0005
0
0
5
10
15
20
Host number


100 Thousand Nematodes m-2
per capita Transmission
0.002
0.0015
0.001
0.0005
0
0
5
10
15
20
Host Number

To evaluate the effect of the observed transmission rates on the dynamics of the system, the approach suggested by Hochberg (1991) was adopted. A non-linear least squares regressions was performed to fit his equation to the full data set presented here, producing estimates of $\beta' = 0.0028$ (SE = 0.0026), $p = 0.203$ (0.133) and $q = -0.278$ (0.168) (adj. $R^2 = 0.34$) suggesting that *per capita* transmission increases slightly with host density, but decreases with the nematode density. The following model was then constructed:

$$\frac{dX}{dt} = r X - f(\beta,X,L) \qquad \text{eqn 9}$$

$$\frac{dY}{dt} = f(\beta,X,L) - \alpha Y \qquad \text{eqn 10}$$

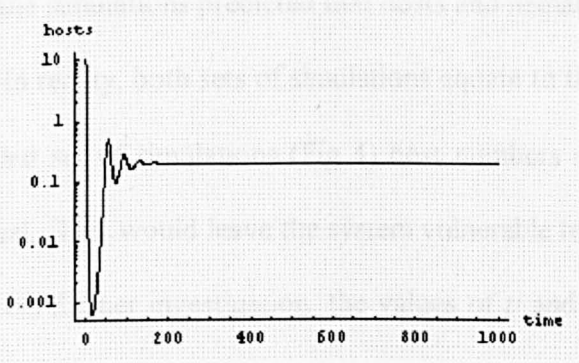
$$\frac{dL}{dt} = \Lambda\alpha Y - f(\beta,X,L) - \mu L \qquad \text{eqn 11}$$

where $f(\beta,X,L)$ is the transmission function ,$\beta'[X^pL^q]XL$. The other parameters used were previously estimated values typically observed for the entomopathogenic nematode system (Fenton *et al*, 2000).

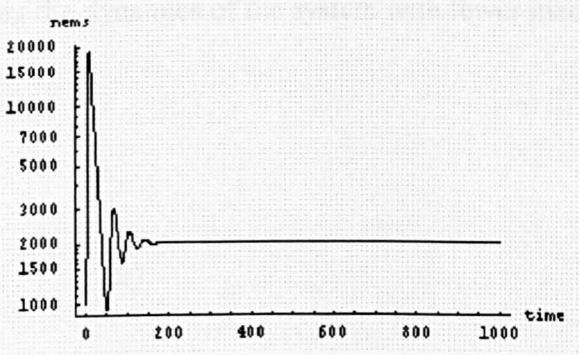
Simulations of this model show that, following an initial reduction in host numbers, both hosts and nematodes reach equilibrium with host numbers suppressed to a very low level (Fig 4).

Figure 4. Temporal simulation plots of changing host (a) and nematode densities (b) with time using Hochberg's (1991) formulation of the transmission term as applied to *Heterorhabditis megidis* with changing host densities. Transmission parameter values used ($\beta' = 0.0028$, $p = 0.203$, $q = -0.278$) were determined from experimental data. For full details of the model see Fenton *et al* (2000; Appendix A)

a)



b)



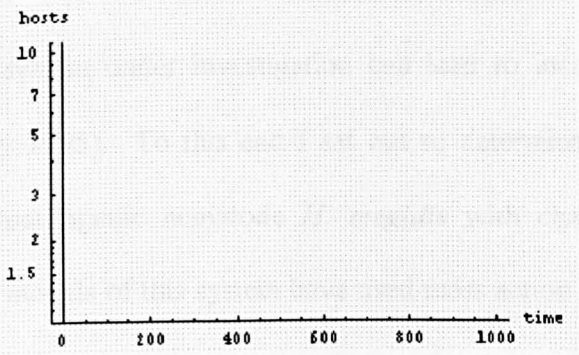
I have shown statistically that mass action cannot be discounted as a mechanism describing the rate of infection. I used Hochberg's formulation of transmission, with the mean β value from the experiment and carried out simulations of the case where $p = 0$, and $q = 0$ to investigate the effect of using mass action on the population dynamics. Unlike non-linear transmission used above, the simulations predicted that hosts and nematodes rapidly became extinct (Fig 5).

In reality, both sets of simulations equate to the same end result: host extinction, as in the first set of simulations (Fig 4) host numbers reach levels of only a fraction of one individual. This would leave the system vulnerable to stochastic fade-out and extinction.

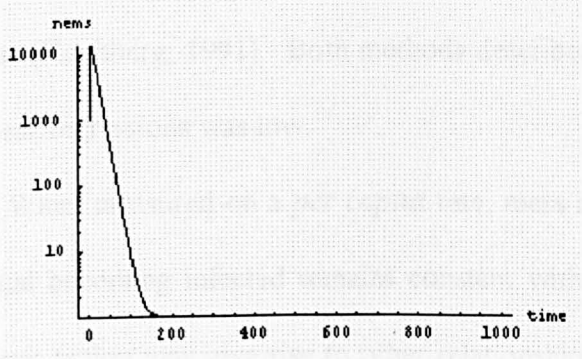
On further investigation, the values of p and q are not significantly different from zero at the 5% confidence level (df=4, t=1.52, t=1.65 respectively). Therefore simple mass action may be the most parsimonious transmission term to apply to these models, capturing the dynamics of the system with fewer mathematical complexities.

Figure 5. Temporal simulation plots of changing host (a) and nematode densities (b) with time using Hochberg's (1991) formulation of the transmission term as applied to *Heterorhabditis megidis* with changing host densities. Transmission parameter values were used to represent mass action (β'=0.0028, p = 0, q = 0). For full details of the model see Fenton *et al* (2000).

a)



b)



DISCUSSION

With any applied model, it is essential to fully understand the implications of the main underlying assumptions and the limitations they impose on the predictive power of the model. Of particular concern in host-parasite models is the nature of the transmission function. Use of a transmission term with implicit assumptions that are not representative of the system under investigation can lead to incorrect control predictions (Briggs & Godfray 1995). To this end I set out to determine the dynamics of transmission in the entomopathogenic nematode *H. megidis* with changing host and nematode densities. Simple models of this system have used mass action to represent the transmission process, where increases in host and pathogen density result in a linear increase in the rate of transmission. I compared model fits using this form of transmission to a form which allows for non-linearities in the transmission function with changing host and parasite densities (Hochberg, 1991). Both methods described the data, although the power of the non-linear regressions was low.

When measured on a *per capita* rate, mass action predicts that the chances of an individual becoming infected remains constant with increasing host density. Deviations from mass action may give rise to other relationships which are typically non-linear. The different responses are analogous to functional responses, where a type one response represents mass action. A type two response would best describe Sexually Transmitted Diseases or predation rate from predator prey theory. Here the individual chance of a susceptible host becoming infected is high at low densities and decreases at higher densities due to a limitation on the number of susceptible hosts which a predator can deal with in any one time period. Non-linearities in the transmission of microparasites may

arise from increased susceptibility at high densities (Hochberg, 1991; Knell *et al*, 1998), or non-homogenous mixing of pathogen and parasite. Importantly, Hochberg's (1991) representation of the transmission rate is such that many different functional responses, from linear through to extremely non-linear may be modelled by adapting the same equation. Therefore, the transmission term described by Hochberg (1991) represents a sliding scale of interactions between changing host and pathogen densities where linear mass action may be represented as one end of the continuum and not a simple linear/non-linear cut off.

When model simulations were performed to determine the predicted dynamics of the system under the two methods, the long-term dynamics differed. Technically the fitted non-linear transmission function stabilises the interaction, leading to stable equilibrium of both host and nematodes. However, the initial transient dynamics suggest that both populations had a high probability of going extinct at an early stage. Simulations of the linear model predict that both host and nematode populations crash to extinction very quickly. Realistically then, both formulations predict early extinction of both host and nematode. For the sake of model accuracy and simplicity I suggest that the more parsimonious mass action approach should be adopted for our model.

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Chapter 5

Testing non-linear transmission in the entomopathogenic nematode *Steinernema feltiae*

INTRODUCTION

In the previous chapter I investigated the dynamics of transmission of *Heterorhabditis megidis* against *Galleria mellonella* with changing host densities. I used two approaches to represent the functional response of the transmission rate to changing densities of host. The first of these was a function described by Hochberg (1991), which allows many different interactions between changing host and parasite densities to affect the rate of transmission. This approach is tailored to describe non-linear interactions, although I also used it to investigate the second form of transmission: linear mass action.

A priori knowledge of the system may allow us to determine the best formulation as determined from the biology of the interaction. The predicted dynamics of disease spread through a host population can be altered by the formulation of the transmission rate in the model (Briggs & Godfray, 1995; Hochberg, 1991).

The main assumption of mass action, which is of interest in our system, is that of random mixing. Hosts and nematodes are assumed to occupy the same space, interacting with every other individual with equal probability, regardless of the numbers of individuals present. In chapter 4 I demonstrated that transmission of the nematode *H. megidis* could best be described by mass action in the experimental system used. Random mixing may therefore be assumed to take place over all host and nematode densities I looked at. Comparing the biology of *H. megidis* and *S. feltiae* may predict that the form of transmission differs between the two. Whereas *H. megidis* is hermaphroditic and only requires one individual to be present in the host in order to reproduce, *S. feltiae* is gonochoristic and requires both sexes be present. This may lead to clumping in hosts infected by *S. feltiae*. In addition, Hay & Fenlon (1995) showed that infective juveniles of

S. feltiae could be placed into one of three sub-populations according to their infective strategy. They classed these behaviour types as 'individuals with the behavioural propensity to initiate infection in unparasitized insects, a second that only invaded infected hosts, and a third group of non-invaders'. Following on from this work, Campbell *et al* (1999), found that nematodes of *S. feltiae* were not randomly mixed amongst hosts, whereas an hermaphroditic nematode species, *H. bacteriophora* was. Together, these two findings demonstrate that random mixing of hosts, and therefore mass action, may not occur in *S. feltiae*. Carrying out experiments with different host and nematode densities it becomes possible to identify the correct formulation of transmission, and the dynamics of the interactions can be investigated.

I showed in chapter 3 that *S. feltiae* have a phased infectivity period over which infective juveniles move into an infective state, although the effect this phenomenon has on the transmission rate of *S. feltiae* has not previously been investigated.

As in chapter 4, a Susceptible Infected model similar to that applied by Dwyer (1991) and by Fenton *et al* (2000) was used to model the system, which collapses to the following form when the experimental design is such that host reproduction (r), nematode mortality (μ) and nematode recycling (Λ and α) are discounted:

$$\frac{dX}{dt} = -\beta L X \qquad \text{eqn 1}$$

$$\frac{dY}{dt} = \beta L X \qquad \text{eqn 2}$$

$$\frac{dL}{dt} = -\beta L X \qquad \text{eqn 3}$$

Solving these differential equations and rearranging we can determine β as:

$$\beta = -\frac{1}{t\,(L_o - X_o)} \ln \left[\frac{L_o \,.\, X_t}{X_o(L_o + X_t - X_o)} \right] \qquad \text{eqn 4}$$

where L_o is the initial density of nematodes, t is the time over which the hosts are exposed to nematodes, X_t is the density of susceptible hosts alive at the end of the exposure period and X_o is the initial density of susceptible hosts.

The transmission term describes the rate at which one susceptible individual becomes infected by one individual pathogen. This term accurately describes transmission for the hermaphroditic nematode species *H. megidis* which requires only one nematode to enter a host in order to successfully reproduce (Chapter 4). However, nematodes of the family Steinernematidae are dioecious and require at least two nematodes to infect for reproduction within the host to occur. For microparasites such as viruses the loss of infectious units may be impossible to quantify, although it is interesting to note that Knell *et al* (1998) found the loss of infectious particles of *Plodia interpunctella* granulosis virus was significant during their experiments. However, it is possible to determine the number of invading entomopathogenic nematodes in *Galleria* due to high susceptibility of the host (Chapter 2). For the purposes of determining the effects of different nematode densities on the rate of transmission it is essential that the numbers of infecting nematodes are included in estimates of β.

This becomes possible using the following formula:

$$\beta = \frac{1}{t\,(L_o - nX_o)} \ln \left[\frac{L_o.X_t}{X_o(L_o + n(X_t - X_o))} \right] \qquad \text{eqn 5}$$

where n is the number of nematodes invading the host. If n=1, equation 4 is recovered and if n=0, we obtain the equation used by Dwyer (1991). In this way we can gain an accurate estimate of the transmission rate and investigate the dynamics of the system with regards to changing nematode and host densities.

METHODS

All experiments used late instars of the host *Galleria mellonella* and infective juveniles of the entomopathogenic nematode *S. feltiae*. Nematodes were collected from Whitehead traps and used within 6 days post-emergence.

Experiment 1: Evaluating how transmission changes over time.

From the data in Chapter 3 we can estimate the transmission coefficient where we assume one host is in contact with one nematode. This can be regarded akin to mass action, as each host is forced to contact one nematode. As the contact times differed it is possible to observe any changes in β over time.

The temporal dynamics of transmission were investigated for 5 days post application. Individual nematodes were applied to moist sand in Eppendorf tubes using a Hamilton micro syringe. They were then left for 6 hours to allow the nematodes to acclimatise before a single *Galleria* was added to the tube. Ventilation was provided by creating a hole in the lid of the tubes. The experimental set up was designed in such a way as to ensure maximum contact between host and nematode so that conditions for infection were as near as possible to optimal. Control tubes consisted of hosts and no nematodes and nematodes without hosts. Every twelve hours 50 *Galleria* were removed, washed and their mortality 48 hours following removal recorded. 20 control tubes were also removed to assess nematode (10 tubes) and host (10 tubes) mortality.

Experiment 2: Evaluating how transmission changes with host and nematode densities.

Nematodes were point injected onto moist sand in plastic plant-pots of 3.8cm radius and hosts were placed into the pots and held against the sand using plastic lids. This ensured that hosts were always in contact with the sand surface whilst allowing free movement across it. All experiments were run at 20°C for 24 hours after which the hosts were removed, washed to detach any nematodes adhering to the surface and stored for 48 hours to determine the number that died from infection. All dead *Galleria* were dissected to determine the number of nematodes which infected. *Galleria* that did not contain nematodes or the symbiotic bacteria were assumed to have died from other causes

whereas *Galleria* which contained the distinctive bacteria, but where no nematodes were recovered were recorded as containing one nematode.

Host densities used were $3528m^{-2}$, $1764m^{-2}$, $882m^{-2}$ and $441m^{-2}$, equivalent of 16, 8, 4 and 2 hosts per pot respectively. Each different host density was run with each density of nematodes in a factorial design. The nematode densities used were 25,000 m^{-2}, $50,000m^{-2}$, $75,000m^{-2}$ and $100,000m^{-2}$.

RESULTS

Experiment 1: Evaluating how transmission changes over time.

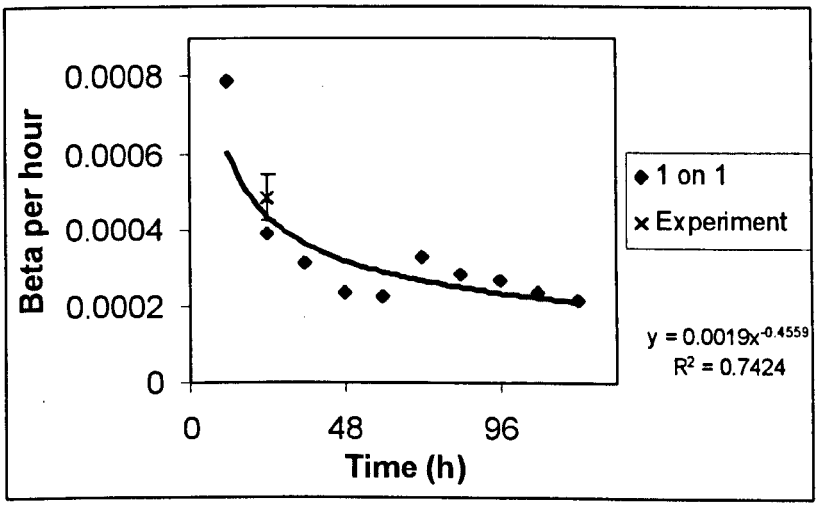
Time of exposure had a negative effect on β (Fig 2. $\beta = 0.0019t^{-0.4559}$, $R^2=0.74$) so that the probability of an interaction between a nematode and an insect after 5 days resulting in an infection was ¼ of that at the start of the experiment. It can be seen that the estimate for β after 24 hours corresponds well with those obtained independently in experiment 2.

The initial number of nematodes which can infect at the beginning of the experiment is large compared to the number which come into infectivity later on, and as a result the *per capita* transmission rate declined with time.

This is consistent with Anderson and May's (1979) formulation of transmission. As β in couple differential equation models represents instantaneous transmission, experiments should be carried out over zero time units. This is obviously not possible for many systems, which will therefore never determine the value of β represented in the

model. However, transmission is constant with time and experiments that are carried out over several time points would show an exponential decrease in the estimates. A departure from an exponential decline in β estimates with time would represent a system where the transmission process is not representative of the Anderson and May (1979) transmission process.

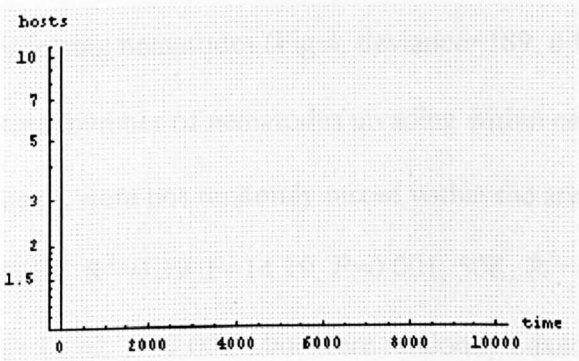
Figure 2. Effect of time of exposure on the transmission rate. Individual hosts were challenged with individual nematodes in sand filled Eppendorf tubes for up to five days (1 on 1). The average transmission rate determined for 24 hours in experiment 2 is represented with its standard error.



When model simulations with mass action were performed using the maximum and minimum values of β, the dynamics of the system appeared to be different (Fig 3). At the highest value of β ($\beta = 0.000784$), the system crashes at an early stage and first hosts, then nematodes become extinct (Fig 3a). At the lowest value of β, ($\beta = 0.000212$), the system appears to enter a series of unstable cycles, finally resulting in extinction of both host and nematode (Fig 3b). In reality the host numbers become so low that it may only be an artefact of the model that these fractions of individuals survive, and in effect the system goes extinct almost immediately, as before.

Figure 3. Temporal simulations of hosts densities over time for β values determined from experiment 1 (β= 0.000784, (a); β= 0.000212, (b)). For details of the model see Fenton *et al* (2000; Appendix A)

a)



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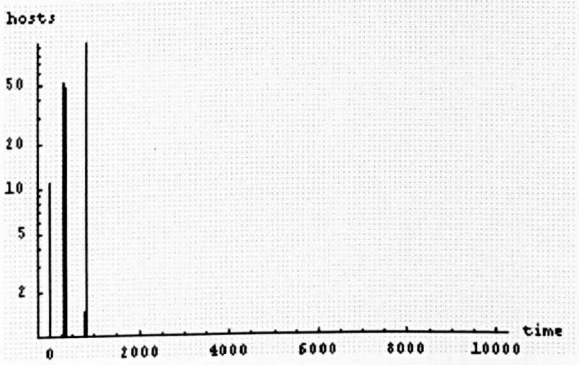
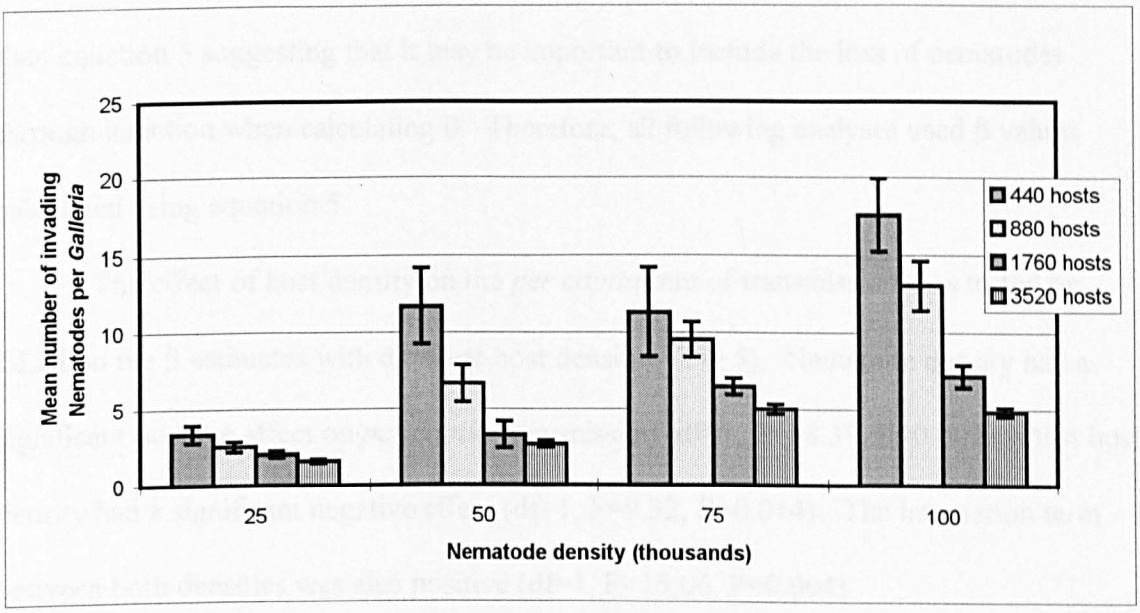


Experiment 2: Evaluating how transmission changes with host and nematode densities.

The frequency distribution of nematodes infecting *Galleria* conformed to a negative binomial distribution (chi-squared test; $\chi^2=18.08$, d.f.=19, $P=0.52$). The number of hosts placed onto the pots (X_o) showed a significant difference in the number of infecting nematodes (Fig 4, deviance=189, d.f.=3, $P<0.001$). Linear regressions of the total number of nematodes invading within each replicate indicated that nematodes and hosts were not randomly mixed within the arenas for 25, 75 and 100 thousand nematodes (25K, $R^2=0.39$, F=14.19, $P=0.001$; 50K, $R^2=69.4$, F=50.0, $P<0.001$; 75K, $R^2=39.9$, F=14.62, $P=0.001$), but were randomly mixed for 50 thousand ($R^2=0.06$, F=1.54, $P=0.227$).

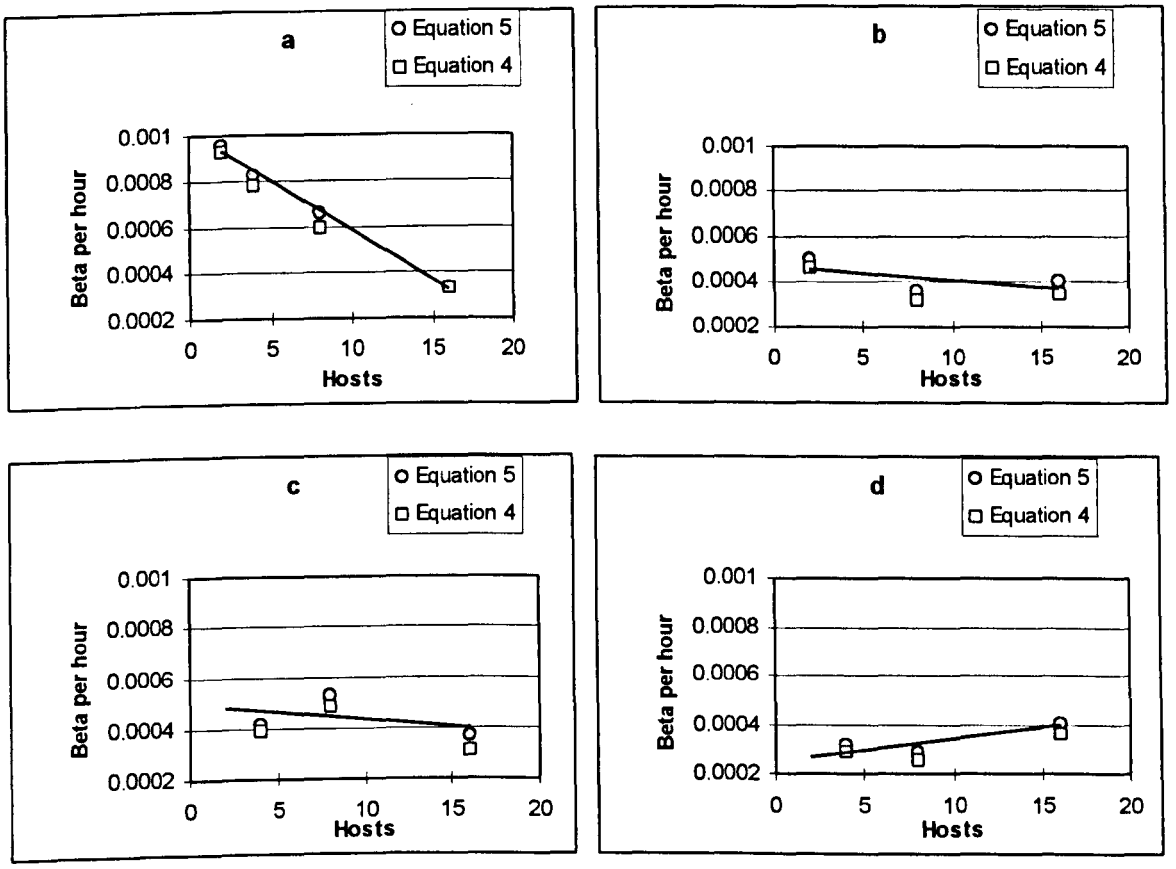
Figure 4. Mean (± standard error) number of nematodes infecting all *Galleria* in different treatment groups. Nematodes and *Galleria* were placed on moist sand filled pots 3.8cm radius for 24 hours at 20°C and all hosts which died were dissected. Nematode and host values are densities m^{-2}.



Testing between the different methods of estimating β (including nematode loss or not) I performed a paired t-test between the two sets of values (Fig 5.). The transmission coefficient using equation 4 gave significantly lower values ($t = 8.36$, d.f.=12, $P<0.001$) than equation 5 suggesting that it may be important to include the loss of nematodes through infection when calculating β. Therefore, all following analyses used β values calculated using equation 5

The effect of host density on the *per capita* rate of transmission was tested by GLM on the β estimates with different host densities (Fig 5). Nematode density had a significant positive effect on *per capita* transmission (df=1, F=18.38, P=0.002) whilst host density had a significant negative effect (df=1, F=9.32, P=0.014). The interaction term between both densities was also positive (df=1, F=15.06, P=0.004).

Figure 5. *Per capita* transmission calculated including one nematode lost per infection (equation 4) and with the total number of nematodes lost to each infection (equation 5). Densities of 25 thousand nematodes m^{-2} (a), 50 thousand m^{-2} (b), 75 thousand m^{-2} (c) and 100 thousand m^{-2} (d).



Model

To evaluate the effect of the observed transmission rates on the dynamics of the system, we adopted the approach proposed by Hochberg (1991). A non-linear least squares regression was used to fit the equation:

$$\beta = \beta' X^p L^q$$

to the full data set presented here, producing estimates of $\beta' = 0.145$ (SE = 0.086), $p = -0.304$ (0.082) and $q = -0.482$ (0.127) (adj. $R^2 = 0.75$). Both p and q are significantly different from zero (df=4, t=3.7, t=3.79 respectively). The following model explored:

$$\frac{dX}{dt} = r X - f(\beta,X,L) \qquad \text{eqn 6}$$

$$\frac{dY}{dt} = f(\beta,X,L) - \alpha Y \qquad \text{eqn 7}$$

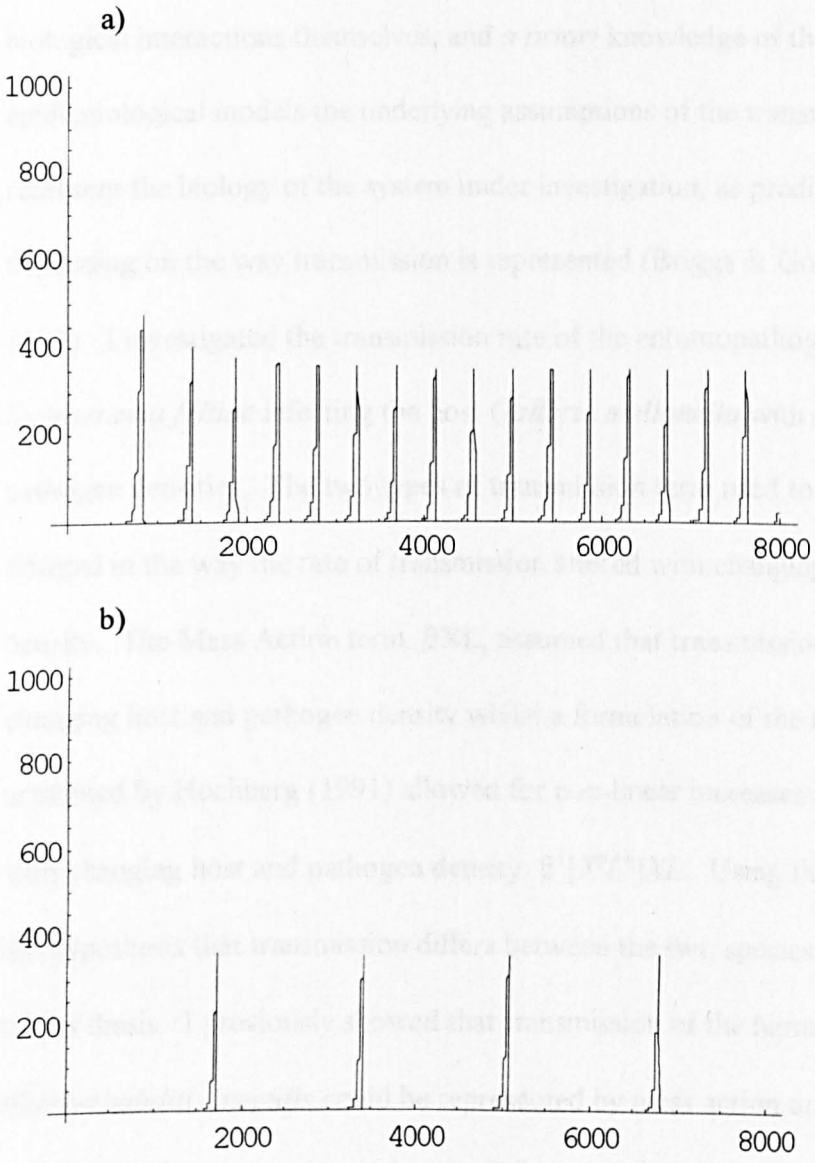
$$\frac{dL}{dt} = \Lambda\alpha Y - f(\beta,X,L) - \mu L \qquad \text{eqn 8}$$

where $f(\beta,X,L)$ is the transmission function which, for the linear transmission model is βXL (as in equations 2-4) and for the non-linear transmission model is $\beta'[X^pL^q]XL$. The model was parameterised using previously estimated values typically observed for the entomopathogenic nematode system (Fenton *et al*, 2000) and, for the linear transmission model, the overall mean value of β estimated from the data here (0.00047).

Simulations of the simple, linear transmission model show the dynamics to be highly unstable, undergoing extreme oscillations (Fig. 6a). It should be noted that, although continuous cycles are observed under the deterministic conditions of the model, during the troughs only fractions of individuals remain, indicating a very high probability of the population becoming extinct. Interestingly, when the non-linear transmission

function is incorporated into the model, the system exhibits even more extreme cyclic behaviour (Fig. 6b). This is in agreement with Hochberg (1991), who said that values of $p< 0$ tend to be destabilizing, so the incorporation of the destabilizing force into an already unstable model leads to the extreme cycling behaviour observed and the same qualitative outcome; extinction of host and nematode.

Figure 6. Temporal simulations of the host population over time with the linear Mass Action function (a) and the non-linear Hochberg (1991) transmission function (b).



DISCUSSION

Mathematical modelling of any biological process should be driven by the biological interactions themselves, and *a priori* knowledge of the system. In epidemiological models the underlying assumptions of the transmission process must represent the biology of the system under investigation, as predictions can vary greatly depending on the way transmission is represented (Briggs & Godfray 1995, Hochberg, 1991). I investigated the transmission rate of the entomopathogenic nematode *Steinernema feltiae* infecting the host *Galleria mellonella* with different host and pathogen densities. The two types of transmission term used to describe the system differed in the way the rate of transmission altered with changing host and pathogen density. The Mass Action term, βXL, assumed that transmission increased linearly with changing host and pathogen density whilst a formulation of the transmission term presented by Hochberg (1991) allowed for non-linear increases in the transmission rate with changing host and pathogen density: $\beta'[X^pL^q]XL$. Using this formula I investigated the hypothesis that transmission differs between the two species of nematodes investigated in this thesis. I previously showed that transmission of the hermaphroditic nematode *Heterorhabditis megidis* could be represented by mass action due the apparent random mixing of nematodes amongst hosts. This may be due to the sexual biology of the nematode which does not require that it infects hosts containing, or likely to contain conspecific nematodes. *S. feltiae* is a gonochoristic species and as such requires that a host contains a nematode of the opposite sex in order for reproduction to occur. Hay & Fenlon (1995) found that *S. feltiae* did have a clumped distribution amongst hosts, and I

tested the hypothesis that mass action, with random mixing implied, would not be an accurate transmission process to describe *S. feltiae*.

In order to account for clumped distributions of nematodes in hosts I used a method for estimating the transmission coefficient, β, that included the number of nematodes which infected hosts. I compared this method with one which included loss of only one infective juvenile per infection. The two different methods of estimating β were significantly different, with the method including the actual measured loss of pathogen giving higher values.

Although nematodes were not randomly mixed amongst hosts at three of the nematode densities investigated (25, 75 and 100 thousand nematodes m^{-2}), mass action was an accurate description for all nematode densities apart from 25 thousand m^{-2}. At this density of nematodes, *per capita* transmission decreased with increasing host density. There are two possible explanations for this result. Firstly it may be due to the biology of the nematode, which can detect the infected status of hosts (Hay & Fenlon, 1995; Glazer, 1997; Campbell *et al*, 1999; Fairbairn *et al* 2000). The requirement for sex within the host may drive *S. feltiae* to invade in groups, thus enhancing the probability of successfully completing the life-cycle. Alternatively, this result may be explained in terms of the small-scale interactions between nematodes and susceptible hosts. At low host densities individual hosts distribute themselves freely around the arena. As the density of hosts is increased, the degree of host aggregation within the pots increases such that some hosts are forced to settle behind others closer to the nematodes in the centre of the pot. In this way, the hosts nearest to the centre will 'soak up' nematode infections and those hosts

behind will not be challenged by infection. Thus, an increase in host density will increase the total population size but not the number of available hosts.

Fitting the estimates of β to Hochberg's transmission term (1991), control predictions were simulated with non-linear interactions between transmission rate and changing host and pathogen density. Comparing the non-linear model with simulations of mass action models, the non-linear case predicted more unstable cyclic oscillations.

This is one of the first experimentally derived results to show that, for a non-sexually transmitted disease, infective individuals do not interact equally with all members of the host population and an increase in total population size does not always imply an increase in local density, even if the arena size remains constant. In this respect, the entomopathogenic nematode system shows similar dynamics to vector-borne diseases, in which the number of infections (bitten hosts, in the case of vectors) remains constant under a wide range of host population sizes even though the bites per individual host rises.

When host and nematodes were placed in contact with each other, removing the spatial aspect of transmission, transmission decreased over the five day period investigated. Model simulations which used the different transmission coefficient estimates appeared to show different dynamics, but essentially the host and nematode population went extinct within a very short period of time. I suggest that this reduction in β over time does not have a discernible affect on the overall dynamics of control. After 24 hours exposure the transmission estimate for these 'non-spatial' encounters was similar to the average value determined from the sand pot experiments (eqn 4). This is more evidence that transmission as estimated without loss of pathogen particles follows the laws of mass action, with apparent random mixing.

The implications of this study to biological control are that current model predictions will under-estimate infection rates in situations where both host and nematode densities are low. Decreasing per capita transmission as a function of host densities at low nematode densities would suggest that transmission is destabilising (Hochberg, 1991) which is in agreement with model predictions when this term is added to our model of entomopathogenic nematodes. However, although infection rates may alter significantly with host and parasite densities, this does not always translate to marked effects on the overall dynamics of the system and there may be more important stabilising mechanisms determining persistence in the field.

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Chapter 6

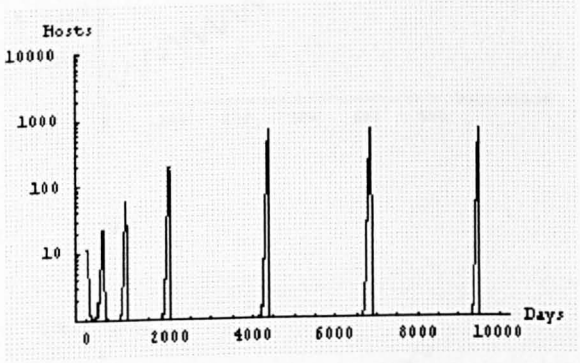
Spatial interaction of *Steinernema feltiae* and the host *Galleria mellonella.*

Mathematical models of parasite-host relationships can allow us to recreate complex dynamics from simple processes. Temporal simulations of the Anderson and May (1978) coupled differential equation models, which implicitly assume homogenous spatial interactions, can capture complex dynamics and accurately describe disease systems (Anderson & May, 1991). However, the addition of space to these models can alter their predictions, often adding stability to the systems that were previously described as unstable (Comins *et al* 1992; Comins & Hassell 1996; White *et al* 1996). These theoretical predictions have also been borne out in experimental systems that have shown space is an important dimension, which enables persistence (Huffaker, 1958). Non-spatial models of the dynamics of entomopathogenic nematodes and their hosts exhibit extremely unstable dynamics when parameterized for a typical host species (Fig 1a; Chapter 4; Fenton *et al* 2000; Appendix A). As we would expect, the addition of space using a coupled map lattice model can alter long term predictions of control (Fig 1b, c.), such that 'classical biological control' may be predicted when parameterized from an arbitrary set of host and nematode dispersal values (Fig 1b) or else hosts may escape regulation (Fig 1c). Coupled map lattice models iterate a version of the model within each cell, each of which is in contact with neighbouring cells to form a grid. Each cell interacts with its neighbours by allowing movement of host and/or pathogen between them. In this way individual populations have an immigration and emigration term that can be made to be dependent on the surrounding populations. Although the preliminary model simulated in figure 1b is parameterized from our temporal model system, the level of dispersal of nematodes or host is unknown and therefore the predictions of such simulations is limited without an accurate estimate of these vital parameters.

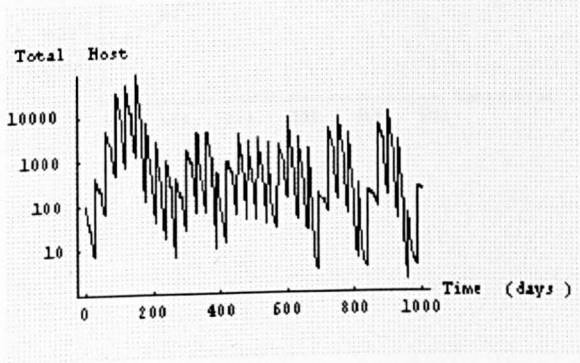
Figure 1. Simulation plots of entomopathogenic nematode/host interactions. a) A Non-spatial model simulation run with typical host and nematode parameter values showing extremely unstable cycles of host population numbers. Parameter values used Λ = 10 000, α = 0.08 day^{-1}, β = 0.000005 day^{-1}, μ = 0.01 day^{-1}, r = 0.05 day^{-1}; initial number of hosts = 10, initial number of nematodes = 10 000 (see Fenton et al (2000, Appendix A, for model details).

b) Host (i) and nematode (ii) populations against time from spatial simulations from a coupled map lattice model with a grid size of 50x50 and reflective boundaries. Parameter values used were β = 0.0005, μ = 0.1, Λ = 10 000, r = 150, with 100 initial hosts and 1 million nematodes. (see Fenton et al Appendix A for details). With high host and nematode dispersal b) and with very low nematode and host dispersal c).

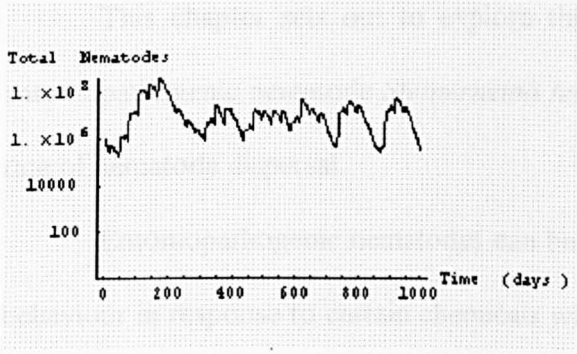
a)



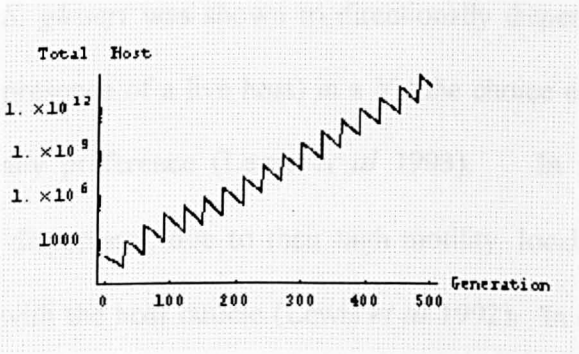
bi)



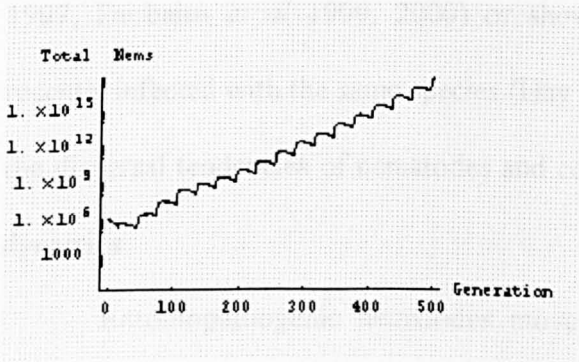
bii)


Total Nematodes
1. ×10 8
1. ×10 6
10000
100
0
200
400
600
800
1000
Time (days)

ci)


Total Host
1. ×10 12
1. ×10 9
1. ×10 6
1000
0
100
200
300
400
500
Generation

cii)


Total Nems
1. ×10 15
1. ×10 12
1. ×10 9
1. ×10 6
1000
0
100
200
300
400
500
Generation

This chapter sets out to explore the role of spatial structure in models of the entomopathogenic nematode *Steinernema feltiae* and to do this I must first determine the rate of nematode dispersal.

Entomopathogenic nematodes can be affected by their environment and alter their behaviour in response to certain chemicals which they encounter (Lewis *et al* 1992; Lewis *et al* 1993; Lewis *et al* 1995; Grewal *et al* 1993; Glazer 1997; Fairbairn *et al* 1999, 2000). *S. glaseri* was shown to directionally disperse towards carbon dioxide (representing the presence of a live host) in a Y-tube choice experiment, but *S. carpocapsae* did not show any preference (Lewis *et al* 1993). In some cases, nematodes that are classed as 'dispersers', due to their high motility, localise their search behaviour following contact with the host cuticle (Lewis *et al* 1992). In other cases some species were repelled from hosts infected with their own or other species of nematodes (Lewis *et al* 1992; Glazer 1997; Fairbairn *et al* 1999, 2000) or showed an increased rate of infection in hosts recently infected with the same species (Hay & Fenlon 1995). These behaviours may alter the dispersal tendencies of nematodes and could alter the dynamics of the nematode host dynamics.

Entomopathogenic nematodes move using backwards body waves that propel them forwards (Kaya 1993). Infective juveniles have finite lipid reserves, laid down by the feeding stage 2 juvenile, prior to developing into the infective stage 3. This energy resource cannot be replenished and it has been suggested that dispersing nematodes trade-off their infectivity with optimising their search for a host (Westerman & Stapel 1992).

Key behaviours of importance for the addition of space to our models of *S. feltiae* and its hosts are the rate of infective juvenile dispersal and how this may be altered by the

presence of hosts and the effect that dispersal has on the their capability of infection. This chapter sets out to answer three questions that are required for the production of spatial models of this system.

1) Do *S. feltiae* infective juveniles alter their infection rates following dispersal?
2) What is the dispersal rate of *S. feltiae*?
3) Do *S. feltiae* infective juveniles alter their dispersal rate in the presence of susceptible hosts?

METHODS

Infective juveniles of *S. feltiae* were collected from Whitehead traps (Woodring & Kaya 1988) less than six days prior to their inclusion in the experiment. Where mentioned, last instar larvae of the greater waxmoth *Galleria mellonella* were used in experiments.

Experiment 1: Do infection rates alter following dispersal?

Ten thousand infective juveniles were point inserted into the centre of square plastic trays (60cm x 60cm) filled with moist sand (8%v/v) to a depth of 4cm. The trays were covered with polythene to retain moisture, and stored at 20°C for 24 hours with six replications performed. One core of sand (r = 1.5cm) was then taken at 4, 8, 12, 16 and 20 cm distances from the point of nematode insertion. These cores were each placed into petri dishes and three *Galleria* placed onto them for a further 24 hours. The *Galleria*

were then removed, washed and stored for a further 48 hours to allow infecting nematodes to develop. The sand and water used to wash the *Galleria* were examined to count the number of infective juveniles (dead or alive) present. Sand washing followed the technique presented by Bohan (1995) which gives a high efficiency in yield of infective juveniles (Chapter 2). All *Galleria* that died were dissected to determine the number of nematodes that successfully invaded.

Experiment 2a: What is the dispersal rate of S. feltiae?

1000 infective juveniles were point inserted into the centre of plastic tubes 14cm x 3cm filled with moist sand (8%v/v) and stored at 20°C for 12, 24 or 36 hours. Six replicates were performed for each time step. Following storage the sand in the tubes was divided into 2cm sections (Fig 2), the sand washed and infective juveniles counted as in experiment 1.

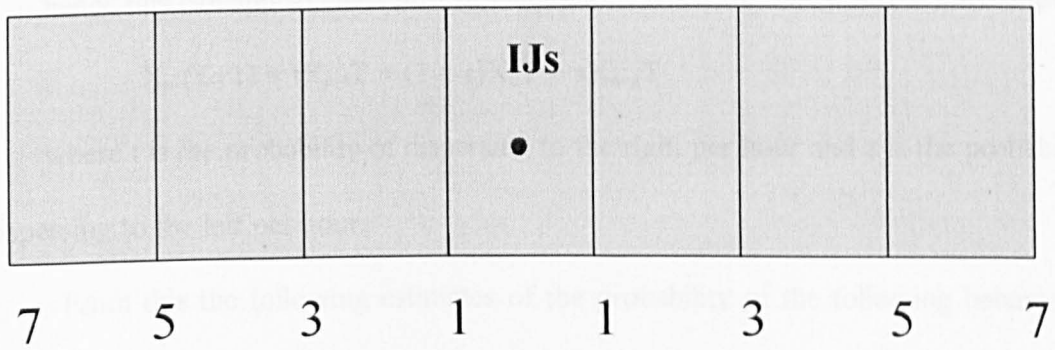
Experiment 2b: Do S. feltiae infective juveniles alter their dispersal rate in the presence of susceptible hosts?

Plastic tubes 14cm x 3cm were filled with moist sand (8%v/v) and 3 *Galleria* held inside wire cages were placed 4 cm to the right of the centre and stored for 6 hours to allow host volatiles to diffuse through the sand. Control tubes consisted of sand and wire cages, but no caterpillars. 1000 infective juveniles were then point inserted into the centre of the tubes which were stored at 20°C for 12 or 36 hours with each treatment replicated 6

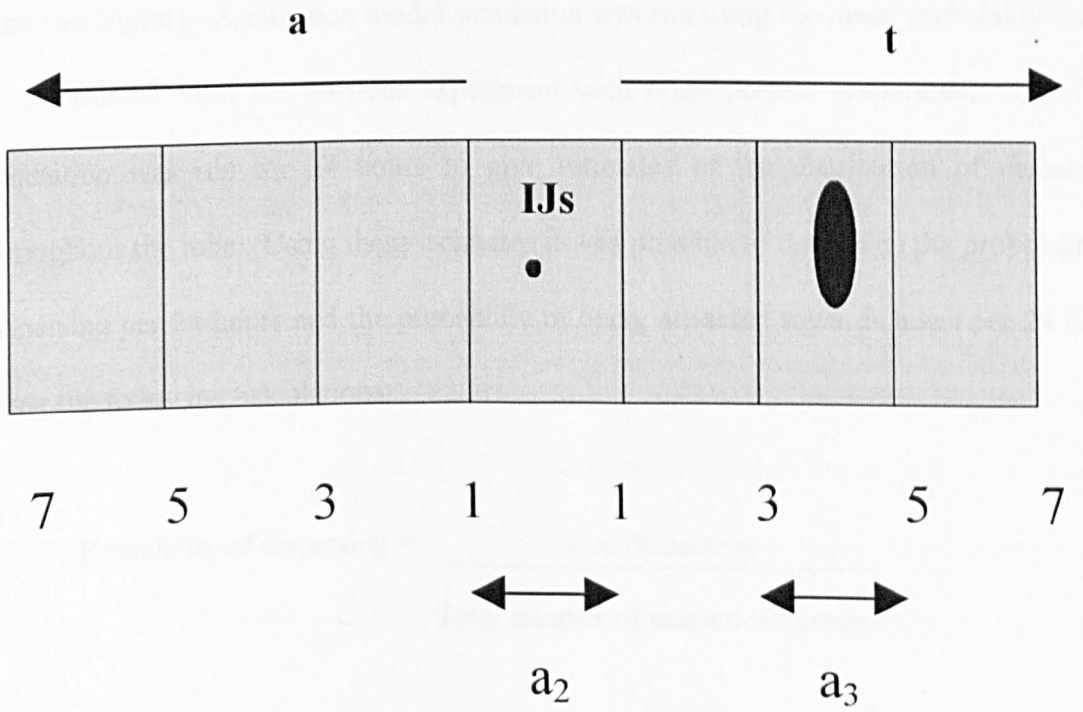
times. Following the allocated period of storage the sand was divided up into 2cm sections (Fig2 b), washed in water and the infective juveniles were counted.

Figure 2. Diagrammatic representation of methods employed. Tubes of sand 14cm x 3cm were filled with moist sand and 1000 infective juveniles inserted into the centre (IJs). Following storage the sand was divided into 2cm sections 1-1, 1-3, 3-5 and 5-7. Three last instar *Galleria* were placed 4cm from the centre of the tubes of experiment 2b(b). Probability per hour of dispersing away from hosts (a), towards hosts (t), leaving the centre (a_2) and leaving the section containing the caterpillar (a_3) from the diffusion equations fitted to the data.

a)



b)



Diffusion Model

To determine the rate of dispersal and to quantify the level of attraction towards hosts, models of simple random walks which approximate to diffusion equations with reflective boundaries, were fitted to the data from experiment 2. At any time step, T, the number of nematodes present at position n was:

$$X_n = tX_{n-1} + (1-t-a)X_n + aX_{n+1}$$

hence this can approximate diffusion equations when we take discrete time steps:

$$X_n (T+1) = tX_{n-1}T + (1-t-a)X_nT + aX_{n+1}T$$

where t is the probability of dispersing to the right per hour and a is the probability of dispersing to the left per hour.

From this the following estimates of the probability of the following behaviours occurring each hour: moving 1cm towards hosts per hour (t), moving 1cm away from hosts (a), leaving the section containing the caterpillar (a_2), and leaving the centre of the tube (a_3; Fig2**b**). A diffusion model simulation was run using the mean probability values as determined from the 36 hour experiment with hosts present (experiment 2b). This simulation was run for 24 hours to give estimates of the distribution of nematodes throughout the tube. Using these estimates it was possible to determine the probability of dispersing per 24 hours and the probability of being attracted towards hosts per 24 hours using the following calculations:

$$\text{Probability of dispersing} = \frac{\text{total dispersing}}{\text{Total number of nematodes present}}$$

$$\text{Probability of attraction} = \frac{\text{number dispersing towards host} - \text{number dispersing away from hosts}}{\text{Total number which dispersed}}$$

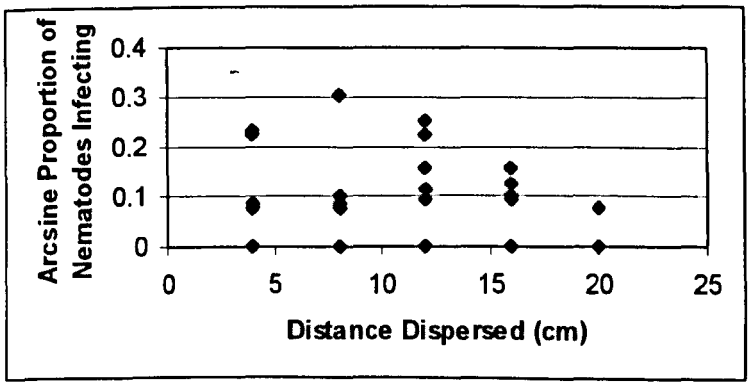
These parameters were then entered into a spatially explicit coupled map lattice model. This model is based on published models of entomopathogenic nematodes (Fenton *et al*, 2000, 2001; Appendix), but with added stage structuring. Essentially, the model iterates on a daily basis, during which the nematodes infect hosts at rate β and hosts and nematodes die at rates b and μ respectively. After each period of infection (each day) the surviving nematodes disperse in proportions determined by the values derived from the diffusion equations and a constant number of hosts disperse. Dispersal takes place to the surrounding 8 cells only, with preferential dispersal towards hosts if they are present in one of these cells. Two weeks after infection, each infected host produces Λ new nematodes and every T days surviving hosts produce r new susceptible hosts. This model was used to investigate the dynamics of infection of hosts under four different scenarios.

RESULTS

Experiment 1: Do infection rates alter following dispersal?

As the data were non-independent, linear regressions were performed for each replicate (sand tray). The whole data set were then analysed, weighting the slope with the reciprocal of the error variance from the individual regressions. The overall slope was not significantly different from zero (df=1, F=1.359, P=0.25(. Therefore, nematodes that had dispersed did not show any significant difference in their infectivity with respect to the distance dispersed.

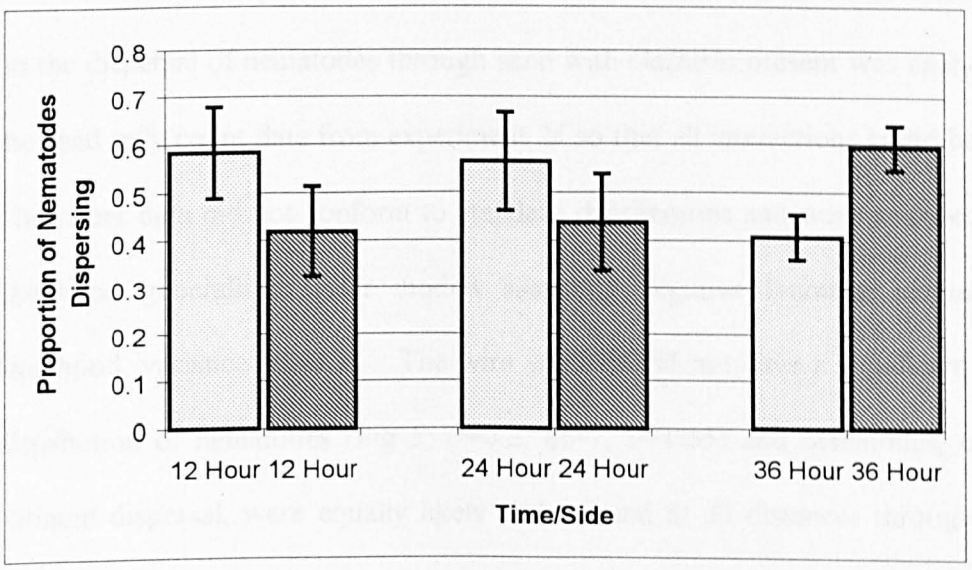
Figure 3. Proportion of nematodes infecting *Galleria mellonella* following dispersal through sand to different distances. No significant difference was detected ($P = 0.089$, n=6).



Experiment 2a: What is the dispersal rate of S. feltiae?

The proportion of nematodes dispersing to the right was used to determine if this differed from 0.5, the probability that dispersal was equal in both directions. Using a binomial GLM the proportion was not different from the expected 0.5 ($df=1$, $F=0.024$, $P=0.878$) and did not change over time ($df=1$, $F=2.39$, $P=0.14$). The diffusion model fitted to the data showed that the probability of leaving the centre of the tube per hour decreased with time (table 1a). Also, the probability of dispersing in either direction remained equal whilst decreasing over time. The rate of dispersal as determined for the coupled map lattice model was 0.589 day^{-1}.

Figure 4. Proportion of nematodes which dispersed to the left (open bars) and to the right (hatched bars) in both directions from the centre of plastic tubes 14x3cm filled with moist sand. Tubes were left at 20°C for 12, 24 or 36 hours. Standard error bars shown.

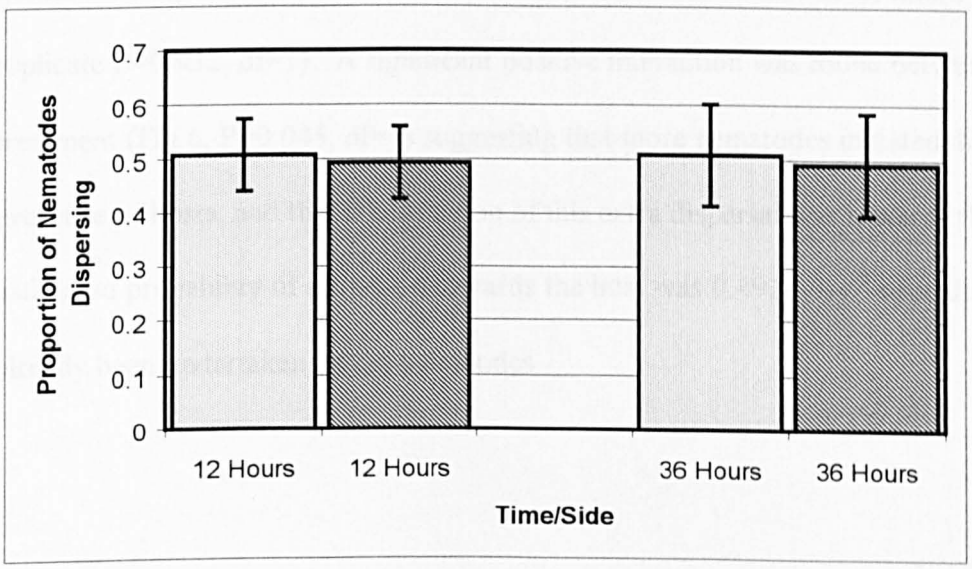


Experiment 2b: Do S. feltiae infective juveniles alter their dispersal rate in the presence of susceptible hosts?

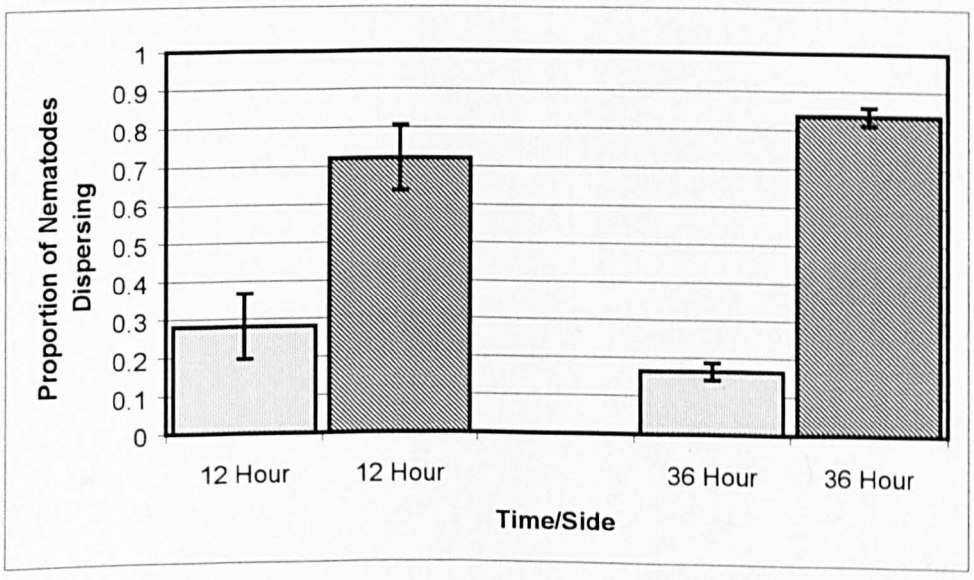
All *Galleria* present in treatment tubes died from nematode infection. Count data on the dispersal of nematodes through sand with *Galleria* present was analysed alongside the sand only count data from experiment 2a so that all interactions could be investigated. The count data did not conform to standard distributions and were analysed using quasi-likelihood generalised linear models assuming negative binomial variance (link=log-likelihood, variance=mean). The wire controls did not have a significant effect on the distribution of nematodes (Fig 5; P=0.2, df=1, F=1.65) and nematodes, once they had initiated dispersal, were equally likely to be found at all distances throughout the tubes (P=0.669, df=1, F=0.18)

Figure 5. Proportion of nematodes which dispersed in both directions from the centre of plastic tubes 14x3cm filled with moist sand. a) Wire cages present 4 cm from the point of nematode insertion (hashed bars). b) Three *Galleria* were placed in wire cages 4 cm from the point of nematode insertion (hashed bars). Tubes were left at 20°C for 12 or 36 hours. Standard error bars shown.

a)



b)



A negative binomial generalised model was performed on the proportion (arcsine transformed) of nematodes dispersing towards or away from hosts (or wire in the case of control tubes) or remaining in the centre whilst controlling for each replicate in order to determine the direction dispersing nematodes take. None of the individual factors in the model were significant (treatment P=0.846, df=1; side P=0.124, df=1; time P=0.893, df=1; replicate P=0.932, df=1). A significant positive interaction was found between side and treatment (Fig.6, P=0.045, df=1) suggesting that more nematodes initiated dispersal in the presence of hosts, and that the direction of this extra dispersal was towards the host. The estimated probability of attraction towards the host was 0.4928 day^{-1} once dispersal has already been undertaken by the nematodes.

Figure 6. Interaction plot of the mean number of nematodes dispersing towards hosts/cages (right), away from hosts/cages (left) or staying in the centre of tubes (middle). Treatments 1) sand only, 2) wire cages 4cm to the right of the nematodes, 3) three *G. mellonella* larvae 4cm to the right of the nematodes.

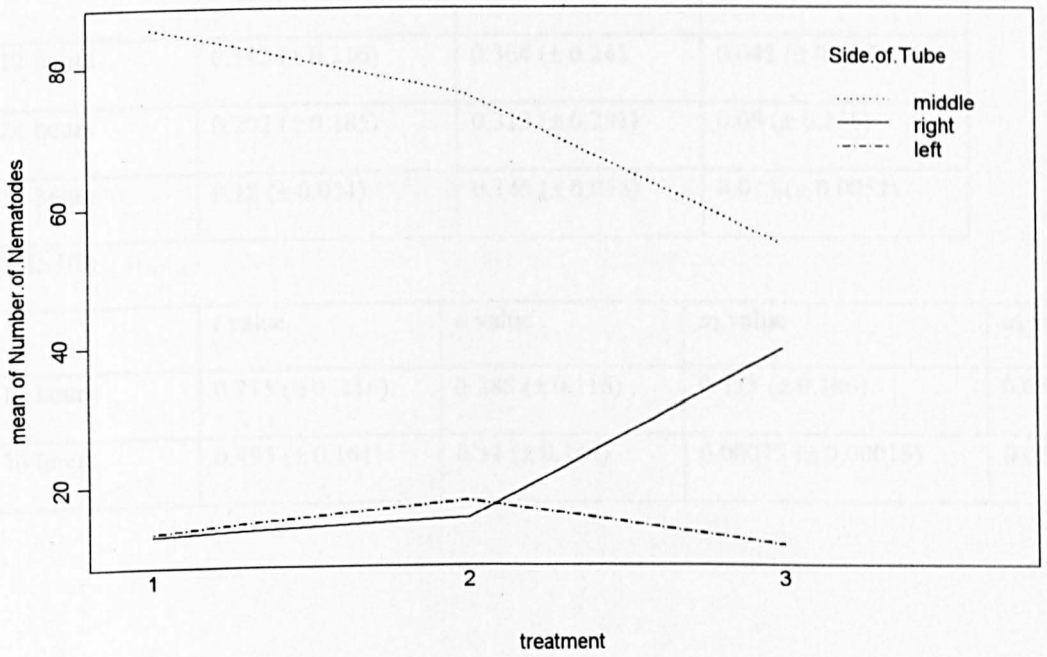


Table 1. Mean t (probability of dispersing 1cm to the right per hour) a (probability of moving 1cm to the left per hour), a_2 (probability of leaving the host) and a_3 (probability of leaving the centre) values for sand only tubes (table 1(a)) and tubes containing 3 *Galleria* larvae (table 1(b)).

Table 1(a)

	t value	a value	$a3$ value
12 hours	0.342 (± 0.216)	0.364 (± 0.241	0.042 (± 0.029)
24 hours	0.202 (± 0.185)	0.313 (± 0.291)	0.08 (± 0.156)
36 hours	0.18 (± 0.034)	0.145 (± 0.056)	0.013 (± 0.0052)

Table 1(b)

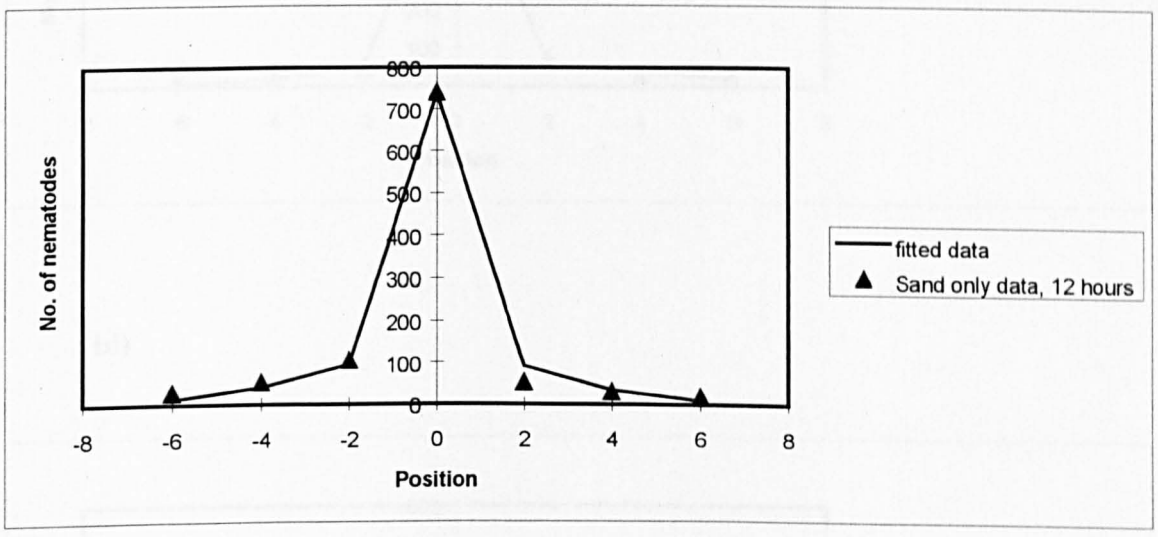
	t value	a value	a_2 value	a_3 value
12 hours	0.715 (± 0 .116)	0.285 (± 0.116)	0.123 (± 0.186)	0.058 (± 0.026)
36 hours	0.495 (± 0.161)	0.34 (± 0.144)	0.00075 (± 0.00015)	0.039 (± 0.054)

Model analysis

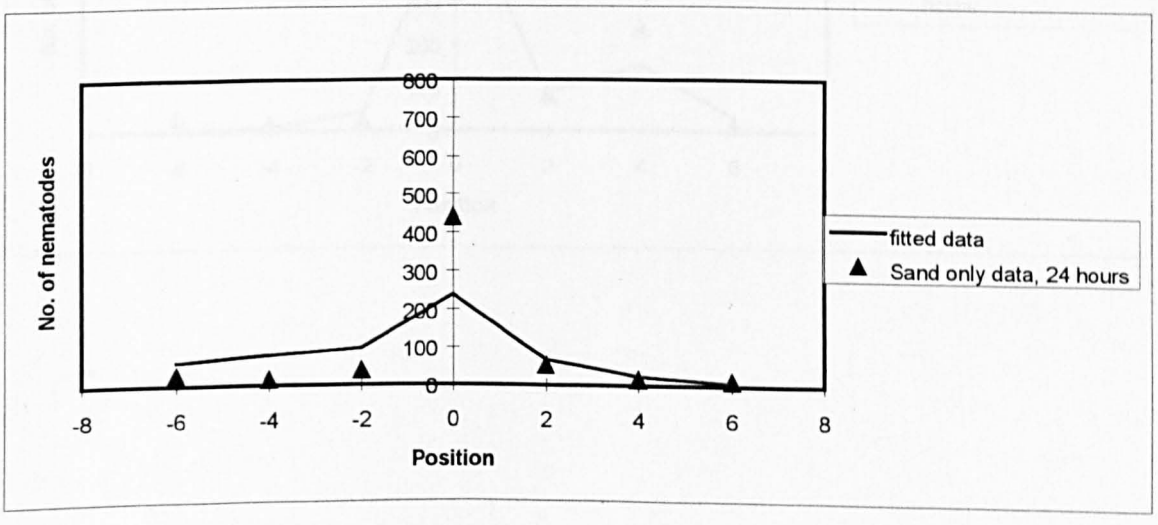
The random walk model simulations provide a good fit to the data (Fig 7, paired t-test; 12 hours sand P=0.96, n=7; 24 hours sand P=0.95, n=7; 36 hours sand P=0.82, n=7; 12 hours with host P=0.95; 36 hours with host P=0.99, n=7) and parameter estimates are shown in table 1. In the absence of hosts, the probability of dispersing declined over time. In the presence of hosts, the probability of dispersing towards hosts (t) was greater than when hosts were not present, being almost three times as likely to move in this direction than away from hosts at 12 hours. There was a greater probability of leaving the centre when hosts were present at both 12 and 36 hours than in the absence of hosts. The probability of leaving the section containing the host decreased greatly over time, although this may influenced by nematodes infecting the host.

Figure 7. Random walk equations fitted to the average data from experiments 2a and 2b. 1000 infective juveniles were inserted into the centre of plastic tubes 14cmx3cm filled with moist sand. Sand only tubes stored at 20°C fro 12 hours (ai), 24 hours (aii) and 36 hours (aiii). Tubes containing three Galleria larvae 4cm from the centre of the tubes stored at 20°C for 12 hours (bi) and 36 hours (bii).

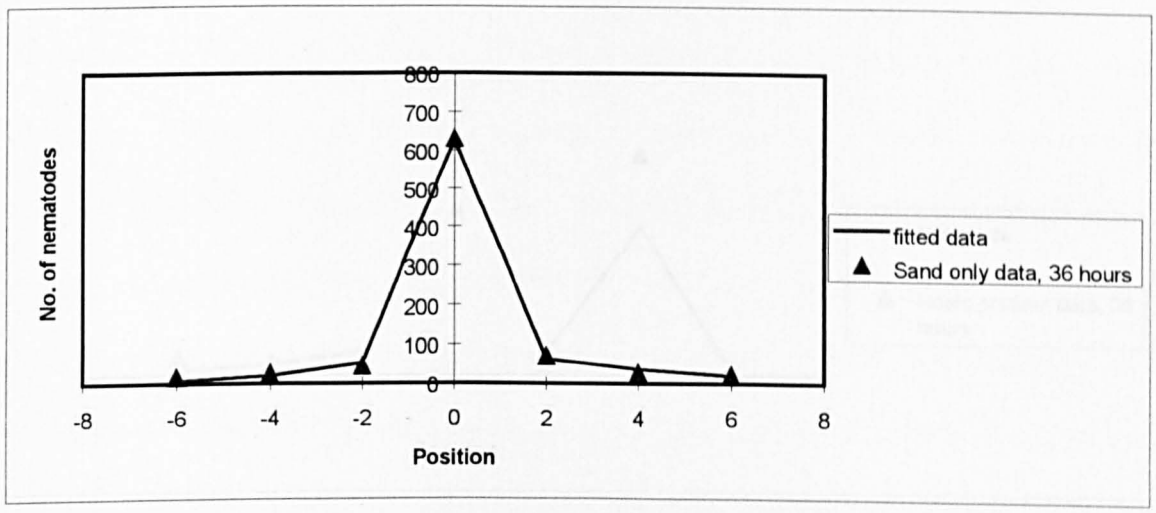
ai)



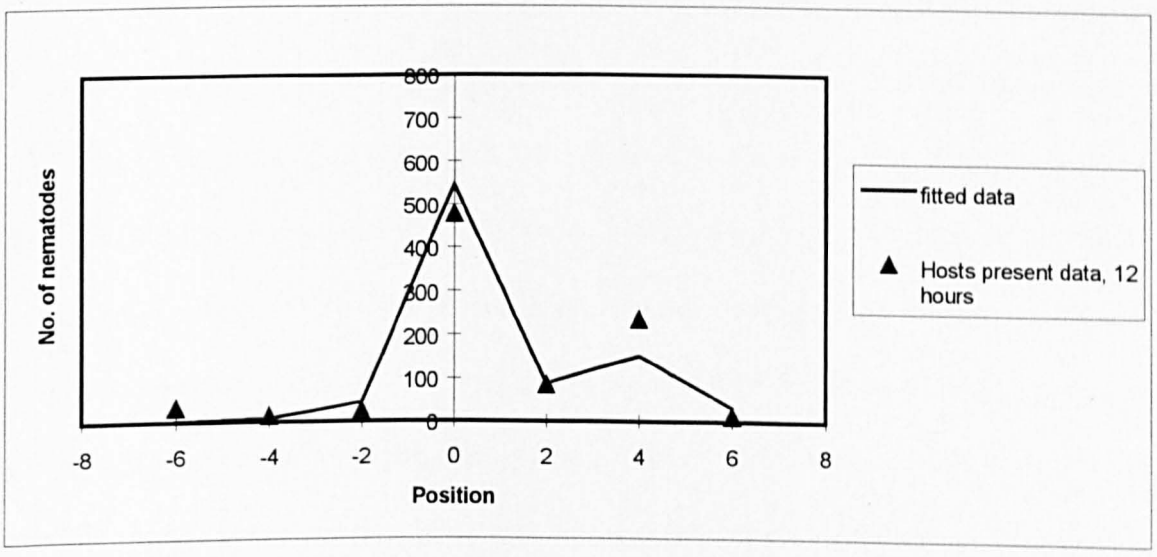
aii)



aiii)


No. of nematodes
800
700
600
500
400
300
200
100
0
-8
-6
-4
-2
0
2
4
6
8
Position
fitted data
Sand only data, 36 hours

bi)


No. of nematodes
800
700
600
500
400
300
200
100
0
-8
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0
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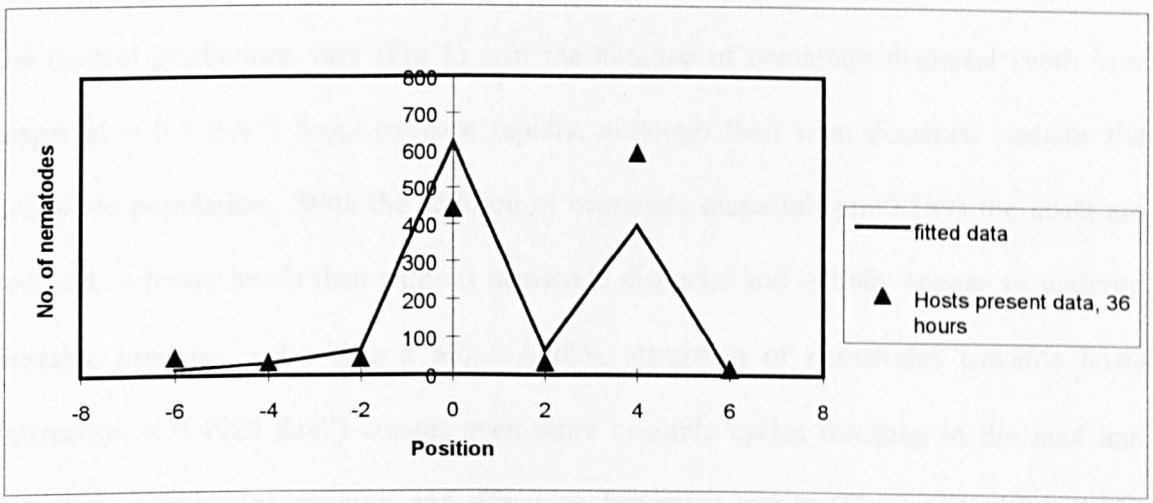
8
Position
fitted data
Hosts present data, 12 hours

bii)

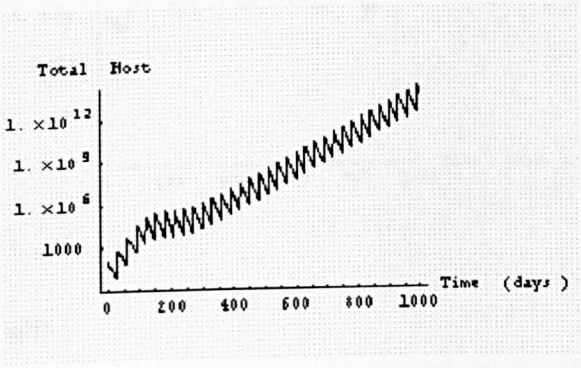

No. of nematodes
800
700
600
500
400
300
200
100
0
-8
-6
-4
-2
0
2
4
6
8
Position
fitted data
Hosts present data, 36 hours

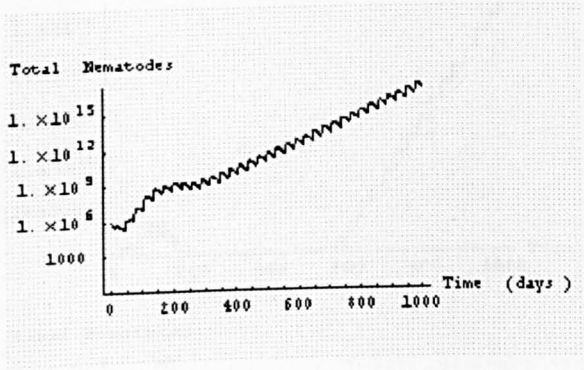
When the parameters of dispersal rate and the effect of host attraction from experiment 2 are placed into a coupled map lattice model of this system we can see that the control predictions vary (Fig 8). In the absence of nematode dispersal (with host dispersal = 0.3 day^{-1}) hosts increase rapidly, although their own dispersal sustains the nematode population. With the addition of nematode dispersal (pn=0.589) the hosts are reduced to lower levels than without nematode dispersal and initially appear to undergo unstable periodic cycles (Fig 8 aii). Adding attraction of nematodes towards hosts (attraction = 0.4928 day^{-1}) creates even more unstable cycles resulting in the host and nematode populations crashing, and effectively becoming extinct (Fig 8 aiii), although the model simulation allows the fractions of individual hosts to recover. Interestingly, the rate of host dispersal has a major influence on the predicted dynamics of the system. When host dispersal = 0.4 day^{-1} the hosts no longer escape regulation in the absence of nematode dispersal (Fig. 8 b) and the system becomes more susceptible to extinction with the addition of nematode dispersal and attractiveness towards hosts (Fig 8 bii, biii). The failure of hosts to escape regulation is possibly a consequence of host dispersal crossing a critical level above which the model approximates homogenous mixing. Although encounters in coupled map lattice models are with nearest neighbours only, a threshold limit of dispersal by hosts and parasites can be reached, below which random mixing is not achieved, and hosts can escape parasitism through the creation of refugia. However, a combination of high host and nematode dispersal can mix the system sufficiently to remove heterogeneous clumping and the system can approximate random mixing, where space becomes less important in affecting the dynamics. Where hosts have a high dispersal rate, this system may be effectively described by non-spatial models. However,

when host dispersal rates are below this critical level coupled map lattice models of this system become important descriptive tools.

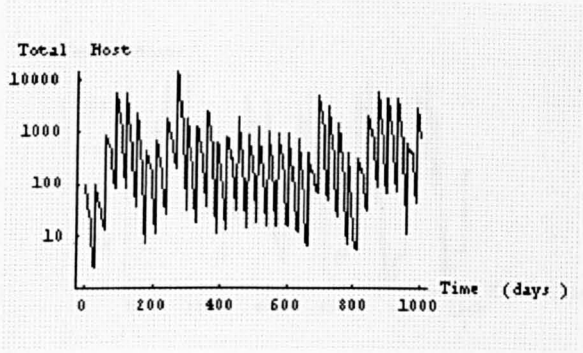
Figure 8. Coupled map lattice simulations representing host and nematode population densities through time. The model iterates on a daily basis, with one host generation = 30 days. Parameters of the system are β = 0.0005, Λ= 10 000, nematode death rate = 0.1 day^{-1}, host reproduce 150 new individuals per surviving host with 1000 000 nematodes applied to an arena of 2500 cells containing 100 hosts randomly placed in 10 sites. a) Host dispersal = 0.3 day^{-1}, i) no nematode dispersal or attraction, ii) nematode dispersal = 0.589 day^{-1}, no attraction, iii) nematode dispersal = 0.589 day^{-1}, attraction = 0.4928 day^{-1}. b) Host dispersal = 0.4 day^{-1}, i) no nematode dispersal or attraction, ii) nematode dispersal = 0.589 day^{-1}, iii) nematode dispersal = 0.589 day^{-1}, attraction = 0.4928 day^{-1}.

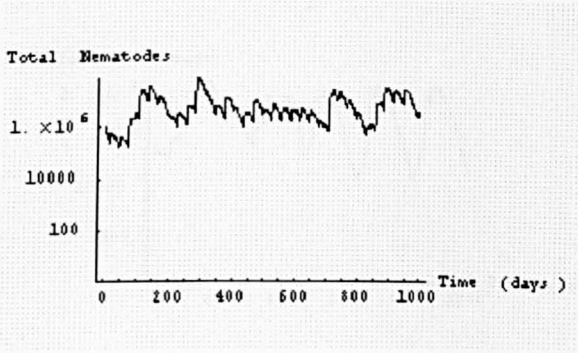
ai)



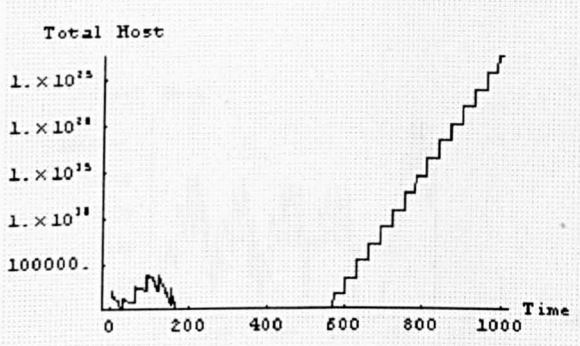


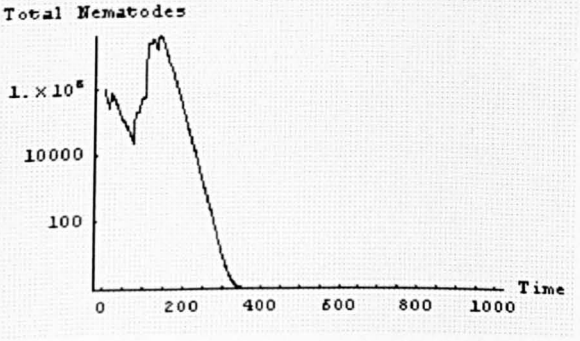
aii)


Total Host
10000
1000
100
10
0
200
400
600
800
1000
Time (days)

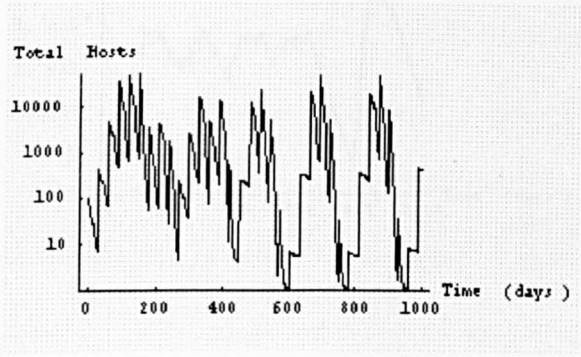

Total Nematodes
1. ×10^6
10000
100
0
200
400
600
800
1000
Time (days)

aiii)


Total Host
1. ×10^25
1. ×10^20
1. ×10^15
1. ×10^10
100000.
0
200
400
600
800
1000
Time


Total Nematodes
1. ×10^6
10000
100
0
200
400
600
800
1000
Time

bi)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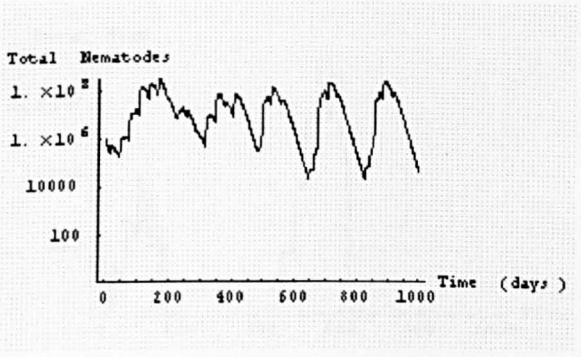
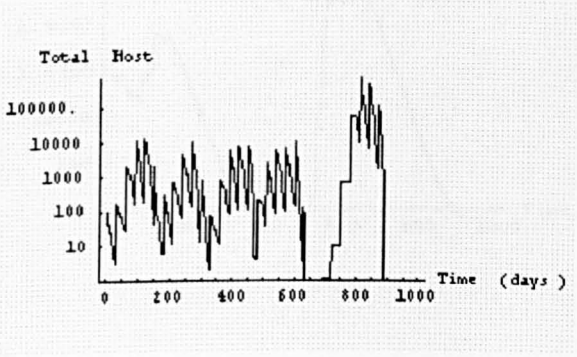
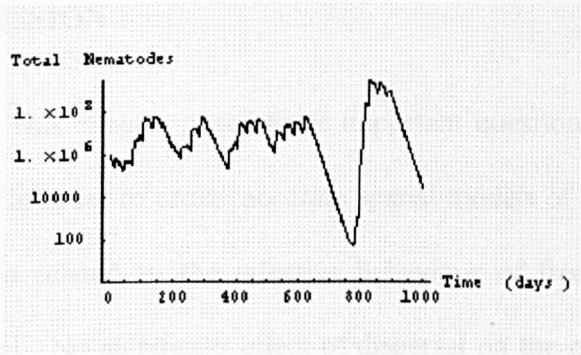
1000
100
10
0
200
400
600
800
1000
Time (days)

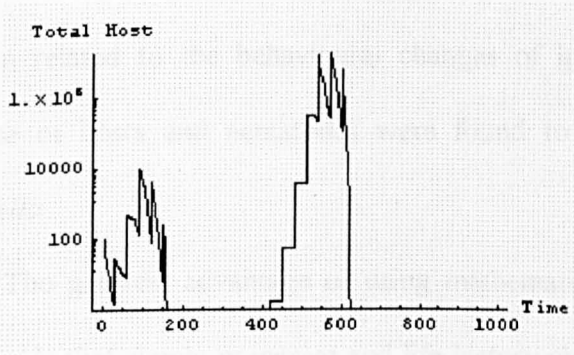

Total Nematodes
1. ×10 2
1. ×10 6
10000
100
0
200
400
600
800
1000
Time (days)

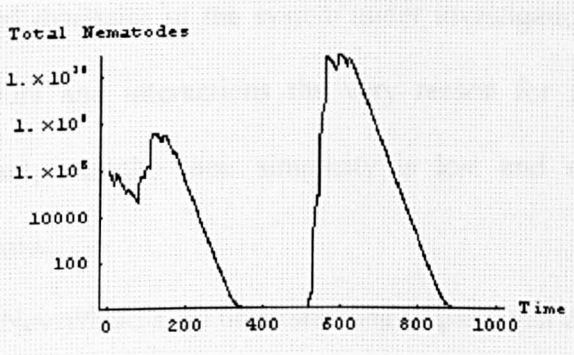
bii)


Total Host
100000.
10000
1000
100
10
0
200
400
600
800
1000
Time (days)


Total Nematodes
1. ×10 8
1. ×10 6
10000
100
0
200
400
600
800
1000
Time (days)

biii)


Total Host
1. ×10 5
10000
100
0
200
400
600
800
1000
Time


Total Nematodes
1. ×10 10
1. ×10 8
1. ×10 6
10000
100
0
200
400
600
800
1000
Time

DISCUSSION

This chapter posed three important questions about the dispersal behaviour of *S. feltiae*, in order to create accurate spatial models of nematode/host interactions. The first question related to the infective behaviour of free living infective juveniles following dispersal. No significant effect of dispersal on the proportion of nematodes that infected *G. mellonella* was detected. Secondly the rate of dispersal of infective juveniles through sand was determined as a daily rate, estimated from sand tube bioassays. The third question related to the behavioural changes of infective juveniles in response to the presence of hosts and nematodes were found to preferentially dispersed towards *G. mellonella*.

The greatest advantage of using mathematical models to investigate population dynamics is that simple processes and life-history characteristics can often encompass the observed dynamics of the system under investigation. By including ever-more complex behaviours and interactions the very reason for using mathematical models becomes redundant; namely, their simplicity is lost and so too the generic nature of their predictions.

Nevertheless, an understanding of pathogen dispersal has been shown to be vital to the effective understanding of disease spread (Dwyer, 1992; Dwyer & Elkinton, 1995; White *et al* 2000). In terms of biological control, the rate at which a pathogen disperses can affect the dynamics and determine success or failure (White *et al*, 2000). Dwyer (1992) demonstrated that Douglas Fir Tussock Moth (*Orgyia pseudotsugata*) and the spread of its Nuclear Polyhedrosis virus depended on several of the disease parameters

including the transmission rate. Here the pathogen was spread by infected individuals in the form of a travelling wave. Dwyer and Elkinton (1995) found the rate of spread of a nuclear polyhedrosis virus through its host population, Gypsy Moth (*Lymantria dispar*), was greater than predicted had infected hosts caused the spread alone. Instead they hypothesised that parasitoids vectored the virus through the moth population. For entomopathogenic nematodes, free living infective stages disperse without assistance from their hosts and therefore will have dispersal rates which are very different from the hosts.

Entomopathogenic nematode infective juveniles have a finite lipid reserve and we may expect the utilisation of this energy store through dispersal activity to affect their infectivity through some form of trade-off. I failed to detect such interaction and found that nematodes which dispersed different distances all infected hosts to the same degree. Nematodes may disperse different distances due to different allocations in these lipid levels. If this leaves nematodes at different distances with the same levels of lipids after dispersal we may expect similar proportions of nematodes to infect hosts. However, the lipid levels were not estimated and there appears to be no link between dispersal and infectivity from this study. Therefore the possibility of such an interaction in a spatial model of the system was not investigated.

The two other aims of this chapter were to determine the rate of dispersal of *S. feltiae* infective juveniles both on their own and in the presence of hosts. Entomopathogenic nematodes have previously been shown to preferentially move towards host cues (Lewis, Gaugler & Harrison, 1993; Lewis, Ricci & Gaugler, 1996; Grewal, Lewis & Gaugler, 1997; Kakoule-Duarte, 1995), but experiments reported here show that the presence of hosts increases the total number of nematodes dispersing. Similar findings

for *Trichostrongylus tenuis*, the parasitic nematode of Red Grouse (*Lagopus lagopus scoticus*), showed that the infective stages preferentially disperse towards the grouse food plant, heather, and that more nematodes were recovered when this was present (Saunders *et al* 2000). The number of nematodes dispersing different distances did not change between the time intervals in my experiment, indicating that once dispersal was initiated the nematodes distributed themselves quickly through the sand, and either ceased moving further or else followed a random dispersal pattern. Through a simple diffusion model this behaviour was quantified and a spatial model of the system was created to investigate the subsequent effects. The predicted dynamics of the system could be altered with the addition of both nematode dispersal rate and attraction of nematodes towards hosts. However, an overriding influence on the system dynamics was the rate of host dispersal. Intuitively, when hosts dispersed at a low rate, the effect of nematode dispersal became important but when hosts dispersed at a high rate the effect of nematode dispersal became unimportant in the model. In other words, when the hosts and nematodes are homogenously mixed, the system approaches the, non-spatial model where homogeneity is described.

Although the aim of our models has been to incorporate the fewest number processes to achieve a simple parsimonious model, here we have investigated a trait where infective stages become activated and preferentially disperse towards adjacent hosts leading to prediction that pest control is increased. Previous models of this system have therefore lacked important aspects of nematode behaviour.

Mathematical models of parasite-host relationships can allow us to recreate complex dynamics from simple processes. Temporal simulations of the Anderson and May (1978)

coupled differential equation models, which implicitly assume homogenous spatial interactions, can capture complex dynamics and accurately describe disease systems (Anderson & May, 1991). However, the addition of space to these models can alter their predictions, often adding stability to the systems that were previously described as unstable (Comins *et al* 1992; Comins & Hassell 1996; White *et al* 1996). These theoretical predictions have also been borne out in experimental systems that have shown space is an important dimension, which enables persistence (Huffaker, 1958). Temporal models of the dynamics of entomopathogenic nematodes and their hosts exhibit extremely unstable dynamics when parameterized for a typical host species (Fig 1a; Chapter 4; Fenton *et al* 1999; Appendix A). As we would expect, the addition of space using a coupled map lattice model can alter long term predictions of control (Fig 1b, c.), such that 'classical biological control' may be predicted when parameterized from an arbitrary set of host and nematode dispersal values (Fig 1b) or else hosts may escape parasitism (Fig 1c). Coupled map lattice models iterate a version of the model within each cell, each of which is in contact with neighbouring cells to form a grid. Each cell interacts with its neighbours by allowing movement of host and/or pathogen between them. In this way individual populations have an immigration and emigration term that can be made to be dependent on the surrounding populations. Although the preliminary model simulated in Figure 1b is parameterized from our temporal model system, the level of dispersal of nematodes or host is unknown and therefore the predictions of such simulations is limited without an accurate estimate of these vital parameters.

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Chapter 7

General Discussion

Theoretical investigations of populations and the spread of disease have become pivotal to the understanding of many applied aspects of ecology. Mathematical models offer the opportunity to investigate systems in ways which are often not possible using experimental approaches. Often, large-scale field experiments cannot be performed due to logistical problems and when considering endangered species there is a risk involved with experimental manipulation (Steinberg & Kareiva 1997). Performing model analyses and simulations can allow us to investigate the system in ways not possible by empirical methods and can also produce testable hypotheses. However, to create genuinely useful predictions, the creation of such models must go hand in hand with empirical studies which can accurately parameterise, test and validate model predictions. This thesis therefore represents the interplay of empirical data collection with the production of models of biological control using the entomopathogenic nematodes *Steinernema feltiae* and *Heterorhabditis megidis*. Although the remit of this thesis was not to produce the models themselves, the experiments described here have been part driven by the need for such parameters in the models, whilst also driving the model production itself. Experiments have shown the system to have important phenomena that must be understood for theoretical advances to take place.

First and foremost I justified the use of both parasite and host species used here. The parasites represent pathogens commercially available for the control of insect soil pests of many crops. Currently, however, their use has had limited success, where failure or success has been described by methods of trial and error. Theoretical understanding of the parasites has allowed us to predict those pests and scenarios where we can expect control to be successful (Fenton *et al* 2001).

The test host used throughout this study was late instar larvae of the greater wax moth (*Galleria mellonella*) which is highly susceptible to nematode infection (Fairbairn *et al* 2000). This host was chosen because the behaviour of the nematode infective juvenile was the main point of interest in this study. Previously information was lacking on many of the parameters used to model the dynamics of infection. Using *Galleria* I was able to determine the maximum levels of infection (Chapters 3, 4 & 5) achievable under the conditions we imposed. For example, the precise value of the transmission coefficient has been shown to greatly affect the predicted dynamics of the system (Fenton *et al* 2000). This was just one of the parameters of immediate interest.

The use of pathogenic organisms as control agents requires that they are safe both to endemic non-target organisms and the consumer. Entomopathogenic nematodes of the Families Steinernematidae and Heterorhabditidae have a ubiquitous distribution and have been identified from all continents of the world with the exception of Antarctica (Downes & Griffin, 1996). The identification of endemic species avoids many of the stringent requirements for licensing biological control agents. However, in order for an alternative method of control to become widely used it must be competitively priced and above all, effective. A major limitation with entomopathogenic nematodes of the family Steinernematidae is that only a small proportion of the potential infective population is infective at any one time (Bednarek & Nowicki 1986; Fan & Hominick 1991; Bohan & Hominick 1995; Bohan & Hominick 1996; Glazer 1997). Previous efforts have concentrated on identifying a new species of nematode with a higher proportion of infective juveniles (Downes & Griffin 1996). This thesis provides evidence that this

proportion is not a static one, and that nematodes enter an infective state over time, with over half of the population becoming infective within a 5 day period (Chapter 3).

One of the most important and yet elusive parameters in epidemiological models is the transmission coefficient, β (Anderson & May 1981; Dobson & Hudson 1992; McCallum 2001). Fenton *et al* (2000) demonstrated that entomopathogenic nematode system dynamics could be greatly affected by the precise value of the transmission coefficient. Previous estimates of β for entomopathogenic nematodes assumed a fixed sub-population, which overestimated its precise value (Bohan 1995; Westerman & Werf 1998). The workers assumed that only a small proportion of the whole infective juvenile population was able to infect hosts, and their calculations considered this sub-population to be the whole infective population. Other work has shown the importance of changing host and parasite density in order to assess the correct transmission term in models (Hochberg 1991; Knell *et al* 1996, 1998; McCallum 2000). Mass action has often been used to describe the rate of transmission in Anderson & May (1981) type models, where the individual chance of a susceptible host becoming infected remains constant as the number of susceptible hosts in the population increases. The dynamics of host/parasite interactions can alter if this assumption is not met (Hochberg 1991; Fenton *et al* in prep). This thesis presents experimental investigations designed to estimate the transmission coefficient, β, and the interaction between the rate of transmission and changing host densities for both *H. megidis – G. mellonella* and *S. feltiae – G. mellonella* systems. *A priori* knowledge of the two systems led to predictions of how the biology of the host/nematode interactions could lead to two different transmission functions.

Free living infective juveniles drive the dynamics of transmission by their requirement for a suitable environment in which to reproduce. For entomopathogenic nematodes this translates as an insect host and (in the case of gonochoristic *S. feltiae*) another nematode of the opposite sex. This is likely to cause foci of infections within one host insect where more than one nematode infects.

H. megidis is an hermaphroditic nematode, which does not require other nematodes to be present in an infection and so may transmit itself more evenly through the host population. In this case, the individual probability of becoming infected as the number of susceptible hosts increases may be expected to be constant. In other words, it was hypothesised that mass action would be an accurate predictor of disease transmission in hermaphroditic species, but non-linear transmission may better describe the transmission of gonochoristic parasites. This was indeed found to be the case.

Experiments demonstrated that the hermaphroditic *H. megidis* transmission could best be described by invoking linear mass action. However, although *S. feltiae* showed non-linear responses in the rate of transmission with changing host densities and low nematode densities, mass action provided a good fit to the data. This result was found in spite of the fact that nematodes were not randomly mixed amongst infected hosts at all nematode densities investigated. A possible explanation for this finding is that only one entomopathogenic nematode is required to kill *G. mellonella*. Calculations of β which use the number of susceptible hosts alive at the start and finish of an experiment would not capture the distribution of nematodes amongst the host population. For the purposes of modelling then, the most parsimonious function of transmission in both the *H. megidis* – and *S. feltiae*- *G. mellonella* system is mass action.

The last chapter investigates the extensions of differential equation models into spatial coupled-map-lattices. The addition of space to such models has been recognised as adding stability to systems that are otherwise unstable. Non-spatial models of entomopathogenic nematodes exhibit highly unstable dynamics (Fenton *et al* 2000). Rohani and Miramontes (1995) investigated the effects of discrete dispersal by hosts and parasitoids, where the parasitoids dispersed preferentially towards hosts. They demonstrated that large parasitoid dispersal rates inhibited persistence of the system. Here, it has been demonstrated with coupled-map-lattice models of entomopathogenic nematodes that control predictions can alter due to the addition of host and nematode dispersal. In the absence of nematode dispersal, predictions of host and nematode exponential growth became predictions of nematode and host cyclic dynamics, when the proportion of hosts which disperse each day was changed from 0.3 to 0.4. At low host dispersal rates, the hosts effectively escaped control of nematodes within their patch and because nematodes could not move into these patches, refugia were created. Dispersal of hosts from these patches maintained the nematode population in surrounding cells by providing them with an increasing number of susceptible hosts over time and both host and nematode populations increased exponentially. At the slightly higher rate of host dispersal, the system became more homogenously mixed, and the dynamics became similar to those of the non-spatial models which implicitly assume random mixing. The system is therefore highly sensitive to rates of dispersal, and although dispersal rates for hosts were not investigated here, estimating nematode dispersal rates creates an important starting point to which specific host systems can be added later.

Experiments involved the dispersal of infective juveniles through a highly porous substrate, sand, which provides estimates of the maximum achievable dispersal rates for nematodes. It is important to describe the optimum behaviours of nematodes when we have no other information on these behavioural aspects, as this provides us with the full spectrum over which models should be investigated.

This thesis combined experimental investigations into several aspects of nematode dispersal and infection, with model simulations predicting their effect on the dynamics of host/nematode interactions. The addition of nematode dispersal and attraction towards hosts predicted much lower numbers of hosts or even extinction than when such parameters were not included in spatial simulations.

Models were created and examined to simulate the use of entomopathogenic nematodes as biological control agents. The culmination of experimental and modelling investigations has provided us with informative models which can be used to predict host traits, scenarios and application methods which would result in effective control by these nematodes. Moreover, provided with information on likely target systems we can now rapidly provide cheap predictions of the outcome of using entomopathogenic nematodes as biological control agents in these cases.

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