Experiments and simulation studies on the system
Lolium perenne / *Tylenchorhynchus dubius*

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Abstract

Den Toom, A.L., 1990. Experiments and simulation studies on the system *Lolium perenne/Tylenchorhynchus dubius.* Thesis, Agricultural University Wageningen.

The host/parasite system *Lolium perenne* L./*Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936 was studied in experiments and by simulation, to elucidate the nature and functioning of the relationship between pasture and ectoparasitic nematodes.

The influence of temperature and soil moisture on the system and the incidence of tolerance and resistance in the host were studied in experiments under controlled conditions.

A mathematical simulation model was developed for the host/parasite system; preliminary models for the population dynamics of the nematode and the influence of the nematode on plant growth are presented.

Measurements and simulations indicate that the ratio of nematode numbers to young roots is a main determinant of the effect of the nematodes on plant growth. The role of *T. dubius* and other ectoparasitic nematodes in the failure of reseeded pastures is discussed.

Additional keywords: nematodes, pasture, reseeding

Stellingen

1   Het vermijden van de noodzaak tot herinzaai van grasland is de beste methode om herinzaaiproblemen te voorkomen.

    M. Hoogerkamp, 1984. Changes in productivity of grassland with ageing. Veehouderij 72 (1987), Themanummer Graslandverbetering.

2   Als grasland opnieuw ingezaaid wordt, is keuze van de zaaidatum een bepalende factor voor het slagen van de herinzaai.

    dit proefschrift

3   In proeven bedoeld om de schadelijkheid van planteparasitaire nematoden te bepalen, moet het stadium waarin de planten blootgesteld worden aan de invloed van nematoden gelijk zijn aan het plantstadium waarin dat onder teeltomstandigheden gebeurt.

    dit proefschrift

4   Veldproeven zijn niet geschikt om de schadelijkheid van ectoparasitaire nematoden te bepalen.

5   Het ontwerpen van een verklarend model voor een waardplant/ parasiet systeem is een uitstekende methode om er achter te komen over welke onderdelen van de interactie de kennis ontoereikend is.

    dit proefschrift

6   Zoeken naar drempelwaardes voor dichtheden waar beneden schadelijke organismen geen effect hebben is zinloos.

    R. Cousens, 1985. A simple model relating yield loss to weed density. Annals of Applied Biology 107: 239-252.

7   Redacties van wetenschappelijke tijdschriften zouden aangeboden artikelen zonder naam van de auteur ter beoordeling moeten geven aan hun referenten.

8   De controle op naleving van impopulaire maatregelen dient niet te berusten bij het ministerie dat traditioneel de belangen van de getroffen groep behartigt.

9   De geringe beschikbaarheid van vaste banen in het wetenschappelijk onderzoek bemoeilijkt voor vrouwen die een wetenschappelijke carrière ambiëren de keuze voor het moederschap.

10   De in het tweede structuurschema Verkeer en Vervoer voorgestelde verbeteringen van het streekvervoer kunnen pas goed tot hun recht komen als er ook geld uitgetrokken wordt om de rijstijl van de chauffeurs te verbeteren.

11   Voor wie eenmaal aan een promotieonderzoek begonnen is, is promoveren het minste van twee kwaden.

Stellingen behorende bij het proefschrift van A.L. den Toom:
Experiments and simulation studies on the system *Lolium perenne/Tylenchorhynchus dubius*.
Wageningen, 18 september 1990.

Dankwoord

Contents

Account

The chapters 2 to 5 have been included into the following publications:

Chapter 2   Toom, A.L. den, 1988. Influence of temperature and soil moisture on the relation between *Tylenchorhynchus dubius* and *Lolium perenne*. Netherlands Journal of Plant Pathology 94: 33-44.

Chapter 3   Toom, A.L. den, 1988. Response of ten cultivars of Lolium perenne to the ectoparasitic nematode *Tylenchorhynchus dubius*. Netherlands Journal of Plant Pathology 94: 105-110.

Chapter 4   Toom, A.L. den, 1989. Simulation of the host/parasite system *Lolium perenne/Tylenchorhynchus dubius* 1. Population dynamics of *T. dubius*. Netherlands Journal of Plant Pathology 95: 15-26.

Chapter 5   Toom, A.L. den, 1990. Simulation of the host/parasite system *Lolium perenne/Tylenchorhynchus dubius* 2. The effect of *T. dubius* on *L. perenne*. Netherlands Journal of Plant Pathology, in press.

1 General introduction

Renewal of pastures is common practice in the Netherlands. Yearly approximately 150 000 ha of permanent pasture - 13% of the total grassland area - are reseeded (Sibma and Ennik, 1988). The decision to reseed a pasture is mainly based on its botanical composition. If the fraction of *Lolium perenne* is too low and too many weed grasses (*Agropyron repens* and *Poa annua*) are present, reseeding is recommended. The seed mixtures used at reseeding consist almost exclusively of varieties of *L. perenne*.

Farmers are advised to reseed in the period from the end of July until mid-September or, second-best, in early spring (Van Dijk, 1987). However, in practice many pastures are reseeded after mid-September in autumn.

Reseeding does not always have the desired effect; it fails in about 10% of the area and less severe problems occur on much of the reseeded area. For instance, on 9% of 69 reseeded pastures included in a survey, the reseeding was so unsatisfactory that it had to be repeated; on 21% reseeding was not completely successful, but another reseeding was not required (Van Bezooijen, unpublished results). Reseeding failures seem to occur most frequently on sandy soils, but may also occur on clay and peat soils (Van Bezooijen, 1986). On the latter two soil types the risk of failure increases rapidly when reseeding is carried out after mid-September (Van Dijk, 1987).

When the initial growth of grass sown after arable crops was compared with that of reseeded grass it was found that soilborne pests and diseases were responsible for the failure of reseeding (Clements et al, 1982; Hoogerkamp, 1984b). Nematicide application at reseeding often results in grass establishing better (Van Bezooijen, 1979; Hoogerkamp, 1984a; Labruyère, 1979; Spaull, 1985; Spaull et al, 1985). This suggests that nematodes may be involved in reseeding failures.

Many parasitic nematode species occur in pasture soils (Van Bezooijen, 1986; Bridge, 1971). Table 1.1 shows the predominant species in soil samples taken from pastures on sandy soil in August. In spring large numbers of infective larvae of *Heterodera mani* and *Meloidogyne naasi* may be present in the soil (Van Bezooijen, 1986; Maas and Brinkman, 1977). These species and also large numbers of the migratory endoparasite *Pratylenchus* can be detected in the roots during the growing season.

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Table 1.1 Numbers and species of the most abundant plant parasitic nematode genera in 32 samples of sandy soil collected in the Netherlands. Survey 1977 (Van Bezooijen, 1986 and unpublished results)

Genus	μ	σ	Species
Tylenchorhynchus and *Merlinius*	850	276	*T. dubius*
			T. maximus
			M. nanus
			M. microdorus
Paratylenchus	961	751	*P. nanus*
			P. projectus
Helicotylenchus	198	104	*H. pseudorobustus*
Pratylenchus	161	49	*P. crenatus*
Trichodorus	40	21	*T. similis*

μ average number per 100 ml soil recovered with an Oostenbrink elutriator.

Table 1.2 shows the effect of nematicide application at reseeding on the dry matter yield of the first cut of 16 experimental fields. On 3 of these fields - Hurley (UK), Maarheeze 82 and Rutten - the yield was considerably higher when nematicides were applied. For the fields in Maarheeze and Rutten, this could be attributed to the elimination of *Meloidogyne naasi* and *Heterodera mani*, respectively. The yield increase on the field in Hurley resulted partly from control of stem-boring *Diptera* (Spaull et al, 1985). On the other fields, where endoparasitic species were not predominant, yield increase as a result of nematicide application was less or even absent.

The endoparasitic species seem to be capable of causing more damage than the ectoparasites. On the other hand, ectoparasitic species are always abundant in pastures, whereas the endoparasitic species *Heterodera mani* and *Meloidogyne naasi* seem to occur less frequently. Therefore, the influence of both ectoparasites and endoparasites on the growth of reseeded pastures needs to be studied.

In view of their abundance and pathogenicity to *Lolium perenne* the following ectoparasitic nematode species and genera have been identified as being responsible for reseeding failures : *Tylenchorhynchus dubius* (Brinkman, 1979), *Longidorus elongatus* (Brinkman, 1979) and *Tylenchorhynchus* together with *Helicotylenchus* (Spaull, 1985). The pathogenicity of *Helicotylenchus pseudorobustus, Tylenchorhynchus maximus*

and *Paratylenchus spp* to *L. perenne* has been shown in inoculation experiments (Van Bezooijen, 1977).

No clear relation has been found between density and composition of the nematode population and the degree of reseeding failure. Reseeding failure occurs with completely different populations of ectoparasitic nematodes. Indications for interactions between nematodes and fungi have been found (Labruyère, 1979), but these aspects were not investigated further in the present study.

Even when problems can be largely attributed to one organism, it is almost impossible to define generally applicable relations between numbers of the parasite and yield (Ferris, 1986; Rabbinge and Bastiaans, 1989). Defining

Table 1.2 Yield of plots treated with nematicides, as percentage of yield of untreated plots. First cut in the spring following reseeding.

location	seeding time	soil type	yield %	nematicide[1]	reference
Hurley (UK)	8-80	sandy loam	188[2]	aldicarb	Spaull et al (1985)
Achterberg	9-77	sandy soil	115	aldicarb	Van Bezooijen (1979)
Maarheeze	8-82	sandy soil	270[3]	oxamyl	Van Bezooijen (1984)
Maarheeze	8-84	sandy soil	110	oxamyl	Van Bezooijen and
Maarheeze	8-85	sandy soil	87	"	Murray (1989)
Bruchem	8-84	clay	103	"	"
Bruchem	8-85	clay	100	"	"
Zegveld	8-85	peat	111	"	"
Heino	8-85	sandy soil	118	"	"
Rutten	8-85	sandy soil	303[4]	"	"
-	8-77	sandy soil	111	oxamyl or	Hoogerkamp (1984a,b)
-	9-79	sandy soil	106	aldicarb	"
-	9-76	clay	107	"	"
-	10-78	clay	89	"	"
-	10-78	clay	119	"	"
-	9-79	sandy soil	99	"	"

[1] if different rates and formulations were applied the percentual yield is calculated from thee mean yield of these treatments
[2] yield determined 8 weeks after seeding, stem boring Diptera caused damage to the control.
[3] *Meloidogyne naasi*
[4] *Heterodera mani*

such relations is even more difficult if several pathogens and parasites are involved (Zadoks, 1985). Because of the large variability of the environmental conditions at reseeding and the possible involvement of anumber of species, generally applicable relations between density and composition of the ectoparasitic nematode population and yield of reseeded grass cannot be defined.

It might be possible to determine empirical relations for a range of environmental conditions. This would require much experimentation and the applicability of such relations is limited. Nevertheless such relations are used to determine the need to apply nematicides. In the Netherlands it is prohibited to apply nematicides when reseeding pastures. This situation is unlikely to change, because of the government's policy of reducing the use of pesticides (Anonymus, 1989). Thus, nematode problems at reseeding have to be prevented or minimized by means of other agronomic practices. For this to succeed the nature and functioning of the nematode/pasture system and its dependence on environmental conditions need to be known, rather than descriptive empirical relations.

Mathematical simulation of host/parasite systems can be a helpful tool to elucidate these systems. Combination models, in which knowledge of the biology of parasite and host is incorporated, can help to clarify the quantitative interpretation of changes caused by the presence of parasites in various plant physiological processes (Rabbinge and Bastiaans, 1989). This insight may help to develop strategies to minimize the effect of the parasite.

The study described in this thesis was aimed to explore the nature and functioning of the system comprising pasture and ectoparasitic nematodes. The subsystem *Lolium perenne* L./ *Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936 was studied.

T. dubius is one of the most numerous and common ectoparasitic nematodes in pastures (Van Bezooijen, 1986). Its biology is relatively well known (Sharma, 1971; Wyss, 1973).

The *L. perenne/T. dubius* system was approached in two ways. First, by studying its behaviour in pot experiments. Secondly, by developing simulation models of the population dynamics of the nematode and the influence of the nematode on its host.

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2 Influence of temperature and soil moisture on the relation between *Tylenchorhynchus dubius* and *Lolium perenne*

ABSTRACT   The relation between the ectoparasitic nematode *Tylenchorhynchus dubius* and the growth and production of *Lolium perenne* during the first month after sowing, was studied in pot experiments at several temperatures and moisture levels.

The nematode reduced the growth of the grass, mainly as a result of activity during the first weeks after seeding. The percentage decrease of dry matter yield was smaller when temperature was more favourable for plant growth. Decreased moisture content of the soil enhanced the effect of nematodes at 10 °C.

2.1 INTRODUCTION

The problems encountered when reseeding pastures in the Netherlands might be due to nematodes (Van Bezooijen, 1979). A survey of Dutch pastures in 1977 showed that grassland contains large numbers of plant-parasitic nematodes (J. van Bezooijen, personal communication). The populations consist mainly of ectoparasitic nematodes. Short periods without a host hardly influence the population density of ectoparasitic nematodes (Simons, 1973). So these populations will also be present at reseeding. The repercussions of these ectoparasitic nematodes on the growth and production of newly sown grass are not well defined. For example, in experiments with the nematode *Tylenchorhynchus dubius* on *Lolium perenne*, Seinhorst and Kozlowska (1979) did not find yield reduction at densities lower than 1.8 nematodes per gram of soil at 18 °C, whereas Sharma (1971) found a decrease in dry matter production of 35% at a density of 0.2 nematodes per gram at 25 °C. These conflicting results may be due to differences in environmental conditions between the experiments.

In this chapter the relation between nematode density and growth and yield reduction is described in the host/parasite system *L. perenne/T. dubius*. *L. perenne* is the prevailing species in seed mixtures used for reseeding grassland. *T. dubius* is one of the most common and numerous ectoparasitic nematodes in Dutch pastures (J. van Bezooijen, personal communication). This nematode feeds on epidermal cells of the apical meristem, the zone of elongation and the root hair zone and on the root hairs (Klinkenberg, 1963;

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Wyss, 1973). It has been found that on various hosts, reduction of dry matter yield is accompanied by effects on shoot/root ratio, morphology, or development (Laughlin and Vargas, 1972; Sharma, 1971). Given the location of nematode attack, effects on water balance and uptake of nutrients might be expected. By quantifying the aforementioned effects and evaluating their consequences for crop growth, the relation between *T. dubius* and *L. perenne* can be elucidated.

2.2 MATERIALS AND METHODS

At three temperatures and at various moisture treatments, grass growing on soil inoculated with nematodes was compared with grass growing on non-inoculated soil (moisture experiments; T10, T18 and T25). In another experiment (density experiment, N25) the influence of different initial densities of the nematode population on the plant was studied. An overview of these experiments is given in Table 2.1.

Soil and nematodes
A population of *T. dubius* was multiplied on *L. perenne* grown in containers of a steam-sterilized sandy soil. The containers were kept in a greenhouse, at 18 °C. Similar containers of non-inoculated soil were treated in the

Table 2.1. Experimental conditions and factors. All experiments were carried out in eight replications and had a randomized block design. Daylength was 16 h in all experiments.

Experiment code	T^1 (°C)	Irradiance[2] (W m^{-2})	RH (%)	Moisture treatments[3]	Nematode treatments[4]	Sampling times (days after emergence)
T10	10	43	73	W,D	N,C	20, 43
T18	18	108	70	W,D,E	N,C	14, 22, 36, 48
T25	25	108	70	W,D	N,C	22, 30
N25	25	92	70	D	C,N1,N2,N3,N4	16, 30

[1] Day temperature equal to night temperature.
[2] Measured at soil level.
[3] W= wet; D= dry; E= extra dry.
[4] N= soil inoculated with *T. dubius* (N1...N4= increasing initial densities of the nematode); C= non-inoculated control.

same way to provide soil for the controls. Before the soil was used in an experiment, the plants were removed from the containers and the soil was sieved. The moisture content was determined by measuring the weight loss of a sample after drying in an oven for 24 h at 100 °C. Moisture content of the soil was expressed as mass percentage water per unit dry soil. The relation between moisture content and pF is shown in Fig. 2.1.

Moisture experiments
At the start of the experiments, pots (9x9x9.5 cm³) with a net volume of 400 ml were filled with either inoculated or non-inoculated soil at a dry bulk density of 1.2 g ml⁻¹ and 16 seeds of *L. perenne* cv. Pelo were sown per pot at a depth of 0.5 cm.

NPK 20-20-20 fertilizer (458 mg) containing trace elements was supplied to each pot as a watery solution. In addition, in T18 a second application of half of this fertilizer dose was given the day after the third sampling time, because of the fast growth of the plants and the relatively long duration of this experiment. The soil was covered with a layer of gravel to reduce evaporation. The pots were placed under controlled conditions (Table 2.1). After emergence, the plants were thinned to 12 per pot in experiment T18. Because this number was found to be large relative to the volume of the pot, in the other experiments the number of plants was reduced to 9.
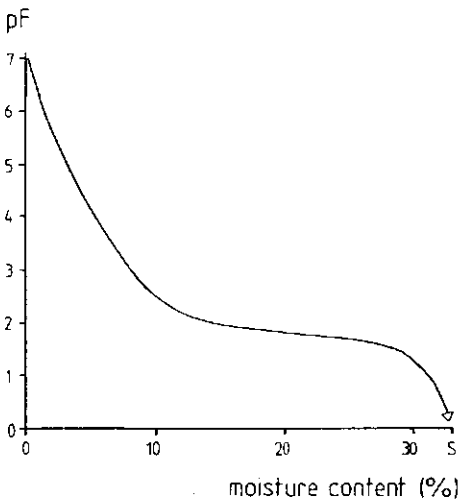
Fig. 2.1   pF curve of the soil used in the experiments. Horizontal axis: moisture content of the soil (mass of water as a percentage of dry mass of the soil).

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At the start of the experiment the moisture content of the pots was determined by weighing each pot (with known mass of dry soil, pot and gravel) and adjusted to the moisture levels mentioned in Table 2.2. As soon as the moisture content in one of the pots in the wet treatment fell below the minimum values mentioned in Table 2.2, all pots of all treatments were watered in the same way as at the start of the experiment. The frequency of watering ranged from once in four days to once in three days in experiment T10 and from once in two days to twice a day at higher temperatures. In experiment T18 the initial moisture contents of the soil in treatments D and E exceeded 16 and 9%, respectively. In these treatments, half of the dose needed to compensate for evapotranspiration was supplied, until moisture content was at the levels mentioned above. It took about a week to achieve this.

Density experiment

To create a series of nematode densities, inoculated soil and non-inoculated soil were mixed. The resulting densities corresponded to 0, 1, 10, 50 and 100% of the density of the inoculated soil. The experiment was prepared and conducted in the same way as described for the moisture

Table 2.2. Moisture treatments.

Experiment	Treatment[1]	Maximum[2]		Minimum[3]	
		%	pF	%	pF
T10	W	25	1.6	15	1.9
	D	16	1.9		
T18	W	25	1.6	11	2.3
	D	16	1.9		
	E	9	2.7		
T25	W	25	1.6	12	2.2
	D	12	2.2		
N25	D	12	2.2	8	3.0

[1] See Table 2.1 for treatment codes.
[2] Maximum: moisture content of the soil immediately after watering.
[3] Minimum: if moisture content in the wet treatments of the moisture experiments or the control of the density experiment fell below the values indicated, frequency of watering was increased for all treatments of the experiment.

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experiments, but to ensure that enough plants would emerge at the high
nematode densities used in this experiment, 20 seeds were sown per pot
instead of 16.

Nematode counts
The population density of the nematodes was determined on the day of
emergence (initial density) and at each sampling time. For this purpose
four pots were elutriated with an Oostenbrink elutriator on the day of
emergence, two pots from each treatment at the intermediate sampling times
and three pots from each treatment at the final sampling time. In
experiment N25, juveniles and adult males and females were counted
separately on the day of emergence and at the final sampling time.

Crop observations
At the sampling times mentioned in Table 2.1, destructive sampling took
place. The aboveground parts were severed at the soil surface. The number
of leaves per pot was counted. Fresh mass per pot was determined and a
subsample was taken to measure leaf area. The rest of the shoot was dried
in an oven at 100 °C for 16 hours.
   Soil was washed of the underground parts (roots and stembases) -
henceforth called roots - and they were dried. At the third sampling time
of T18 and the final sampling time of the other experiments the N, P and K
contents were measured in a sample from each treatment.

2.3 RESULTS

Nematodes
The nematode population increased exponentially in all nematode treatments,
except for the dry treatment of experiment T18 in which no significant
multiplication was found. The relative growth rate of the populations
increased more than proportionally with temperature (Table 2.3). In
experiment T18, decreased moisture content of the soil led to a lower
relative growth rate.
   In the density experiment, the relative growth rate (RGR) of the
nematode population was lowest at the highest density (Table 2.4). The
percentage of juveniles increased from about 60 to about 80 at all
densities.

Table 2.3. Initial density, final density and relative growth rate (RGR) of the nematode population under different moisture conditions.

Experiment	Treatment[1]	Initial density[2]	Final density[3]	RGR[4] (d^{-1})
T10	NW	5.3a[5]	12.4b	0.020
	ND		12.8b	0.021
T18	NW	6.2a	31.7b	0.033
	ND		23.5c	0.028
	NE		8.1a	0.006
T25	NW	3.3a	35.1b	0.079
	ND		27.4b	0.070

[1] See Table 2.1 for treatment codes.
[2] Mean of four replicates, expressed as thousands per pot (480 g dry soil in a volume of 400 ml)
[3] Mean of three replicates, expressed as thousands per pot (480 g dry soil in a volume of 400 ml)
[4] Based on four observations in time for T18 and three for T10 and T25.
[5] For each experiment values followed by a different letter differ significantly at p<0.05 (Student's t-test).

Table 2.4. Initial and final population densities of the nematodes and relative growth rate of the nematode population (RGR) in soils inoculated with different concentrations of nematodes.

Trea-ment[1]	Initial density[2]				Final density[2]				RGR
	m	f	j	tot	m	f	j	tot	
C	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.000
N1	0.1	0.2	0.4	0.7	0.2	0.4	1.8	2.3	0.042
N2	1.3	1.3	4.6	7.3	1.8	3.2	29.8	34.7	0.052
N3	5.0	7.3	22.2	34.5	6.2	8.6	70.3	84.8	0.030
N4	12.5	16.1	43.8	72.4	9.9	11.1	86.0	106.7	0.013

[1] See Table 2.1 for treatment codes.
[2] Number of males (m), females (f), juveniles (j) and total number of nematodes (tot) expressed in thousands per pot. The differences between the treatments are significant (Student's t-test, p<0.05).

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Crop measurements
Emergence Time of emergence varied with temperature: 15 days at 10 °C, 5 days at 18 °C and 4 days at 25 °C. Neither nematodes nor moisture conditions influenced the time of emergence or number of emerged plants.

Dry matter production In the density experiment, dry matter yield decreased significantly in all nematode treatments except the one with the lowest initial density. Fig. 2.2 shows the relation between the logarithm of initial density and dry matter yield at the final sampling time. The correlation coefficient found by linear regression was 0.99; whereas a correlation coefficient of 0.94 was found for regression of dry matter yield on final density of the population.

In the moisture experiments, nematodes had significantly decreased dry matter yield at all sampling times of all experiments, except for the last sampling time of experiment T18. At this sampling time no negative effect of nematodes was found in the wet treatment, and the negative effect was small in the medium treatment. The decline of the effect of nematodes with increasing water content was probably due to growth reduction in the controls, caused by the restricted room for growth in the pots. To facilitate evaluation of the influence of temperature, moisture and time on
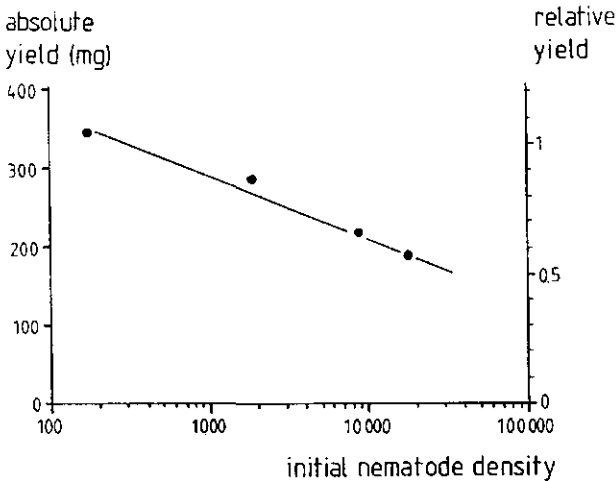

Fig. 2.2 Relation between initial nematode population per 100 ml soil and final yield of the nematode treatments in the density experiment N25. Dry matter yield is expressed in mg per pot on the left-hand vertical axis and on the right hand vertical axis as a fraction of dry matter yield of the control.

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the effect of nematodes, dry matter yield of the nematode treatments was expressed as a fraction of dry matter yield of the corresponding controls (relative yield). In experiment T10 both moisture treatments had a relative yield of 0.70 at the first sampling time. Relative yield decreased with time to 0.40 in the wet treatment and 0.33 in the dry treatment. At the first three sampling times of T18 the relative yield was about 0.80 in all moisture treatments. At the final sampling time it was 1.00 in the wet treatment, 0.93 in the medium treatment and 0.80 in the dry treatment. In T25 the relative yield was about 0.60 in both moisture treatments at both sampling times. The relative yield was constant with time in N25 and ranged from 1.00 at the lowest nematode density to 0.55 at the highest nematode density (Fig. 2.2).

Growth analysis Growth was analysed to elucidate the effect of nematodes on dry matter yield. The relative growth rate (RGR) of the plants could not be calculated directly from data on dry matter yield, because insufficient data were available. Therefore, the RGR of the plants was calculated from transpiration data. The transpiration of the plants was calculated from the transpiration of the pots, by subtracting the evaporation. Daily evaporation was assessed from transpiration of the pots before emergence. Initially, cumulative transpiration increased exponentially with time (Fig. 2.3). By plotting the logarithm of cumulative transpiration against time, the RGR of the plants can be calculated as the regression coefficient of the linear part of this curve. This procedure is only valid if there is a constant ratio between transpiration and leaf area and between leaf area and dry mass (i.e. a constant transpiration coefficient, TC). Although this was not the case for the total duration of the experiments, the differences in TC between the first two sampling times of T18 were so small that this assumption might be reasonable for a shorter period.

Relative rate of increase of the plant mass at the later sampling times was calculated from the increase in the logarithm of cumulative transpiration between the watering times preceding and following the sampling time. Leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) were calculated from measurements at the sampling times; net assimilation rate (NAR) was derived from RGR and LAR. The results of growth analysis for the first sampling time are shown in Tables 2.5 and 2.6.

The largest RGR was found in experiment T18, indicating that this temperature was most favourable for growth of perennial ryegrass. Nematodes caused a lower RGR during exponential growth. After the period of exponential growth the relative rate of increase of plant mass decreased

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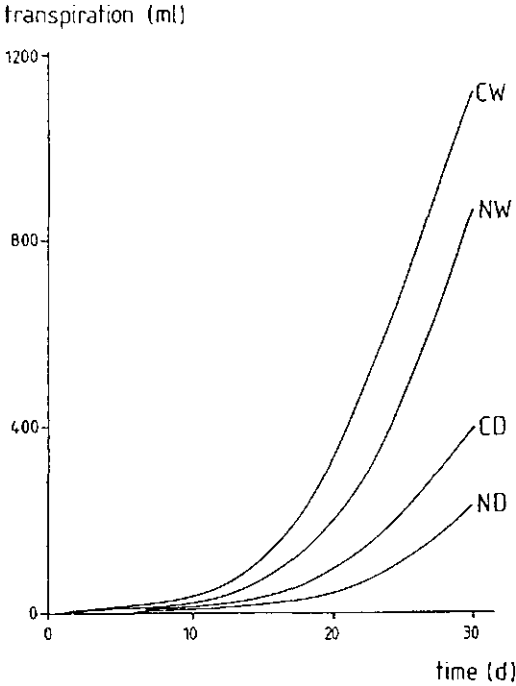
transpiration (ml)

Fig. 2.3 Course of cumulative transpiration in time in experiment T25. Cumulative transpiration is expressed in ml per pot, and time in days after emergence. CW: wet control, NW: wet nematode treatment, CD: dry control, ND: dry nematode treatment.

with time in all treatments. The relative rate of increase of dry mass of the nematode treatments was generally higher than in the controls after the period of exponential growth, but in T10 it was still lower than in the controls at the final sampling time.

The lower RGR during exponential growth was due to decreased LAR in experiments T18 and N25. In experiment T25 the first sampling took place about a week after the end of the period of exponential growth. At this time no causes of reduced RGR could be found any more. The decrease in LAR was caused by a decrease in LWR in T18 and by a decrease in SLA in T18 and T25. In T10, LWR was reduced by nematodes, but this did not result in a decrease of LAR in the wet treatment, because SLA was increased by nematodes. Generally, the influence of lower moisture content on RGR, LAR, LWR and SLA at the first sampling had the same direction as that of nematodes. In the experiments at T25 and N25 , LWR was highest in the nematode treatments at the later sampling times. In experiments T18 and

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Table 2.5. Growth analysis of the moisture experiments for the first sampling time, and relative growth rate during exponential growth.

Experiment	Treatment[1]	Time[2]	RGR[3]	NAR[4]	LAR[5]	LWR[6]*	SLA[7]*	TC[8]*
T10	CW	20	0.18	0.7	241	0.75	323	139
	NW	20	0.16	0.6	284	0.73	389	152
	CD	20	0.16	0.6	254	0.70	360	123
	ND	20	0.16	0.7	240	0.67	365	101
T18	CW	14	0.35	2.6	133	0.62	216	192
	NW	14	0.35	3.1	112	0.60	188	210
	CD	14	0.33	2.9	113	0.61	186	141
	ND	14	0.32	3.8	93	0.56	167	140
	CE	14	0.31	3.1	111	0.61	184	112
	NE	14	0.26	3.4	77	0.52	148	86
T25	CW	16	0.31					
		22	0.15	1.0	149	0.62	242	227
	NW	16	0.30					
		22	0.16	0.9	175	0.64	273	232
	CD	16	0.25					
		22	0.16	1.0	149	0.59	253	141
	ND	16	0.24					
		22	0.18	1.2	152	0.60	254	122

[1] See Table 2.1 for treatment codes.
[2] Duration of period of exponential growth (d) and sampling time in days after emergence (if a second value is indicated, this is the sampling time).
[3] Relative growth rate (d^{-1}).
[4] Net assimilation rate (mg cm^{-2} d^{-1}).
[5] Leaf area ratio (cm^2 g^{-1}).
[6] Leaf weight ratio (g g^{-1}).
[7] Specific leaf area (cm^2 g^{-1}).
[8] Transpiration coefficient (g g^{-1}).
* Results of analysis of variance for the effect of nematodes ($p<0.05$):
   LWR   T10, T18, T25: significant;
   SLA   T10, T25: interaction, T18: significant;
   TC   T10, T18: not significant, T25: interaction.

Table 2.6. Growth analysis of the density experiment N25 for the first sampling time, and relative growth rate during exponential growth.

Treatment[1]	Time[2]	RGR[3]	NAR[4]	LAR[5]	LWR[6]*	SLA[7]*	TC[8]*
C	16	0.23	2.5	186	0.67	276	142
N1	16	0.23	3.0	160	0.68	240	138
N2	16	0.22	2.7	162	0.66	252	129
N3	16	0.19	2.7	140	0.69	206	117
N4	16	0.17	2.7	124	0.70	176	113

[1] See Table 1 for treatment codes.
[2] Duration of period of exponential growth (d) and sampling time in days after emergence.
[3] Relative growth rate (d^{-1}).
[4] Net assimilation rate $(mg\ cm^{-2}\ d^{-1})$.
[5] Leaf area ratio $(cm^2\ g^{-1})$.
[6] Leaf weight ratio $(g\ g^{-1})$.
[7] Specific leaf area $(cm^2\ g^{-1})$.
[8] Transpiration coefficient $(g\ g^{-1})$.
* Results of analysis of variance for the effect of nematodes ($p<0.05$):
LWR : not significant
SLA : significant
TC : significant

T25, SLA was significantly higher in the nematode treatments at the final sampling times. This effect was caused by a higher dry matter content in the controls, which in turn was probably caused by the overcrowding of the roots.

The influence of nematodes on TC at the first sampling time varied with moisture content of the soil. In the dry treatments, nematodes caused a decrease, whereas a tendency towards increase was found in the wet treatments. Lowering the moisture content caused the TC to decrease both in the controls and in the nematode treatments. At the final sampling times, a significant increase in the TC was found in experiment T10 and the wet treatment of experiment T25. This does not necessarily imply an increase in transpiration per unit dry matter produced; it may also mean that part of the dry matter produced had died.

Development Generative development could not occur because the plants were not vernalized. If assessed from the number of leaves, development was reduced to the same extent as growth; the relation between number of leaves and dry mass of the shoot was described by the same curve for all treatments and all experiments. This means that the mass invested in each successive leaf is not influenced by environmental conditions. The number of leaves per tiller was not influenced. Adventitious roots were formed at about the same time as tillering. This process had started by the first sampling of all experiments, except for the nematode treatments of experiment T10.

NPK content Both nematodes and lower moisture content caused the contents of N, P and K to increase. The slower the growth rate of the plant, the higher the contents of N, P and K.

2.4 DISCUSSION AND CONCLUSIONS

Tylenchorhynchus dubius causes growth reduction of *L. perenne*. Clear symptoms do not occur. Growth retardation is accompanied by a proportional reduction of vegetative development. The influence of nematodes on the contents of N, P and K resembles the general effect of growth retardation. Growth analysis showed that growth retardation is mainly connected with a decrease in LWR and SLA during the period of exponential growth. The effect on LWR was most important at the lower temperatures, whereas the effect on SLA occurred at higher temperatures. In the dry treatments, including the density experiment, TC was initially decreased by nematodes, whereas no effect occurred in the wet treatments. According to the functional equilibrium theory of Brouwer (1962), a decrease in the LWR is a response to decreased uptake of water and/or nutrients. Nematodes reduce water uptake in another way than reduced moisture content of the soil; in the controls, decreased moisture level always caused a decrease of the TC and sometimes a decrease of the SLA. The reduction of water uptake by the nematodes is most severe in the first period of growth of the host, since the effects of nematodes on LWR, SLA and TC disappear later on, or even change direction.

The percentage decrease of dry matter yield in the nematode treatments did not increase after the formation of adventitous roots had started. So the plant seems to be most susceptible to damage by nematodes in the period in which it depends completely on the seminal roots. This may be because of differences in the morphology of the two root systems; possibly the death

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of epidermal cells hampers root elongation more in the thin seminal roots than in the thicker adventitious roots. Because the nematodes can only feed on young roots, the increase over time in the fraction of old roots may also explain the decrease in the plant's susceptibility.

In the density experiment a linear relation was found between the logarithm of initial density of the nematode population and final dry matter yield of the plant. This relation, which indicates that the effect per nematode decreases with nematode density, has been found for many nematode/host combinations, both in pots and in the field (Oostenbrink, 1966). So it is reasonable to assume that such a relation also applies to the *T. dubius/L. perenne* nematode/host combination at environmental conditions other than those in the density experiment, although the values of the parameters will change.

The relation between nematode density and dry matter production is influenced not only by environmental conditions, but also by the condition of the nematode population at the time of sowing. In the dry treatment of T25 both effect per nematode and RGR of the nematode population were larger than at comparable initial densities in the density experiment, whereas environmental conditions were about the same. The main difference between the two experiments was the much higher density in the stock population of the density experiment. Probably, high nematode density causes irreversible changes in reproductive potential and food requirements of the nematodes involved. Since population density in the stock populations did not vary much for the moisture experiments, it is assumed that differences in effect on dry mass between these experiments are due solely to differences in nematode numbers and environmental conditions and not to differences in condition of the stock populations.

The effect of *T. dubius* on dry matter production was smaller when temperature was nearer to the optimum for growth of *L. perenne*. A decrease in the moisture content of the soil hardly increased the effect of nematodes, except in T10. In this experiment the effect of nematodes on relative yield at the second sampling was enhanced by suboptimal water supply, whereas no difference between the moisture treatments was found at the first sampling. This suggests that the greater damage in the dry treatment resulted from the longer duration of the susceptible period. The same explanation may hold for the influence of sub- and supra-optimal temperatures on damage. But the lack of effect of suboptimal water supply at the higher temperatures shows that conditions that decrease the growth rate of the plant do not necessarily enhance the effect of nematodes. A smaller susceptibility may have compensated for the longer duration of the

susceptible period in the dry treatments. Because water supply in the dry treatments was regular, the plants were able to adapt to suboptimal moisture content of the soil. As nematodes cause an effect comparable to moisture stress, this adaptation may have reduced the plant's susceptibility to the effect of nematodes. Incidental moisture stress will probably have more influence on the effect of nematodes.

Environmental conditions affect the relation between nematodes and their host also by directly influencing the nematode. At the first sampling time the effect in T25 was more marked than in T10. Since the feeding rate of nematodes increases with temperature (Boag, 1980), this may be caused by the nematodes having a higher feeding rate at higher temperatures. However, the results at the final sampling suggest that the influence of environmental conditions on the host is more crucial for the amount of damage than their direct influence on the nematode. It is therefore concluded that a given population of *T. dubius* will cause the smallest percentage decrease in the dry matter yield of *L. perenne* when environmental conditions are most favourable to the growth of the grass.

3 Response of ten cultivars of *Lolium perenne* to the ectoparasitic nematode *Tylenchorhynchus dubius*

ABSTRACT   The interaction between 10 cultivars of *Lolium perenne* and *Tylenchorhynchus dubius* was studied to investigate the incidence of tolerance and resistance to ectoparasitic nematodes in *L. perenne*.

Differences in tolerance were small; the nematode treatments yielded 67 to 76 % of the dry shoot mass of the controls. Cultivars with a low leaf weight ratio and a low transpiration coefficient tended to have a somewhat better tolerance. Differences in resistance occurred, but resistance is of minor importance to reduce damage in the *L. perenne/T. dubius* host/parasite system.

3.1 INTRODUCTION

Nematodes contribute to reseeding problems of pastures (Van Bezooijen, 1979). The major part of the population of plant-parasitic nematodes in pastures consists of ectoparasitic species. It is not known whether cultivars of the predominant grass species Lolium perenne differ in tolerance and resistance to ectoparasitic nematodes. To ascertain the incidence of tolerance and resistance in *L. perenne*, the interaction between the ectoparasite Tylenchorhynchus dubius and ten cultivars of *L. perenne* was studied. *T. dubius* is one of the commonnest and most numerous nematode species in Dutch pastures.

The reaction of dry matter production of the host on nematode infestation determines the degree of tolerance, and the reaction of the nematode to its host (as measured by the multiplication of the nematode population) determines the degree of resistance (Oostenbrink, 1972). Degree of tolerance and degree of resistance usually do not correlate, and a different experimental design is needed to assess both characteristics accurately. To measure tolerance, the population density must be sufficiently high to cause measurable damage, whereas resistance can be determined best when initial density is low in relation to the available root mass and the duration of the experiment is long enough to permit the formation of several generations. In chapter 2 it was shown that *L. perenne* is most susceptible to damage by *T. dubius* during the first weeks of growth. Multiplication of the population during this period is small. So, susceptibility of the plant seems to be more determinant for the amount of

Table 3.1. Name, type and ploidy of the cultivars of *L. perenne* used in the experiment.

Cultivar	Type	Ploidy
Gremie	production	2n
Hora	production	2n
Perma	production	2n
Pelo	production	2n
Meltra	production	4n
Artal	production	4n
Royal	turf	2n
Manhattan	turf	2n
Ensporta	turf	2n
Idole	turf	2n

damage than the nematode's ability to reproduce on the plant. Therefore, the experiment described in this chapter concentrated on tolerance and its correlation with other plant characteristics.

3.2 MATERIALS AND METHODS

Table 3.1 gives an overview of the cultivars of *L. perenne* used. In a pot experiment under controlled conditions each cultivar was grown in soil containing *T. dubius* and in non-infested soil (control). The experiment contained eight replications and was carried out as described in chapter 2. Per pot, 25 seeds were sown and the number of plants was reduced to 16 after emergence. Daylength was 16 h, temperature 25 °C (day and night), irradiance 92 W m^{-2} and relative humidity 70%. At watering, the moisture content of the soil was adjusted to 25%. The method of watering was comparable with the method described in chapter 2, but generally the water need of each cultivar/nematode treatment combination was determined by measuring water loss in two replicate pots; each third time of watering all pots were weighed.

In the period from 29 to 31 days after seeding, destructive sampling took place. The aboveground parts were severed at the soil surface. Fresh mass was determined and in four replicates a subsample was taken to measure leaf area. The rest of the shoot was dried in an oven at 100 °C for 24

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hours. In the other replicates total dry mass of the shoot was determined. The soil was washed off the underground parts and they were then dried.

Initial density of the nematode population was determined on the day after the start of the experiment by elutriating four pots with an Oostenbrink elutriator. Final density in the nematode treatments was determined in three pots of each cultivar. Both times, adults and juveniles were counted separately.

3.3 RESULTS

Nematodes

Initial density was 5.9×10^3 nematodes per pot. Ninety % of the population were adults. Final density ranged from 6.1×10^3 to 13.8×10^3 nematodes per pot with a mean of 10.7×10^3. For 'Ensporta' and 'Idole' final population density was significantly smaller than the mean, and for 'Pelo', 'Artal' and 'Manhattan' it was significantly larger. The percentage juveniles increased in all cultivars, although less so in 'Ensporta' and 'Idole'. Since the growth of the nematode population was found to be exponential in comparable experiments with *T. dubius*, the relative growth rate of the population was calculated (Table 3.2).

Crop observations

T. dubius decreased the dry matter yield of all cultivars. Except for 'Gremie' both shoot mass and root mass were decreased (Fig. 3.1). Because the coefficient of variation for root dry mass was large (about 40%), the effect on root dry mass was not significant in an analysis of variance.

The dry matter yield of the nematode treatment of each cultivar was expressed as a fraction of the dry matter yield of the control of that cultivar. Using t-tests the resulting relative yields were all compared with the mean relative yield. The mean relative yield was 0.71 for the shoot (Table 3.3), 0.88 for the roots·and 0.76 for total dry mass. 'Gremie' differed significantly from the other cultivars, because of the absence of an effect of nematodes on the root mass of this cultivar. The relative yield of the shoot of 'Gremie' did not differ from the mean.

Transpiration of the plants was calculated from transpiration of the pots by subtracting evaporation, which was assessed from transpiration of the pots before emergence. Leaf weight ratio (LWR), transpiration coefficient (TC) and specific leaf area (SLA) of all cultivar/treatment

Table 3.2. Effect of 10 cultivars on final density, relative growth rate and composition of the population of *T. dubius*. Initial density was 5.9×10^3 *T. dubius* per pot for all cultivars and initial percentage of juveniles was 10.

Cultivar	Final density[1]	RGR[2]	J[3]
Gremie	9.9	0.017	79
Hora	8.8	0.013	72
Perma	10.6	0.020	77
Pelo	13.7*	0.028	77
Meltra	12.5	0.025	77
Artal	13.8*	0.028	79
Royal	11.3	0.022	77
Manhattan	13.3*	0.027	73
Ensporta	6.1*	0.001	60
Idole	7.8*	0.009	65
mean	10.7	0.020	

[1] Expressed in thousands per pot (480 g dry soil in a volume of 400 ml). Values followed by * are significantly different from the mean (t-test, $p<0.05$).
[2] RGR= relative growth rate (d^{-1}). A period of 30 days was taken to calculate RGR.
[3] J = percentage juveniles in the final population.

combinations were calculated (Table 3.4). An analysis of variance revealed a significant cultivar effect on the magnitude of these characteristics. Nematodes significantly influenced LWR, TC and SLA. To ascertain whether the effect of nematodes on relative yield of the shoot correlated with LWR, TC and SLA, Spearman's correlation test was carried out. The same was done to investigate the relation between the decrease in relative yield of the shoot and the magnitude of the effect of nematodes on LWR, TC and SLA. Nematodes generally decreased LWR. The magnitude of the decrease did not correlate with the effect of nematodes on the relative yield of the shoot, but a lower LWR in the control correlated with a smaller effect of nematodes on relative yield. TC was either increased or not influenced by nematodes. When expressed per unit shoot mass, transpiration was increased in all cultivars. A smaller TC in the control correlated with a smaller effect of nematodes on relative yield. Nematodes increased SLA for all cultivars. No correlation was found between SLA in the control and relative yield.
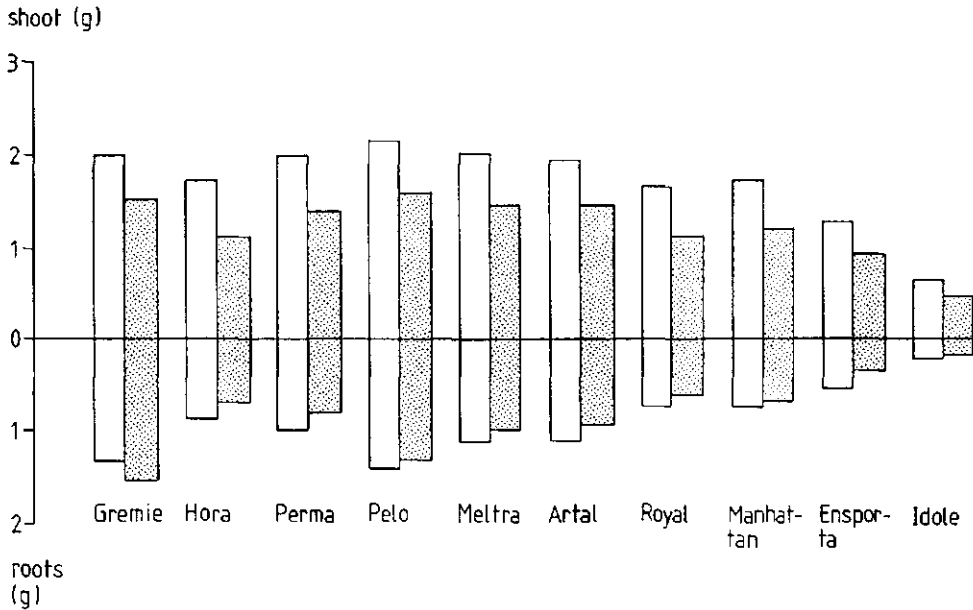
shoot (g)

Fig. 3.1 Shoot dry mass and root dry mass per pot of 10 cultivars of *L. perenne* after 30 days growth in soil containing *T. dubius* (grey bars) and in non-infested soil (white bars)

3.4 DISCUSSION

As the effect of nematodes on shoot yield shows, no large differences in tolerance were found between the cultivars. The shoot mass of the nematode treatments was 67 to 76 % of that of the controls. Cultivars with a relatively large root system (low LWR) and a low TC tended to have a somewhat greater tolerance.

T. dubius generally decreased LWR and increased the TC and SLA. The magnitude of these effects did not correlate with the degree of tolerance. In experiments with *T. dubius* on *L. perenne* cv. Pelo it was shown that the effect of the nematode on the dry mass of the plant was mainly connected with a decrease in LWR and SLA in the first week of growth. The magnitude of the effects on LWR, SLA and TC varied with time and even changed direction (Chapter 2). So, it is unlikely that there will be a strong correlation between tolerance and the magnitude of the effect of nematodes on the above-mentioned characteristics 30 days after sowing.

Although the experiment was not designed to measure resistance, an indication of differences in resistance was obtained from the figures on

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Table 3.3. Relative yield of the shoot (RS) of 10 cultivars in response to a nematode treatment.

Cultivar	RS[1]	C[1]
		(g/pot)
Gremie	0.74	2.03
Hora	0.67	1.73
Perma	0.70	1.99
Pelo	0.73	2.15
Meltra	0.73	2.02
Artal	0.76	1.92
Royal	0.67	1.66
Manhattan	0.68	1.76
Ensporta	0.72	1.27
Idole	0.73	0.66
mean	0.71	1.72

[1] For each cultivar dry matter yield of the nematode treatment is expressed as a fraction of dry matter yield of the corresponding control (C). None of the relative yields differs significantly from the mean (t-test, $p < 0.05$)

Table 3.4. Leaf weight ratio (LWR), transpiration coefficient (TC) and specific leaf area (SLA) of control (C) and nematode (N) treatments of 10 cultivars.

Cultivar	LWR ($g\ g^{-1}$)		TC ($g\ g^{-1}$)		SLA ($cm^2\ g^{-1}$)	
	C	N	C	N	C	N
Gremie	0.62	0.55	178	177	196	202
Hora	0.69	0.66	190	208	204	210
Perma	0.69	0.69	176	193	190	219
Pelo	0.63	0.58	170	180	182	192
Meltra	0.66	0.62	175	198	168	186
Artal	0.66	0.63	170	199	178	199
Royal	0.70	0.69	208	215	189	217
Manhattan	0.71	0.64	193	190	187	208
Ensporta	0.72	0.76	213	248	207	242
Idole	0.74	0.71	218	239	313	361

final nematode density and RGR (Table 3.2). 'Pelo', 'Artal' and 'Manhattan'seem to be less resistant than the other cultivars. Nematode reproduction on 'Ensporta' and 'Idole' was small, but the increase of the percentage juveniles in these cultivars showed that it did occur.

This small reproduction is not necessarily caused by a high degree of resistance of 'Ensporta' and 'Idole'. From the results of Sharma (1971), it may be concluded that the part of the population that contributes to reproduction is determined more by the expansion of the root system than by the mobility of the nematodes. The root growth of 'Ensporta' and 'Idole' was relatively slow, and this may be why the nematode reproduced poorly on these cultivars.

4 Simulation of the host/parasite system *Lolium perenne/ Tylenchorhynchus dubius* 1. Population dynamics of *T. dubius*

ABSTRACT An explanatory dynamic simulation model of the population dynamics of the ectoparasitic nematode *Tylenchorhynchus dubius* during the first month after the grass *Lolium perenne* was seeded has been developed, using published data.

Experimental results were simulated to estimate certain parameters and to test the model. The results of experiments at temperatures between 18 and 25 °C in which no moisture stress occurred, were described well by the model. However, the population increase was underestimated for low temperatures and overestimated at sub-optimal water supply for the host. To improve the explanatory value of the model, better data are needed on nematode fecundity and on the relation between temperature and nematode development.

To simulate population dynamics for periods exceeding a few weeks, the effect of root growth on nematode development and oviposition rate has to be incorporated. This is probably also needed to explain the effect of soil moisture on population increase.

4.1 INTRODUCTION

Every year about 10% of the grassland in the Netherlands is reseeded. On 5 to 10% of the reseeded area problems are encountered during the establishment phase (De Jong and Boeken, 1985). Nematodes are assumed to be at least partly responsible for these problems (Van Bezooijen, 1984; Spaull et al, 1985). Ectoparasitic nematodes are always abundant in old pastures (Van Bezooijen, 1986). Whether they cause problems at reseeding depends on environmental conditions (Van Bezooijen, 1985).

To determine the effect of environmental conditions on the relation between ectoparasitic nematodes and grass hosts, the ectoparasitic nematode/grass system *Tylenchorhynchus dubius* (Bütschli,1873) Filipjev, 1936/*Lolium perenne* L. was studied (Chapter 2) and an explanatory dynamic simulation model of these interactions is being developed. The model consists of submodels for the population dynamics of the nematode and the growth of the host. This chapter describes a preliminary submodel for the population dynamics of *T. dubius*.

The model is based on published data and has been constructed according to the state variable approach (De Wit and Goudriaan, 1978).

4.2 THE MODEL

Description
The structure of the model is represented in a relational diagram in Fig. 4.1.

The life cycle of *T. dubius* is divided into five development classes: eggs (including the first juvenile stage which moults inside the egg),
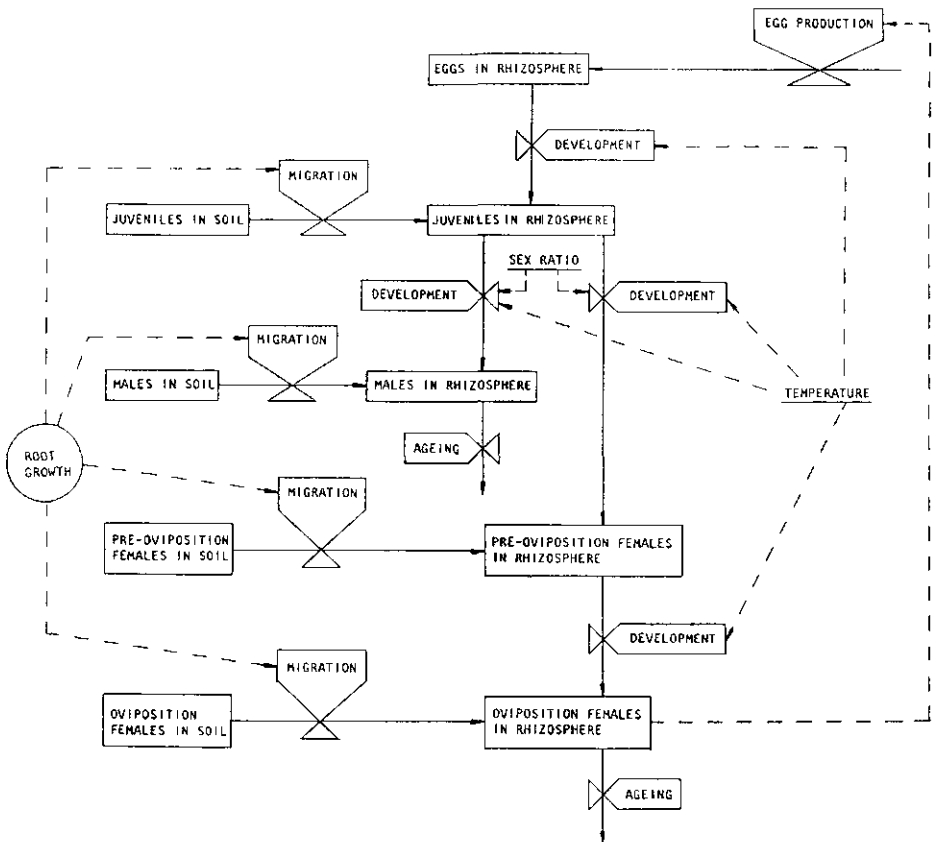

Fig. 4.1 Relational diagram for the population dynamics of *Tylenchorhynchus dubius*. Rectangles: state variables; Valve symbols: rate variables; Underlined: driving variables; ⎯⎯▶ flow of material; ⎯ ⎯ ⎯▶ flow of information.

juveniles (J2 to J4), adult males, pre-oviposition females and reproductive females. Apart from the eggs, all stages are mobile and parasitic, even when moulting (Sharma, 1971). The model simulates the changing numbers of individual nematodes in each of the development classes, both in soil and on the roots, as a function of temperature and root growth. A direct effect of soil moisture on the nematodes is not included in the model.

The nematode population in the soil outside the rhizosphere is assumed to be homogeneously distributed. These nematodes enter the rhizosphere as a result of rhizospere expansion, which is calculated from the increase of root length and the rhizosphere radius. The rhizosphere radius is defined as the distance over which nematodes are attracted to the roots, plus the radius of the root. Nematodes in the rhizosphere are assumed to stay there. So, when the rhizosphere volume is equal to the pot-volume, the nematode population in the soil is assumed to be depleted and migration stops. In this preliminary model the population in the soil is assumed to consist of mobile stages only. So, eggs only occur in the rhizosphere as a result of oviposition and not as a result of migration. No quantitative data are available on the eggs of *T. dubius* in the soil, because the eggs are lost in the usual extraction procedures. Ignoring them is not a major shortcoming in the structure of the model, since the eggs of *T. dubius* do not function as a resting stage (Simons, 1973). Mortality of the nematodes in the soil is ignored. No data are available on the mortality of *T. dubius* during the first weeks after removal of a host, but it is expected to be limited during this period.

In the model, development and oviposition occur only on the roots. It is assumed that no overpopulation occurs on the roots during the first weeks of the growth of the host. Therefore, development and oviposition of nematodes in the rhizosphere are assumed to be independent of food supply.

To enable the introduction of age-dependent processes such as mortality in a later phase of model development, development of the nematode is simulated with an escalator boxcar train (Goudriaan and van Roermund, 1989). Each stage is divided into a number of age classes of equal length. The content of each age class is transferred to the next class whenever the residence time in a class is exceeded. The residence time equals the average duration of the development stage, divided by the number of age classes. The duration of development depends on temperature. The outflow from the last age class of the eggs is quantitatively transferred to the first age class of the juveniles. The outflow from the juveniles is divided into males and pre-oviposition females, according to the sex-ratio. Since fertilization of *T. dubius* is not hampered by low densities (Seinhorst, 1966), all pre-oviposition females are assumed to develop into reproductive

females, even at low population densities. The outflow from the last age class of adult males and reproductive females represents mortality by ageing. Mortality during development is ignored, because there are no published data on this. The juveniles and adults migrating into the rhizosphere are assumed to be equally distributed over their respective age classes.

Fecundity is assumed to be independent of temperature, as is the case for *Aphelenchus avenae*, (Fisher, 1969), *Tylenchus emarginatus* (Gowen, 1970) and *Pratylenchus penetrans* (Mamiya, 1971). The oviposition rate of *T. emarginatus* and *P. penetrans* at constant temperatures is about constant during the first weeks of the oviposition period, and declines thereafter. Since the estimated length of the oviposition period of *T. dubius* is shorter than that of the above-mentioned species, the oviposition rate of *T. dubius* is assumed to be independent of the age of the female during the total oviposition period in this preliminary model.

Quantification
Conversion of root mass into root length Root mass is introduced as a function of time. Root mass is converted into root length with a conversion factor of 6.67 cm root length per mg root mass on the basis of data on *L. perenne* cv Pelo (J.G. Mulder, personal communication).

Rhizosphere radius *T. dubius* is not specifically attracted to the roots of *L. perenne* (Sharma, 1971). The maximum distance over which non-specific attractants are assumed to act is 2 cm (Prot, 1980). Since the radius of the roots of *L. perenne* is negligible compared with this figure, a maximum rhizosphere radius of about 2 cm is realistic. To test the importance of this parameter, the effect of values of 2, 1, 0.5 and 0.1 cm on the simulated results will be evaluated.

Development and ageing At 20 °C the duration of the development stages is 8 days for the egg, 26 days for the juveniles, 8 days for the males, 4 days for the pre-oviposition females and 14 days for the reproductive females (estimated from Sharma, 1971). The influence of temperature on the duration of the stages is derived from data on the life cycle of *Tylenchus emarginatus* (Gowen, 1970) and *Pratylenchus penetrans* (Mamiya, 1971). Because there are no reasons to assume that any of the stages of *T. dubius* is more sensitive to temperature than the others, it is assumed that the duration of each stage is influenced in the same way as the length of the total life cycle (Fig. 4.2).
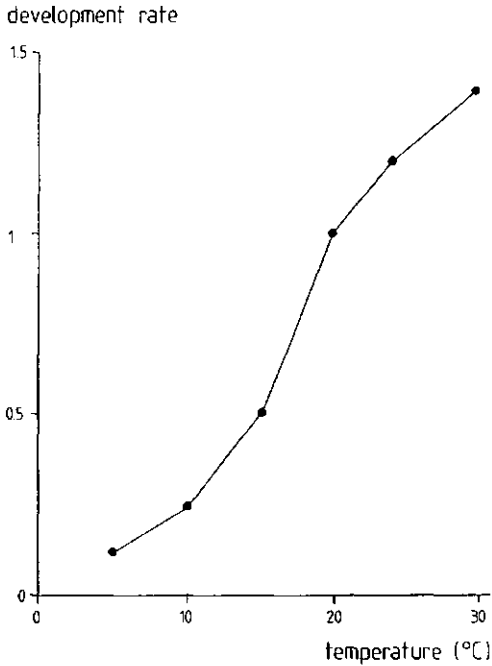
development rate

Fig. 4.2 Effect of temperature on the development rate of the nematode. The rate is expressed as a fraction of the development rate at 20 °C.

Fecundity Sharma (1971) found a maximum fecundity of 12 eggs for *T. dubius* and Bridge (1971) found an average fecundity of 6 eggs for *T. maximus*. These values are low compared with the averages of 180 and 50 found for *Tylenchus emarginatus* (Gowen, 1970) and *Pratylenchus penetrans* (Mamiya, 1971) respectively. The low values found for *Tylenchorhynchus* are probably due to experimental conditions. To estimate fecundity, experimental results will be simulated using values of 10, 20, 30 and 40 eggs per female. The value of 40 is derived from the average egg production of 3 eggs per female of *T. dubius* in 24 hours at 22 °C. found by Brzeski and Dowe (1969), assuming a constant oviposition rate during two weeks.

Oviposition rate The oviposition rate is calculated as the quotient of the fecundity and the temperature dependent duration of the reproductive stage.

Sex ratio *T. dubius* is an obligatory amphimictic species. It is generally expected that such species have a strong genetic mechanism of sex determination that will insure the development of approximately equal

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numbers of males and females in each generation (Triantaphyllou, 1973). Sharma (1971) found no clear influence of environmental conditions on sex ratio, and intersexes are not known for this species. Therefore, a juvenile sex ratio of 50 % is assumed.

The model is written in Fortran. It uses Euler's rectilinear integration method and a time step of integration of 0.05 day.

4.3 SIMULATIONS

The most uncertain parameters in the model are fecundity and rhizosphere radius. First, the fecundity was estimated by simulating an experiment in which migration could be ignored. Using the resulting value, other experiments were simulated to estimate the rhizosphere radius.

The ability of the model to explain the effects of moisture stress and host cultivar on the nematode population was then tested.

Initial nematode population
The initial numbers of juveniles, males and females in the simulated experiments were known. From these figures the initial concentration of the stages in soil was calculated. The females were divided over the pre-oviposition and the oviposition stage according to the estimated duration of these stages, so 23% in the pre-oviposition stage and 77% in the reproductive stage. The initial population in the rhizosphere was calculated from the nematode concentration in soil and the initial rhizosphere volume, assuming a homogeneous distribution over the age classes within each stage.

Fecundity
Data on the increase of *T. dubius* on *L. perenne* at 18 °C (J.G. Mulder, personal communication) were used to estimate the fecundity. The simulation was set to start about a month after the grass had been seeded. By this time migration has ended, so the total population was assumed to reside on the roots.

Population increase was simulated with values for fecundity of 10, 20, 30 and 40 eggs per female (Fig. 4.3). The total active population (juveniles and adults on the roots) was described reasonably well when values for fecundity were 30 and 40. The number of females was underestimated with both values, but the value of 30 gave a better
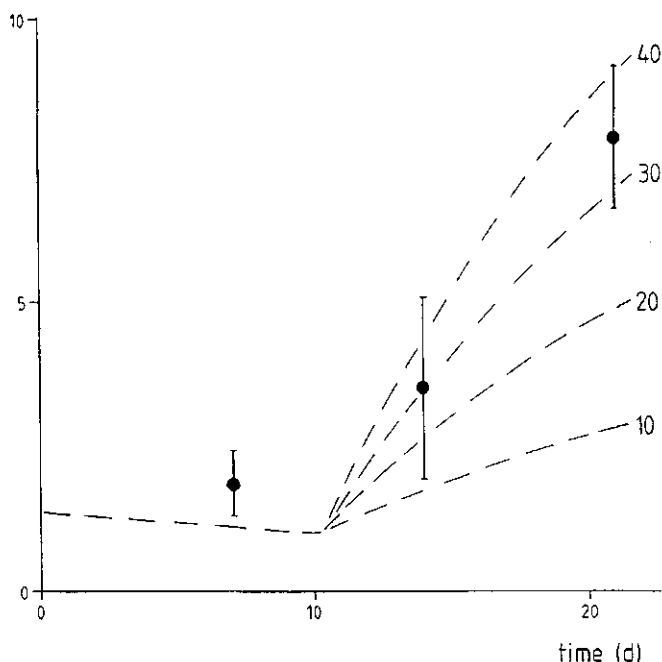
32

population density

Fig. 4.3 Effect of various levels of fecundity (10 to 40 eggs per female) on population increase.
------: Simulated;   ● : Observed by J.G. Mulder (unpublished results), vertical bars represent the standard deviations of the measurements.

prediction of the number of juveniles (Table 4.1). Therefore, the value of 30 was used for the rest of the simulations.

The initial decrease of the simulated population can be attributed to the assumed absence of eggs on the roots at the start of the simulation.

Rhizosphere radius
The population increase of *T. dubius* on *L. perenne* in moist soil in pot experiments at 10, 18 and 25 °C (Chapter 2) was simulated, to estimate the rhizosphere radius.

When the total initial population was assumed to reside on the roots (not realistic for these experiments), the active population was overestimated at 18 and 25 °C, but the active population at 10 °C was greatly underestimated (Table 4.2). There is no reason to expect a higher fecundity at low temperatures, therefore the development rate at 10 °C was probably underestimated.

Table 4.1. Effect of fecundity on the predicted composition of the population. Numbers in brackets represent standard deviations of the measurements.

Stage	Code[1]	Time[2]					
		7		14		21	
Juveniles[3]	M	1294	(680)	2961	(1561)	6607	(846)
	S30	750		3262		6626	
	S40	750		4135		8696	
Females[3]	M	431	(89)	465	(182)	1146	(455)
	S30	258		160		191	
	S40	258		160		191	
Males[3]	M	105	(48)	86	(62)	137	(51)
	S30	97		74		137	
	S40	97		74		137	

[1] M : measured by J.G. Mulder (personal communication); S30 : simulated with a fecundity of 30 eggs per female; S40 : simulated with a fecundity of 40 eggs per female.
[2] Time in days from the start of the simulation (33 days after the host was sown).
[3] Numbers per 100 ml soil.

When migration was not ignored, the population increase at 25 °C was best simulated with a rhizosphere radius of 2 cm, corresponding with a migration period of 4 days. The intermediate population density at 18 °C was best simulated with a rhizosphere radius of 1 cm, corresponding with a migration period of a week, but the final density was overestimated. This indicates that the assumption of non-limiting food supply might not hold for the total duration of the experiment.

Moisture stress
The population increase at 25 °C in soil with sub-optimal water supply was overestimated when a rhizosphere radius of 2 cm was used (Table 4.3). When a rhizosphere radius of 1 cm was assumed, the prediction improved.

Effect of root growth on population increase
The population increase on the fast growing cultivar Pelo and the slow growing cultivar Idole in a pot experiment (Chapter 3) was simulated. The

Table 4.2. Effect of assumptions on nematode migration on the predicted active population on the roots of *L. perenne*.

Temperature (°C)	Code[1]	Intermediate population[2]	Final population[3]
10	M	5.7	12.6
	S no migration	5.1	8.4
18	M	11.1	31.7
	S no migration	18.3	61.9
	S radius 1. cm	11.9	50.5
25	M	18.6	35.1
	S no migration	23.7	38.6
	S radius 2. cm	21.2	35.9

[1] M measured (Chapter 2), S simulated
[2] Total number of juveniles and adults on the roots in thousands per pot 21 days after emergence of *L. perenne* at 10 and 18 °C, and 22 days after emergence at 25 °C
[3] Total number of juveniles and adults on the roots in thousands per pot 43 days after emergence of *L. perenne* at 10 °C, 42 days after emergence at 18 °C and 30 days after emergence at 25 °C.

Table 4.3. Influence of moisture stress on increase of the active nematode population at 25 °C (in thousands per pot).

Moisture treatment	Code[1]	Time[2]	
		22	30
wet	M	18.6	35.1
	S radius 2. cm	21.2	35.9
dry	M	15.7	27.4
	S radius 2. cm	18.9	33.3
	S radius 1. cm	12.5	26.1

[1] M measured (Chapter 2), S simulated
[2] Time in days after emergence of *L. perenne*

environmental conditions during this experiment were comparable with the dry treatment of the aforementioned experiment at 25 °C, therefore a rhizosphere radius of 1 cm was used.

The simulations show that differences in root growth during the migration period may cause large differences in population density (Fig. 4.4). However, the predicted final density was much too high for both cultivars. For 'Pelo' the difference between this prediction and the one for the moisture experiment can be explained from the higher initial nematode density and the higher number of plants in the cultivar experiment. The higher number of plants initially caused a higher growth rate of the plant mass per pot, resulting in a higher migration rate. It also caused earlier and stronger competition between the plants, resulting in slower growth of the individual plants. So, the differences between the predicted final density of the nematodes in the moisture experiment and the cultivar experiment and between the measured and the predicted final density in the cultivar experiment might be explained by the effect of limited food supply on the growth of the nematode population.
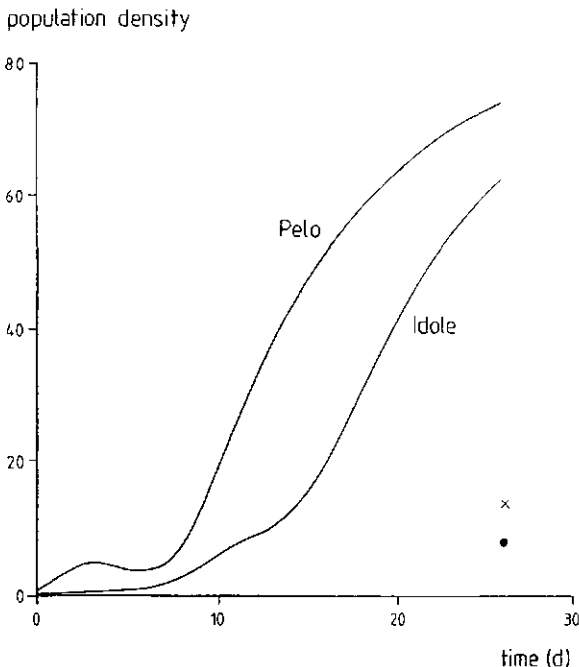
population density

Fig. 4.4 Influence of the *Lolium perenne* cultivars Pelo (fast growth) and Idole (slow growth) on population increase of *T. dubius*. ————: Simulated; ✕ : measured for 'Pelo'; ● measured for 'Idole'.

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4.4 DISCUSSION

For a limited set of environmental conditions - no moisture stress and temperatures between 18 and 25 °C - the model describes experimental results during the first month after seeding of the grass reasonably, using a value for fecundity of 30 eggs per female and a rhizosphere radius of 1 or 2 cm. However, at low temperatures the increase of the population on the roots is underestimated, and it is overestimated when moisture stress occurs.

The underestimation of the population increase at low temperatures is caused by underestimation of the development rate of the nematode. It is necessary to determine the relation between temperature and development rate for *T. dubius*, instead of using a relation determined for other species; the temperature threshold of development seems to be lower for the population of *T. dubius* used in the experiments than for *Tylenchus emarginatus* and *Pratylenchus penetrans*. At the same time, the effect of temperature on mortality should be determined. At high temperatures, mortality during development is certainly not negligible; although the development rate of *T. dubius* is probably highest at 30 °C, the population declines at this temperature and only little reproduction occurs (Malek, 1980).

The overestimation of population increase at moisture stress may have various causes. In the model, soil moisture acts indirectly by its influence on root growth. Since root growth only influences migration, the influence of soil moisture is limited to the migration period. The results of population increase under moisture stress were better described with a lower value for the rhizosphere radius. However, it is questionable whether the effect of moisture stress can be explained by a direct relation between soil moisture and the rhizosphere radius. Nematode mobility is influenced by soil moisture, but the mobility is highest at intermediate moisture levels (Vrain, 1986). Therefore it is doubtful whether the mobility of *T. dubius* was lower in the simulated experiments under moisture stress than at optimal water supply for the host. In any case, a rhizosphere radius dependent on soil moisture cannot explain any effects of soil moisture that occur after the migration period, as its effect is limited to this period. Extreme moisture conditions did not occur in the simulated experiments. Therefore, a direct effect of soil moisture on development and reproduction of the nematodes in the rhizosphere is not expected (Vrain, 1986).

The overestimation of population increase at moisture stress may also be due to the assumption that no overpopulation occurs. The overestimation of the final population in the experiment at 18 °C and the cultivar experiment

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indicates that this assumption only holds during a short period. When root growth is slower, as is the case at lower soil moisture levels, food shortage is likely to occur earlier and at lower initial population densities. Development rate, oviposition rate and mortality are probably all affected by food shortage. Fecundity is probably not affected, as Fisher (1969) showed for *Aphelenchus avenae*. To incorporate food shortage in the model, detailed information is needed about the availability of young roots. The applicability of the model will be much greater when this information is incorporated, as more situations and longer periods may be simulated. However, it is questionable whether this is necessary for an accurate simulation of the damage caused by *T. dubius*, because experimental results suggest that damage to *L. perenne* is mainly caused by nematode activity during the first weeks after sowing (Chapter 2).

Before extending the population model, the length of the period during which population increase determines the amount of damage to the host plant should be estimated. This can be done by simulating the growth of the host in a model in which population density is introduced as a forcing function. Various density curves may be evaluated.

To improve the explanatory value of the model, certain parameters need to be determined more accurately.

Fecundity is the most important parameter in the model. The population increase of *T. dubius* cannot be explained with the low values for fecundity reported for *Tylenchorhynchus* in literature. The value of 30 used in the simulations is only a rough estimate. Since this estimate was obtained from an experiment in which the growth of the host was probably already limited by pot volume, and given the assumed absence of mortality, a still higher value might be expected.

The estimate of the rhizosphere radius depends on fecundity; a higher value of the fecundity would have caused a lower estimate for the rhizosphere radius. Although measurement of this parameter is preferable, it might be sufficient to fit the rhizosphere radius when fecundity and the relation between temperature and development rate have been determined more accurately.

In chapter 3 it was suggested that the differences in population increase of *T. dubius* on fast- and slow-growing cultivars of *L. perenne* might be caused by the effect of slow plant growth on nematode migration to the roots, rather than by differences in degree of cultivar resistance. The simulations of population increase on 'Idole' and 'Pelo' support this hypothesis, but they also indicate that growth of the individual roots might be an important factor determining the growth rate of the nematode

population. Therefore it might be better to hypothesize that differences in population increase on fast- and slow-growing cultivars of *L. perenne* are determined by quantitative rather than qualitative differences between the cultivars.

The model underestimates the number of females, but the numbers of juveniles and males are described reasonably well. This indicates that the juvenile sex ratio and the longevity of the males are probably well estimated and that the duration of the period in which oviposition mainly occurs is about equal to the longevity of the reproductive females assumed in the model. However, the lifetime of the adult female is probably much longer than assumed in the model. The life time of the adult females of other species of nematode may approximately equal the length of their life cycle or even be 6 to 10 times longer, whereas oviposition mainly occurs during the first part of adulthood (Mamiya, 1971; Gowen, 1970). A better estimation of the longevity of the females and the course of the individual oviposition rate during ageing is necessary when periods in the order of a few times the generation time are simulated.

The quality of the model output greatly depends on the quality of the input relations. Therefore, the number of eggs in the initial populations deserves attention, because quantitative information on this is lacking.

The model presented here can be adapted for other species of ectoparasitic nematodes and host species by changing the input relations and values of the parameters.

5   Simulation of the host/parasite system *Lolium perenne/ Tylenchorhynchus dubius* 2. The effect of *T. dubius* on *L. perenne*

ABSTRACT   The effect of the ectoparasitic nematode *Tylenchorhynchus dubius* on various processes that determine the growth of the grass *Lolium perenne* was studied using dynamic simulation. An equation was formulated for the relation between nematode density in the rhizosphere and the penetration rate per nematode. Various hypotheses on the effect of cell penetration on plant growth processes were formulated and then introduced in a SUCROS-type growth model of the host. The plausibility of the hypotheses was tested by their ability to simulate experimental results with given population data.

The simulations showed that it is unlikely that all cells penetrated by *T. dubius* will die. The effect of *T. dubius* cannot be attributed to the nematodes' consumption of dry matter. Growth reduction may be attributed to the nematode negatively affecting the roots' permeability.

5.1 INTRODUCTION

The   nematode/grass   system   *Tylenchorhynchus dubius/Lolium perenne*   was studied to elucidate the effect of the nematode on the grass under various environmental conditions (Chapter 2). An explanatory dynamic simulation model comprising submodels of the population dynamics of the nematode and the growth of the host plant is being developed. The submodels are linked by the effect of the nematode on the processes that determine the growth of the host and by the effect of the host on the population dynamics of the nematode.

The latter effect was touched on in chapter 4 in which a preliminary model of the population dynamics of *T. dubius* was presented. The present chapter deals with the simulation of the effect of the nematode on the host; it describes attempts to couple data on the nematode population to a SUCROS-type model of the growth of the host.

Two basic relations were needed for this coupling: 1) the relation between nematode density in the rhizosphere and the daily feeding activity per nematode, and 2) the effect of individual feeds on the carbon flow processes in the host. As knowledge on these relations is incomplete for *T. dubius* (as for most other plant parasitic nematodes), various hypotheses were formulated and their plausibility was evaluated.

5.2 PLANT MODEL

The plant model used in this study is based on SUCROS (Van Keulen et al, 1982; Spitters et al, 1989). Photosynthesis is calculated from incoming radiation as a driving force. The assimilates are partitioned over shoot and root according to constant partitioning coefficients. The model was adapted to simulate the growth of grass in pots in a climate room. It was extended with a relation that accounts for the effect of reduced soil moisture content on photosynthesis. It was assumed that the reduction of the rate of photosynthesis is equal to the reduction in transpiration rate and that the photosynthetic properties of the leaves and assimilate partitioning are not affected by reduced soil moisture content. This assumption only holds for a reduction of less than about 40% in water uptake (Van Keulen, 1982). The relation between soil moisture and the reduction factor for transpiration is based on Feddes et al. (1978).

Aging of the roots was introduced in the model because young roots are attractive to *T. dubius* and old roots are not attacked. Aging was simulated with an escalator boxcar train (Goudriaan and van Roermund, 1989). It was assumed that epidermal cells of *L. perenne* live for 16.5 days at 21 °C unless killed by external factors (Kirk and Deacon, 1986). The temperature dependency of root aging was approximated by a Q10 value of 2.

To calculate the number of epidermal cells per unit root mass a value of 2.21×10^4 mg^{-1} was used, based on the epidermal cells having an outer surface area of 3.8×10^{-3} mm^2 (measured on cv Pelo), a root diameter of 0.4 mm (Kutschera et al, 1982) and a root length per unit dry mass of 66.7 mm mg^{-1} (H. Mulder, unpublished results).

The model is written in CSMP.

5.3 RELATION BETWEEN NEMATODE DENSITY AND PENETRATION RATE

Description

The active stages of *T. dubius* (second stage juveniles to adult males and females) feed mainly on epidermal cells in the root hair zone and the zone of elongation and to a lesser extent on root hairs and root tips (Bridge and Hague, 1974; Laughlin and Vargas, 1972; Sharma, 1971; Wyss, 1973). The nematodes penetrate the cells with their stylet and partly remove the cell contents. The feeding period is short, in the order of ten minutes, and the searching time, i.e. the time between retraction of the stylet and successful penetration of the next feeding site, is in the order of seconds to minutes (Bridge and Hague, 1974; Wyss, 1973).

Nematode density was assumed to have no influence on the mean feeding period, because feeding nematodes are usually not disturbed by encounters with searching nematodes (Wyss, 1973). The mean searching period was assumed to increase with increasing nematode density.

The penetration rate per nematode, PR_n, was defined as the number of cells penetrated per unit of time by one nematode. It is the inverse of the sum of the feeding period and the searching period.

The ratio between feeding period and searching period at a given moment is equal to the ratio between the number of feeding nematodes and the number of searching nematodes. So, the searching period can be calculated when feeding period and the numbers of feeding and searching nematodes are known.

The following method for calculating the number of feeding nematodes is analogous to the kinetic derivation of the Langmuir isotherm equation. This equation describes the relation between the number of occupied adsorption sites and the concentration of molecules at adsorption/desorption equilibrium.

A cell can be occupied by only one nematode at a time. Therefore, the number of occupied cells equals the number of feeding nematodes N_f (1). The rate VR (d^{-1}) at which occupied cells are vacated is proportional to the number of feeding nematodes.

$$VR = k_v \cdot N_f \tag{5.1}$$

The proportionality constant, k_v (d^{-1}) is the inverse of the feeding period per cell.

The occupation rate, OR (d^{-1}), of consumable epidermal cells, E_h (1), is proportional to the concentration of searching nematodes on the young roots and the number of consumable epidermal cells not occupied by feeding nematodes. The nematode concentration in the rhizosphere is defined as the ratio between the number of searching nematodes, N_s (1), and the total number of epidermal cells, E_t (1).

$$OR = k_o \cdot \frac{N_s}{E_t} \cdot (E_h - N_f) \tag{5.2}$$

The proportionality constant, k_o (d^{-1}) is equal to the inverse of the searching period at unlimited food supply. It is assumed that the kinetics of the feeding and searching processes are so fast that equilibrium exists

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between the two processes. Then VR equals OR and equations (5.1) and (5.2) may be combined to give:

$$N_f = \frac{K \cdot E_h \cdot N_s / E_t}{1 + K \cdot N_s / E_t} \qquad (5.3)$$

In which K $(d.d^{-1})$ is the ratio between k_v and k_o.

N_t is the total number of nematodes in the rhizosphere of young roots. Substituting ($N_t - N_s$) for N_f in equation 5.3 leads to a quadratic equation for N_s with the solution:

$$N_s = \frac{\sqrt{(1 + \dfrac{K}{E_t} (E_h - N_t))^2 + \dfrac{4K}{E_t} N_t} - (1 + \dfrac{K}{E_t} (E_h - N_t))}{2K / E_t} \qquad (5.4)$$

The other root of the quadratic equation (with the minus sign before the square root) is not valid because it gives a negative value for N_s.

Quantification

The feeding period per cell at room temperature on *L. perenne* averages just under 7 minutes. The searching period at low nematode density is in the range of a few seconds to a few minutes. (Bridge and Hague, 1974). In the calculations a feeding period of 7 minutes and a minimal searching period of 30 seconds were assumed at 20 °C. This corresponds with a K value of 14.

The influence of temperature on feeding period and searching period was calculated with equation (5.5)

$$P_T = P_{20} / (a T - b) \qquad (5.5)$$

In which P_T is the length of the period at temperature T (°C), P_{20} is the length of the period at 20 °C, a is a constant with value 0.062 (°C^{-1}) and b is a constant with value 0.26 (1). This relation was derived from observations made by Boag (1980) on the influence of temperature on the rate of oesophagus contractions in three nematode species. The equation is only valid for temperatures in the range of 10 to 25 °C.

K is temperature-independent, because of the assumption that temperature influences feeding period and searching period in the same way.

Figure 5.1 shows the sensitivity of PR_n to K at various values for the nematode concentration in the rhizosphere (N_t/E_t). When the nematode concentration is high, the penetration rate is hardly sensitive to the value of K. At lower nematode concentrations the penetration rate is mainly sensitive for K values up to 15.
The fate of penetrated cells influences the fraction of consumable epidermal cells in the young roots (E_h/E_t). Figure 5.2 shows how PR_n is influenced by the fraction of consumable epidermal cells. Figure 5.3 shows that PR_n decreases rapidly when the number of nematodes surpasses the number of epidermal cells, especially when the consumable fraction is low.

5.4 EFFECT OF INDIVIDUAL FEEDS ON PLANT GROWTH PROCESSES

Fate of penetrated cells
Wyss (1973) and Brzeski (1971) both studied the effect of penetration by *T. dubius* on cells of a *Brassica* species through a light microscope and observed no leakage of cell contents as a consequence of penetration.
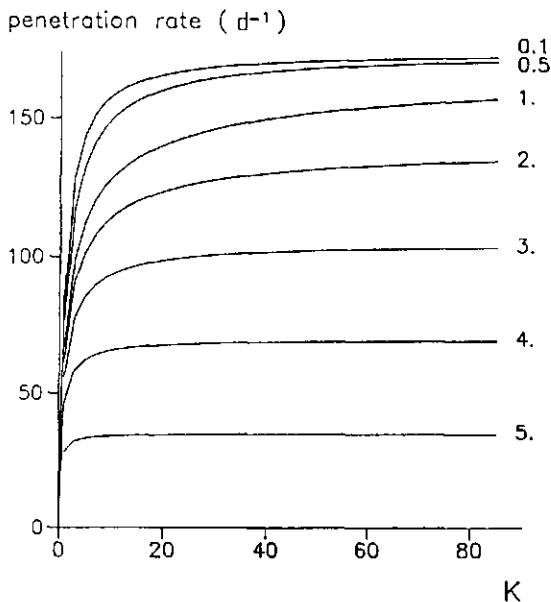

Fig. 5.1 Effect of the ratio K between the feeding period and the minimal searching period per nematode per cell on the calculated number of cells a nematode penetrates daily, for nematode concentrations in the rhizosphere of young roots ranging from 0.1 to 5 nematodes per epidermal cell.
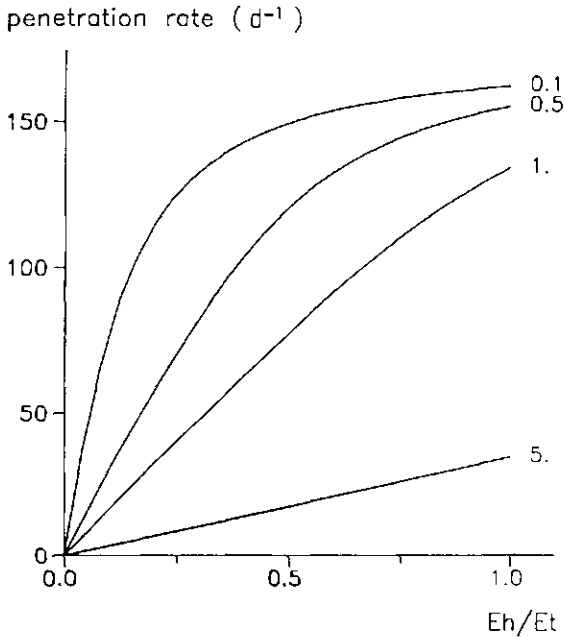
penetration rate (d^{-1})

Fig. 5.2 Effect of the fraction of healthy epidermal cells E_h/E_t on the calculated number of cells a nematode penetrates daily, for nematode concentrations in the rhizosphere of young roots of 0.1, 0.5, 1., and 5 nematodes per epidermal cell.

Brzeski (1971) observed that the cytoplasm had regenerated within a few hours after the nematode left the cell, and no visible traces of feeding activity remained. In contrast, Wyss (1973) observed that cyclosis of penetrated cells slowed down and stopped within a few hours after the nematode had left.

As the literature is inconclusive on this point, the death of all penetrated cells, the recovery of all penetrated cells, and - an intermediate option - the death of some of the penetrated cells were all considered in the hypotheses on the effect of nematodes on plant growth processes.

It was assumed that the number of affected cells equals the number of penetrated cells, because there are no reports of visible influences of penetration on neighbouring cells.

Effect on growth processes
Bridge and Hague (1974) and Wyss (1973) observed no changes in the branching pattern of the roots, nor other morphological effects on root
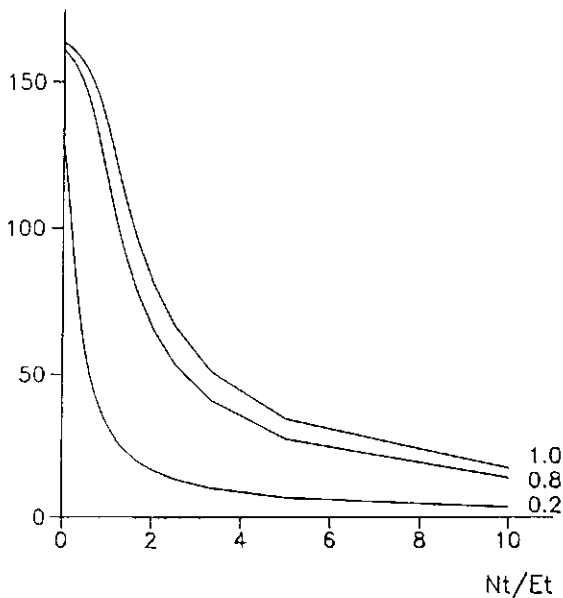
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penetration rate (d^{-1})

Fig. 5.3 Effect of the nematode concentration in the rhizosphere of young roots (N_t/E_t) on the calculated number of cells a nematode penetrates daily, when the fraction of healthy epidermal cells is 1., 0.8, or 0.2.

growth. It is therefore unlikely that *T. dubius* introduces or induces hormones that affect root morphogenesis.

Boote et al (1983) have classified pest effects on carbon flow processes. Nematodes are classified as assimilate sappers or turgor reducers.

T. dubius acts as an assimilate sapper when the cell mass removed by nematodes is replaced by assimilates to repair the cells. Additional assimilates are probably needed to account for the costs of repair.

Unless *T. dubius* affects the plant by injecting or inducing toxic substances that act directly upon stomatal behaviour and as a consequence on transpiration, it can only act as a turgor reducer if the resistance to water uptake is located at least partly in the epidermis and if the permeability of the epidermis depends on the condition of the epidermal cells. The traditional view is that the main resistance to radial water flow in the roots is located in or near the endodermis, but this has not yet been proved conclusively (Passioura, 1988). According to Drew (1987) the location of the main resistance might depend on the physical environment.

The relative importance of the symplastic and apoplastic pathways for water transport across the root is not yet clear either (Drew, 1987, Passioura, 1988). Cyclosis is needed for the symplastic pathway. The conductive properties of the cell walls, in which the apoplastic pathway is located, might be influenced by the condition of the adjacent plasma membranes.

Thus *T. dubius* may act as a turgor reducer through its direct effect on epidermal cells, both if cells die and if the penetrated cells recover.

Hypotheses
The following hypotheses on the effects of *T. dubius* on crop growth processes were tested.
1) All penetrated cells die.
2) All penetrated cells recover. The growth rate of the host plant decreases, because part of the assimilates are used to repair penetrated cells (respiration hypothesis).
3a) All penetrated cells recover. The permeability of the penetrated cells is reduced during recovery. Consequently, the rate of photosynthesis falls via a fall in transpiration.
3b) Some of the penetrated cells die. The permeability of dead cells is reduced. Consequently the rate of photosynthesis falls via a fall in transpiration.

5.5 SIMULATIONS

Each hypothesis was introduced separately in the model, and its ability to simulate the effect of *T. dubius* on dry matter production of *L. perenne* as measured in pot experiments (Chapter 2) was evaluated.

General information
Table 5.1 gives an overview of the simulated treatments. For a first screening of each hypothesis the treatments of experiment T18 were simulated. In treatment T18NW the measured effect of *T. dubius* on shoot growth of *L. perenne* was only moderate; a 30% reduction after 14 and 22 days and a 10% reduction after 36 days from the start of the experiment at an initial population density of 13 nematodes per g soil. Seinhorst and Kozlowska (1979) found a 20% reduction of shoot growth at a comparable initial nematode density; a maximum reduction of 90% was found at an initial nematode density of 200 nematodes per g soil. Therefore, the hypotheses tested are only plausible if they permit a range of initial

Table 5.1 Explanation of the codes of the simulated treatment (Chapter 2)

Experiment code	Temperature (°C)	Nematode treatments[1]	Moisture treatments[2]
T18	18	N, C	W
T25	25	N, C	W, D

[1] N= soil inoculated with *T. dubius*; C= non-inoculated control
[2] W= wet (maximum soil moisture content 25%); D= dry (maximum soil moisture content 12%)

densities of nematodes higher than that of T18NW to do more damage than found in T18NW.

If the results of the first screening justified further simulations, the treatments at 25 °C were simulated to examine the effects of temperature and moisture stress.

Unless indicated otherwise, the parameters on assimilate partitioning and specific leaf area at 18 °C and 25 °C were based on data from the controls T18CW and T25CW, respectively. Soil moisture content was introduced as a function of time; temperature and radiation as constants.

Figure 5.4 shows the course of the nematode population in the rhizosphere of the young roots as used as input in the simulations. The initial population of nematodes in the rhizosphere was calculated from the initial density in the pots and initial root mass, assuming a rhizosphere radius of 1 cm (Chapter 4). For T18 the nematode population was determined by this initial value and the data on population density on the sampling dates, assuming that the total population was residing in the rhizosphere of the young roots at the sampling dates. For T25 the first sampling date was 22 days after emergence of the grass. To estimate intermediate population density, it was assumed that growth was exponential between the calculated initial density and the first sampling date.

Unless indicated otherwise, the penetration rate in the simulations was calculated with the method described above.

Hypothesis 1
The hypothesis that all penetrated cells die was tested without making assumptions on the effect of cell death on plant growth processes. The effect of nematodes was introduced by basing the values for the parameters on assimilate partitioning and specific leaf area on data measured in the nematode treatment T18NW instead of the control T18CW.
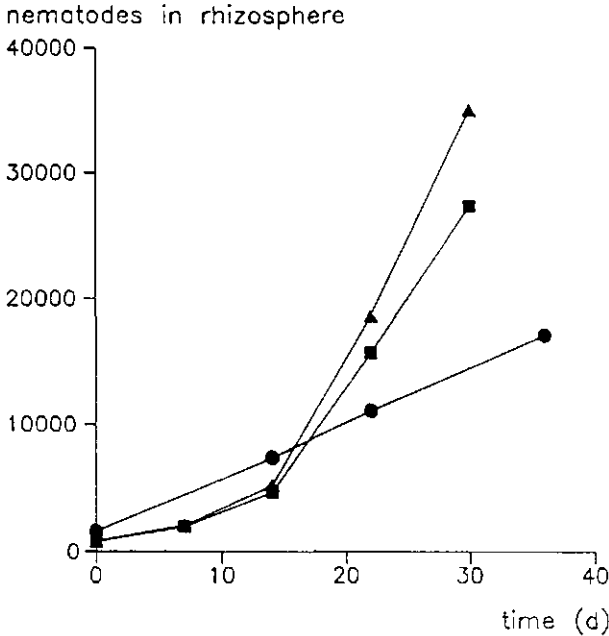
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nematodes in rhizosphere

Fig. 5.4 Course of the *Tylenchorhynchus dubius* population in the rhizosphere of young roots in time, used for the simulation of T18NW (●), T25NW (▲), and T25ND (■).

The fraction of living epidermal cells on the young roots and the PR_n are simulated with population data from T18 NW. The fraction of living cells indicates to what extent a higher population can cause more damage; if this fraction is zero at a certain population density, a higher population can't cause more damage, because no feeding sites are available. Figure 5.5a shows that the simulated fraction of living epidermal cells was initially so low that a higher nematode population could hardly affect more epidermal cells. However, differences between various densities could already be observed on the seventh day in an experiment (Seinhorst and Kozlowska, 1979).

In the case of unlimited food supply PR_n should be about 160 cells per day. Figure 5.5b shows that PR_n is less than a quarter of this value during the first two weeks of the simulation. Because the reduction of PR_n would be even larger at higher nematode densities, it is doubtful whether initially higher populations could remain higher than the population of T18NW. This suggests that differences between T18NW and higher population densities might not even occur at later sampling times. The simulations show that it is unlikely that all penetrated cells die.
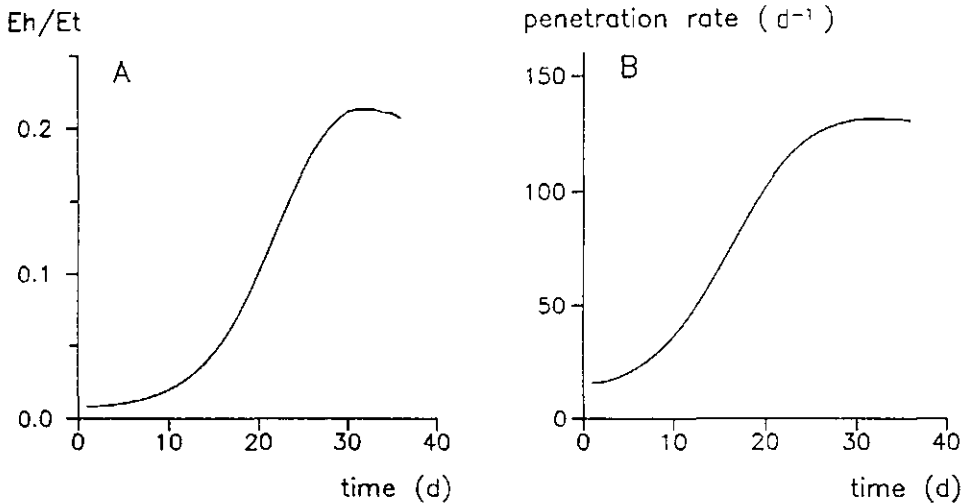
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Fig. 5.5 Consequences of death of all penetrated cells on the fraction of living epidermal cells (A) and the penetration rate per nematode (B) for T18NW.

Hypothesis 2
All penetrated cells recover. The costs of recovery were calculated as the dry mass consumed by the nematodes multiplied by a conversion factor to account for the costs of transforming assimilates into dry matter.

The daily dry matter consumption per nematode at 20 °C was estimated to be 0.02 µg per day. This value was calculated from dry matter production, sex ratio and longevity of males and females, assuming that consumption is six times production (Petersen and Luxton, 1982). At 20 °C, the longevity of females is 44 days including an oviposition period of 14 days during which 2 eggs per day are produced, the longevity of males is 34 days (Chapter 4). At a sex ratio of 0.5, a stable population consists of 56% females and 44% males, because of the differences in longevity. The dry mass of the nematodes was calculated from data on the length and width of *T. dubius* (Sharma, 1971) using Andrassy's formula for the calculation of fresh mass (Andrassy, 1956) and assuming a dry matter content of 25 % (Yeates, 1979). The resulting values were 0.09, 0.14 and 0.005 µg for males, females and eggs, respectively.

In the calculations for T18NW the value for daily dry matter consumption per nematode at 20 °C was used. It was assumed that the daily dry matter consumption per nematode was not influenced by nematode density. Thus, the daily dry matter consumption per nematode was certainly not underestimated.

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However, simulated shoot growth was hardly affected (Table 5.2). The extra maintenance respiration caused by the nematodes was about 1% of the total assimilates available for growth and maintenance at the start and decreased with time to 0.1%.

When a daily dry matter consumption of 0.4 μg per nematode was used, the simulated nematode effect was comparable with the measured effect (Table 5.2). However, it is unlikely that the daily dry matter consumption per nematode was underestimated by a factor of 20. Therefore, the effect of *T. dubius* on the production of *L. perenne* cannot be wholly attributed to an effect on maintenance respiration.

Hypotheses 3a and 3b
It was assumed that the permeability of non-functioning epidermal cells equals the permeability of old root cells. It was assumed that the permeability of old roots is 0.3 times that of young roots (De Wit et al, 1978). The level to which the permeability of the total root system is reduced by nematodes (R_{nem}) was calculated with equation 5.6:

$$R_{nem} = \frac{Y_h \cdot 1.0 + (Y_a + 0) \cdot 0.3}{(Y_h + Y_a) \cdot 1.0 + 0 \cdot 0.3} \tag{5.6}$$

in which Y_h is the mass of young roots with healthy epidermal cells, Y_a the mass of young roots with epidermal cells affected by nematodes and 0 is the mass of old roots. R_{nem} and the reduction factor resulting from reduced moisture content in the soil were multiplied to compute the total reduction of photosynthesis.

Hypotheses 3a and 3b differ with respect to the relation between the nematode population and the affected root mass.

For hypothesis 3a it was assumed that all penetrated cells are less permeable during a recovery period that is in the order of a few hours. During the recovery period the cells cannot be penetrated. Table 5.3 shows the effect of the length of the recovery period on shoot production of T18NW. The results are within the limits of the measurements when recovery periods of 2, 3 and 4 hours were used. The correspondence between simulations and measurements was best with a recovery period of 3 hours. Therefore, this value was used in the subsequent simulations.

For hypothesis 3b the percentage penetrated cells that die as a result of penetration depends on the physiological age of the cells. It was assumed that 0, 5%, 10%, 20%, 40% and 80% of the penetrated cells in the

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Table 5.2 Respiration hypothesis. Measured and simulated shoot mass in the nematode treatment as a fraction of shoot mass of the control on the sampling dates of experiment T18W.

	Time[1]		
	14	22	36
Measured	0.70	0.68	0.90
Simulated $DCN^2 = 0.02$	0.98	0.99	0.99
Simulated $DCN = 0.40$	0.63	0.69	0.90

[1] Time in days after emergence = days after start of simulation
[2] DCN= daily dry matter consumption per nematode ($\mu g.d^{-1}$)

Table 5.3 Sensitivity analysis for the period in which penetrated cells do not function in hypothesis 3a for experiment T18. Simulated shoot mass. Figures in parentheses represent standard deviations of the measurements.

		Time[1]		
		14	22	36
Measured		141 (46)	724 (191)	2868 (532)
Simulated				
NP^2	1	192	892	3299
	2	166	792	3153
	3	139	681	2978
	4	112	557	2755
	5	92	459	2550

[1] Time in days after emergence = days after start of simulation
[2] NP= period during which the cells do not function (hours)

successive age classes die. All living cells can be penetrated.The consequence of both hypotheses on shoot production of T18 NW is shown in Fig. 5.6, together with the shoot production of the control T18 CW. The difference between the two hypotheses is illustrated by the course of R_{nem}

in time (Fig. 5.7). The irregularities in the curve of R_{nem} at hypothesis 3a are the result of young root mass being simulated with a boxcar train; if more boxcars had been used the curve would have been smoother. For both hypotheses, the effect of *T. dubius* on water uptake was highest during the first half of the experiments. With hypothesis 3a all penetrations are equally effective and their effect is short-lived. With hypothesis 3b the effect per penetration initially increases with time. The effect per killed cell lasts until the cell becomes part of the old root mass. Therefore, the maximum decrease of water uptake was higher and occurred earlier with hypothesis 3a than with 3b. In both cases the minimum value of R_{nem} was much higher than the theoretical minimum of 0.3, which occurs when all young roots are affected. The penetration rate decreased by no more than 3% in both cases and the fraction of non-affected roots did not fall below 70% for hypothesis 3a and 74% for hypothesis 3b. So, under both hypotheses higher initial populations can cause more damage and can multiply to higher densities than the population in T18NW.
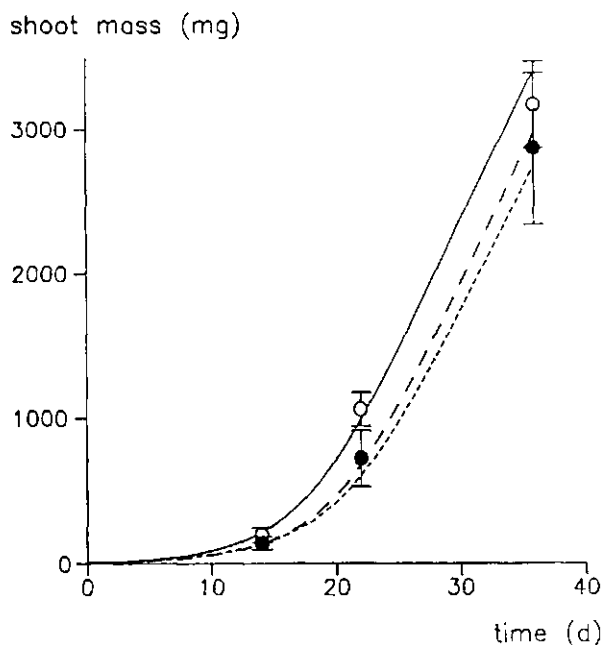

Fig. 5.6 Shoot production of T18NW simulated with hypotheses 3a (– – – – –) and 3b (– – – – – – –) and shoot production of the control T18CW (————). Points represent measurements; vertical bars represent standard deviations of the measurements. ● T18NW, ○ T18CW.
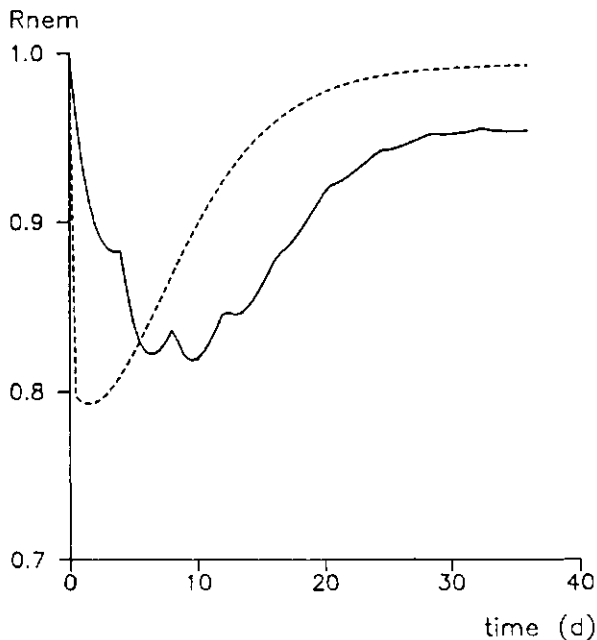
Fig. 5.7 Course of R_{nem} with time for T18NW simulated with hypotheses 3a (-------) and 3b (————).

As both hypotheses gave reasonable results for T18, the experiment at 25 °C was simulated to examine the effects of temperature and moisture stress.

For hypothesis 3a the effect of temperature on the length of the recovery period needs to be explicitly incorporated. The rate of repair is expected to increase with temperature. Therefore, penetrated cells should have a shorter recovery period at higher temperatures. However, the shoot production of T25NW was overestimated instead of underestimated when the recovery period was 3 hours (Fig. 5.8).

In hypothesis 3b temperature influences death of the penetrated cells through its effect on root senescence. This hypothesis gave better results for the shoot production of T25NW (Fig. 5.8). The disparity between measurements and simulations might have been caused by the fact that estimates rather than measured data were used to calculate the effect of temperature on root senescence and the feeding activity of *T. dubius*. In the dry treatments T25CD and T25ND moisture stress occurred. The effect of moisture stress on the control T25CD was simulated reasonably, but the effect of *T. dubius* on shoot production was underestimated with both hypotheses (Figure 5.8). Shoot production of the nematode treatment T25ND
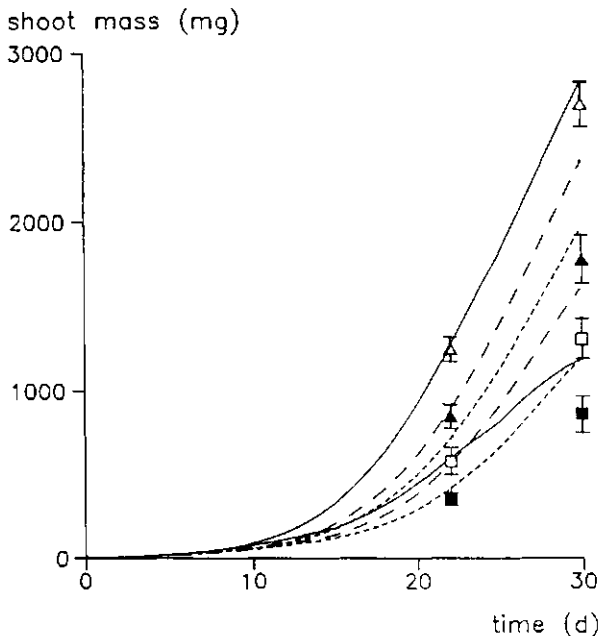
shoot mass (mg)

Fig. 5.8 Shoot production of T25NW and T25ND simulated with hypotheses 3a and 3b, and shoot production of the controls T25CW and T25CD. Points represent measurements; vertical bars represent standard deviations of the measurements. ▲ T25NW, △ T25CW, ■ T25ND, □ T 25CD.
——————— control, ————— 3a, -------- 3b.

even exceeded shoot production of T25CD for hypothesis 3a. The latter effect was permitted by the fact that the daily fall in moisture content in the nematode treatment was somewhat smaller than in the control.

An overestimation of the shoot production of T25ND with hypothesis 3a could have been expected, given the overestimation for T25NW with this hypothesis. With hypothesis 3b, the effect of the nematodes on shoot production of treatment T25NW on the first sampling date was slightly overestimated. Therefore, it is unlikely that the negative effect of the nematodes on the permeability of the roots was underestimated for T25ND. Probably, the assumption that assimilate partitioning and photosynthetic properties of the leaves are not affected by reduced water uptake is not valid for treatment T25ND; in this treatment, the reduction of the water uptake as a result of moisture stress was more than 40% only incidentally, but the combined effect of nematodes and moisture stress surpassed this critical value for longer periods. Therefore, the model might be improved

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by incorporating a (reversible) effect of prolonged water stress on assimilate partitioning and photosynthetic properties of the leaves.

R_{nem} under hypothesis 3b was simulated for population densities of 1x, 2x and 4x the population density of T18NW at non-limiting water supply (Fig. 5.9). The two higher nematode densities not only caused a lower R_{nem}, but the period during which R_{nem} was low was also longer.

5.6 CONCLUSIONS AND GENERAL DISCUSSION

Although Wyss (1973) observed that penetrated cells died, the simulations show that it is unlikely that all cells penetrated by *T. dubius* die. Wyss worked with isolated root systems, and therefore lack of assimilates may have precluded cell recovery.

Although important for the recovery of the cells, the assimilates needed for repair constitute such a small part of the daily assimilate production
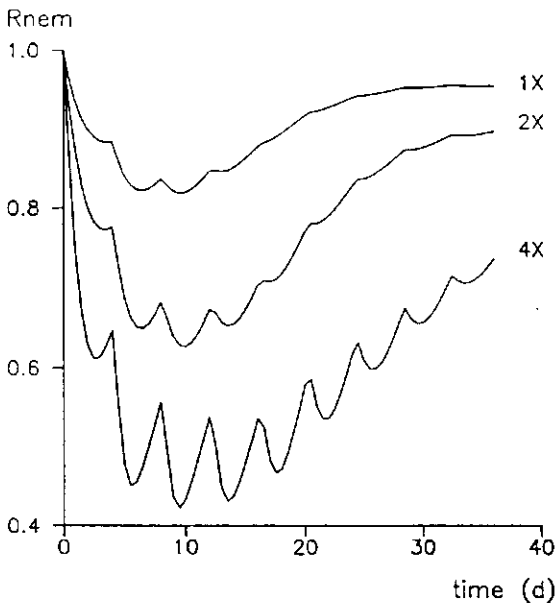

Fig. 5.9 Influence of population density of *T. dubius* on R_{nem} calculated with hypothesis 3b. From top to bottom, normal population density of T18NW, double density, and four times the normal density, respectively. Other circumstances as for T18NW.

that they cannot explain the effect of *T. dubius* on the growth of *L. perenne*.

Seinhorst (1981) states that *T. dubius* does not affect water uptake of *L. perenne* at initial nematode densities up to 45 individuals per g soil. He postulated that at lower nematode densities the growth rate should be affected without water consumption being affected.

The simulations show that the effect of *T. dubius* on plant growth at initial nematode densities as low as 13 (T18) and 7 (T25) individuals per g soil could be attributed to a reduction of water uptake. The course of the simulated reduction factor R_{new} in time (Fig. 5.9) shows that the length of the period during which a pronounced effect of the nematode on plant properties is likely to be found, increases with increasing initial population density of the nematode. The longer the first sampling is postponed, the less likely it is to find that nematodes at low densities have a pronounced effect on plant properties. As Seinhorst based his theory on an experiment in which the first sampling took place 37 days after the start of the experiment (Seinhorst and Kozlowska, 1979), his observations do not indicate that different mechanisms are operating at low and high nematode densities.

Of the two simulated possibilities of nematode influence on water uptake, the possibility that the permeability of the roots is reduced as a result of the death of a some of the penetrated cells is the most likely. To explain the effect in terms of the decreased permeability of recovering cells, requires that these cells have a recovery period of about 3 hours. This is a rather long recovery period, although it falls within the vague boundaries set by Brzeski (1971). Moreover, contrary to the expectations a longer recovery period was needed in my simulations at 25 °C than at 18 °C. A more accurate measurement of the recovery period would show whether temporary disfunctioning of the penetrated cells could contribute to the effect of *T. dubius* on plant growth, or whether growth reduction is only caused by the death of some of the penetrated cells.

My assumptions on the permeability of young and old roots and of affected and non-affected cells were arbitrary. The difference in permeability between various regions along the roots has not been completely quantified (De Willigen and Van Noordwijk, 1987; Drew, 1987). The difference need not be as large as assumed here, but if the reduction in permeability of affected epidermal cells and old parts of the roots becomes less than about 50%, the hypothesis that the effect of *T. dubius* is mainly caused by a direct effect on water uptake is no longer plausible. In that case the nematode density at which the maximum effect is reached is

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too close to the density at which the moderate effect measured in the simulated experiments was obtained.

An effect of the nematodes on the permeability of the roots might also affect plant growth by influencing nutrient uptake. This hypothesis could not be tested with the present model, because the model assumes there is no nutrient shortage. If such an effect did occur in the simulated experiments, it was so slight that no clear symptoms of nutrient shortage occurred.

Hypotheses implying that the effect of nematodes is caused by induction of hormones or introduction of growth-reducing substances (Seinhorst, 1981) are meaningless unless the crop growth process affected is indicated. Only hypotheses that are quantified at the process level can be quantitatively tested.

6 Evaluation

6.1 MODELLING OF THE SYSTEM *L. PERENNE* / *T. DUBIUS*

A combination model to simulate the system *L. perenne/T. dubius* should consist of two models: one of the population dynamics of the nematode and the other a model of growth of the host plant. These two models are linked by their mutual relations. The models presented in this study considered the population dynamics of the nematode as affected by root growth (Chapter 4), and the growth of the host as affected by the nematode population (Chapter 5). It is premature to couple the two models, because they are not yet sufficiently reliable, especially with respect to the interactions between host and parasite.

Parameters and input relations of the nematode model
The parameters used in the model of the population dynamics of *T. dubius* were estimated from literature data on this nematode or, if these were lacking, from data on other nematodes. The simulations done with the model showed the need for a more accurate estimation of the parameter values, especially of the fecundity of the females and the influence of the temperature on the development rate. This could be done by establishing cohort life tables from experiments in which groups of individuals of the same age (development stage) are followed throughout their development (Poole, 1974). Such life tables would provide data on nearly all parameters occurring in the population model and would show whether juvenile mortality needs to be incorporated.

Food shortage
In the nematode model it was assumed that overpopulation of the roots and an associated food shortage would not occur during the first weeks of growth of the host (Chapter 4). The overestimates of the final nematode populations produced by the nematode model show that the effect of food shortage could not be ignored. The decrease of the ratio of nematodes to young roots with time found in the simulations on the effect of the nematode on the host (Chapter 5) shows that food shortage is most likely to occur during the early growth of the host. Slow root growth and high initial nematode density prolong the food shortage and the period during which the nematode directly influences plant growth. Therefore, food

shortage should be incorporated in the model of the population dynamics of the nematode.

The mean penetration rate per nematode, calculated from root data and magnitude of the nematode population as described in chapter 5, may be used as a measure of food shortage. Various hypotheses on the effect of food shortage on development rate, oviposition rate and mortality may be formulated.

Pre-emergence growth of the grass

The model of the population dynamics of *T. dubius* and the plant model were both initialized at the day of emergence of the grass, but in reality the nematodes attack the seedling from the moment the roots appear. In the experiments the number of emerging seedlings and the time of emergence were not noticeably influenced by the nematodes (initial nematode density ca. 10 individuals per g of soil), but this does not imply that the roots were not affected. *T. dubius* can prevent seedling emergence at high initial nematode densities. For instance, 50 % of the seedlings did not emerge at an initial density of 450 nematodes per g of soil (Seinhorst & Kozlowska, 1979).

The period from seeding to emergence should not be overlooked in the plant model and data on pre-emergence root growth should be introduced in the model for the population dynamics of *T. dubius*.

Effect of T. dubius at process level and cell level

The simulation studies (Chapter 5) showed that the nematode probably affects water uptake. This needs to be studied in process experiments.

The fate of the cells penetrated by *T. dubius* has to be studied to determine whether death of the cells or inactivity during recovery is the primary cause of reduced water uptake, and to permit a better estimate of the recovery period of the cells or the fraction of penetrated cells that die. The latter information is needed to quantify the relation between penetration rate and consequences for the host and to calculate the food availability for the nematode.

The results of the simulations of the hypothesis that all penetrated cells die (Chapter 5), indicate that the death of the penetrated cells observed by Wyss (1973) might have been caused by a lack of assimilates resulting from excision of the shoots. To avoid artifacts, the conditions during experiments intended to determine the fate of penetrated cells should permit the grass to grow normally.

Experiments in which the effect of penetration on the cells is determined can also be used to assess the length of the searching period. (This period is defined as the period between stylet retraction and

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successful penetration of a new feeding site.) The length of this searching period can be assessed by direct observation or by frequently counting the numbers of feeding and searching nematodes.

Root function and functional balance
The plant model used is descriptive with respect to root function, the consequences of moisture stress on photosynthesis and assimilate partitioning. For the simulation of the effect of *T. dubius* and root parasites and pathogens in general, explanatory simulation of plant water relations and functional equilibrium is preferable and should be pursued. However, the lack of a completely explanatory model does not justify avoiding simulating the effect root pathogens and parasites have on plant growth; the conceptual phase and the first steps of the comprehensive modelling phase (Rabbinge and De Wit, 1989) are by no means impeded.

With respect to the simulation of the presumed effect of *T. dubius* on water uptake, the reliability of the plant model can also be improved by introducing the descriptive effect of prolonged water stress (Van Keulen, 1982) as proposed in chapter 5.

6.2 DYNAMICS OF THE EFFECT OF *T. DUBIUS*

Susceptibility of the young plant
The results of the experiments (chapter 2) indicate that *T. dubius* affects the growth of *L. perenne* mainly during the seedling period, before the nodal roots appear.

Thus, the effect of *T. dubius* on plant growth processes is expected to be greatest during the seedling period. This is confirmed by the results of the simulations for the experiment at 18 °C in chapter 5. Whether the nematodes were assumed to affect respiration (hypothesis 2) or water uptake (hypotheses 3a and 3b), the effect on both processes decreased with time. The decrease started immediately if it was assumed that the effect of penetration did not depend on cell age (hypothesis 3a), and occurred later if the effect of penetration was assumed to increase with cell age (hypothesis 3b ,Fig 5.7). The decrease in the nematode effect was a result of the decrease in the ratio between the number of nematodes in the rhizosphere of the young roots to the quantity of young roots (ratio of nematodes to young roots). Seinhorst and Kozlowska (1977) reached the same conclusion for *Rotylenchus uniformis* on carrot.

Judging from the relative growth rates of the total nematode population and total plant mass during exponential growth in the temperature/moisture

experiments (Tables 2.3 to 2.6), the ratio of nematodes to young roots decreased with time in all nematode treatments. The smallest effect of the nematode on relative yield (dry matter yield of the nematode treatment as a fraction of dry matter yield of the control) occurred when the ratio of nematodes to young roots decreased most rapidly (at 18 °C), whereas the largest effect on relative yield corresponded with the slowest decrease of this ratio (at 10 °C).

The decrease of the ratio of nematodes to young roots does not necessarily continue during the growing season. In periods when root growth is small, e.g. after mowing, the ratio of nematodes to young roots may reach a harmful level again. Figure 6.1 shows a hypothetical course in time of this ratio.

The susceptibility of the young plant seems to result mainly from its small size. Therefore, in experiments intended to assess the importance of nematodes like *T. dubius* at population densities that occur in the field, the nematodes should be present in the soil or applied to it no later than on the day the host is sown. If the nematodes are inoculated later, or if older plants are used (e.g. Bridge, 1971), the absence of a nematode effect in such experiments does not prove that the nematodes are not harmful in the field.

Although the diameter of the roots may have some influence on the quantitative relation between the feeding activity on cells and plant growth, the decrease in the effect of nematodes on plant growth does not
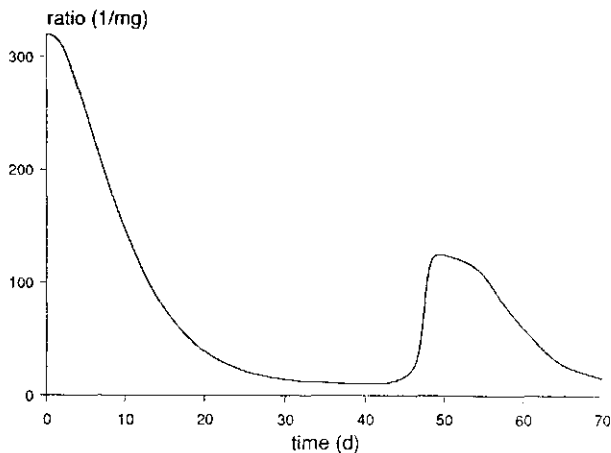

Fig. 6.1 Hypothetical course in time of the ratio of nematodes to young roots (nematodes/mg dry mass of the roots). Based on experimental and simulated results of T18NW during the first 36 days.

result from the appearance of nodal roots. When the nodal roots appear, the ratio of nematodes to young roots is already much smaller than at the emergence of the plants. Moreover, the decrease of the nematode effect with time also occurs in non-gramineous hosts (Seinhorst and Kozlowska, 1977).

Interpretation of results of experiments at the system level

At a given moment t the relative yield (RY_t) of plants exposed to nematodes is determined by the initial dry masses (dimension M) of the nematode treatment (y_{ni}) and the control (y_{ci}) and relative growth rates (dimension T^{-1}) of the nematode treatment (x_n) and the control (x_c) (eq. 6.1).

$$RY_t = \frac{y_{ni}}{y_{ci}} \cdot e^{(x_n - x_c)t} \tag{6.1}$$

If $x_n < x_c$ the relative yield decreases;
if $x_n = x_c$ the relative yield remains constant;
if $x_n > x_c$ the relative yield increases.

With constant conditions the relative growth rate of the control remains constant (exponential growth) until it is increasingly reduced as a result of inter plant competition. The relative growth rate in the nematode treatment may be reduced from the outset. Consequently, reduction as a result of competition occurs later in the nematode treatment than in the control. As long as the growth in the control is exponential, the relative yield can decrease or remain constant from the moment that the influence of the nematode on plant growth processes is negligable. Once inter-plant competition occurs, a decrease in the relative yield shows that the nematodes are still influencing plant growth processes. However, an increase in the relative yield does not necessarily indicate that their effect is negligable. Only if the relative growth rates of the nematode treatment and the control can be compared for equal plant masses can it be concluded when the nematodes are no longer important. This requires constant experimental conditions and frequent sampling.

The same applies to the specific leaf area (SLA) and the leaf weight ratio (LWR). Both features changed with plant mass during the temperature/moisture experiments (Chapter 2). In these experiments growth retardation during exponential growth was connected with a decrease of LWR and SLA. At a later stage these effects disappeared. This does not necessarily indicate that the parameters were no longer influenced by the nematode after the period of exponential growth.

Nature of tolerance and resistance

Measures that reduce the initial ratio of nematodes to young roots or that stimulate its decrease with time reduce the effect of *T. dubius* on *L. perenne*. At a given ratio of nematodes to young roots the effect of the nematodes is modified by the activity of the nematodes and the extent to which plant growth can be influenced by the nematode. Both can be influenced by the host variety.

Tolerance A tolerant host variety shows a faster absolute growth at a given nematode density than a less tolerant variety.

Tolerance can be a result of plant characteristics that also cause a high growth rate in the absence of nematodes, such as high values for SLA, LWR, initial light use efficiency and photosynthesis at light saturation, and a low value for dark respiration. A high growth rate resulting from a high value of the LWR may be less profitable than the same growth rate caused by a high SLA. In the former case the roots initially take less advantage of the high growth rate.

Tolerance can also be based on characteristics concerning the interaction between nematodes and host. For example, a variety may be more tolerant the smaller the fraction of epidermal cells that die when penetrated. The percentual effect of nematodes on yield is smaller in varieties showing this type of tolerance.

Resistance The slower the net reproduction rate of the nematode population, the more resistant the host variety. Figure 6.2 shows the simulated effect of the course in time of the nematode population on the relative yield of the shoots. In chapter 3 it was assumed that resistance is not important, because of the small reproduction of the nematodes during the period in which they influence plant growth. However, figure 6.2 shows that resistance may be more important than assumed. The impact of resistance increases with increasing initial nematode density, unless the initial density is so high that root growth is severely reduced.

Resistance may be a result of low mean penetration rate per nematode, high mortality, or low efficiency of food uptake and use by the nematode. Reduction of the mean penetration rate could be a result of larger cells or thicker cell walls. However, searching time and the time needed for penetration of the cell wall are small compared with the feeding time (Chapter 5). Consequently, their increase has little effect on the mean penetration rate. This suggests that differences in resistance to *T. dubius* are physiologically based. In general, hosts are resistant to nematodes because they do not supply the proper quantity or quality of food for the
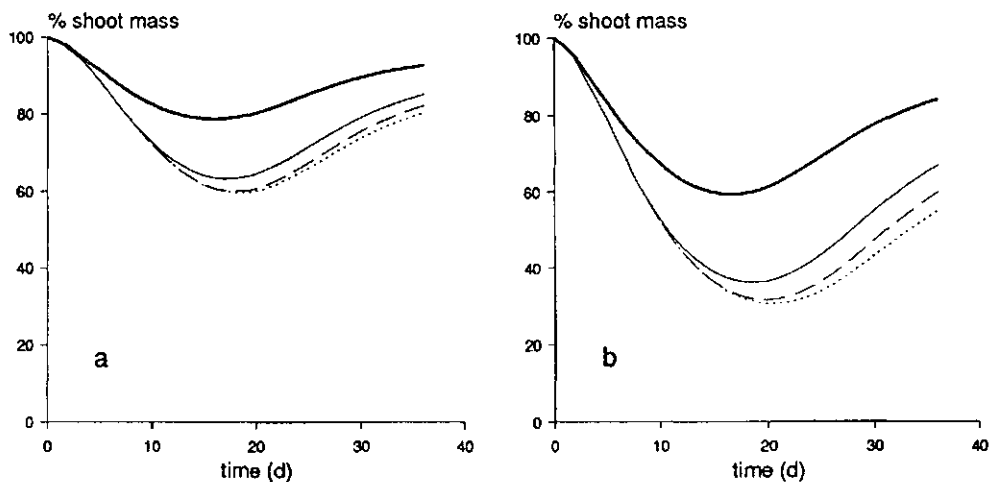
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Fig. 6.2 Influence of course in time of the population density of *T. dubius* on yield of the shoot as a percentage of the nematode-free control, simulated with hypothesis 3b (Chapter 5). A: growth of the nematode population as in T18NW, assuming that the density remained constant from emergence (————) and after 6 (————), 12 (——————) and 24 (················) days. B: the same for double the population density.

nematode, contain or produce nematicidal compounds, neutralize the nematode saliva, or isolate the nematode or its feeding site by a hypersensitive reaction (Gommers, 1981). Differences in food quality are the most probable cause of differences in resistance to *T. dubius*. They probably reduce the efficiency of food conversion, resulting in a slower rate of development and a lower rate of egg production.

6.3 INVOLVEMENT OF ECTOPARASITIC NEMATODES IN RESEEDING FAILURES

The ectoparasitic nematodes that occur most frequently in pastures (Table 1.1) differ from each other in their feeding behaviour and the consequences of the feeding on the roots. So the quantitative relations between nematode numbers and feeding activity and feeding activity and consequences on plant growth processes are unique for each species.

However, these species also show some similarities. All stages can be found throughout the year and they all feed on parts of the young roots (Berge et al, 1973; Bridge and Hague, 1974; Rhoades and Linford, 1961;

Wyss, 1971) They attack the roots almost from the moment they appear and reproduction can start almost immediately.

The application of the nematicide/insecticide thionazin to established swards resulted in a 10% increase of the dry matter production (Ennik and Baan Hofman, 1977). So, the numbers of ectoparasitic nematodes that generally occur in pastures have only a limited effect on the growth of established swards. Therefore, just as with *T. dubius*, the effect of the other ectoparasitic nematodes on plants is greatest during the first weeks after seeding. In this period nematode numbers are high relative to the amount of roots they feed on (high ratio of nematodes to young roots).

In pot experiments the effect of nematodes on relative yield may decrease with time. In pastures the consequences of growth retardation are more severe than in pot experiments, because the invasion of weeds in open spaces may prevent the effect on relative yield of *L. perenne* from decreasing with time.

Measures that stimulate vigorous seedling growth enhance the decrease of the ratio of nematodes to young roots. In practice this is probably the best way to minimize problems caused by ectoparasitic nematodes; nematicide application on pastures is prohibited in the Netherlands, but reduction of the population by rotation with non-hosts is difficult, because most ectoparasites have a large host range. Seedbed preparation, seed quality, fertilization, moisture conditions and temperature all influence seedling growth.

Temperatures that promote seedling growth also stimulate growth and activity of the nematode population, but the ratio of nematodes to young roots will probably decrease fastest at temperatures that are optimal for growth of the grass. So, reseeding from July until mid-September gives the best chance of minimizing nematode problems, provided that water supply is not limiting.

Grass resistance and tolerance based on characteristics concerning the interaction of a nematode and its host are expected to be specific. If the density and composition of the nematode population are not known, grass varieties that show a vigorous growth of the seedling will give the best chances for minimizing the effect of ectoparasitic nematodes.

Reseeding failure mainly occurs if conditions are not optimal for vigorous growth of the young seedlings. Ectoparasitic nematodes reinforce the negative effect of sub-optimal conditions. A combination model of the system that comprises grass and ectoparasitic nematodes can be helpful for assessing the risk of reseeding when conditions are sub-optimal for plant growth, depending on the composition and density of the population of

ectoparasitic nematodes. However, the best way to minimize the effect of ectoparasitic nematodes is to optimize the conditions for plant growth.

Summary

Every year approximately 13% of the pasture area in the Netherlands is reseeded to improve pasture composition and productivity. Reseeding is not always successful; it fails on 10% of the reseeded area. Experiments in which nematicides were applied at reseeding have shown that nematodes may be responsible for this failure.

Both ectoparasitic and endoparasitic nematodes may cause problems. Ectoparasites are always abundant; the effect of endoparasites is more severe, but these species occur less frequently.

This study was intended to elucidate the nature and functioning of the system that comprises pasture and ectoparasitic nematodes. For this purpose the host/parasite system *Lolium perenne/Tylenchorhynchus dubius* was studied. (Chapter 1)

In pot experiments the growth of *L. perenne* sown in soil inoculated with *T. dubius* was compared with that of grass sown in non-inoculated soil at constant temperatures of 10, 18 and 25 °C and under various moisture treatments.

The nematodes did not influence the time of emergence of the grass or the number of plants that emerged. They significantly decreased the dry matter yield. The percentage decrease of dry matter yield was smallest at 18 °C and largest at 10 °C; at 10 °C the effect increased with time, whereas it remained constant or tended to decrease with time at the other temperatures. Only at the final sampling of the experiment at 10 °C did moisture stress enhance the effect of the nematodes.

Growth analysis showed that the reduction of the relative growth rate during exponential growth in the nematode treatments was mainly connected with a lower leaf weight ratio and a smaller specific leaf area. These effects decreased with time.

The results of these experiments suggested that the nematodes reduce growth mainly during the first weeks after seeding and that the better the conditions for plant growth, the shorter the period in which the nematode effect is greatest.

An experiment was carried out to monitor the growth of *L. perenne* seeded in inoculated soil at a range of initial nematode populations. A linear relation was found between the logarithm of the initial density of the nematode population and dry matter production of the host. However, this relation did not apply to the dry treatment of the experiment at 25 °C

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although this was conducted under similar environmental conditions. This indicates that in addition to environmental conditions the viability of the nematode population may influence the effect of the nematodes on dry matter production of the host. (Chapter 2)

The reaction of the dry matter production of the host to nematode infestation determines the degree of tolerance of the host. To investigate the incidence of tolerance in commercial varieties of *L. perenne*, the effect of *T. dubius* on growth and production of ten *L. perenne* varieties with different origins was studied under controlled conditions. The relative shoot yield 30 days after emergence of the plants ranged from 0.67 to 0.76, with a mean of 0.71. Varieties with a relatively large root system (low leaf weight ratio) and a low transpiration coefficient tended to have a somewhat greater tolerance. However, the differences between the varieties were not significant, possibly because of the large variability in the measured values.

Differences in multiplication rate of the nematode population indicated differences in the resistance of the host varieties. However, the experiment was not suitable for measuring host resistance; the multiplication of the population on the varieties depended on the growth rate of the roots as well as on the quality of the host as food source. (Chapter 3)

In this research a start was made with the development of a combination model for the system *L. perenne/T. dubius*. Knowledge of the growth processes of *L. perenne* and of the biology of *T. dubius* can be incorporated in explanatory models to simulate the growth of the grass and the population dynamics of the nematode. Such a combination model can be used to predict the behaviour of the pathosystem under various circumstances and to predict the effect of various control strategies.

The preliminary model of the population dynamics of *T. dubius* was based on published data on the biology of *T. dubius* and if necessary on data on other species. The effect of food scarcity was not included in the model; the root growth of the host only affected the migration of the nematodes to the roots.

Experiments were simulated to estimate the value of certain parameters and to test the model. The results showed that certain parameters need to be determined more accurately and that food scarcity should be included in the model. However, for a limited set of environmental conditions the results of the simulations corresponded reasonably with the experimental results. (Chapter 4)

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An existing model for the simulation of plant growth (SUCROS) was adapted to simulate the growth of *L. perenne* as influenced by temperature and availability of soil moisture under controlled conditions.

An equation was formulated for the relation between nematode density in the rhizosphere of the young roots and the rate at which the nematodes penetrate epidermal cells.

To calculate the nematode effect from the penetration rate, various hypotheses on the effect of cell penetration on plant growth processes were formulated and included in the plant model. The plausibility of the hypotheses was tested by their ability to simulate experimental results with given population data. Data on some of the treatments of the experiments at 18 and 25 °C (Chapter 2) were used to do this.

The simulations showed that it is unlikely for all cells penetrated by *T. dubius* to die as a result of one penetration. Growth reduction may be attributed to the nematode negatively influencing the roots' permeability. (Chapter 5)

Both models need to be refined before coupling is meaningful. (Chapter 6.1).

The effect of *T. dubius* on the growth of *L. perenne* is largest in the first weeks of growth of the host, because the number of nematodes per unit young root mass is largest in this period. Consequently, the effects of the nematode on plant growth parameters are expected to be largest during this period.

Factors that stimulate the decrease of the ratio of nematodes to young roots decrease the effect of *T. dubius*. (Chapter 6.2)

This also holds for other ectoparasitic nematodes that occur in pastures. If the conditions at reseeding are optimal for growth of the host, the effect of the ectoparasitic nematodes is expected to be limited. Ectoparasitic nematodes reinforce the negative effect of sub-optimal conditions. A combination model of the system that comprises grass and ectoparasitic nematodes could be helpful for assessing the risk of failure of reseeding at conditions that are sub-optimal for plant growth, depending on the composition and density of the population of ectoparasitic nematodes. However, the best way to minimize the effect of ectoparasitic nematodes at reseeding is to reseed in the period from July until mid-September, providided that water supply is non-limiting. (6.3)

Samenvatting

Experimenten en simulatie studies aan het systeem *Lolium perenne/Tylenchorhynchus dubius*

Elk jaar wordt ongeveer 13% van het nederlandse grasland opnieuw ingezaaid om de botanische samenstelling en de produktiviteit te verbeteren. Dit heeft niet altijd het beoogde resultaat; op 10% van het ingezaaide oppervlak mislukt de herinzaai. Proeven waarin bij herinzaai nematiciden werden toegepast, toonden aan dat nematoden een rol kunnen spelen bij het mislukken van herinzaai.

Zowel ectoparasitaire als endoparasitaire nematoden kunnen problemen veroorzaken. Ectoparasitiare nematoden zijn altijd in grote aantallen aanwezig. Endoparasieten kunnen grotere problemen veroorzaken, maar ze komen minder algemeen voor.

Dit onderzoek had tot doel om meer inzicht te krijgen in aard en functioneren van het systeem grasland/ectoparasitaire nematoden. Hiertoe werd het waardplant/parasiet systeem *Lolium perenne/Tylenchorhynchus dubius* bestudeerd. (Hoofdstuk 1)

De invloed van *T. dubius* op de groei van *Lolium perenne* werd bij temperaturen van 10, 18 en 25 °C en bij verschillende vocht behandelingen in potproeven onderzocht.

Het opkomsttijdstip van het gras en het aantal opgekomen planten werden niet door de nematoden beinvloed. De nematoden veroorzaakten wel een significante opbrengstverlaging. Het procentuele effect op de droge stof opbrengst was het kleinst bij 18 °C en het grootst bij 10 °C; bij 10 °C nam het effect toe met de tijd, terwijl het constant bleef of enigszins afnam bij de andere temperaturen. Vochtstress versterkte het procentuele effect van de nematoden alleen op de laatste bemonsteringsdatum van de proef bij 10 °C.

Groeianalyse liet zien dat in de fase van exponentiele groei de verlaging van de relatieve groeisnelheid in de nematodenbehandelingen vooral samenhing met met een lagere spruit/wortel verhouding en een kleiner specifiek bladoppervlak. De effecten op spruit/wortel verhouding en specifiek bladoppervlak namen af met de tijd.

De uitkomsten van de potproeven suggereerden dat nematoden de groei van de plant vooral in de eerste weken na inzaai beinvloeden en dat de periode

waarin zij het meeste effect hebben korter is naarmate de groeiomstandigheden voor de plant gunstiger zijn.

In een proef waarin het effect van een reeks nematoden dichtheden in de grond op de groei van het gras bestudeerd werd, werd een lineair verband gevonden tussen de logaritme van de initiële nematoden populatie en de droge stof produktie van de waardplant. Deze relatie gold niet voor de uitkomsten van droge behandeling van de proef bij 25 °C, hoewel deze proef onder vergelijkbare omstandigheden uitgevoerd was. Dit wijst er op dat niet alleen milieufactoren maar ook de vitaliteit van de nematodenpopulatie het effect van de nematode op de droge stof produktie van de waardplant beïnvloeden. (Hoofdstuk 2)

De mate van tolerantie van een waardplant voor nematoden wordt afgemeten aan de grootte van het effect van de nematoden op de groei van de plant. Om het voorkomen van tolerantie in bestaande rassen van *L. perenne* te onderzoeken, werd het effect van *T. dubius* op groei en produktie van 10 variëteiten met verschillende herkomst onderzocht onder gecontroleerde omstandigheden. De relatieve spruitopbrengst 30 dagen na opkomst van de planten varieerde van 0.67 tot 0.76 met een gemiddelde van 0.71. Variëteiten met een relatief groot wortelsysteem (lage spruit/wortel verhouding) en een lage transpiratie coeffiënt vertoonden een wat grotere tolerantie. De verschillen tussen de rassen waren echter niet significant, mogelijk als gevolg van de grote variatie in de metingen.

Verschillen in vermenigvuldigings snelheid van de nematoden populatie op de variëteiten wezen op mogelijke verschillen in resistentie tussen de variëteiten. Dat kon echter niet met zekerheid geconcludeerd worden omdat de proef niet was opgezet om verschillen in resistentie te bepalen; de vermenigvuldiging van de nematoden populatie hing namelijk niet alleen af van de kwaliteit van de wortels als voedselbron maar ook van de groeisnelheid van de wortels. (Hoofdstuk 3)

In dit onderzoek werd een begin gemaakt met het ontwikkelen van een combinatiemodel voor de simulatie van het systeem *L. perenne/T. dubius*. Kennis van groeiprocessen vam *L. perenne* en de biologie van *T. dubius* kan ingebouwd worden in verklarende simulatiemodellen voor de groei van het gras en de populatiedynamica van de nematode. Zo'n combinatiemodel kan gebruikt worden om het gedrag van het systeem onder verschillende omstandigheden en het effect van mogelijke bestrijdingsstrategiën te voorspellen.

Het voorlopige model voor de populatie dynamica van *T. dubius* werd gebaseerd op literatuur gegevens; zo mogelijk voor *T. dubius* en anders voor andere soorten.

In het model beïnvloedde de wortelgroei alleen de migratie van de nematoden naar de wortel; er werd aangenomen dat er geen voedselgebrek optrad.

Om de waarde van sommige parameters te schatten en om het model te testen werden proeven gesimuleerd. De resultaten van de simulaties lieten zien dat de waarde van een aantal parameters nauwkeuriger bepaald moet worden en dat voedselschaarste in het model opgenomen moet worden. Desondanks kwamen de resultaten van de simulaties voor een beperkte set milieu condities redelijk met de gemeten waarden overeen. (Hoofdstuk 4)

Een bestaand model voor de simulatie van plantgroei (SUCROS) werd aangepast om de de groei van *Lolium perenne* onder invloed van temperatuur en bodemvocht onder gecontroleerde omstandigheden te simuleren.

Er werd een vergelijking opgesteld om de snelheid waarmee *T. dubius* epidermiscellen aanprikt te berekenen uit de nematodendichtheid in de rhizosfeer van de jonge wortels.

Er werden verschillende hypotheses opgesteld voor het gevolg van het aanprikken van cellen op de groei van de plant. Deze hypotheses werden elk afzonderlijk ingebouwd in het plantmodel. De aannemelijkheid van de hypotheses werd getoetst door na te gaan in hoeverre ze het in proeven gevonden effect van *T. dubius* op grond van de populatie gegevens konden verklaren. Hiervoor werden gegevens van enkele behandelingen van de proeven bij 18 en 25 °C (Hoofdstuk 2) gebruikt.

De resultaten van de simulaties laten zien dat het onwaarschijnlijk is dat alle door *T. dubius* aangeprikte cellen na eenmaal aanprikken dood gaan. De groeireductie van de plant die optreedt in aanwezigheid van *T. dubius* waarschijnlijk een gevolg van het negatieve effect van de nematoden op de doorlatendheid van de wortels. (Hoofdstuk 5)

Zowel het populatiemodel als het model voor de groei van *L. perenne* o.i.v. *T. dubius* moeten verbeterd worden voor het zinvol is om ze samen in een combinatiemodel op te nemen. (Hoofdstuk 6.1)

Het effect van *T. dubius* op de groei van *L. perenne* is het grootst in de eerste weken na inzaai, omdat in deze periode het aantal nematoden per eenheid jonge wortelmassa het grootst is. In deze periode zijn dan ook de grootste effecten op groeiparameters van de plant te verwachten.

Factoren die de afname van de verhouding tussen de nematoden en de jonge wortels stimuleren, verminderen het schadelijke effect van *T. dubius*.(6.2)

Dit geldt ook voor de andere ectoparasitaire nematoden die in grasland voorkomen. Als de condities bij herinzaai optimaal zijn voor de groei van de waardplant, is te verwachten dat het negatieve effect van ectoparasitaire nematoden beperkt is. Ectoparasitaire nematoden vergroten het negatieve effect van suboptimale condites. Een combinatie model van het systeem gras/ectoparasitaire nematoden kan nuttig zijn om het risico op mislukken van herinzaai onder minder gunstige condities in afhankelijkheid van samenstelling en dichtheid van de nematoden populatie te berekenen.

Het is echter beter om de condities bij herinzaai te optimaliseren en in te zaaien in de periode van juli tot midden september, vooropgesteld dat de vochtvoorziening in orde is.

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In 1974 behaalde zij het diploma gymnasium B aan het Stedelijk Gymnasium te Haarlem. In hetzelfde jaar begon zij haar studie planteziektenkunde aan de Landbouwhogeschool te Wageningen. Het doctoraal examen werd behaald in 1981 (met lof). Het doctoraalexamen omvatte de vakken Fytopathologie, Nematologie en Landbouwplantenteelt.

Van 1981 t/m 1985 was zij wetenschappelijk assistent bij de vakgroep Nematologie (28 uur per week). In deze periode werd de basis gelegd voor het in dit proefschrift beschreven onderzoek.

In de periode 1986 t/m 1990 werden de simulatiemodellen ontwikkeld en werd het materiaal tot vier artikels (zie p. ix) en dit proefschrift bewerkt.