

## **Modelling nematode behaviour to identify the most promising traits and strategies to improve biocontrol by *Heterorhabditis* spp.**

**P.R. Westerman, W. van der Werf**

*Wageningen Agricultural University, Department of Theoretical Production Ecology,  
P.O. Box 430, 6700 AK Wageningen, The Netherlands*

### **Summary**

Control success with heterorhabditid nematodes varies with nematode species, isolate, production and storage conditions, and environmental conditions after application. These factors affect nematode behaviour. A model that simulates numbers of nematodes in space and time from the moment of application on a sand column until penetration into a host was used to identify, (1) which nematode traits can best be used for improvement, and (2) what is the most promising strategy of improvement. The sensitivity of simulated mortality of larvae of *Otiorynchus sulcatus* and *Galleria mellonella* by *Heterorhabditis* spp. to changes in nematode behavioural parameters was quantified and related to genetic and environmental variation found in the nematodes. Parameters characterizing nematode movement had little influence on simulated mortality. Parameters characterizing aggregation and arrestment had a large effect on simulated mortality, but no variation has been found for these traits in *Heterorhabditis*. Parameters characterizing penetration (rate and proportion infectious) had a moderate effect on insect mortality. The most promising strategy to enhance insect mortality by *Heterorhabditis* would be to exploit the genetic (*G. mellonella*) and non-genetic (*O. sulcatus*) variation in the proportion infectious nematodes.

### **Introduction**

The control of soil-inhabiting pests by insect parasitic nematodes, *Heterorhabditis* Poinar, and *Steinernema* Travassos, can be very effective. To infect an insect, the nematodes have to move towards the target insect (movement) and penetrate into the haemocoel (penetration). Inside the haemocoel, they release a symbiotically associated bacterium from the intestine. These bacteria are insect pathogenic and are mainly responsible for killing the insect host (virulence). The success of a soil application with nematodes is influenced by a combination of factors, including pest species, environmental factors (mainly temperature and moisture), soil factors (e.g. type and soil structure), and nematode isolate (Georgis and Gaugler, 1991) and pre-treatment of the nematodes (production and storage).

In this study a model is used (1) to identify which nematode traits can be best used for improvement of biocontrol, and (2) which strategy of improvement is most promising. The best strategy for improvement depends on the amount and source of variation in behaviour. Potential improvement strategies are screening species or isolates, breeding, improving production and storage conditions, or controlling environmental conditions after application. The model integrates the available knowledge of the processes underlying the nematode-host-soil system. It simulates the spatio-temporal dynamics of a nematode population from the moment of application on a sand column until penetration into a host. Sensitivity of

simulated control success to parameters characterizing behavioural traits is quantified and related to available genetic or environmental variation in the parameters. There is scope for improvement if there is both sensitivity and variability. The model was parameterized for the NW European HF85 in the presence of a highly susceptible host, *Galleria mellonella* (L.), or the less susceptible black vine weevil, *Otiorhynchus sulcatus* F., at 20 °C.

## Materials and methods

**Model.** A dynamic simulation model was developed using the state variable approach. It was written in FST (Rappoldt & Van Kraalingen, 1996). Details are given in Westerman (1997). The following behavioural processes are included: temporary accumulation near the soil surface, dispersal in soil, arrestment near hosts, and penetration into hosts. Mortality of nematodes is not considered. The model represents a system consisting of a 9 cm high sand column with (optional) an insect host (*O. sulcatus* or *G. mellonella*) at the bottom. Nematodes are applied on top of the cylinder. The effect of aggregation of nematodes among hosts was accounted for by translating the mean number of penetrated nematodes per insect into proportions infected insects using fitted negative binomial distributions with density dependent  $k$  (characterized by the parameters  $a$  and  $b$ ) (Westerman, 1997).

**State variables.** State variables characterizing the change of the system in time are the numbers of nematodes in six soil layers (1.5 cm), temporarily immobilized nematodes in the top layer, arrested nematodes in the layer containing a host insect, and penetrated nematodes. Penetration is irreversible. A nematode population consists of a proportion infectious ( $C$ ) and non-infectious individuals (Bohan & Hominick, 1996). Non-infectious nematodes are assumed to behave in exactly the same way as infectious nematodes, except that they do not penetrate. Non-infectious nematodes are accounted for in a separate array of state variables and all processes are calculated for both groups separately.

**Parameters.** The rates at which the numbers of nematodes, accounted for by state variables, change per unit time depend on the number of nematodes and the relative rate of change, i.e. the relative rate of dispersal ( $M$ ), immobilization ( $I$ ), remobilization ( $EI$ ), arrestment ( $A$ ), escape from arrestment ( $EA$ ), and penetration ( $B$ ). Parameter values with respect to immobilization, movement and host localization were determined by controlled random search using the software package SENECA (De Hoop *et al.*, 1992) on data from experiments similar to those described by Westerman (1995). Arrestment was virtually absent in the presence of *O. sulcatus* ( $A/(EA+A) \rightarrow 0$ ) and practically complete in the presence of *G. mellonella* ( $A/(EA+A) \rightarrow 1$ ).

**Sensitivity analysis.** The sensitivity of the model was tested for changes of 50% in the parameters controlling immobilization, movement, and penetration, starting from parameter values for HF85 (batch 1; Westerman, 1997). The effect of arrestment was tested by changing the proportion arrested nematodes ( $A/(EA+A)$ ) from 1 to 0.66 ( $A = 2*EA$ ) in case of *G. mellonella*, and from 0 to 0.66 in case of *O. sulcatus*. The effect of these changes on numbers penetrated nematodes was evaluated after a simulation period of 2 days and 3 weeks for *G. mellonella* and *O. sulcatus*, respectively.

**Genetic and environmental variation.** Data from this and previous studies (Westerman, 1995; 1997) on the behaviour of a number of heterorhabditid isolates were used to estimate genetic and environmental variation in parameter values. It is unknown if there is genetic variation in the relative penetration rate,  $B$ , because it was assessed for HF85

only. There was little or no environmentally induced variation in this parameter (Westerman, 1997). Genetic or environmental variation in the degree of aggregation was assumed absent.

## Results and discussion

Sensitivity analysis (Table 1) shows that the model for *G. mellonella* is especially sensitive to changes in the proportion infectious nematodes ( $C$ ) and arrestment ( $A/(EA + A)$ ), relatively insensitive to changes in the relative penetration rate ( $B$ ), and virtually insensitive to changes in parameters characterizing immobilization ( $I$  and  $EI$ ) and dispersal ( $M$ ). The model for *O. sulcatus* is (highly) sensitive to changes in arrestment, the proportion infectious nematodes and the relative penetration rate, less sensitive to the changes in parameters characterizing immobilization, and insensitive to changes in relative dispersal rate. Although not visibly included in the model, the model is highly sensitive to changes in the degree of aggregation of nematodes.

**Table 1.** Percentage change in the number of penetrated nematodes after a 50% change in parameter values for the heterorhabditid HF85 at 20 °C, starting from 61.06 nem. per *G. mellonella* after 2 days and 12.70 nem. per *O. sulcatus* after 3 weeks per 100 nematodes applied, at standard parameter values.

input	dimension	<i>G. mellonella</i>			<i>O. sulcatus</i>		
		standard value	+50%	-50%	standard value	+50%	-50%
$I$	[1/min.]	$3.6 \times 10^{-3}$	-0.0	0.0	$58.5 \times 10^{-3}$	-18.7	29.1
$EI$	[1/min.]	$22.7 \times 10^{-3}$	0.1	-0.3	$6.0 \times 10^{-3}$	18.0	-31.7
$M$	[1/min.]	$96.4 \times 10^{-3}$	0.2	-1.0	$89.0 \times 10^{-3}$	0.4	-1.1
$A/(EA+A)$	[min./min.]	1	n.a.	-29.0*	0	74.4*	n.a.
		1	n.a.	-43.4**	0	57.8**	n.a.
$B$	[1/min.]	$1.3 \times 10^{-3}$	2.6	-14.8	$0.3 \times 10^{-3}$	31.4	-42.4
$C$	[infect. nem./total nem.]	0.63	50.0	-50.0	0.29	50.0	-50.0

n.a. not applicable

\*  $A = 1 \times 10^{-2}$ [1/min.];  $EA = 0.5 \times 10^{-2}$ [1/min.]; \*\*  $A = 1 \times 10^{-3}$ [1/min.];  $EA = 0.5 \times 10^{-3}$ [1/min.]

The best strategy for improvement depends on the amount and source of variation in behavioural traits. Sensitivity in model parameters was related to available genetic and non-genetic variation, as illustrated in Table 2. It shows that three categories of behavioural traits can be distinguished: 1) Those with influential but invariable parameters, such as aggregation and arrestment. They have a large influence on model outcome and are therefore most important to control success. However, they vary little among nematodes and are typical for the host. 2) Those with relatively influential and variable parameters, such as penetration. These are eligible to improvement. 3) Those with variable parameters that have little influence on model outcome, such as movement. Control success would benefit little from improvement of this trait.

The behavioural trait with the largest potential for improvement of mortality of both

*G. mellonella* and *O. sulcatus* is the proportion infectious nematodes, *C*, which has a considerable influence on model outcome and which shows genetic and environmentally induced variation. Appropriate strategies for improvement would be selective breeding and collecting new heterorhabditid isolates with enhanced proportion infectious nematodes. However, heritability would be low, especially in case of *O. sulcatus*, because the proportion non-genetic variation is relatively large. Therefore, an alternative strategy would be to raise the proportion infectious nematodes first by optimizing production and storage conditions (responsible for non-genetic variation). In case of *O. sulcatus* as a host, the relative penetration rate, *B*, may similarly be improved by selective breeding or collecting new isolates, if there is genetic variation for this trait (which is unknown). In this case heritability would be high, because so far no environmentally induced variation has been found for this parameter.

**Table 2.** Summary of the available information on sensitivity of behavioural traits of *Heterorhabditis* spp. (see Table 1) and on the level of genetic and environmental variation in these traits for biocontrol of *G. mellonella* and *O. sulcatus* at 20 °C.

		<i>G. mellonella</i>			<i>O. sulcatus</i>		
		Sensitivity to	Source of variation		Sensitivity to	Source of variation	
			50% change	genetic		non-genetic	50% change
% immobile	<i>EI/I</i>	very low	high	interm.	low	high	interm.
movement	<i>M</i>	very low	high	interm.	low	high	interm.
% arrested	<i>EA/A</i>	low-interm.	none	none	interm.-high	none	none
penetration	<i>B</i>	low	unknown	none	interm.	unknown	none
% infectious	<i>C</i>	interm.	high	interm.-high	interm.	interm.	interm.
aggregation	<i>a, b</i>	high	none	none	high	none	none

low  $\approx$  0-35% ; intermediate  $\approx$  35-70%; high  $\approx$  70-100%

## References

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