Population dynamics of potato cyst nematodes and associated damage to potato

Promotoren: dr. ir. R. Rabbinge
hoogleraar in de produktie-ecologie
met bijzondere aandacht voor de plantaardige produktie

dr. ir. A.F. van der Wal
hoogleraar in de nematologie

Jan Schans

Population dynamics of potato cyst nematodes and associated damage to potato

Proefschrift

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STELLINGEN

1. Alleen resistentiemechanismen tegen aardappelcysteaaltjes, die aangrijpen op het initiëren van reuzecellen, zijn gekoppeld aan tolerantie voor deze aaltjes.

   Dit proefschrift.

2. Opbrengstverhoging door aardappelcysteaaltjes berust op een tijdelijk gunstiger balans tussen bruto assimilatie en onderhoudsademhaling van een geinfekteerd gewas in vergelijking met een gezond gewas.

   Dit proefschrift.

3. Don't count your *Globoderas* until they hatch.

4. Een verhoogde bladtemperatuur van aardappelplanten op met aardappelcysteaaltjes besmette grond, als gevolg van een verminderde openingstoestand van huidmondjes tijdens aanwezigheid van tweede-stadium juvenielen in de wortels, kan worden benut voor grondmonster-vrije opsporing van besmettingshaarden met behulp van infrarood remote-sensing technieken.

5. Bij gelijke initiële dichtheid veroorzaakt *Globodera pallida* een grotere opbrengstderving bij aardappel dan *G. rostochiensis*.

   Dit proefschrift.

6. Wetenschappelijke en niet-wetenschappelijke inspanningen met betrekking tot duurzaamheid zullen geen resultaat geven zolang (inter)nationaal overheidsbeleid wordt gedomineerd door de doelstelling van maximale bedrijfs- en staatswinsten.

7. Bij een onzekere toekomst dient op duurzaamheid gericht beleid een voortdurend zicht te hebben op alternatieve ontwikkelingspaden en hun begaanbaarheid.

8. Beperking van agrarische produktie tot het niveau van nationale zelfvoorziening, zoals volgt uit de omschrijving van ekologische landbouw volgens Goewie, gaat voorbij aan verschillen in produktiemogelijkheden tussen landen, bevordert ongelijkheden tussen landen en remt derhalve de ontwikkeling naar stabiele internationale verhoudingen.

   E.A. Goewie. Ecologische landbouw: een duurzaam perspectief? Inaugurele rede, Landbouwuniversiteit, Wageningen, 1993.

9. Rock & Roll als jeugdcultuur is de belangrijkste kracht achter de snelle emancipatie in de westerse samenleving, begonnen in de tweede helft van de twintigste eeuw.

10. De snelle groei van het aantal leden van de Nederlandse Voedsel-Allergie Stichting (NVAS) gedurende de afgelopen vijf jaar is vooral het gevolg van gebrek aan belangstelling, erkenning en deskundigheid bij medici voor een sinds 60 jaar goed onderzocht en gedocumenteerd ziektebeeld.

    A.H. Lowe. Food allergy, its manifestation, diagnosis and treatment. Philadelphia, Lei and Fabiger, 1931.

11. De duidelijkheid van lezingen wordt vaak negatief beïnvloed door oneigenlijk gebruik van de 'over-head' projector als 'behind-back' projector.

Stellingen behorend bij het proefschrift van Jan Schans: 'Population dynamics and associated damage to potato'.

Wageningen, 27 april 1993.

Abstract

Schans, J. 1993. Population dynamics of potato cyst nematodes and associated damage to potato. Ph.D. Thesis, Wageningen Agricultural University, The Netherlands. 115 pages, 16 tables, 36 figures.

Population dynamics of potato cyst nematodes (PCN; *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone) and their interactions with potato plants are insufficiently understood to explain variations of population increase and yield reduction among years and locations. This thesis describes experiments and simulation studies to elucidate mechanisms of PCN population increase and associated damage to potato. Models of potato crop growth and PCN population development, driven by radiation and ambient and soil temperatures, were linked through effects of second-stage juveniles on photosynthesis and through interactions among root growth, hatching and root invasion of second-stage juveniles. Photosynthesis reduction by second-stage juveniles during syncytial initiation could fully explain yield loss due to PCN. Population increase was explained by effects of the density of second-stage juveniles in roots on the sex ratio and on root death and concomitant death of the nematodes within. Simulated mechanisms of crop resistance and tolerance agreed with experimental observations. Tolerance was enhanced by resistance, acting upon or before the stage of syncytial initiation. The potential use of the model for development and evaluation of PCN control strategies in sustainable production systems is discussed.

Additional keywords: *Globodera rostochiensis*, *Globodera pallida*, *Solanum tuberosum* L., stomata, photosynthesis, tolerance, resistance, root growth, spatial root distribution, host-parasite interactions, hatching, development, production ecology

Voorwoord

Het in dit proefschrift beschreven onderzoek werd uitgevoerd in een samenwerking tussen de vakgroepen Nematologie en Theoretische Produktie-ecologie van de Landbouwuniversiteit Wageningen, in de periode 1984-1988. Mijn promotoren, prof. dr. ir. A.F. van der Wal en prof. dr. ir. R. Rabbinge, wil ik bedanken voor hun begeleiding. Van hun kritische kommentaar op de concept-hoofdstukken heb ik geleerd, hoe ik mijn gedachten ook voor anderen inzichtelijk kan maken. Rudy, je begrip en persoonlijke belangstelling voor zaken, die de afronding van dit proefschrift hebben vertraagd, en het daarbij gestelde vertrouwen in de goede afloop hebben mij zeer gestimuleerd.
Ik bedank de collega's van beide vakgroepen, Nol Mulder, Hans Mulder en Jans Roosjen (Hilbrands Laboratorium voor Bodemziekten), en Marcel van Oijen, Daniël van Kraalingen en Peter van Leeuwen (CABO-DLO) voor adviezen, hulp en inspirerende discussies bij opzet, uitvoering en verslaglegging van dit onderzoek. Ik heb goede herinneringen aan de samenwerking met Frits Arntzen (CPRO-DLO), die heeft geleid tot hoofdstuk 3 van dit proefschrift.
Een aantal mensen hebben met een doctoraalonderzoek bijgedragen aan dit proefschrift. Gerrie Tuitert, Leendert Molendijk, Gert Sikken, Ludo Koenders en Jan van Esch wil ik bedanken voor de enthousiaste wijze, waarop dit is gebeurd.
Ik ben dr. ir. J.H.J. Spiertz en dr. F.W.T. Penning de Vries zeer erkentelijk voor de geboden gelegenheid om het proefschrift op het CABO-DLO af te ronden.
Ik bedank mijn schoonmoeder, mevrouw Leemans-Seggers, hartelijk voor al die dagen, waarop ze de verzorging van Laura en Marleen van me heeft overgenomen. En Alet, tenslotte wil ik jou bedanken voor de ruimte, die je aan mijn avond- en weekendwerk hebt gegeven. Toekijken vanaf de zijlijn is wellicht vaak moeilijker geweest dan het spelen zelf en ik waardeer het zeer, dat je dit hebt kunnen opbrengen.

Contents

Account

The chapters 2 - 5 in this thesis have been published or are submitted to the following journals:

2. Schans, J. 1991. Reduction of leaf photosynthesis and transpiration rates of potato plants by second-stage juveniles of *Globodera pallida*. Plant, Cell and Environment 14: 707-712.

3. Schans, J. and Arntzen, F.K. 1991. Photosynthesis, transpiration and plant growth characters of different potato cultivars at various densities of *Globodera pallida*. Netherlands Journal of Plant Pathology 97: 297-310.

4. Schans, J. 199x. Temperature effects on hatching, juvenile development and reproduction of potato cyst nematodes: experiments and simulation. Fundamental and Applied Nematology, submitted.

5. Schans, J. 199x. Simulation of population dynamics of potato cyst nematodes and associated damage to potato plants. Agricultural Systems, submitted.

Chapter 1

General introduction

Potato cyst nematodes (PCN; *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone) are highly persistent, soil-borne pests of potato, causing yield losses of upto 80 % (Seinhorst, 1982a). PCN control has relied heavily on chemical soil disinfestation and, to a lesser extent, cultivar resistance to maximize cropping frequency of potatoes. However, intensive chemical control did not prevent increase of PCN population densities in infested fields and dispersion of PCN to other fields. This was caused by PCN multiplication on volunteer potatoes, loss of resistance to virulent populations, and insufficient effectiveness of chemical disinfestation and sanitary measures. Growing public awareness of environmental problems of chemical PCN control pressured the government and the potato production sector to substitute chemical control by (combinations of) other control measures, including resistance and tolerance to PCN, crop rotation and biological control (Anonymous, 1990; Bakker, 1990). Development of such cropping systems requires insight in the mechanisms of population dynamics and associated damage, as influenced by cultivar and population characteristics in fluctuating, site-specific environments.

Thusfar this insight has been insufficient, even for susceptible and intolerant cultivar-population combinations. In field experiments, multiplication rates of PCN on susceptible cultivars varied between 2.5 x and 150 x (Seinhorst, 1986c), and maximum yield loss due to PCN varied between 20 % (Seinhorst, 1982b) and 97 % (Greco *et al.*, 1982) of control yields. This variation is little understood and might be attributed to variation in weather and soil structure, experimental error, genetic variability of the functional response of PCN populations to environmental variables and differences in seed potato vigour, but the relative contribution of each factor remains largely unexplained. Descriptive models of PCN population increase and yield loss due to PCN have been formulated (e.g. Jones & Perry, 1978; Seinhorst, 1986b). These models describe the variation in multiplication rates and yield losses among sites and years with hindsight only, by fitting curves through historical data. Understanding of this variation can be gained with models, that explain population density and crop

yield through knowledge of the underlying physiological processes as affected by their environment (Rabbinge & de Wit, 1989). A first approach towards such an explanatory model of PCN population dynamics and associated damage was developed by Ward, Rabbinge & den Ouden (1985). With this model, several aspects of the potato - PCN system could be analyzed, but also the lack of knowledge of interactions between nematode invasion and crop growth processes was demonstrated.

In this thesis, research was focussed on these interactions at the earliest possible stage, i.e. second-stage juveniles just after their penetration in roots, and for the principal process of crop growth, i.e. photosynthesis (Chapter 2). Photosynthesis rate was strongly reduced by PCN at this stage. To investigate the consequence of this interaction for plant growth, the time course of photosynthesis was analyzed in relation to growth of four cultivars in soil, containing PCN eggs at several densities (Chapter 3). Reduction of total dry weight correlated with reduction of both leaf area and photosynthesis rate, but was not related to tolerance and resistance of the cultivars. To analyze the significance of this damage mechanism for yield reduction of field-grown crops, dynamic models of potato crop growth and PCN population dynamics were linked. The effect of temperature on the rate of development in all stages in the PCN life cycle was quantified in order to calculate the density of PCN in each developmental stage at any time during the potato growing season (chapter 4). These experimental results were supplemented with published data to develop a model of hatching in response to spatial root growth dynamics and the subsequent development of PCN to cysts (chapter 5). Yield reduction was largely explained by photosynthesis reduction caused by second-stage juveniles in roots. Population increase was regulated by the density of second-stage juveniles in roots, through effects on the sex ratio of the developing population, and by mortality of roots containing second-stage juveniles at densities exceeding the root 'carrying capacity'. Formulated mechanisms of tolerance and resistance affected yield loss and population increase in conformity with experimental observations. Possible applications of this modelling approach for analysis of the efficacy of PCN control measures in relation to variable environmental factors, required for development of sustainable potato cropping systems, are discussed in Chapter 6.

Chapter 2

Reduction of leaf photosynthesis and transpiration rates of potato plants by second stage juveniles of *Globodera pallida*

Abstract

Net photosynthesis and transpiration rates of potato plants, grown in pots in the greenhouse, were measured at various light intensities and ambient CO_2 concentrations, three days after inoculation with second stage juveniles of *Globodera pallida*. Gas exchange rates, both in darkness and in light, and the initial light use efficiency were strongly reduced by nematodes. Stomatal conductance of infected plants was lower than that of control plants and showed little response to decreasing ambient CO_2 concentration. The maximum internal CO_2 concentration of infected plants was lower than that of control plants. *G. pallida* reduced photosynthesis also by apparent non-stomatal effects.

The effects of *G. pallida* on gas exchange rates are similar to the effects of abscisic acid in the transpiration stream and of abiotic stresses in the root environment. Apparently there is a general response of plant roots to adverse conditions. The reduction of photosynthesis may be an important factor in yield reduction by potato cyst nematodes.

Introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone, cause serious yield losses in regions where potatoes are grown frequently. Empirical relations between PCN density and yield loss, averaged over several experiments, have been established by Seinhorst (1965) and Brown (1969). The parameter values in these relations vary strongly among years, locations and cultivars (e.g. Greco *et al.*, 1982; Seinhorst, 1982a; Brown, 1983). This variation may be due to effects of variable environmental factors on the

physiological processes that determine the behaviour of the potato - PCN system. These effects are not described by the Seinhorst and Brown equations.

Understanding of the effects of PCN on the physiological processes which determine the growth rate of a potato crop (e.g. photosynthesis, respiration and assimilate partitioning) is a prerequisite for explanation of the variation in yield reduction by PCN under field conditions. Several studies have been focused on effects of PCN on weight, dry matter content and mineral composition of plant organs and on plant water relations (reviewed by Trudgill, 1986). However, various disorders can cause the observed phenomena and the experiments do not explain which plant physiological processes are affected by PCN.

Disturbance of potato growth processes by PCN may occur during several phases of nematode development, in particular the invasion of roots by second stage juveniles and the withdrawal of plant metabolites by following nematode stages. In this paper the effects of penetration into roots and subsequent formation of syncytia by second stage juveniles of *G. pallida* on photosynthesis and transpiration rates of potato plants are investigated.

Materials and methods

Two experiments are described: one using the cultivar 'Irene' (susceptible) and nematode densities 0 and 15 juveniles g^{-1} soil; and one using the cultivar 'Darwina' (with PCN resistance derived from *Solanum vernei*) and nematode densities 0 and 65 juveniles g^{-1} soil.

Plants, nematodes and inoculation

Tubers (size 28-35 mm, with one sprout) of potato cultivars 'Irene' and 'Darwina' were surface sterilised in a hypochlorite solution and planted in pots containing 4 kg fertilized sandy loam soil to which 15 % by weight of water was added (pF=2; approximately field capacity). The pots were weighed and watered twice a day to keep soil moisture close to this level. The plants were grown in the glasshouse with 16 hour daylength. Minimum temperature was 14 °C and maximum temperature varied between 20 and 26 °C.

Cysts of *G. pallida* pathotype Pa2 were placed in root exudate solution at 20 °C after being soaked in water for 5 days. The root exudate solution was

changed each day and the emerged second stage juveniles were collected and stored at 5 °C. Hatching continued over a period of 10 days. Plants were inoculated 3 weeks after emergence. A suspension of the juveniles was injected into the soil using a veterinary syringe to approximate an even vertical distribution of juveniles. Eight uniformly distributed injections per pot were made. The control plants were inoculated with water.

Measurement of nematode density in roots

Roots were carefully separated from the soil, air-dried on filter paper for 15 hours, after which fresh weight was determined. The roots of control plants and 75 % (by fresh weight) of infected roots were placed at 80 °C for three days to determine dry weight. The remaining 25 % (by fresh weight) of the infected roots was used to estimate the number of penetrated juveniles. The roots were macerated with a blender. The suspension was poured over three sieves (350, 175 and 45 μ). The debris from the 45 μ and 175 μ sieves were collected separately and each was suspended in 80 ml. From each suspension two 5 ml samples were taken and stained with acid fuchsin. The nematodes present were counted. The debris from the 350 μ sieve was macerated again and the procedure was repeated to collect remaining nematodes. This appeared sufficient to collect all nematodes.

Gas exchange measurements

Three days after inoculation, rates of photosynthesis and transpiration were measured on the fifth leaf from the top. Leaves were counted starting from the first leaf with terminal leaflet > 1 cm^2. The laboratory assembly used is similar to the type described by Louwerse & van Oorschot (1969). The CO_2 concentration and water vapour content of the air stream entering and leaving the leaf chamber, irradiance, temperature and air humidity were recorded by a micro-computer. From these data net photosynthesis and transpiration rates, stomatal resistance and internal CO_2 concentration were calculated. For both cultivars the light response of gas exchange rates at a constant ambient CO_2 concentration of 620 mg m^{-3} was measured at irradiances ranging from 0 to 250 J m^{-2} s^{-1} (photosynthetically active radiation, PAR). These measurements continued 3 - 5 hours. The light response was characterised by three parameters: the net

photosynthesis rate at high irradiance (PHOTHI, in μg CO_2 m^{-2} s^{-1}), the respiration rate in the dark (RD, in μg CO_2 m^{-2} s^{-1}), and the initial light use efficiency (EFF, in μg CO_2 J^{-1}). Values of PHOTHI and RD were measured directly. Values of EFF were calculated as the increase in photosynthesis rate per unit of irradiance between darkness and the first light level. To separate between stomatal and non-stomatal effects on gas exchange (Rabbinge, Jorritsma & Schans, 1985; Kropff, 1987), the CO_2 response of gas exchange rates at a constant irradiance of 250 J m^{-2} s^{-1} (PAR) was measured for 'Darwina' at ambient CO_2 concentrations ranging from 1600 mg m^{-3} at the beginning of the measurements to 80 mg m^{-3} at the end. These measurements, performed immediately after measuring the light response, lasted 4 - 5 hours. The CO_2 response was characterised by the net photosynthesis rate at high ambient CO_2 concentration (PHOTHC, in μg CO_2 m^{-2} s^{-1}) and the initial slope of the CO_2 response curve, i.e. the mesophyll conductance (MC, in mm s^{-1}). Values of PHOTHC were measured directly. As a criterion of MC the increase in photosynthesis rate per unit of CO_2 concentration between the two lowest internal CO_2 concentration levels was employed. In all series, air temperature was approximately 20 °C and air humidity was approximately 10 mbar. The area and fresh and dry weights of the measured leaves were recorded immediately after the gas exchange measurements.

Gas exchange rates of plant leaves are controlled by the relative opening of stomata. Stomatal opening may be affected directly by a stimulus, or indirectly as a reaction on changes in the carboxylation rate (Schulze, 1986). Accordingly, effects of *G. pallida* on gas exchange rates may be caused by direct and indirect effects on stomatal conductance. In darkness carboxylation processes are absent, therefore an effect of *G. pallida* on H_2O and CO_2 exchange rates indicates a direct influence on stomatal conductance. An effect on CO_2 exchange rate only would indicate an effect of *G.pallida* on the leaf respiration rate. When gas exchange rates of irradiated leaves are affected by *G. pallida*, then direct and indirect effects on stomata may be present simultaneously. Indirect effects can be demonstrated by measuring the CO_2 response of gas exchange rates at constant high irradiance, under the assumption that the measured gas exchange is uniformly distributed over the leaf. Differences in stomatal resistance are eliminated by relating the photosynthesis rate to the internal CO_2 concentration.

The mesophyll conductance for CO_2 can then be calculated. If this conductance is reduced by *G. pallida*, then indirect effects on stomatal opening must exist. However, if the distribution of transpiration and photosynthesis over the leaf is non-uniform (Downton, Loveys & Grant, 1988), then indirect effects can not be distinguished.

Results

Photosynthesis and transpiration measurements

Dark respiration and transpiration rates (RD and TRANSD, respectively) of 'Darwina' plants infected with *G. pallida* were significantly lower than RD and TRANSD of 'Darwina' control plants (Table 2.1). This indicates that stomatal conductance was already reduced three days after inoculation with second stage juveniles of *G. pallida*. The dark respiration and transpiration rates of 'Irene' were not affected, probably because of the lower inoculum level used for this cultivar.

The light response of the net photosynthesis rate for both cultivars with and without nematodes is presented in Figures 2.1 and 2.2. The initial light use efficiency (EFF, Table 2.1) of 'Darwina' plants was significantly reduced by *G. pallida* infection, due to a reduction of the dark respiration rate in combination with a reduction of the net photosynthesis rate at the first light level (± 20 J m^{-2} s^{-1} PAR). At both irradiance levels stomatal conductance of 'Darwina' plants was more than halved by *G. pallida* (COND0 and COND1, respectively, Table 2.1), which limits CO_2 concentration at the carboxylation site and thus reduces the net photosynthesis rate. Similar effects of *G. pallida* on 'Irene' plants were observed, but significant differences occurred in light only (Table 2.1). At high irradiance, the net photosynthesis rate (PHOTHI, Table 2.1) was strongly reduced by *G. pallida*. This effect occurred on both cultivars, but was largest for the high nematode density on 'Darwina'. Transpiration rates were more strongly reduced by penetrating nematodes than the photosynthesis rates. This is reflected in a higher WUE (i.e. the ratio of photosynthesis rate to transpiration rate) of infected plants at all irradiance levels (Figure 2.3).

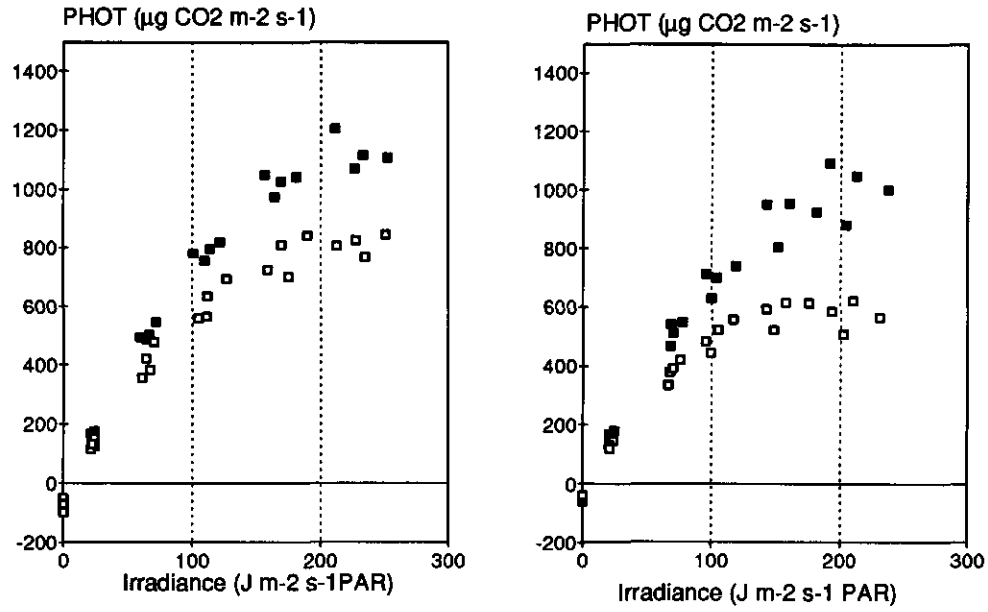


Figure 2.1. (left) Response of net photosynthesis rate (PHOT; µg m^{-2} s^{-1}) of leaves of potato cultivar 'Irene' to irradiance (J m^{-2} s^{-1}), three days after inoculation with hatched juveniles of *G. pallida* (density 15 juveniles g^{-1} soil), and of leaves of control plants. Symbols: (□) *G. pallida* infected plants; (■) control plants.

Figure 2.2. (right) Response of net photosynthesis rate (PHOT; µg CO_2 m^{-2} s^{-1}) of leaves of potato cultivar 'Darwina' to irradiance (J m^{-2} s^{-1} PAR), three days after inoculation with hatched juveniles of *G. pallida* (65 juveniles g^{-1} soil), and of leaves of control plants. Symbols: (□) *G. pallida* infected plants; (■) control plants.

The reduction of photosynthesis and transpiration rates by *G. pallida* may be caused by direct or indirect effects on stomatal closure. The response of stomatal conductance to decreasing ambient CO_2 concentration was analysed (Figure 2.4). At high CO_2 concentrations stomatal conductance of nematode infected plants was much lower than that of control plants. When the ambient CO_2 concentration was gradually decreased, stomatal conductance of control plants increased strongly until the CO_2-compensation point was reached at approximately 80 mg m^{-3}. This reflects the negative feedback of CO_2 supply for carboxylation processes on stomatal conductance (Goudriaan & van Laar, 1978). Stomatal conductance of nematode infected plants remained low when

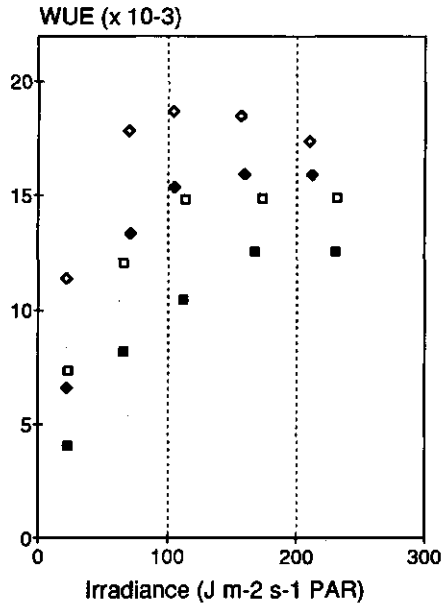


Figure 2.3. Response of water use efficiency (WUE, x 10^{-3}) of leaves of potato cultivars 'Irene' and 'Darwina' to irradiance (J m^{-2} s^{-1}), three days after inoculation with hatched juveniles of *G. pallida*, and of leaves of control plants. Symbols: (◊) 'Darwina' infected with *G. pallida*; (♦) 'Darwina' control plants; (□) 'Irene' infected with *G. pallida*; (■) 'Irene' control plants.

ambient CO_2 concentration decreased. This indicates a direct inhibition of stomatal opening by *G. pallida*.

To investigate whether *G. pallida* influenced carboxylation processes of leaves as well, the CO_2 response of net photosynthesis at light saturation of control and infected plants was analysed (Figure 2.5). Here photosynthesis rate is related to internal CO_2 concentration to eliminate differences in stomatal resistance. The values of PHOTHC and MC are given in Table 2.2. PHOTHC is strongly reduced by *G. pallida*. The internal CO_2 concentration at which PHOTHC is reached is lower for infected plants than for control plants. The calculated mesophyll conductance for CO_2 (MC) is significantly reduced by nematodes as well. This indicates either a non-uniform distribution of stomatal closure over the leaf, or a reduction of the carboxylation rate by *G. pallida*, which indirectly reduces stomatal aperture.

The water content of the measured leaves of infected plants tended to be higher than that of control plants for both cultivars, but this effect was not significant (Table 2.3).

Nematode density in roots

The estimated total number of juveniles present in the root system was 7160 for 'Irene', 12 % of inoculum, and 18820 for 'Darwina', 7 % of inoculum (Table 2.3). A fraction of the juveniles had slightly swollen bodies, indicating that initiation of syncytia had occurred. The root system of 'Darwina' plants contained 3.3 times

Table 2.1. Measured values of respiration rate in darkness (RD), transpiration rate in darkness (TRANSD) initial slope of light response (EFF), stomatal conductance in darkness and at ± 20 J m^{-2} s^{-1} PAR (COND0 and COND1, respectively) and net photosynthesis rate at high irradiance (PHOTHI), for plants infected with *G. pallida* and control plants of the potato cultivars 'Irene' and 'Darwina'. The P-value refers to the probability of false rejection of differences between infected and control plants.

	'Irene'			'Darwina'		
Variable	Control	Infected		Control	Infected	
RD (μg CO_2 m^{-2} s^{-1})	58.3	59.5	(P=0.815)	55.3	43.5	(P=0.012)
TRANSD (mg H_2O m^{-2} s^{-1})	12.6	9.9	(P=0.385	23.3	10.6	(P=0.006)
EFF (μg CO_2 J^{-1})	9.9	8.1	(P=0.007)	10.0	8.2	(P=0.009)
COND0 (mm s^{-1})	0.9	0.8	(P=0.374)	2.3	0.9	(P=0.006)
COND1 (mm s^{-1})	3.6	1.5	(P=0.006)	2.5	1.0	(P=0.004)
PHOTHI (μg CO_2 m^{-2} s^{-1})	1123.5	810.9	(P<0.001)	1006.1	570.0	(P<0.001)

as many nematodes per gram dry root as the root system of 'Irene' plants. The number of recovered juveniles, expressed as a fraction of the total number inoculated, was low. Turner & Stone (1984) observed, that the recovery efficiency of early development stages of *G. pallida* from potato roots is lower than that of later stages. Therefore it is likely that the actual number of juveniles penetrated in the roots was higher.

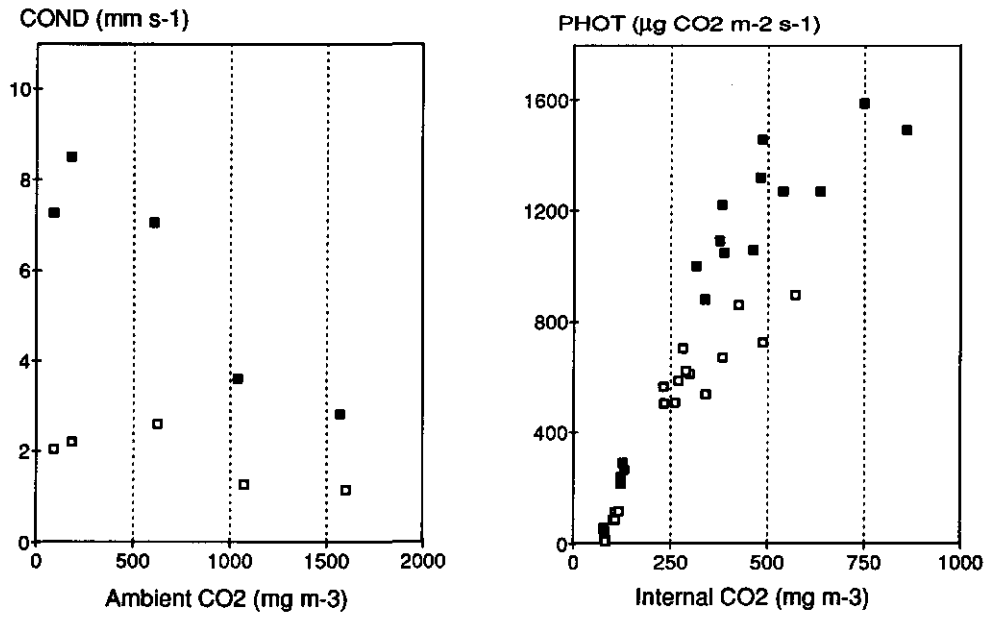


Figure 2.4. (left) Response of stomatal conductance (COND; mm s^{-1}) to ambient CO_2 concentration (mg m^{-3}) at high irradiance of leaves of potato cultivar Darwina, three days after inoculation with hatched juveniles of *G. pallida*, and of leaves of control plants. Symbols: (□) *G. pallida* infected plants; (■) control plants.

Figure 2.5. (right) Response of net photosynthesis rate (PHOT; µg m^{-2} s^{-1}) to internal CO_2 concentration (mg m^{-3}) at high irradiance of leaves of potato cultivar Darwina, three days after inoculation with hatched juveniles of *G. pallida*, and of leaves of control plants. Symbols: (□) *G. pallida* infected plants; (■) control plants.

Discussion

Three days after inoculation second stage juveniles of *G. pallida* reduced leaf photosynthesis and transpiration rates of potato plants by an effect on stomatal resistance and a apparent independent effect on carboxylation processes. The latter effect is likely to be an artifact due to the erroneous assumption of uniform distribution of gas exchange over the leaf (Downton *et al.*, 1988). Similar effects on photosynthesis have been observed after application of abscicic acid (ABA) to the transpiration flow through leaves of various plant species (Fischer, Raschke & Stitt, 1986; Seemann & Sharkey, 1987). The process of penetration and initiation of syncytia by *G. pallida* in potato roots might therefore increase the level of ABA in the leaves. Seemann & Sharkey (1987) suggest that ABA production in leaves is the common link between any abiotic stress and reduction in photosynthesis rate. The results of my work suggest that this may also occur with *G. pallida* on potato. This is supported by Fatemy *et al.* (1985), who showed elevated ABA levels in leaves of potato plants, 47 days after planting in soil infested with cysts of *G. rostochiensis.*

The stomatal closure in nematode infected plants may be explained by two possible mechanisms: induction of water stress in leaves due to reduced water uptake capacity of roots, or interference with production and transport of hormones synthesised in roots. The first mechanism is unlikely, because the leaf water content of infected plants was not lower than that of infected plants. The second mechanism is supported by Gollan, Passioura & Munns (1986), who show that stomata close in response to a signal from the roots, independent of leaf water potential. The nature of the messenger substance, released by roots

Table 2.2. Measured values of the net photosynthesis rate at high ambient CO_2 concentration (PHOTHC) and of the initial slope of the CO_2 response (MC), for plants infected with *G. pallida* and control plants of the potato cultivar 'Darwina'. The P-value refers to the probability of false rejection of differences between infected and control plants.

Variable	Control	Infected
PHOTHC ($\mu g\ CO_2\ m^{-2}\ s^{-1}$)	1452.1	797.1 (P<0.001)
MC ($mm\ s^{-1}$)	4.6	3.6 (P=0.041)

in drying soil, is not yet elucidated (Munns & King, 1988; Zhang & Davies, 1990). A reduction of photosynthesis rate not linked to water stress has been reported for several plants infected with nematode species from the families *Heteroderidae* and *Meloidogynidae* (e.g. Poskuta, Dropkin & Nelson, 1986; Melakeberhan *et al.*, 1985). Brueske & Bergeson (1972) observed reduced cytokinin levels in root xylem sap of tomato plants infected with *Meloidogyne incognita*. Cytokinin may be the messenger for stomatal response to nematode infection.

It is unknown which actions of *G. pallida* specifically induce the closure of stomata. Rice, Stone & Leadbeater (1987) observed that susceptible and resistant potato plants react similarly to penetration and movement of *G. pallida* juveniles through the root cortex. Within 48 hours after inoculation of second stage juveniles on potato roots grown in Petri dishes, syncytial formation was initiated in roots of all clones tested. Subsequent development of syncytia was checked by resistance. In my work the average temperature was approximately equal to the constant temperature of 20 °C in the experiments of Rice *et al.* (1987). On the assumption that the period, necessary for movement of nematodes through the soil to the penetration site on the root surface, was 1 - 2 days (Oostenbrink, 1950), nematode - plant interactions had advanced to the stage of syncytial formation at the time of the gas exchange measurements. This

Table 2.3. Water content (1.0 - dry weight/fresh weight) of measured leaves, immediately after gas exchange measurements, and root fresh and dry weights of plants infected with *G. pallida* and of control plants of the potato cultivars 'Irene' and 'Darwina' and the estimated number (± SE; n=4) of nematodes in the root system of infected plants. The P-value refers to the probability of false rejection of differences between infected and control plants.

	'Irene'		'Darwina'	
Variable	Control	Infected	Control	Infected
Leaf water cont.	0.872	0.877 (P=0.055)	0.874	0.881 (P=0.059)
Root fresh w. (g)	8.7	7.9 (P=0.505	7.5	6.7 (P=0.550)
Root dry w. (g)	1.6	1.5 (P=0.580)	1.4	1.2 (P=0.440)
Nematodes		7160 ± 1930		18820 ± 6790

is confirmed by the recovery of slightly swollen second stage juveniles from infected root systems. Therefore stomatal closure must result from nematode actions just before or during formation of syncytia. Probably the disturbance of cell development just behind the root tips, which preceeds syncytial formation (Seinhorst, 1986a), interferes with the production of hormones which act as messengers for stomatal closure (Munns & King, 1988).

This work demonstrated stomatal closure and reduction of leaf photosynthesis rate of potato plants due to root invasion by hatched second stage juveniles of *G. pallida*. The resistance of 'Darwina' did not prevent the nematode effects. This fits with field observations where resistant cultivars suffer as much damage as susceptible cultivars (Velema & Boerma, 1987). However, in the natural situation with cyst inoculum stomatal function may be affected differently, because invasion of roots by PCN is dispersed over several weeks and the root density of second stage juveniles at each moment is much lower than that in my experiments. The response of stomatal function to nematode infection may also be influenced by the tolerance level of the cultivar (Fatemy *et al.*, 1985). The consequences of these considerations for photosynthesis and stomatal functioning in relation to plant growth are reported in Chapter 3.

Chapter 3

Photosynthesis, transpiration and plant growth characters of different potato cultivars at various densities of *Globodera pallida*

Abstract

Plants of four potato (*Solanum tuberosum* L.) cultivars were grown in pots in a greenhouse at five densities of *Globodera pallida* between 0 and 300 eggs per gram of soil. Photosynthesis and transpiration of selected leaves were measured at 30, 37, 49 and 60 days after planting. Stem length was recorded at weekly intervals. Plants were harvested 70 days after planting and various plant variables were determined.

At 30 days after planting, when second and third stage juveniles were present in roots, both photosynthesis and transpiration rates were severely reduced by *G. pallida.* In the course of time these effects became less pronounced. Water use efficiency was reduced by *G. pallida* between 30 and 49 days, but not at 60 days after planting. The results suggest independent effects of *G. pallida* on stomatal opening and on photosynthesis reactions. There were no consistent differences among cultivars in the response of leaf gas exchange rates and water use efficiency to nematode infection. Reduction of photosynthesis by *G. pallida* appeared additive to photosynthesis reduction due to leaf senescence.

Total dry weight was reduced by 60 % at the highest *G. pallida* density. Weights of all plant organs were about proportionally affected. Shoot/root ratio was not affected and dry matter content was reduced. Stem length and leaf area were most strongly reduced during early stages of plant-nematode interaction. The number of leaves formed was only slightly reduced by *G. pallida*, but flowering was delayed or inhibited. Reduction of total dry weight correlated with reduction of both leaf area and photosynthesis rate. Leaf area reduction seems the main cause of reduction of dry matter production. Tolerance differences among cultivars were evident at 100 eggs per gram of soil only, where total dry

weight of the intolerant partially resistant cv. Darwina was lower than that of the tolerant partially resistant cv. Elles and of the tolerant susceptible cv. Multa. The tolerance differences were not correlated with leaf photosynthesis and transpiration. Apparently these processes are not part of tolerance of plants.

Introduction

Potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone, cause reduction of growth rate and tuber yield of potato plants. Under the same environmental conditions, the amount of damage due to PCN varies among susceptible as well as among resistant cultivars (Huijsman *et al.*, 1969; Dale *et al.*, 1988). The term 'tolerance' has been used for several aspects of this phenomenon. Here tolerance is defined as the complex of 'physiological mechanisms that enable the plant, passively or actively, to counteract the stresses caused by environmental constraints including parasitic nematodes'. (Wallace, 1987). Although effects of PCN on plant growth characteristics have been shown in many studies (summarized by Trudgill, 1986), the causal mechanisms of damage and the nature of tolerance remain largely unclear. Insight in these phenomena can be gained from the effects of PCN on the physiological processes that determine plant growth and yield, e.g. photosynthesis and dry matter distribution. This may identify the key factors of tolerance and improve selection methods for higher tolerance levels.

Plant growth reduction due to nematode infection was associated with reduction of photosynthesis rate for *Phaseolus* bean infected with *Meloidogyne incognita* (Melakeberhan *et al.*, 1985) and for soybean infected with *Heterodera glycines* (Poskuta *et al.*, 1986). In Chapter 2 a strong reduction of photosynthesis and transpiration rates of potato plants, due to invasion into roots by second stage juveniles of *G. pallida* was demonstrated, but effects of nematodes on plant growth were not investigated.

In order to investigate whether the photosynthesis response of different potato cultivars to infection by *G. pallida* contributes to their tolerance of damage by *G. pallida*, measurements of leaf gas exchange rates at several times after plant emergence and at several densities of *G. pallida* were combined with measurements of plant growth rate. The experiment was carried out in pots in a greenhouse, where PCN-free treatments could be included as the control series and where root weights could be measured accurately.

Materials and methods

Plants and nematodes

Eye scoops of potato tubers with one sprout were planted on 7 July 1987 in pots containing 4 kg of fertilized sandy loam soil to which 15 mass % water was added (pF=2). Pots were watered twice a day to keep soil moisture approximately at this level. At planting, an egg suspension of *G. pallida*, pathotype Pa3, was added to the pots as described in Chapter 2. Five density levels were established: 0, 10, 30, 100 and 300 eggs g^{-1} soil. Four potato cultivars were used. Two, 'Darwina' and 'Elles', are partially resistant to Pa3, but 'Elles' has a higher tolerance level (Boerma and Velema, 1987). The other two cultivars, 'Multa' and 'Eba', are susceptible to PCN. The high tolerance level of 'Multa' is well documented (Huijsman *et al.*, 1969) and 'Eba' is relatively intolerant (Ir. A. Mulder, pers. comm.). The experiment was done in a randomized block design with seven replicates. The pots were kept in a naturally lit glasshouse at 18 °C for 10 hours and 12 °C for 14 hours per day. Relative air humidity was kept at 80 %. Plants emerged on average 10 days after planting. Analysis of root samples indicated that at 30 days after planting numerous nematodes, mainly second and third stage juveniles, were present in the roots. Tubers were not yet present at that time.

Photosynthesis and transpiration rates

Photosynthesis rate (PHOT; expressed in µg CO_2 m^{-2} s^{-1}) and transpiration rate (TRANS; expressed in mg H_2O m^{-2} s^{-1}) of terminal leaflets of leaves were measured with the LCA-2 gas analysis system (The Analytical Development Company Ltd., Hoddesdon, England). Air was supplied from a gas cylinder at a rate of 400 ml/minute and contained approximately 585 mg m^{-3} CO_2. Air temperature in the leaf chamber varied between 20 and 24 °C. The relative humidity of the air was 40-50 %. The halogen light source delivered 360 J m^{-2} s^{-1} (1800 µEinstein m^{-2} s^{-1}) photosynthetically active radiation at the leaf surface. Water use efficiency (WUE) was calculated as PHOT/TRANS.

At 30 days after planting, the first leaf from the plant top with length > 2.5 cm was labeled A. The leaves, which developed subsequently above leaf A, were labeled B through E respectively. The position of the leaves on the plant at

Table 3.1. Position of leaves used for gas exchange measurements at each observation date (days after planting). The leaf label indicates the relative position of the measured leaf. Leaf A is the first leaf from the plant top with length > 2.5 cm at 30 days after planting, leaves B through E have developed subsequently above leaf A. The leaf number indicates the actual position of the leaf, relative to the plant top, at the time of measurement.

observation date	leaf label	leaf number at measurement time
30	A	5
37	A	7
	B	6
	C	5
49	C	7-8
	D	6-7
60	E	8-10

the time of measurement are presented in Table 3.1. At 30, 37, 49 and 60 days after planting gas exchange rates of selected leaves were measured in light. At 30 and 49 days after planting gas exchange rates were also measured in darkness.

Plant growth

Stem length was recorded at weekly intervals during plant growth. The plants were harvested 70 days after planting, and the number of leaves formed was counted. The highest leaf with a length > 2.5 cm was regarded as leaf 1. Three groups of leaves were distinguished. They are referred to as young (leaf 1-5), intermediate (leaf 6-10) and old leaves (leaf 11-15). Leaf area and length of the stem parts for these groups were recorded. Also, the diameter of the stem 30 cm under the top was measured. Fresh and dry weight of leaves, stems, stolons, roots and tubers were determined. Shoot/root ratio's were calculated on dry weight basis. Dry matter content was calculated as total dry weight / total fresh weight. Specific leaf area was calculated as leaf area/dry weight of leaves and

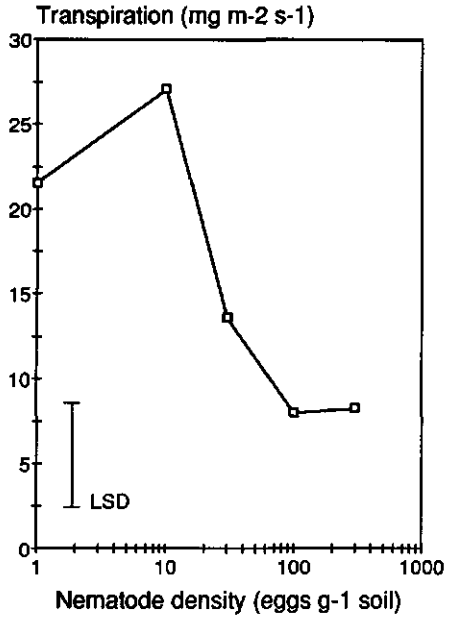


Figure 3.1. Transpiration rate (TRANS, mg H_2O m^{-2} s^{-1}) of leaf A in darkness, averaged over four potato cultivars at various densities of *G. pallida* (eggs per gram of soil), at 30 days after planting. The vertical bar indicates the least significant difference (LSD, P=0.05).

specific stem length as stem length/dry weight of stem. Finally, cysts were extracted and counted. Tolerance differences among cultivars were examined as differences in final total dry weight per nematode density level. Correlations between various characteristics were calculated, based on means per cultivar and density.

Results

Photosynthesis and transpiration rates

Significant differences in gas exchange rates among cultivars were found on each observation date, but these were neither consistent with nematode density nor with time. On each observation date, the relative reduction of photosynthesis and transpiration rates due to *G. pallida* was not different among cultivars.

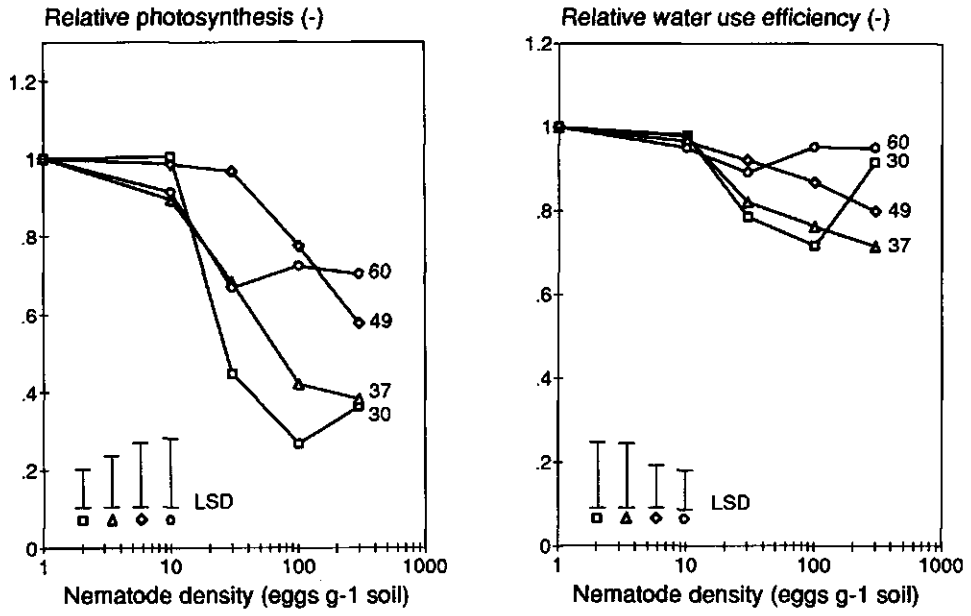


Figure 3.2. (left) Photosynthesis rate in light (PHOT) at each observation date, averaged over four potato cultivars, at various densities of *G. pallida* (eggs per gram of soil), relative to control plants. Symbols: (❑) leaf A; 30 days after planting. (Δ) leaf C; 37 days after planting. (◊) leaf D; 49 days after planting. (O) leaf E; 60 days after planting. The vertical bars indicate the least significant difference (LSD, P=0.05) for each observation date.

Figure 3.3. (right) Water use efficiency (WUE) at each observation date, averaged over four potato cultivars, in light at various densities of *G. pallida* (eggs per gram of soil), relative to control plants. Symbols: (❑) leaf A; 30 days after planting. (Δ) leaf C; 37 days after planting. (◊) leaf D; 49 days after planting. (O) leaf E; 60 days after planting. The vertical bars indicate the least significant difference (LSD, P=0.05) for each observation date.

Therefore no correlation between effects of *G. pallida* on leaf gas exchange and known tolerance or resistance characteristics of the four potato cultivars to *G. pallida* was apparent.

Respiration in darkness at 30 days after planting was not affected by *G. pallida*, but TRANS in darkness was strongly reduced at 30 or more eggs g^{-1} soil (Figure 3.1). In darkness no photosynthesis occurs, therefore these results suggest a direct reduction of stomatal opening by *G. pallida*. Gas exchange

Table 3.2. Absolute values of photosynthesis rate (PHOT) and water use efficiency (WUE, *1000) of indicated leaves of control plants (0 eggs g^{-1} soil) in Figures 3.2 and 3.3, at each observation date (days after planting). The values are averages of the four cultivars.

observation date	leaf measured	PHOT ($\mu g\ CO_2\ m^{-2}\ s^{-1}$)	WUE
30	A	532.2	9.1
37	C	605.2	10.1
49	D	457.4	9.4
60	E	490.9	7.2

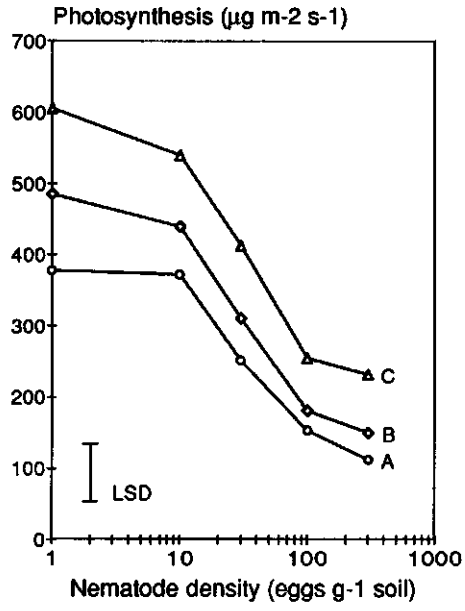
measurements at 49 days after planting revealed no effects of nematode density.

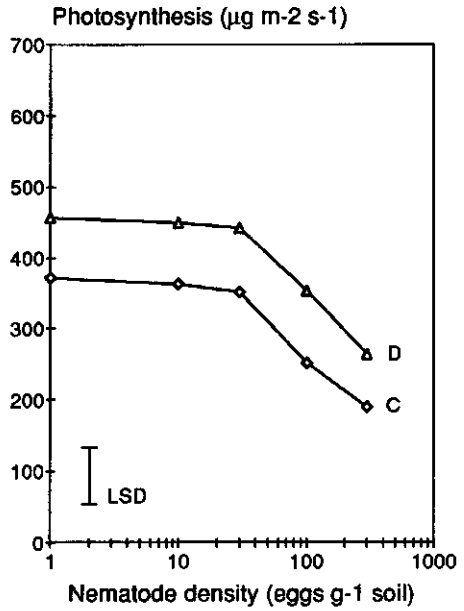
At 30 days after planting, *G. pallida* infection reduced PHOT of irradiated leaves to about 45 % of control values at 30 eggs g^{-1} soil, and to about 30 % of control values at 100 and 300 eggs g^{-1} soil (Figure 3.2). In the course of time, the reduction of PHOT by *G. pallida* became less pronounced. At 37 days after planting, PHOT of plants infected with *G. pallida* at 30 eggs g^{-1} soil was still about 70 % of control values and only at 300 eggs g^{-1} soil a reduction to 30 % of control values was observed. At 49 days after planting, PHOT was reduced to about 80 % and 60 % of control values at 100 and 300 eggs g^{-1} soil, respectively. The trend of diminishing effects of *G. pallida* with time was not continued at 60 days after planting, when PHOT was reduced by *G. pallida* at 30 or more eggs g^{-1} soil to about 70 % of control values. The absolute values of PHOT of control plants are presented in Table 3.2.

Infection by *G. pallida* reduced TRANS of irradiated leaves along with, but not always proportional to the reduction of PHOT. At 30, 37 and 49 days after planting, WUE (= PHOT/TRANS) was increasingly reduced with increasing *G. pallida* densities (Figure 3.3), indicating that PHOT was more reduced than TRANS by *G. pallida*. This suggests that gas exchange rates of infected plants were reduced mainly by limitations on photochemical or biochemical processes of photosynthesis rather than on stomatal functioning. The high WUE 30 days after planting at 300 eggs g^{-1} soil is an inexplicable exception. At 60 days after

planting, WUE was not affected by nematodes. The absolute values of WUE of control plants are presented in Table 3.2.

The interference of effects of *G. pallida* with ageing of leaves was analysed by comparing PHOT of leaves A, B and C at 37 days after planting (Figure 3.4), and PHOT of leaves C and D at 49 days after planting (Figure 3.5). At 37 days after planting, PHOT of leaves A and B was significantly lower than PHOT of leaf C, at all *G. pallida* densities. PHOT of leaf A was significantly lower than that of leaf B only at 0 and 10 eggs g^{-1} soil. At 49 days after planting, PHOT of leaf C was significantly lower than that of leaf D, irrespective of nematode density. Interaction between the effects of leaf age and nematode density on PHOT was absent (F-probability = 0.59 and 0.99, respectively). This indicates that *G. pallida* did not affect senescence processes in potato leaves in this period.





Figures 3.4-3.5. Effect of leaf position on photosynthesis rate (PHOT; µg CO_2 m^{-2} s^{-1}) of potato leaves in light at various densities of *G. pallida* (eggs per gram of soil). Average of four cultivars. The vertical bar indicates the least significant difference (LSD, P=0.05).

Figure 3.4. (left) 37 days after planting. Symbols: (O) leaf A, (◊) leaf B and (Δ) leaf C . **Figure 3.5.** (right) 49 days after planting. Symbols: (◊) leaf C and (Δ) leaf D.

Plant growth

Significant interaction between cultivars and density of *G. pallida* was observed only for dry weight of roots and tubers, total stem length at 50 days after planting and the length of the stem section carrying the oldest leaves at 70 days after planting. The correlation of these characters with total dry weight at 70 days after planting (TDW) was high. Cultivar effects for the other measured plant characters were not important when compared with effects of nematode density.

Stem length was reduced by *G. pallida* from 28 days after planting onwards (Figure 3.6). Between 21 and 42 days after planting, the growth rate of stem length was significantly reduced at 30 and more eggs g^{-1} soil. From 42 to 56 days reduction of stem length growth rate occurred at 100 and 300 eggs g^{-1} soil. Between 63 and 70 days after planting stem length growth rate was reduced at 300 eggs g^{-1} soil only.

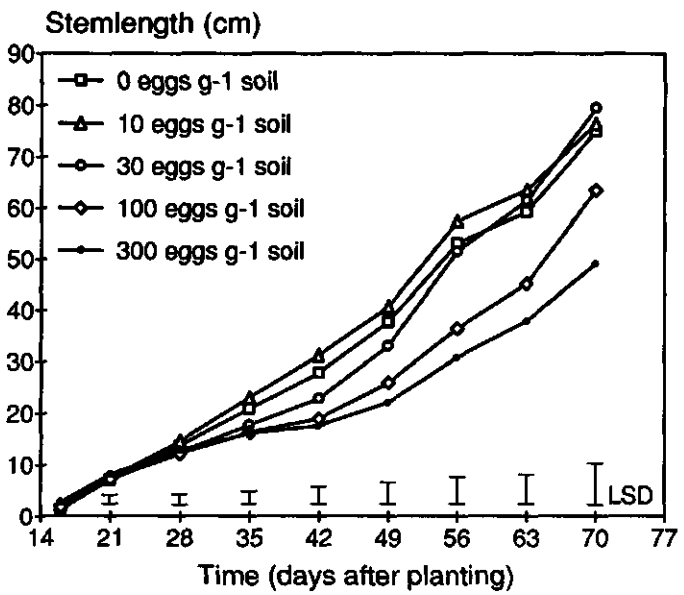


Figure 3.6. Stem length of potato plants (cm), averaged over four cultivars, at weekly intervals for five *G. pallida* densities (eggs per gram of soil). The vertical bars indicate the least significant difference (LSD, P=0.05) for each observation date.

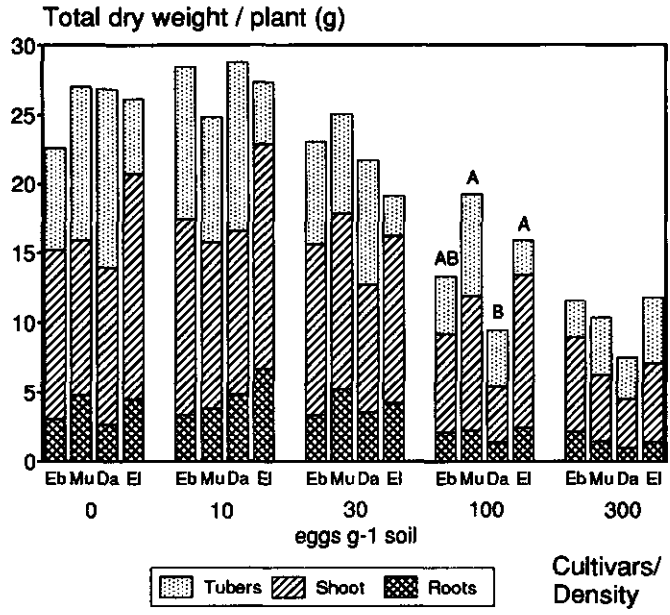


Figure 3.7. Total dry weight (g) and dry weight of roots, sprouts and tubers (g) 70 days after planting for 4 cultivars at 5 *G. pallida* densities (eggs per gram of soil). Different letters indicate significant differences (P=0.05) in total dry weight between cultivars per density level.

Total dry weight at 70 days after planting (TDW), averaged over cultivars, was significantly reduced by *G. pallida* at 100 and 300 eggs g^{-1} soil (Figure 3.7). At 300 eggs g^{-1} soil, average TDW was about 40% of that of control plants. The reduction of TDW was about proportionally distributed over root, sprout and tuber weights. The correlation of root, sprout and tuber weights with TDW was 0.85, 0.95, and 0.79, respectively. Similarly, the decrease in sprout weight due to *G. pallida* infection was caused by about proportionally decreased weights of leaves, stems and stolons. A significant difference in tolerance was observed at 100 eggs g^{-1} soil only, where TDW of 'Darwina' was lower than that of 'Multa' and 'Elles'.

The shoot/root ratio, whether calculated with shoot weight including or excluding stolons and tubers, was not affected by *G. pallida* (Table 3.3; F-probability = 0.15, for both computations). Dry matter content was reduced at 100 eggs g^{-1} soil. The total leaf area per plant was strongly reduced at 100 and 300 eggs g^{-1} soil and was highly correlated with TDW (Table 3.3). The number of

leaves longer than 2.5 cm was only slightly decreased at 100 and 300 eggs g^{-1} soil (Table 3.3). Hence, total leaf area was reduced because of smaller size of individual leaves. Specific leaf area was not influenced by *G. pallida* (Table 3.3). Specific stem length increased at 100 and 300 eggs g^{-1} soil, indicating that stems of infected plants were thinner, and had a highly negative correlation with TDW (Table 3.3). The stem diameter 30 cm under the top highly correlated with specific stem length (r=0.79). The number of plants flowering 70 days after planting was significantly lower at 100 and 300 eggs g^{-1} soil (Table 3.3). 'Darwina' was excluded from these data, because this cultivar seldomly flowers.

Table 3.3. Means per density and correlation with TDW for several plant growth characteristics. Different letters indicate significant differences (P=0.05) of characteristics between densities. Significance of the correlation at the 5 % and 1 % level is indicated by (*) and (**), respectively.

Characteristic	Density (eggs g^{-1} soil)					Correlation with TDW
	0	10	30	100	300	
shoot/root ratio (excl. tubers and stolons)	3.1	3.0	2.7	4.4	3.9	-0.36
shoot/root ratio (incl. tubers and stolons)	6.3	6.2	5.2	7.9	7.2	-0.23
dry matter content (%)	11.3^{a}	11.3^{a}	10.6^{ab}	10.4^{b}	10.7^{ab}	0.32
number of leaves formed	18.9^{ab}	19.4^{a}	19.3^{a}	18.2^{b}	17.2^{c}	0.72**
leaf area $plant^{-1}$ (cm^2)	2160^{a}	2427^{a}	2215^{a}	1558^{b}	1069^{c}	0.92**
specific leaf area ($cm^2\ g^{-1}$)	291^{a}	323^{a}	308^{a}	317^{a}	318^{a}	-0.19
specific stem length ($cm^2\ g^{-1}$)	24^{a}	24^{a}	28^{a}	42^{b}	46^{b}	-0.81**
frequency of flowering	0.60^{a}	0.55^{a}	0.64^{a}	0.29^{b}	0.32^{b}	0.67**

The decrease of total leaf area was mainly attributed to leaf area reduction of the old leaves (Table 3.4), which were formed during early stages of nematode infection. The leaf area of the old leaves at 300 eggs g^{-1} soil was less than half of that of the control, whereas the leaf area of young leaves was not reduced at all (Table 3.4). However, the young leaves were not yet full-grown at harvest time. The correlation between leaf area and TDW increased with age of leaves. The same effects were found for length of different stem parts, but length of young stem parts was still reduced at 300 eggs g^{-1} soil (Table 3.4).

The correlation between PHOT of various leaves at various dates and TDW was quite high (Table 3.5). At 60 days after planting correlation was lowest, but still significant.

There was no correlation between the number of cysts and TDW (r=0.04).

Discussion

Photosynthesis and transpiration rates

PHOT and TRANS of potato leaves were strongly reduced by *G. pallida* at 30 or more eggs g^{-1} soil, at 30 days after planting. The reduction of gas exchange

Table 3.4. Means per density and correlation with TDW for leaf area plant^{-1} (cm^2) and for length of stem parts (cm) in young, intermediate and old leaf layers. Different letters indicate significant differences (P=0.05) of traits between densities. Significance of the correlation at the 5 % and 1 % level is indicated by (*) and (**), respectively.

Trait	Density (eggs g^{-1} soil)					Correlation with TDW
	0	10	30	100	300	
area young leaves	63^a	73^a	66^a	65^a	61^a	0.36
area interm. leaves	152^a	164^a	161^a	134^b	97^c	0.71**
area old leaves	144^a	150^a	139^a	92^b	65^c	0.95**
length young stem	18^a	19^a	20^a	19^a	14^b	0.48*
length interm. stem	26^a	26^a	28^a	22^b	16^c	0.64**
length old stem	17^a	19^a	17^a	12^b	10^b	0.64**

rates persisted throughout the experiment, but its severity decreased with time. In Chapter 2 a reduction of leaf gas exchange rates of potato, three days after inoculation with freshly hatched second stage juveniles of *G. pallida*, was demonstrated. The prolonged reduction of gas exchange rates by *G. pallida* reported here can be explained by the continuous presence of second stage juveniles in roots, because emergence of second stage juveniles from eggs and subsequent penetration of roots is dispersed over time. The dispersion varies from six weeks in small pots (Forrest and Phillips, 1984) to three months in the field (Storey, 1982b). The decreasing severity of the nematode effects with time, up to 49 days after planting, reflects the gradual increase of the fraction of non-infected root tips, due to growth of new roots and diminishing numbers of infective second stage juveniles. The turn of this trend at 60 days after planting might have been caused by second stage juveniles of a second generation of the nematodes, as observed by Evans (1969).

The reduction of PHOT due to *G. pallida* might also be explained by a decrease of sink strength of the tubers (Dwelle, 1985), because *G. rostochiensis* at 189 eggs g^{-1} soil reduced the number of tubers per plant (Trudgill & Cotes, 1983b). However, the strongest reduction of PHOT and TRANS occurred at 30 days after planting, before the start of tuber growth. Therefore, it is more likely that the reduction of tuber number due to PCN is caused by lower PHOT or by

Table 3.5. Correlation of photosynthesis measurements with total dry weight (TDW). Significance of the correlation at the 5 % and 1 % level is indicated by (*) and (**), respectively.

days after planting	leaf label	leaf number at measurement time	correlation with TDW
30	A	5	0.72**
37	A	7	0.73**
37	B	6	0.78**
37	C	5	0.81**
49	C	7-8	0.78**
49	D	6-7	0.67**
60	E	8-10	0.58**

the factors, responsible for the reduction of PHOT.

The reduction of WUE by *G. pallida* in this experiment is different from the observations in Chapter 2, where WUE of potato plants was increased by *G. pallida*, three days after inoculation with freshly hatched second stage juveniles. The increased WUE indicated that PHOT was less inhibited than TRANS, which implies that limitation of gas exchange was due to stomatal closure. The decreased WUE indicated that non-stomatal processes (i.e. photochemical or biochemical processes of photosynthesis) were involved in the reduction of PHOT and TRANS. However, a simultaneous direct reduction of stomatal conductance may have been masked because of feedback of these processes on stomatal opening. Apparently, infection by *G. pallida* reduced gas exchange rates initially by stomatal closure and later on by non-stomatal processes, as observed between 30 and 49 days after planting. Possibly the prolonged release of stomatal inhibitors by infected roots (as discussed in Chapter 2) not only reduces stomatal conductance, but affects the Calvin cycle or the photochemical reactions as well. Schapendonk *et al.* (1989) reported analogous effects of water stress on photosynthesis of potato plants. Photosynthesis rate was reduced by water stress initially as a consequence of stomatal closure, but after three days increasingly by non-stomatal processes. These results support the hypothesis of Seemann and Sharkey (1987), that initial plant reaction to stress in the root environment, resulting in stomatal closure, is independent of the nature of the stress. The effects of *G. pallida* on the WUE of individual leaves agree with effects of *G. rostochiensis* on the transpiration ratio (i.e. water consumption/dry matter growth per period of 7 days) of whole potato plants (Evans, 1982). Initially the transpiration ratio was decreased (WUE increased) by *G. rostochiensis*, but from 32 days onwards the transpiration ratio was increased in the presence of nematodes.

The reduction of leaf photosynthesis by *G. pallida* was additive to the reduction of leaf photosynthesis due to senescence. In ageing potato leaves, photosynthesis reduction is closely correlated with the reduction of leaf nitrogen content due to breakdown of proteins (Vos and Oyarzun, 1987; Thimann, 1980). However, the reduction of nitrogen content of haulms or leaves by PCN is unimportant, when compared with the reduction of potassium content (Trudgill *et al.*, 1975; Trudgill and Cotes, 1983b; Trudgill, 1987b). Leaf potassium content of infected plants is reduced by 75 % of control plant values to about 1 % of leaf dry matter, depending on cultivar and fertilizer level. This is below the potassium

levels that reduced photosynthesis rates of sugar-beet and alfalfa because of increased mesophyll resistance (Terry and Ulrich, 1973; Peoples and Koch, 1979). The results suggest that the reduction of photosynthesis by *G. pallida* is not mediated by processes of leaf senescence, but by the release of stomatal inhibitors in infected roots, probably in combination with reduced potassium uptake rate.

Plant growth

Total dry weight of potato plants was strongly reduced by *G. pallida*. The weight reduction was proportionally distributed over all plant organs and was associated with reduction of leaf area, stem length and stem diameter. The number of leaves longer than 2.5 cm at 10 weeks after planting was only slightly reduced by *G. pallida*. Hence, dry matter production was reduced primarily by equal growth reduction of all plant organs, and only little by reduction of vegetative development rate as concluded by Seinhorst (1986b). The reduced frequency of flowering at 70 days after planting due to *G. pallida* may indicate interference of the nematodes with reproductive development of potato plants.

Dry matter content of plants was reduced by *G. pallida*. In most reports dry matter content is increased by PCN, but Fatemy and Evans (1986) showed that the effect depends on the time of measurement: up to 51 days after planting infected plants had greater dry matter contents than uninfected plants, and smaller dry matter contents at 71 days after planting. Shoot/root ratio was not affected by *G. pallida*. Sometimes a reduction of shoot/root ratio due to PCN infection is observed. This may also depend upon the time of measurement (Trudgill and Cotes, 1983b).

The expected tolerance differences among cultivars were confirmed significantly for 'Darwina', 'Multa' and 'Elles' at 100 eggs g^{-1} soil only. At lower densities tolerance could not be expressed because TDW was not reduced. TDW of 'Darwina' tended to be lower than that of the other cultivars at 300 eggs g^{-1} soil as well, but this was not significant. The intolerance of 'Eba' was not observed in this experiment. However, the experiment was ended when plants were still growing, whereas tolerance differences are usually based on tuber yield of fully matured plants. Possibly final tuber yield of the cultivars is affected differently by nematodes than TDW at 70 days after planting.

The reduction of TDW was not related to the number of cysts formed on the plants, hence resistance to PCN did not influence damage in this experiment. Resistance acts upon the development of third and later juvenile stages, therefore damage was brought about before the third juvenile stage. Apparently feeding on plant roots by third and later juvenile stages and adults has no implication for the expression of damage.

Gas exchange rates related to plant growth

The strong reductions of PHOT and leaf area may both have been caused by stress-induced elevated ABA levels in leaves (Watts *et al.*, 1981). Leaf area and PHOT were both highly correlated with TDW, but this does not necessarily imply causal relationships between TDW and these factors. In several studies, differences among cultivars in dry matter production are highly correlated with leaf area, but not with leaf photosynthesis rate (Dwelle *et al.*, 1981; Shimshi and Susnoschi, 1985; Prange, 1987). This indicates that leaf area is the most important factor in light capture and plant growth. Based on these findings, the reduction of leaf area by *G. pallida* is most likely the causal factor of plant growth reduction, with reduction of gas exchange rates being a parallel effect of the underlying mechanism, i.e. the presumed elevated levels of ABA in leaves.

Apparently damage was caused in the early stages of plant-nematode interaction: the strongest reduction of gas exchange rates was observed 30 days after planting, the strongest reduction of leaf area occurred for the oldest leaves and the increase of stem length was most strongly reduced during 28-42 days after planting. The intensity of the damage effects probably depends on the proportion of root tips invaded by second stage juveniles to nematode-free root tips. In this experiment root weight, stem length, leaf area and leaf gas exchange rates were good indicators of PCN damage. Tolerance differences were significant only at 100 eggs g^{-1} soil and were correlated with root weight, stem length and leaf area, but not with leaf gas exchange rates. Nematodes strongly reduced photosynthesis and transpiration rates, but apparently these processes are not part of the tolerance of plants, as defined by Wallace (1987). Further research using a wider range of cultivars should be carried out to identify tolerance mechanisms, especially in *G. pallida* resistant material, and to find a more simple *G. pallida* tolerance test.

Chapter 4

Temperature effects on hatching, juvenile development and reproduction of potato cyst nematodes: experiments and simulation

Abstract

Hatching of field populations of *Globodera rostochiensis* and *G. pallida* at the time of potato planting was studied *in vitro*. Various constant and alternating regimes of temperature and potato root diffusate (PRD) were examined. Cumulative hatching in constant presence of PRD was fitted to a negative exponential function. Its parameters, i.e. the time to first appearance of hatched juveniles, the maximum fraction of juveniles capable of hatching and the relative hatching rate, were all affected by temperature. The response to temperature of the maximum hatched fraction and the relative hatching rate were different between *G. rostochiensis* and *G. pallida*. The optimum temperature for hatching was lower for *G. pallida* than for *G. rostochiensis*. Hatching was simulated by numerical integration of this function, which agreed with experimental observations. Only when temperature and PRD presence were both alternated, the simulated response was lower than observed.

Development of juvenile stages, cysts and eggs of *G. rostochiensis* and *G. pallida* was studied in potato roots growing in petri dishes. Temperature effects were quantified in terms of development periods, their relative dispersions and the maximum number of offspring per female. Development periods of juvenile stages were not different between between species except at 24 °C, where second-stage juveniles of *G. pallida* developed slower than those of *G. rostochiensis*. The relative dispersion of development to adult females was mainly caused during development of second-stage juveniles. Cyst maturation period was not different between species nor among temperatures. The reproduction rate (offspring female^{-1} day^{-1}) was similar for both species and it was lower at 15 °C than at higher temperatures. The maximum number of eggs cyst^{-1} was extremely variable and not different between species nor among

temperatures. Simulation of development and its dispersion by numerical integration of development rates agreed with experimental observations.
The results indicate, that differences in population dynamics between *G. pallida* and *G. rostochiensis* are mainly caused during hatching and development of second stage juveniles. Prediction of cyst appearance, based on average daily temperatures, is unreliable because of instantaneous responses of hatching and development to temperature.

Introduction

Soil temperature is the major factor controlling hatching and development rates of potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone. Only the movement in the soil and the mortality rates of free-living second stage juveniles (J2s) and males are influenced also by soil moisture content (Wallace, 1955; Robinson *et al.*, 1987a). PCN become active in spring, when J2s hatch from cysts in response to potato root growth. Cumulative hatching *in vitro* follows a saturation type curve (Fenwick, 1950), characterized by the total number of hatched juveniles, the time to first hatching and the slope of the cumulative hatching curve with time. These parameters are all strongly affected by temperature (references in Table 4.1), the season in which the experiment is performed (den Ouden, 1960; Mulder & Vroom-Wolf, 1990) and by the conditions for rearing and storage of the PCN populations used (Oostenbrink, 1967; Van Dongen, 1983). The response of hatching to temperature at planting time of potatoes is essential for understanding of PCN population dynamics, but has not been satisfactorily determined for field populations.

Hatched J2s penetrate potato roots and develop via third and fourth stage juveniles into males and females (designated J3s, J4s, AM and AF, respectively). After mating, males die and females mature and develop into cysts. During female maturation, eggs are fertilized and develop into J2s that remain dormant in the cyst, until activation by potato root growth. The effect of temperature on J2, J3 and J4 development inside potato roots was investigated by Mugniery (1978). However, only the first appearance of each development stage was recorded, except at 15 °C, where the cumulative distribution of appearance was given as well. Development rates and their

Table 4.1. Optimum, minimum and maximum temperatures for total hatch of *G. rostochiensis* (ros) and *G. pallida* (pal) in different experiments reported in the literature.

Reference	Country	species	population history	temperatures studied (° C)	optimum temp. (° C)	minimum temp. (° C)	maximum temp. (° C)
van Dongen (1983)	Netherlands	ros, pal	field *	7, 9, 12, 15, 18, 21, 24, 27	ros: 21 pal: 15-21	ros: 9 pal: 9	ros: > 27 pal: >27
Feldmesser & Fassuliotis (1950)	USA	ros	field	4.4, 10, 21, 27, 38	21	< 4.4	> 38
Franco (1979)	GB, Peru	ros, pal	laboratory	5, 10, 15, 20, 25, 30	ros: 20 pal: 10-20	ros: 10 pal: 10	ros: 25-30 pal: 25-30
Kühn (1972)	D.D.R.	ros	field	6-8, 8-10, 20-21	8	not measured	not measured
Lownsbery (1950)	USA	ros	unknown	unknown	21	15	30
Oostenbrink (1967)	Netherlands	ros	field	continuous 2-40 °C	20	5-10	25-30
Robinson *et al.* (1987b)	GB	ros, pal	laboratory	5, 10, 15, 20, 25, 30	ros:20 pal: 15-20	ros: 10 pal: 10	ros: 25-30 pal: 25-30
Stanton & Sartori (1990)	Australia	ros	unknown	5, 10, 15, 22, 27, 30	22	5	27-30

* Stored in the laboratory at 4 °C for about 18 months

Table 4.2. Temperature and hatching agent treatments.

Treatment number	Treatment code	Temperature (°C)	Hatching agent
1	T12 - PRD	12	PRD
2	T14 - PRD	14	PRD
3	T17 - PRD	17	PRD
4	T20 - PRD	20	PRD
5	T23 - PRD	23	PRD
6	T14/20 - PRD	14 / 20	PRD
7	T17/23 - PRD	17 / 23	PRD
8	T17 - PRD/WATER	17	PRD / WATER
9	T14/20 - PRD/WATER	14 / 20	PRD / WATER
10	T17 - WAT	17	WATER
11	T14/20 - WAT	14 / 20	WATER

relative dispersion might be differentially affected by temperature and both should be quantified for understanding of population dynamics. Embryonic development to the first juvenile stage was analysed in detail by Langeslag *et al.* (1982), but subsequent development to J2 at various temperatures was deduced roughly from one temperature treatment. Moreover, the effects of temperature on rates of cyst development and reproduction and on the maximum offspring were not measured. The present knowledge is insufficient for understanding of population dynamics in response to soil temperature.

A dynamic, mechanistic model of the density-dependent interactions between PCN population dynamics and potato crop growth, POTACYST, is developed (Schans, 199x). Analysis of these interactions requires calculation of nematode numbers in each developmental stage in response to soil temperature. In this paper, effects of temperature on hatching, development and reproduction of PCN are experimentally determined and mathematically formulated for incorporation in the POTPCN model. Hatching of field populations of *G. rostochiensis* and *G. pallida* is investigated in response to constant and alternating temperatures, and constant and alternating presence of potato root

diffusate (PRD). Rates of juvenile and cyst development and reproduction of both species are determined in response to constant and alternating temperatures, in controlled conditions. The models of hatching, development and reproduction are validated with data from various experiments.

Materials and methods

Hatching

Cysts of *G. rostochiensis* and *G. pallida* were collected from fields, where potatoes are grown every second year, one week before planting of potato tubers (April 20). The fields were not fumigated. Four populations were used: 'ros-potato' and 'pal-potato', both with potatoes in the previous year, 'ros-other' with wheat in the previous year and 'pal-other' with sugarbeet in the previous year. Five sets of 10 uniformly sized brown cysts were subjected to various temperature and PRD regimes (Table 4.2). Standard PRD was used (Janzen and van der Tuin, 1956). Hatched juveniles were counted twice a week. The hatching solution was refreshed at each observation. In the treatments with alternating temperatures, temperature was changed every 24 hours.

The cumulative frequency distribution of juvenile emergence from cysts with time was described by a negative exponential function (Spitters, 1989):

$$EM_t = EMMX(1 - \exp(-EMRR(t-EMINC))) \qquad t >= EMINC \qquad \text{Equation 1}$$

where EM_t is the cumulative fraction of emerged juveniles at time t, relative to the total number of viable eggs in the cysts, EMMX is the maximum fraction of juveniles capable of emerging, EMINC is the time to first emergence after induction of hatching by PRD (days), and EMRR is the relative emergence rate (day^{-1}). This function permits easy quantification of temperature effects, by multiplication factors for all function parameters. An alternative approach to describe development in relation to the environment would be the 'boxcar train' method (Goudriaan & van Roermund, 1989), but a reversible effect of temperature on EMMX is less easily simulated with this method. The function parameters were estimated for each set of treatments with constant temperature and PRD regimes (treatments 1-5 in Table 4.2), by non-linear curve fitting (GENSTAT 5 *Reference Manual*, 1988). Significant effects of temperature on

EMINC, EMRR and EMMX were determined. Cumulative emergence of J2s in response to temperature was simulated by numerical integration of two rate variables: the rate of development to first emergence, EMDVR (day^{-1}), which is the inverse of EMINC; and EMRR, i.e. the fraction of unemerged juveniles that emerges in a short time interval dt:

$$EMRR = (dEM_t/dt) / (EMMX - EM_t) \qquad \text{Equation 2}$$

Simulated cumulative hatching was validated with the treatments of fluctuating temperature and/or PRD, and experiments by Bishop (1955).

Juvenile and cyst development and egg fertilization

Populations "Mierenbos" (pathotype Ro1) of *G. rostochiensis* and "HPL1" (pathotype Pa2) of *G. pallida*, reared in the greenhouse on the susceptible cultivar 'Eigenheimer' for several generations, were used in this study. Nematode development from penetration of J2s to adult stages was studied in roots of potato sprouts (cv. 'Eigenheimer'), grown on water agar in Petri dishes (Mugniery & Person, 1976; Mugniery, 1982). Three J2s, hatched in PRD solution no longer than 24 h before inoculation, were inoculated per root tip. Movement of J2s away from the inoculation site was prevented by placing a small plastic ring over the root. The Petri dishes were incubated in darkness at constant temperatures of 10, 15, 18, 21 or 24 °C, or at alternating temperatures of 15 and 21 °C for 24 h per temperature (referred to as 15/21 °C). The development stage of the nematodes was observed at 7-day intervals for the 10 and 15 °C treatments and at 5-day intervals for the other treatments. Monitoring of development of individual nematodes was not possible, because J2s and J3s are visible in roots by destructive staining only. Randomly selected Petri dishes containing 17 inoculated roots (maximally 51 nematodes) were examined per observation date for each population and temperature. Infected root pieces were stained with lactophenol cotton blue 0.1 % (Goodey, 1937) for 6 minutes at 90 °C and stored in plain lactophenol at room temperature for one month. The root pieces were transferred to a 1:1 glycerine-water mixture. The nematodes present in the roots, including those detached from the plant tissues during handling of the root pieces, were scored as J2, J3, J4(female), J4(male), adult female or adult male. The average development period and the relative dispersion for each

developmental stage were derived from the cumulative frequency distribution of each stage (Rabbinge, 1976).

A number of Petri dishes was selected for observation of embryogenesis as soon as adult females appeared on the roots at each temperature except 10 °C, where the number of females was insufficient. Each female mated with two males of the same population. The males were reared on potato plants cv. 'Eigenheimer', grown in pots containing fertilized sand in a climate chamber at 18 °C for 30-50 days. The day before mating, males were extracted from the soil with an Oostenbrink elutriator using a water flow rate of 700 ml per minute (Oostenbrink, 1960). After mating of the nematodes, the Petri dishes were incubated at the temperature at which the females had been reared. The average development periods of cysts and eggs and their relative dispersions were derived from the cumulative frequency distributions, observed with the same time intervals as used with development of juvenile stages. Only cysts containing 10 or more eggs were used for estimation of development rates.

PCN development from penetration of J2s in roots until embryogenesis in relation to temperature was simulated with the boxcar train method (Goudriaan & van Roermund, 1989). The parameters for this routine are the average development period and the relative dispersion of each stage. The model was validated with the fluctuating temperature treatment and with experiments of Mugniery (1978) and Langeslag *et al.* (1982).

Table 4.3. Total number of eggs cyst^{-1}, number of healthy eggs cyst^{-1} and fraction healthy eggs of the populations used in the hatching experiment. ros = *G. rostochiensis*; pal = *G. pallida*.

Species	Previous crop	total eggs cyst^{-1} ± SE; n=55	healthy eggs cyst^{-1} ± SE; n=55	fraction healthy ± SE; n=55
ros	potato	334.29 ± 64.16	263.45 ± 54.63	0.79 ± 0.08
ros	wheat	173.72 ± 63.58	135.79 ± 56.32	0.78 ± 0.11
pal	potato	284.99 ± 79.35	215.70 ± 80.71	0.76 ± 0.11
pal	sugar beet	117.83 ± 37.72	77.10 ± 27.93	0.65 ± 0.14

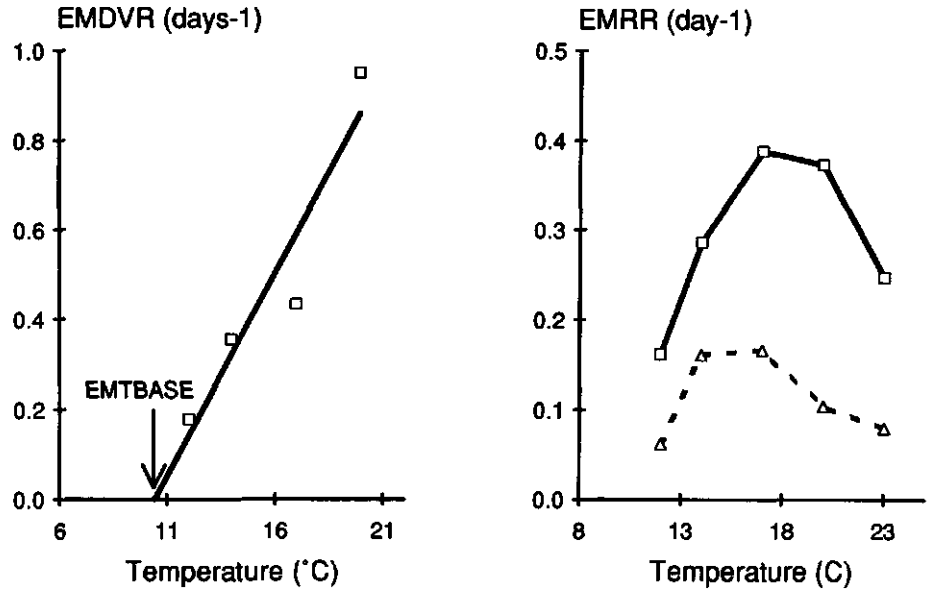


Figure 4.1. (left) Development rate of encysted second-stage juveniles of potato cyst nematodes to first emergence from cysts (EMDVR, days-1) at different constant temperatures (°C). The arrow indicates the base temperature for hatching (EMTBASE).

Figure 4.2. (right) Relative emergence rate (EMRR) of second-stage juveniles from cysts as function of temperature (°C), for *G. rostochiensis* (——□——) and *G. pallida* (- - Δ - -).

Results

Hatching

The initial number of eggs per cyst was not different between the nematode species, but populations from fields with potatoes in the previous year showed a 90 - 180 % larger cyst content (healthy eggs) then populations from fields with other previous crops (Table 4.3). Hatching in water was practically absent with EMMX less than 0.008 at all temperatures, except for 'pal-other' at alternating temperatures, where EMMX was 0.138. The cumulative emergence of J2s in PRD in response to constant temperatures was well described by the negative exponential function (Equation 1). The 'percentage variance accounted for'

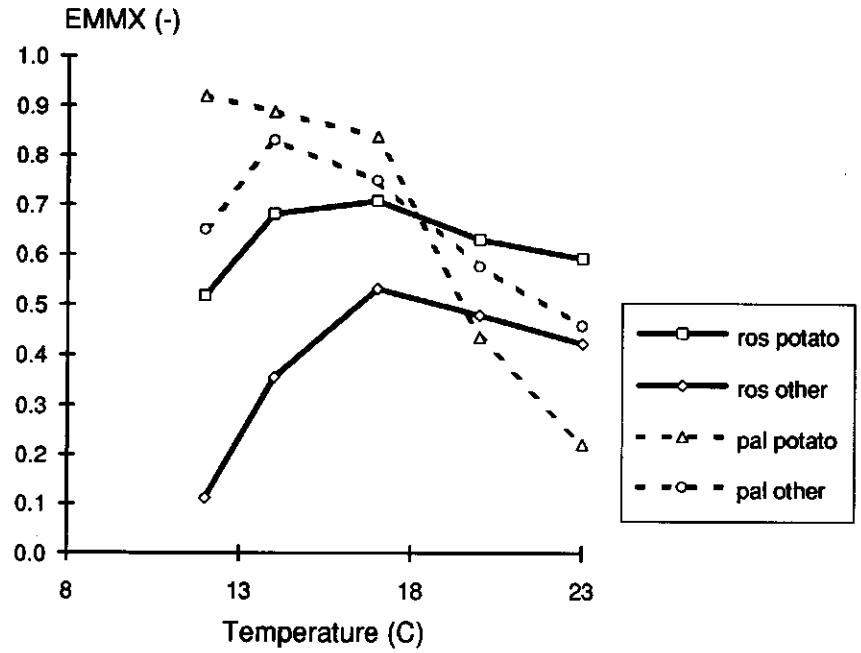


Figure 4.3. Maximum fraction of emerged juveniles after 40 days, relative to initial cyst content (EMMX), as function of temperature for *G. rostochiensis* (ros) and *G. pallida* (pal) with different previous crops.

(%v.a.f.; GENSTAT 5 *Reference Manual*, 1988), which measures goodness of fit, was generally above 97 %. Only for those sets at 20 °C and 23 °C, when the time to first emergence (EMINC) was zero, %v.a.f. varied between 25 and 98 %. In these cases EMMX was reached in less than 10 days and too few observations were available for correct fitting.

The three parameters of the cumulative emergence function were differentially affected by temperature. The time to first emergence (EMINC) was reduced with increasing temperature ($P<0.001$), but it was not affected by nematode species ($P=0.58$) nor by the previous crop ($P=0.24$). The inverse of EMINC, i.e. the development rate to first emergence (EMDVR, $days^{-1}$), was related to temperature between 12 °C and 20 °C (Figure 4.1). The base temperature for hatching, average for both species, was computed as 10.4 °C by extrapolation of the regression line to the X-axis. The relative emergence rate (EMRR), was affected by temperature and by species ($P< 0.001$ for both factors), but not by the previous crop ($P=0.34$; Figure 4.2). EMRR of *G.*

rostochiensis was largest at 17 - 20 °C and significantly smaller at lower temperatures and at 23 °C (P=0.05). EMRR of *G. pallida* was smaller than that of *G. rostochiensis*, with a peak at 14 - 17 °C which differed significantly only from EMRR at 12 °C (P=0.05). The third function parameter, the maximum fraction of emerged juveniles (EMMX), was significantly affected by temperature, species and previous crop (P<0.001 for all factors; Figure 4.3). For *G. rostochiensis*, EMMX after the potato crop was higher than after the non-potato crop at all temperatures, with a peak emergence at 17 °C . For *G. pallida,* interaction between effects of the previous crop and temperature occurred: at 12 °C, EMMX of 'pal-potato' was higher than that of 'pal-other', but at 23 °C the effect was reverse. Peak emergence was between 12 and 17 °C.

Simulation of hatching at alternating temperatures with constant presence of PRD corresponded with observed data for all populations (Figure 4.4a). In most cases there was little difference between simulated hatching in response to

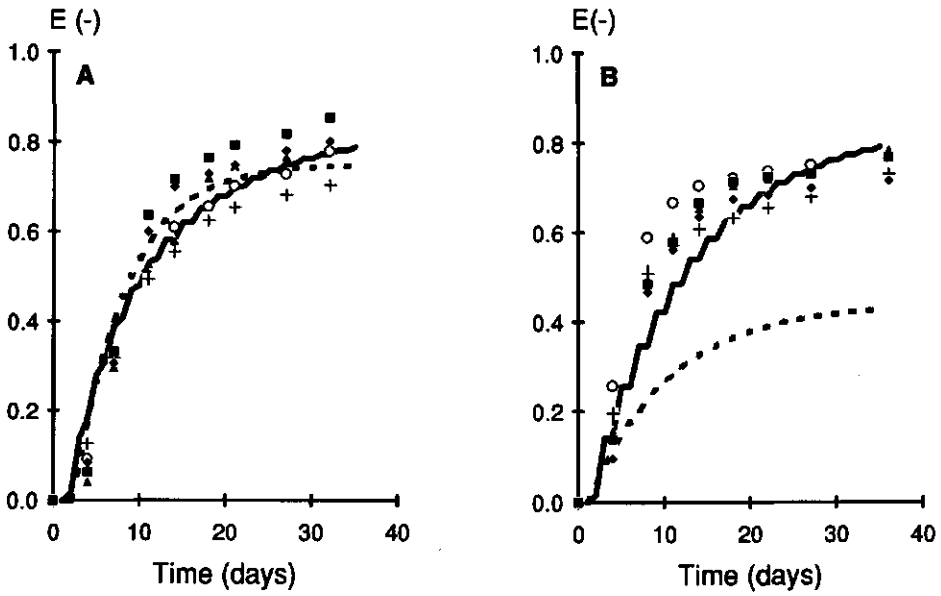


Figure 4.4. Simulated (———) and observed (data points of five replications) cumulative fraction emerged second-stage juveniles (E) at alternating temperature, and simulated E (- - - -) at the corresponding average temperature. (**A**) *G. pallida* with sugarbeet previously, 14-20 °C. (**B**) *G. pallida* with potato previously, 17-23 °C

the fluctuating temperature and its average temperature. Only for the 'pal-potato' population at 17/23 °C the fit to the fluctuating temperature was much better than the fit to the average temperature (Figure 4.4b). In this case, values of both EMMX and EMMR at the average temperature of 20 °C differed from the maximum values at 17°C, indicating an instantaneous response of hatching to temperature. This effect also explains the smaller fraction of hatched J2s at the constant temperature of 25 °C compared with fluctuating temperature regimes in the experiments with PRD by Bishop (1955). Simulated hatching for Bishop's experimental conditions was in good agreement with observed hatching, as illustrated for Bishop's third treatment (Figure 4.5).

In the treatments with alternating presence of PRD, hatching occurred during the periods of PRD presence, and stopped when PRD was absent (Figure 4.6a). Simulated hatching agreed with observations, except at alternating temperatures for 'ros-potato' and 'pal-other', where simulated levels were much lower than observed values (Figure 4.6b).

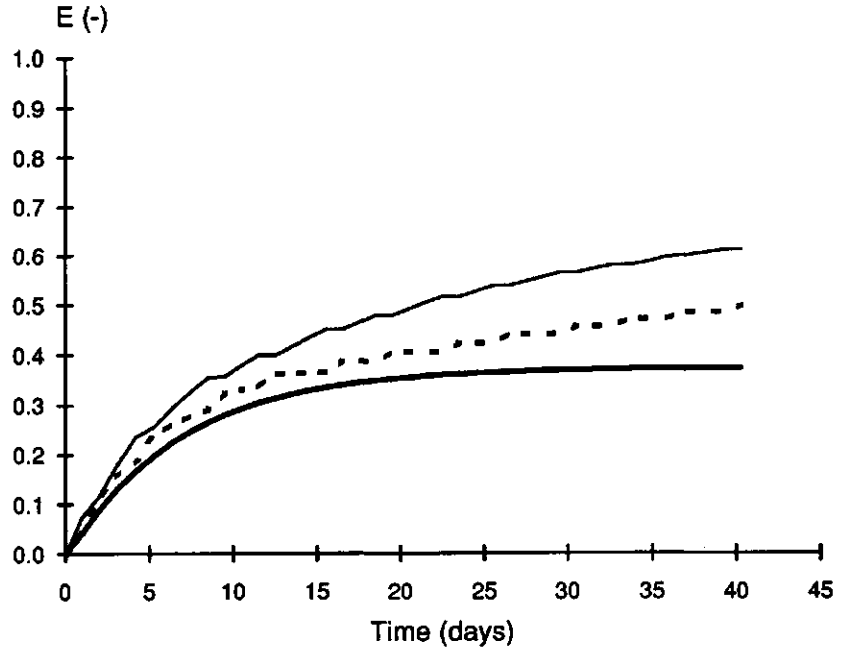


Figure 4.5. Simulated cumulative fraction emerged second-stage juveniles (E), for experimental conditions (treatment 3) of Bishop (1955). (———) : 25 °C constant. (- - - -) : twice per week: 19 hr at 25 °C + 5 hr at 15 °C. (———) : 5 times per week 19 hr at 25 °C + 5 hr at 15 °C.

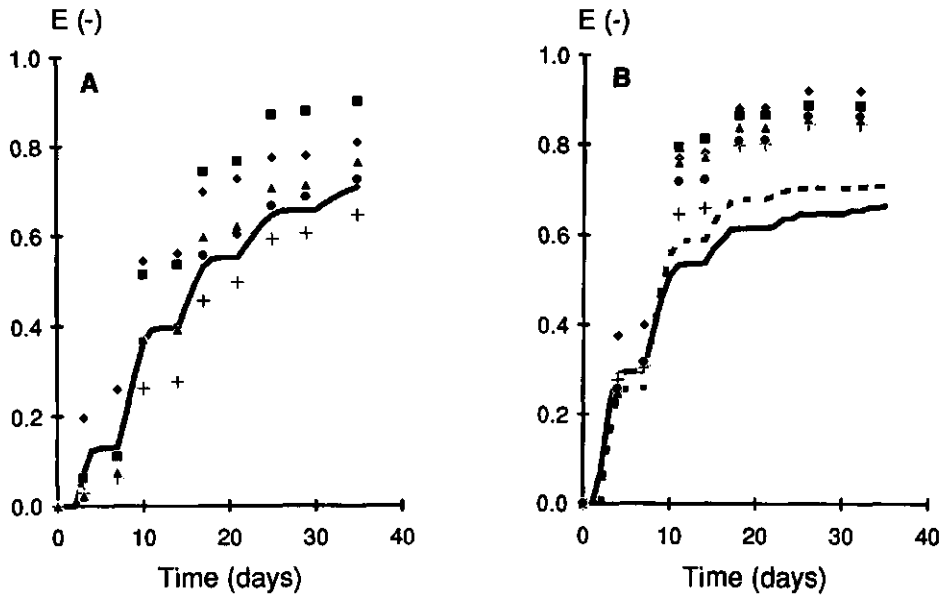


Figure 4.6. Simulated (———) and observed (data points of five replications) cumulative fraction emerged second-stage juveniles (E) at alternating presence of potato root diffusate. (**A**) *G. pallida* with sugarbeet previously, 17 ° C. (**B**) *G. rostochiensis* with potato previously, 14-20 ° C, and simulated (- - - -) E at the corresponding average temperature.

Juvenile and cyst development, and egg fertilization

The maximum number of nematodes reaching adult stages was not affected by temperature, therefore development of second, third and fourth juvenile stages (J2-J3, J3-J4, and J4-AF, respectively) of *G. rostochiensis* and *G. pallida* is characterized by the average development period (ADP, in days) and the relative dispersion (RD, calculated as the ratio of standard deviation SD and ADP) only, assuming a normal distribution of development periods. Distributions of development periods were considered as significantly different, when 2-sided 50 % confidence intervals did not overlap, i.e. a maximum overlap of 25 %. ADP and SD of J2-J3 development were higher for *G. pallida* than for *G. rostochiensis* at all temperatures, but only at 24 °C the distributions were different (Table 4.4).

Table 4.4. Average development period (ADP, in days), its standard deviation (SD, in days) and its relative dispersion (RD, calculated as SD / ADP) for PCN development of J2 to J3, and ADP for subsequent juvenile development stages, at various temperatures (Temp). ros = *G. rostochiensis*; pal = *G. pallida*.

Temp	Species	J2-J3			J3-J4	J4-AF	J2-AF
(°C)		ADP	SD	RD	ADP	ADP	ADP
10	ros	30.9	10.2	0.33	11.3	10.1	52.3
	pal	28.9	10.6	0.37	13.0	13.8	55.7
15	ros	10.0	4.9	0.49	9.0	11.2	30.2
	pal	14.6	6.5	0.45	8.3	3.9	26.8
18	ros	7.9	2.5	0.32	7.0	6.1	21.0
	pal	9.7	3.3	0.34	6.5	6.7	22.9
21	ros	7.1	1.9	0.27	7.1	7.0	21.2
	pal	9.7	5.5	0.57	6.1	5.4	21.2
24	ros	7.2	1.3	0.18	6.6	7.8	21.6
	pal	12.3	6.4	0.52	5.8	5.6	23.7
15/21	ros	9.9	4.9	0.49	5.5	6.1	21.5
	pal	10.4	3.1	0.30	3.7	7.9	22.0

Development at the alternating temperature 15/21 °C was not different from development at the constant average temperature 18 °C, implying an instantaneous response of development to temperature. At all temperatures, the SD was not correlated with development stage ($r=0.132$, 28 df), indicating that the dispersion of development, originated between penetration of J2 into roots and appearance of the J3 stage, did not increase during later stages.

This implies that the processes of penetration site finding and syncytium establishment are mainly responsible for the variation in time to cyst appearance. Hence, RD is calculated as SD/ADP for J2-J3 development and set to zero for J3-J4 and J4-AF development (Table 4.4). The ADP for complete development from J2 to AF was not different between *G. rostochiensis* and *G. pallida* at all temperatures. ADPs for J3-J4 and J4-AF development were calculated as the difference in cumulative ADP of successive developmental

stages. They tended to be smaller for *G. pallida* than for *G. rostochiensis*. Because different distributions of development periods were observed at 24 °C only, the ADPs at lower temperatures were derived from the effect of temperature on development of both species together. The relation between temperature and the development rate of each development stage, calculated as the inverse of its ADP, is presented in Figure 4.7. The base temperature for development of each stage was estimated by linear extrapolation of the initial slope to the temperature axis. The base temperature for J2 development, 6.8 °C, was slightly higher than the value observed by Mugniery (1978) for the most rapidly developing individuals in a batch of nematodes, but the base temperatures for J3 and J4 development, -0.1 °C and 1.2 °C respectively, were much lower.

The processes of egg fertilization and cyst development are here characterized by egg production rate per female, maximum number of eggs per female, development period of fertilized eggs to the dormant J2 stage and rate of cyst browning. At 24 °C no viable eggs were formed. This must be due to

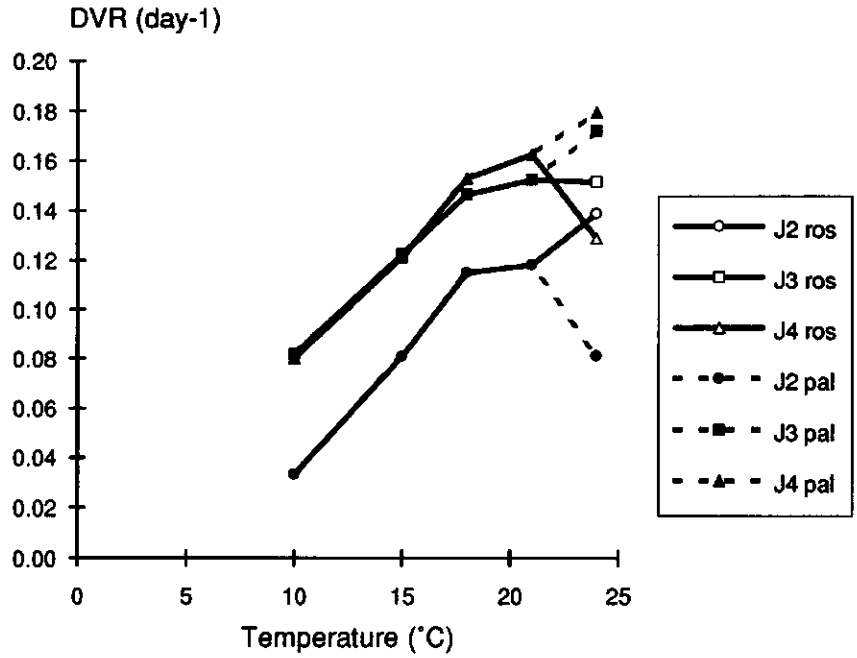


Figure 4.7. Effect of temperature (°C) on development rate (DVR, day-1) of second-stage (J2), third-stage (J3) and fourth-stage juveniles (J4) of *G. rostochiensis* (ros) and *G. pallida* (pal).

inhibited egg production or to inhibited fertilization, because development of embryos, isolated from females reared at lower temperatures, was possible at 24.4 °C (Langeslag *et al.*, 1982). The egg production rate at 18 °C and 21 °C was twice as high as that at 15 °C, but was not different between species (Table 4.5). The maximum number of eggs per female was extremely variable, ranging from 13 to 689 eggs at 40 days after mating, and no significant differences among temperatures or between species were observed.

At 15/21 °C, both the egg production rate and the maximum number of eggs per female tended to be higher than at the average temperature of 18 °C. Cyst and embryonic development, although independent of each other (Langeslag *et al.*, 1982), were characterised by similar ADPs and SDs, not different among temperatures nor between species (Table 4.5). Langeslag *et al.* (1982) estimated a temperature sum of 280-300 degree-days above a base temperature of 4.5-6.8 °C for the ADP of embryonic development, implicating ADPs of 26.7 - 36.6 days, 20.7 -26.8 days and 16.7 - 21.1 days at 15 °C, 18 °C and 21 °C, respectively. These values fit well within the distributions of ADP presented above (Table 4.5).

Table 4.5. Maximum number of eggs formed per female (MAXEG), the rate of egg production (REGBP, eggs female^{-1} day^{-1}) and the average period of embryonic and cyst development (ADPEMB and ADPCYS, respectively, in days), and their standard deviations (SD) per temperature (Temp, in °C), for *G. rostochiensis* (ros) and *G. pallida* (pal).

Temp		MAXEG	SD	REGBP	SD	ADPEM	SD	ADPCY	SD
15	ros	151	110	6.1	0.8	21.1	6.8	24.4	11.3
	pal	116	94	5.5	0.8	18.3	9.2	14.2	8.0
18	ros	169	94	10.6	1.3	17.8	9.9	24.9	11.5
	pal	184	108	10.5	0.8	17.9	6.5	21.7	8.7
21	ros	219	133	9.9	0.8	17.2	7.3	19.6	4.6
	pal	177	101	10.6	1.1	14.9	6.6	18.6	10.2
15/21	ros	240	171	12.9	1.0	20.7	5.6	24.5	9.7
	pal	280	166	12.4	0.6	19.8	9.2	21.9	8.5

Table 4.6. First appearance of *G. rostochiensis* and *G. pallida* individuals in J3, J4 and adult female development stages at different temperatures (Temp), simulated and observed by Mugniery (1978), in days after penetration by J2s in roots.

Temp (° C)		*G. rostochiensis*			*G. pallida*		
		J3	J4	female	J3	J4	female
9.5	simulated	24	37	50	24	37	50
	Mugniery	19.7	35	58.4	19.5	32	52.1
11.5	simulated	14	24	33	14	24	33
	Mugniery	13.7	24.7	39.3	12.6	22.1	34.6
15	simulated	8	16	23	8	16	23
	Mugniery	8.6	14.2	24.2	8.7	15	24.5
18	simulated	6	11	17	6	11	17
	Mugniery	6	10.95	17	6	11.7	19
19	simulated	5	11	17	5	11	17
	Mugniery	5.7	10.2	15.9	5.9	11.6	18.6
24	simulated	5	11	18	3	9	14
	Mugniery	4.7	8.6	13.9	5.6	10.4	16.5

The effect of temperature on juvenile, cyst and egg development of PCN was simulated with the boxcar train method (Goudriaan & van Roermund, 1989), using the significant relations presented above. For comparison of juvenile development with the observations by Mugniery (1978) on the most rapid individual of 50 developing nematodes, the time necessary for development of 2 % of the population in each juvenile stage was computed (Table 4.6). Except for *G. rostochiensis* at low and high temperatures, the simulated values generally agreed with the observations by Mugniery. The disagreements may have been caused by differences in population history or by experimental error.

Simulated development of juvenile stages at the alternating temperature treatment 15/21 °C was slightly faster than development at the constant average temperature 18 °C (Figure 4.8), in agreement with the experimental results. Simulated number of eggs per female, the resultant of the development rate of females to cysts and the egg fertilization rate per female, was strongly influenced

by temperature (Figure 4.9), due to the temperature-dependent rate of embryo production. The maximum final egg content of cysts, about 200 eggs, agrees reasonably with general observations, but its relation with temperature is only partly confirmed by Franco (1979). However, Franco (1979) did not present the statistical significance of his data and standard errors of eggs/cyst might have been large in his work too (e.g. Table 4.3).

Discussion

The populations of *G. rostochiensis* studied here hatched faster than those of *G. pallida* at all temperatures, but the maximum fraction of hatched juveniles was smaller at temperatures below 20 °C. The optimum temperature for hatching was higher for *G. rostochiensis* than for *G. pallida*. These results confirm previous findings (e.g. McKenna & Winslow, 1972; Parrott & Berry, 1976; van Dongen, 1983), and are probably caused by differences in the rate of lipid

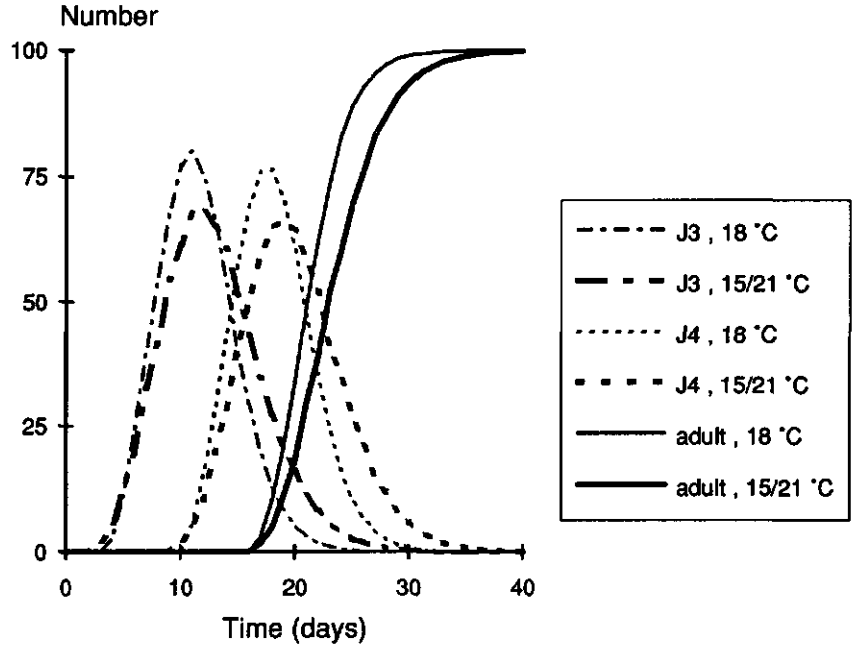


Figure 4.8. Simulated number of third-stage (J3), fourth-stage (J4) and female nematodes at 15/21 °C and 18 °C, developing from 100 penetrating second-stage juveniles at time=0 days.

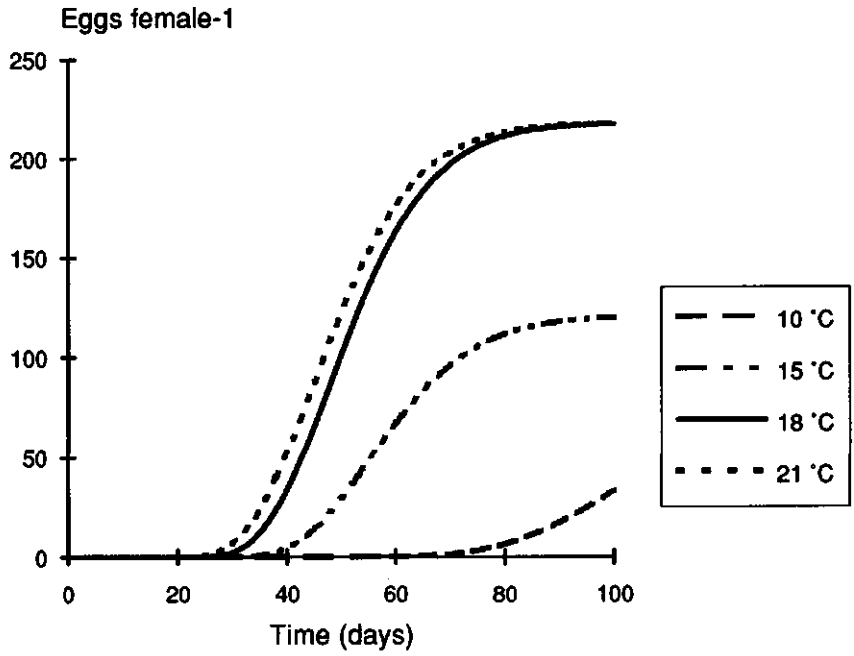


Figure 4.9. Simulated time course of the number of eggs per female at different temperatures, starting from penetration of second-stage juveniles at time=0 days.

depletion (Robinson *et al.*, 1987b). For both species, the optimum temperature for hatching was lower than generally observed (Table 4.1).This might be explained by adaptation of the studied populations to local field conditions (Ellenby & Smith, 1975; Hominick, 1979). A sigmoid cumulative hatching curve at constant temperatures (Fenwick, 1950) was not observed here. This corresponds with previous results obtained with field populations in springtime (Oostenbrink, 1967) and is probably due to absence of diapause and inhibitory factors at this stage (Clarke & Perry, 1967). The absence of hatching in water is probably due to spontaneous hatching in the field, before the cysts were collected (den Ouden, 1960), because average soil temperature has been above 10 °C since the end of March. This indicates that infective J2s of PCN are present in the soil at the time of initial potato root growth (Schans, 1992).

The synergistic effect of alternating temperature and alternating PRD presence was probably also present in experiments of Forrest & Perry (1980) and Perry & Beane (1982). They showed for *G. pallida* and *G. rostochiensis*, respectively, that brief exposures to PRD of 5 minutes per week for 3 or 5 weeks

are sufficient for 40 - 50 % emergence of J2s,much higher than the amount after one continuous period of 15 or 20 minutes of PRD presence. However, storage of hatching eggs at 20 °C was repeatedly alternated with washing under running tap water for 30 minutes, which usually is far below 20 °C. This combination of alternating PRD presence and temperature fluctuation, rather than alternating PRD presence alone, was probably responsible for the high emergence rate. The implications of this synergistic effect for hatching in a natural soil environment are questionable. Little is known about the time course of PRD concentration in a certain soil volume as a function of production, diffusion, breakdown, and distribution rates. Moreover, it is unknown to which extent effects of fast changes from presence to abscence of PRD and reverse, apply to gradual fluctuations in PRD concentration. Apart from the synergistic effect of alternating temperature and PRD presence, the negative exponential model is competent for simulation of hatching of PCN in response to fluctuating soil temperature, at the time of planting of potatoes. The instantaneous response to temperature explains why predictions of hatching based on average daily temperatures are unreliable. The fast adaptation of PCN hatching to field-specific environments impairs the general applicability of model results, obtained with specific populations. However, the explicit formulation of the present model by parameters, that depend on the environment, permits research on the nature of adaptation and its contribution to variability in population dynamics and damage among locations.

Juvenile and cyst development contributed little to the variability of population dynamics of both PCN species, because development rate was almost constant between 18 and 21 °C. Differences between *G. rostochiensis* and *G. pallida* occured above 21 °C only. The larger period for total development from eggs to new cysts of *G. pallida*, compared with *G. rostochiensis* (McKenna & Winslow, 1972), is therefore largely caused by the lower relative hatching rate of *G. pallida*. Relative dispersion of nematode development inside roots was mainly caused at the J2 stage. Since at this stage also a major damage mechanism is operative (Chapter 2), it is likely that any factor influencing density and development of J2s in roots has a major effect on both population dynamics and yield loss. Using the hatching and development models presented here, this is further investigated in Chapter 5.

Chapter 5

Simulation of population dynamics of potato cyst nematodes and associated damage to potato

Abstract

A dynamic, explanatory model was developed to simulate the population dynamics of potato cyst nematodes and the associated damage to potato plants at optimal water supply. Crop growth was simulated from planting to harvest. Root growth was distributed over vertical soil layers, which are separated between potato ridges and furrows, to allow for various cyst distributions. In each soil layer, hatching of second stage juveniles was calculated from infiltration and breakdown of potato root diffusate, and soil temperature. The number of successfully invaded juveniles in roots depended on root growth, free juveniles in the soil and a density-dependent invasion efficiency. Development of invaded juveniles to cysts containing new eggs was calculated in dependence of temperature only. Population increase was regulated by the density of second stage juveniles in roots, through effects on the sex ratio of the developing population, and through mortality of roots containing second-stage juveniles at densities exceeding the 'root carrying capacity'. Crop damage depended only on the relation between the number of juveniles initiating syncytia, relative to leaf area, and crop photosynthesis.

The simulated relations between initial and final population density, and between initial density and tuber yield, agreed with experimental observations. Population increase and yield loss were different between *Globodera rostochiensis* and *G. pallida*, in interaction with initial density and temperature. Preliminary sensitivity analysis indicated, that simulated yield reduction was strongly affected by the parameter of the relation for density-dependent photosynthesis reduction. Simulated effects of tolerance and resistance on yield loss and population increase agreed with independent experimental results. Tolerance was enhanced by resistance, acting upon or before the stage of syncytial initiation, and was apparently independent of resistance acting after this stage. The model provides insight in population dynamics and yield loss, as

affected by environment and crop characteristics at various spatial cyst distributions, and is a tool for evaluation of strategies to control potato cyst nematodes.

Introduction

Population dynamics of potato cyst nematodes (PCN), *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone, and associated yield loss of potato, vary greatly among years and locations (Greco *et al.*, 1982; Seinhorst, 1982a; 1986b). This variation might be attributed to weather (Jones, 1983), soil heterogeneity (Trudgill, 1986), population-specific response of PCN to soil environment (Chapter 4), population- and cultivar-specific variation in host-parasite interactions (Arntzen & Bakker, 1990), or to sampling errors (Seinhorst, 1982b). The processes of nematode development in interaction with crop growth, influenced by weather and soil, are insufficiently understood to explain this variation. Systems analysis of the relations among crop growth, population dynamics, weather and soil environment may improve understanding of PCN population increase and potato yield loss in variable environments, and may identify sources of their variation.

Various empirical models describing the general relations between initial and final PCN density (Seinhorst, 1967; 1986a) and between initial PCN density and yield loss (Seinhorst, 1965; Oostenbrink, 1966; Brown, 1969; Elston *et al.*, 1991) have been developed. In these models, PCN density and potato yield are not related to underlying biological processes, which limits their ability to analyze spatial and temporal variation of population dynamics and yield loss. A preliminary explanatory model of PCN population dynamics and potato yield loss, relating PCN development to average daily air temperature and to potato crop growth, and crop damage to root death in response to PCN invasion, was developed by Ward *et al.* (1985). Although several features of the PCN - potato system can be investigated with this model, the processes of hatching and initial PCN development are formulated too simply for analysis of the interactions between crop growth and invading second stage juveniles, causing crop photosynthesis reduction and variation of the development time to new cysts (Chapter 2; Chapter 4).

In this paper, a dynamic simulation model combining potato crop growth and PCN population dynamics, named POTACYST, is developed. Crop growth

Table 5.1. Effect of temperature on the relative growth rate of sprout dry matter before crop emergence (SPRGR)

Temperature (°C)	SPRGR (day^{-1})
7	0.05
12	0.11
15	0.15
20	0.21
25	0.26

and production, PCN development and interactions between second-stage juveniles and crop growth are investigated at optimal water and nutrient supply, to gain insight in the PCN - potato system in the least complicated environment. The various sections of POTACYST, the sources of parameter values and the simulation results are described here. The formal description of POTACYST, written in standard FORTRAN 77 (Schans, 199x), is available from the author on request.

Model sections

Potato crop growth and development

Growth and development of potato at optimal water and nutrient supply is simulated with a daily time step, using the crop growth model SUCROS and the standard parameter set for potato (Spitters *et al.*, 1989). Daily photosynthesis is calculated from incoming radiation and crop leaf area. The assimilates are partly used for maintenance of the standing biomass, while the remainder is converted to dry matter and partitioned over the various plant organs, as a function of the phenological development stage of the crop. The development stage is a function of the temperature sum after emergence and the maturity class of the cultivar.

SUCROS was extended to initiate crop growth at planting of pre-sprouted tubers, because considerable root growth and associated interactions between potato and PCN occurs before crop emergence. Calculation of pre-emergence growth is based on the following assumptions: exponential growth of sprout dry matter, derived from mother tuber reserves, with a temperature-dependent relative sprout growth rate (Table 5.1); a specific sprout length factor (SPRLN) of 400 m kg^{-1}; and constant partitioning of dry matter growth to shoots (80 %) and roots (20 %). These parameters were derived from experiments by Davies & Ross (1985) and Morris (1967). The partitioning factors agree with the initial dry matter partitioning at crop emergence (van Heemst, 1986). Crop emergence occurs when the average sprout length, calculated from SPRLN and total sprout weight, equals planting depth. Between emergence time and the time at which the leaf area index (LAI) equals 0.1, dry matter growth is derived from mother tuber reserves and photosynthesis combined (Moorby & Milthorpe, 1975).

The extended SUCROS model was validated with field experiments using the late maturing potato cultivar 'Astarte' at optimal water and nutrient supply (K.B.A. Bodlaender & M. van de Waart, Centre for Agrobiological Research CABO, Wageningen, 1979, unpublished). There was good agreement between simulated and measured values for total dry matter weight, tuber dry matter weight , and leaf area index LAI (Figure 5.1). Root weights have not been measured. Simulated crop emergence was 2 days later than observed. Simulated time of tuber initiation, i.e. the time when tuber weight exceeds 50 kg ha^{-1}, was 3 days earlier than observed. Simulation of crop emergence was further validated for the different planting conditions reported by Vos & Groenwold (1986). Planting dates in their experiments were 12 April 1982 and 9 June 1983. Simulated emergence was 2 days earlier than observed for their 1982 experiment and 1 day earlier than observed for their 1983 experiment. In conclusion, the SUCROS model may be used as a reliable potato crop growth model, from planting to harvest, at optimal water and nutrient supply.

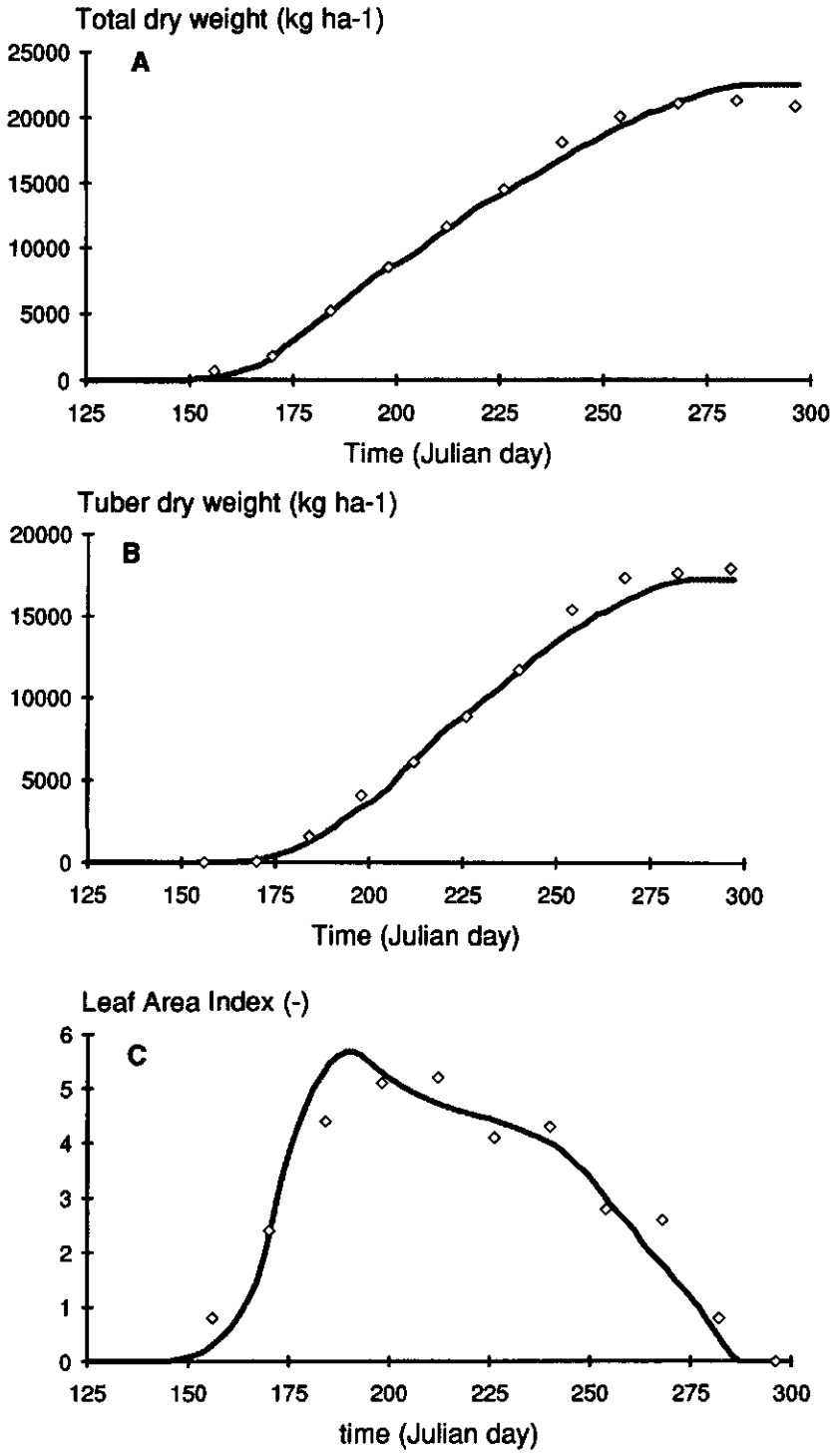


Figure 5.1. Simulated (———) and observed (◊) **(A)** total dry weight (kg ha^{-1}), **(B)** tuber dry weight (kg ha^{-1}) and **(C)** leaf area index (-) of potato cultivar 'Astarte' in 1979.

Root distribution over soil layers

To analyse spatial effects of PCN population dynamics and damage effects, simulated total root growth is distributed over vertical soil layers for soil sections under potato ridges and under furrows (Figure 5.2). The plant and soil interactions governing the spatial distribution of plant roots are unsufficiently understood for an explanatory model of potato root distribution. Instead, distribution of roots is forced over soil layers according to two parameters: the 'critical root length density', above which root growth is initiated in the adjacent soil layer, and the 'root extension fraction' , i.e. the fraction of root growth, which extends into the adjacent layer, when the root length density exceeds the critical density (Figure 5.3). In order to obtain a differentiation in root length density between soil layers under potato ridges and furrows (Vos & Groenwold, 1986), different critical densities for horizontal and vertical root

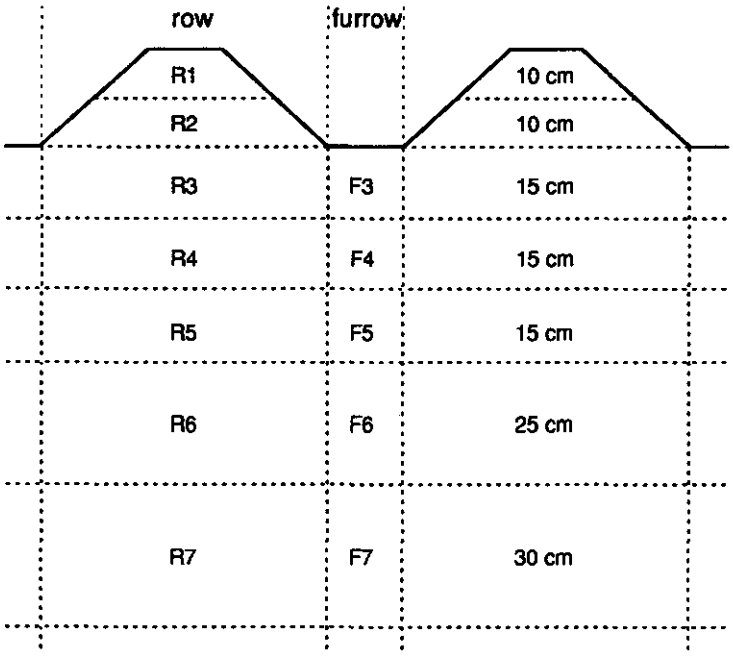


Figure 5.2. Designation of soil layers under potato rows and furrows and thickness of the compartments.

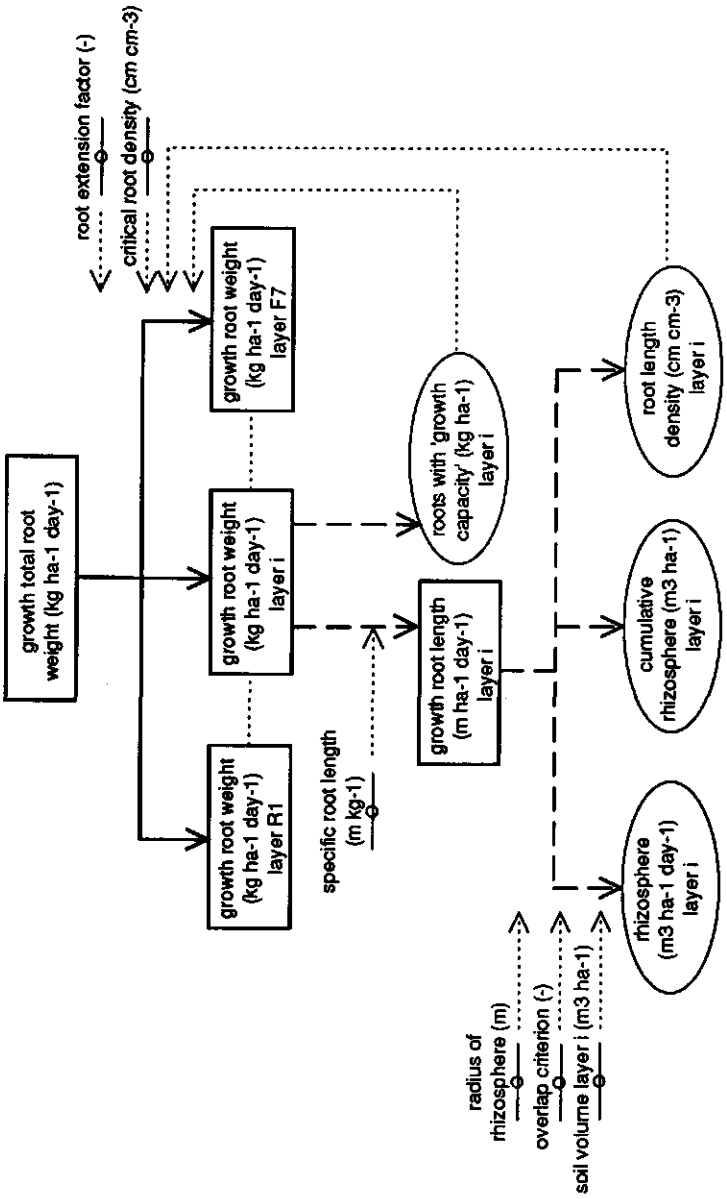


Figure 5.3. Method for distribution of total root dry matter growth (kg ha^{-1} day^{-1}), as calculated by SUCROS, over the various soil layers R1 - F7, and computation of auxilliary root variables.

extension are employed. Various root distribution patterns can be generated by variation of the values of critical root length density values.

Daily total root growth generated by SUCROS is partitioned over soil layers proportional to the weight of growing roots per soil layer (Figure 5.3). Here, growing roots include all roots younger than one day and 1% of the older roots, i.e. an estimate for the fraction of meristematic tissue that forms branch roots. If the root length density exceeds the 'critical root length density' for vertical or horizontal root extension, a fixed fraction of the root growth, allocated to that layer, is extended into the adjacent layer. Per soil layer, root weights are calculated by integration of the resulting net growth rates of roots per layer. Root length is computed from root weight and specific root length. Root length density is calculated from root length and the volume of the soil layer. Root senescence and death due to other factors than PCN is not simulated, because only the interactions of young roots with potato cyst nematodes are considered in this study. Specific root length of potato varies between 75 - 120 m g^{-1} dry weight (Lesczynski & Tanner, 1976; Vos & Groenwold, 1986) and is set at 100 m g^{-1} dry weight in the model. The vertical and horizontal critical root length densities for root growth to adjacent layers are set at values that mimick the root distribution observed by Vos & Groenwold (1986). The root length densities of a PCN-free crop, at various depths under potato ridges and under furrows and at various dates, are presented in Figure 5.4.

Production and persistence of potato root diffusate (PRD)

Potato root diffusate (PRD) is generally required to induce massive hatching of potato cyst nematodes (Perry, 1986; Atkinson & Fowler, 1990). Its concentration affects the fraction of hatched J2s (Fenwick, 1952). Production of PRD is closely correlated with the growth rate of roots (Widdowson, 1958a,b) and the difference in PRD activity between two cultivars with resistance to *G. rostochiensis* is largely explained by different root growth rates (Rawsthorne and Brodie, 1986). These results suggests that PRD is produced by root tips only, which agrees with studies on root exudation (McDougall & Rovira, 1970; McCully & Canny, 1985). Persistence of PRD in soil varies between 4 days (Fenwick, 1956) and over 100 days (Tsutsumi, 1976). Breakdown of PRD depends on soil type, with sand giving the highest and peat the lowest breakdown rate (Fenwick, 1956). In this study, exponential decay of PRD with a constant relative breakdown rate of 0.1

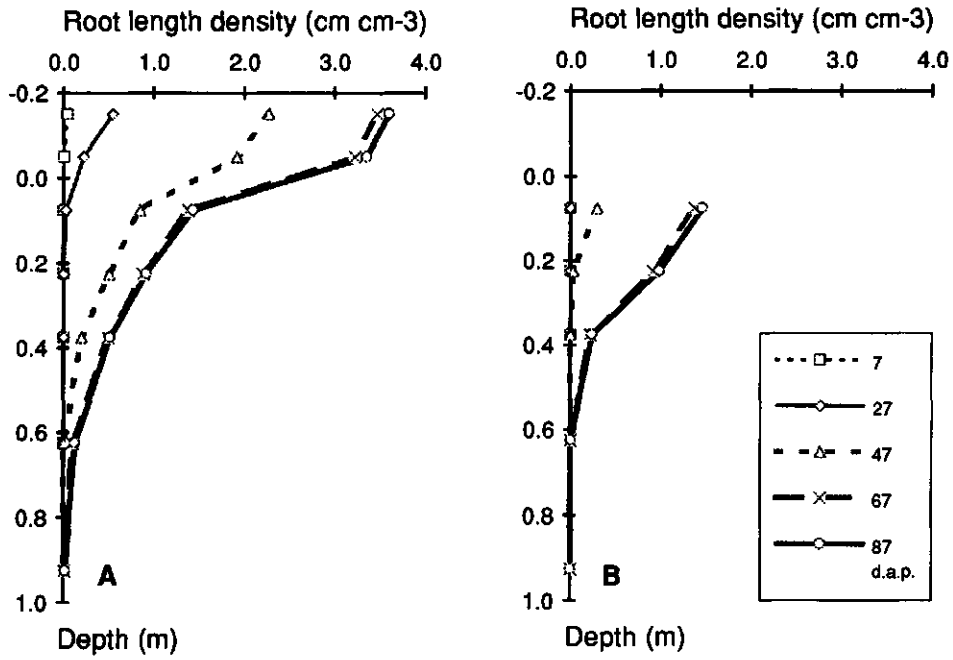


Figure 5.4. Simulated distribution of root length density (cm root cm^{-3} soil) over soil depth under the potato ridge (**A**) and under the furrow (**B**), at various days after planting (d.a.p.). The furrow surface is taken as zero depth.

day^{-1} is assumed. PRD is distributed through the soil by diffusion and soil water flow, but at this stage only diffusion is considered. In each time step, the soil volume infiltrated with PRD (designated here as the 'PRD-sphere'), is calculated from the length of roots, formed in the time step, and the maximum diffusion distance of soluble root exudates. This distance is between 0.5 cm, estimated on theoretical grounds by Darrah (1991), and 1 cm, experimentally observed by Weischer (1959). In this study, a 'PRD-sphere' radius of 0.5 cm is employed.
The rate, at which the soil is infiltrated with PRD, depends on the assumed root length distribution over the soil volume. With a uniform distribution, roots do not overlap and the 'PRD-sphere' in each time step consists entirely of soil, infiltrated by PRD for the first time (designated as 'first PRD-sphere'), upto the moment when the cumulative 'PRD-sphere' exceeds the total soil volume. From then on, the 'PRD-sphere' in each time step consists entirely of repeatedly infiltrated soil (designated as 'overlapping PRD-sphere'). In this study, a random root length

distribution over the soil volume is assumed. This implies, that the probability of repeated infiltration increases with the cumulative 'PRD-sphere'. To distinguish between 'first PRD-sphere' and 'overlapping PRD-sphere' for randomly distributed roots, the cumulative 'first PRD-sphere' is calculated from the cumulative total 'PRD-sphere', by analogy of Gregory's multiple infection transformation for the fraction of diseased plants (Gregory, 1948). The increase of this cumulative value during the latest time step is the 'first PRD-sphere' in this time step. The difference between total 'PRD-sphere' in the time step and 'first PRD-sphere' equals the 'overlapping PRD-sphere'.

To simulate these dynamic interactions of root growth, PRD concentration and hatching of PRD, each soil layer is divided into a number of 'hatch compartments'. Each hatch compartment is a collection of randomly distributed soil elements with identical PRD concentration. The volume of a hatch compartment is set at 1 m^3 ha^{-1}, which approximates the initial 'PRD-sphere' in a soil layer, when roots are extended from the adjacent layer. The initial PRD concentration, expressed in arbitrary units because the concentrations of its active substances are unknown, is zero in all hatch compartments. During crop growth, the PRD concentration in each separate hatch compartment is calculated by integration of the PRD infiltration and breakdown rates over time. The infiltration rate equals one for all hatch compartments that are part of the 'PRD-sphere', and zero for all other compartments. Exponential breakdown of PRD is calculated for all compartments with positive PRD concentration. The 'first PRD-sphere' is distributed over hatch compartments with zero PRD concentration, while the 'overlapping PRD-sphere' is randomly distributed over the hatch compartments with positive PRD concentration. When the 'overlapping PRD-sphere' is less than half the volume of a hatch compartment, the total 'PRD-sphere' is considered as 'first PRD-sphere'. For each separate hatch compartment, emergence of J2s is calculated as a function of the local PRD concentration, according to the relation between PRD concentration and the fraction of hatched J2s described by Fenwick (1952).

Hatching of potato cyst nematodes (PCN)

Hatching of potato cyst nematodes (PCN) starts with activation of second stage juveniles (J2s), confined in egg shells inside the cysts, and ends with the emergence of infective J2s from the cyst in the soil. The cumulative emergence of J2s *in vitro* is described with a saturation type curve (Fenwick, 1950) characterized here by the maximum fraction of juveniles emerging from cysts (EMMX), the time to first emergence (EMINC) and the slope of the cumulative emergence curve with time (EMRR) (Chapter 4). These parameters are affected by soil temperature, PRD-concentration, the fraction of J2s hatching in the absence of PRD and the periodicity of hatching (Figure 5.5). Effects of

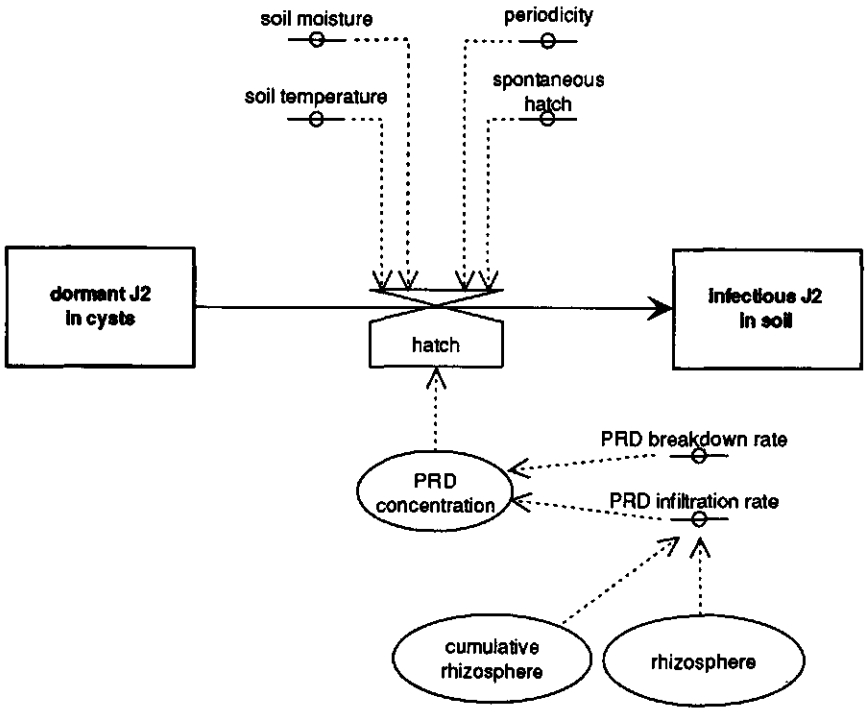


Figure 5.5. Relation diagram for hatching of potato cyst nematodes.

temperature on the hatching parameters of both PCN species is analyzed in Chapter 4.

The effect of PRD-concentration on the hatched fraction of J2s is described by a log-linear relation (Fenwick, 1952), and probably indicates variation among juveniles in the threshold concentration of PRD for initiation of juvenile metabolic activity (Atkinson & Fowler, 1990). The fraction of J2s, hatching in the absence of PRD, may be as high as 60 % for *G. rostochiensis* (den Ouden, 1960), indicating that the requirement for PRD in the hatching process is not absolute. High rates of spontaneous hatching have also been observed for *G. pallida* (J.G. Mulder, pers. comm., 1991), but large variation for this trait exists among populations. Perry (1986) suggested that spontaneous hatching is in fact delayed hatching of those juveniles, that were in diapause at the time of activation by PRD. Emergence then occurs when diapause is completed. For simulation, it is assumed that a fixed fraction of the initial population may hatch in the absence of PRD. The temperature-dependent processes, that determine EMINC in PRD-induced hatching, have already been fulfilled or are by-passed in the spontaneously hatching sub-population (Perry, 1986) and EMINC is set at zero.

During the first autumn and winter after harvest, hatching is impaired due to obligate diapause (Hominick *et al.*, 1985). The length of the diapause is negatively correlated with the photoperiod during growth of the host plant (Hominick, 1987). This relation is probably modified by other factors influencing plant growth, e.g. cultivar characteristics and weather conditions. The subsequent periodicity of the hatching response to PRD, observed after natural storage conditions in outdoor soil (Cunningham, 1960; Oostenbrink; 1967) and after dry storage at 7 °C in darkness (Mulder & Vroom-Wolf, 1990), is caused by facultative-induced diapause (Evans, 1987): periodicity of hatching was absent after storage of cysts at 20 °C in darkness during the summer after harvest (Hominick *et al.*, 1985). For PRD-induced hatching, only EMRR is affected by periodicity (Oostenbrink, 1967; Mulder & Vroom-Wolf, 1990) and for spontaneous hatching only EMMX is affected (Mulder & Vroom-Wolf, 1990).

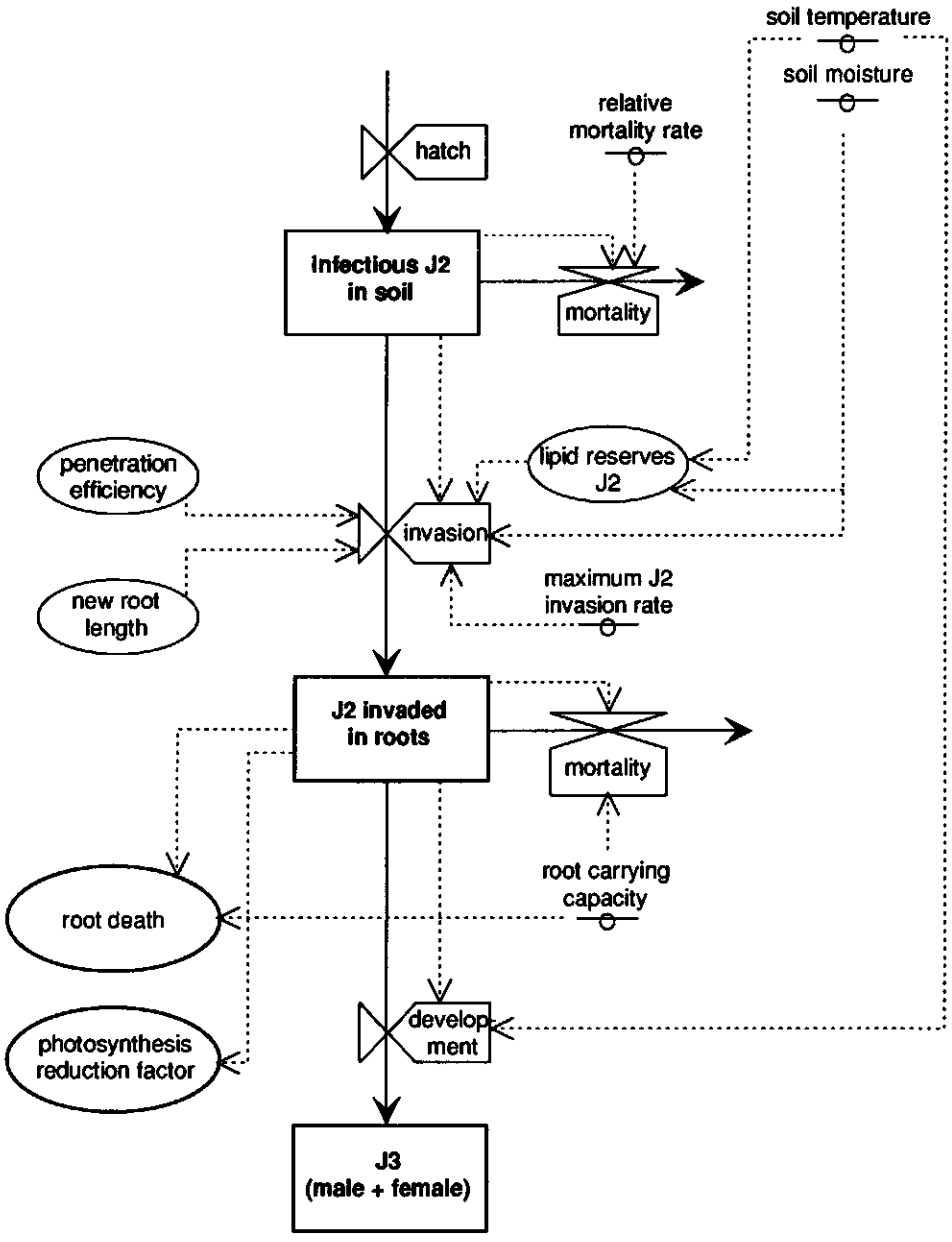


Figure 5.6. Relation diagram for invasion of second stage juveniles of potato cyst nematodes in potato roots.

Root penetration by second stage juveniles

The rate of penetration of J2s into roots depends on the movement rate of J2s to the root tip surface and the maximum penetration rate per unit root length (Figure 5.6). The movement rate of J2s in soil under optimal conditions is 2 - 3 cm day^{-1} (Wallace, 1960). Attraction of J2s to host roots has not been clearly demonstrated (Kuhn, 1959; Weischer, 1959) and penetration site finding is assumed to be random.

Free J2s are assumed to be regularly distributed through the soil. Then, for each day the fraction of J2s penetrating roots equals the soil volume with a radius of 2 cm around roots formed that day, as a fraction of the total soil volume. This fraction, multiplied with the number of free J2s, gives the number of penetrating J2s day^{-1}. Data on the maximum number of penetrating J2s per unit root length are not available. This parameter is estimated as 25 % of the ratio between specific root volume and J2 volume. With a root radius of 0.1 mm (Vos & Groenwold, 1986), the specific root volume is about 3.10^8 μm^3 cm^{-1} root length. The J2 volume is about 5.10^5 μm^3 (Stone, 1973), hence the maximum number of penetrating J2s is 150 cm^{-1} root length.

The infectivity of J2s, expressed as the fraction of penetrating J2s establishing syncytia, is inversely related to the density of penetrating J2s, indicating intraspecific competition for feeding sites (Mugniery & Fayet, 1981). This relation probably includes the emergence of J2s from roots (Forrest, Trudgill & Cotes, 1986). The infectivity of J2s is also related to their lipid content (Storey, 1984; Robinson *et al.*, 1985). The depletion rate of lipid content is influenced by soil temperature and differs between *G. rostochiensis* and *G. pallida* (Robinson *et al.*, 1987b).

Development of PCN juvenile stages, cysts and eggs

Development of penetrated J2s to third and fourth juvenile and adult stages (Figure 5.7) is characterized by average development periods and their relative dispersions, which are differentially influenced by temperature (Chapter 4). The numbers of individuals in each stage at any time are simulated with the 'boxcar train' method (Goudriaan & van Roermund, 1989).

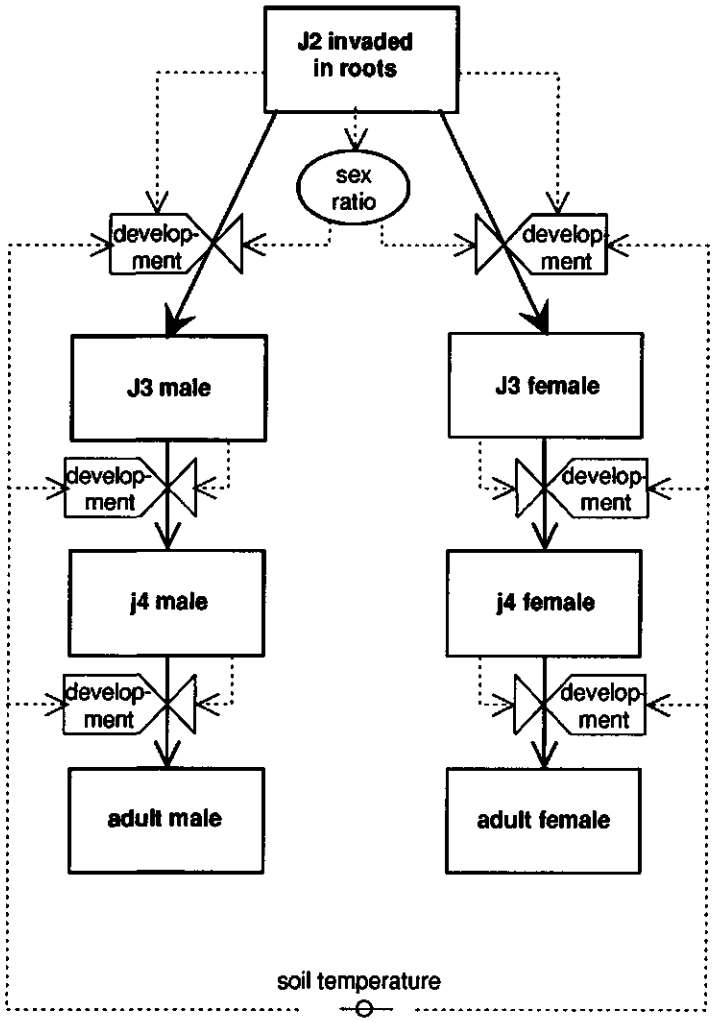


Figure 5.7. Relation diagram for juvenile development of potato cyst nematodes.

The density of penetrated J2s per unit root length determines population dynamics and plant growth (see next section), therefore the amount of J2-infected root length is calculated in parallel with the number of J2s in the roots, also using the boxcar train method. The number of J2s in roots is the integral of the root penetration rate of J2s minus the rate of appearance of third stage juveniles. The J2-infected root length is the integral of the root growth rate at positive J2 penetration rates, minus the rate of development into roots containing third juvenile and later PCN stages. This development rate is equal to the rate of appearance of third stage juveniles. It is assumed that all adult females mate immediately. After mating, rates of female maturation into cysts, fecundation of eggs and subsequent egg development to new J2s (Figure 5.8) are influenced by temperature (Chapter 4; Langeslag *et al.*, 1982). Mortality of third juvenile and later PCN stages and of eggs is not considered.

Interactions between second stage juveniles and crop growth

Interactions between penetrated J2s and plant physiological processes affect population dynamics through the epigenic sex ratio and a 'carrying capacity' of roots for syncytia, and crop yield loss through photosynthesis reduction. The sex ratio is calculated for appearing third stage juveniles as a function of their density per unit root length, with the analytical function by Thornley & Hesling (1972), which agrees with observations of Trudgill (1967) and Mugniery & Fayet (1981). Although not directly investigated, a maximum density of developing females is effectuated through a 'carrying capacity' of roots for syncytia. This 'carrying capacity', defined as the maximum number of syncytia supported per unit root length, depends on root diameter (Trudgill, 1967; Bridgeman & Kerry, 1980). Trudgill (1967) observed a carrying capacity of about 40 juveniles cm^{-1} of selected roots. Storey (1982a) measured 5.19 cysts cm^{-1} for a root system with a range of root diameters, which corresponds to 10 juveniles cm^{-1} root (Thornley & Hesling, 1972). This latter value is applied in the model, because the specific root length is assumed to be average for a root system, representing the range of root diameters. The effects of penetrated J2s on photosynthesis rate, demonstrated in Chapters 2 and 3, are analyzed by simulation, as discussed in a later section.

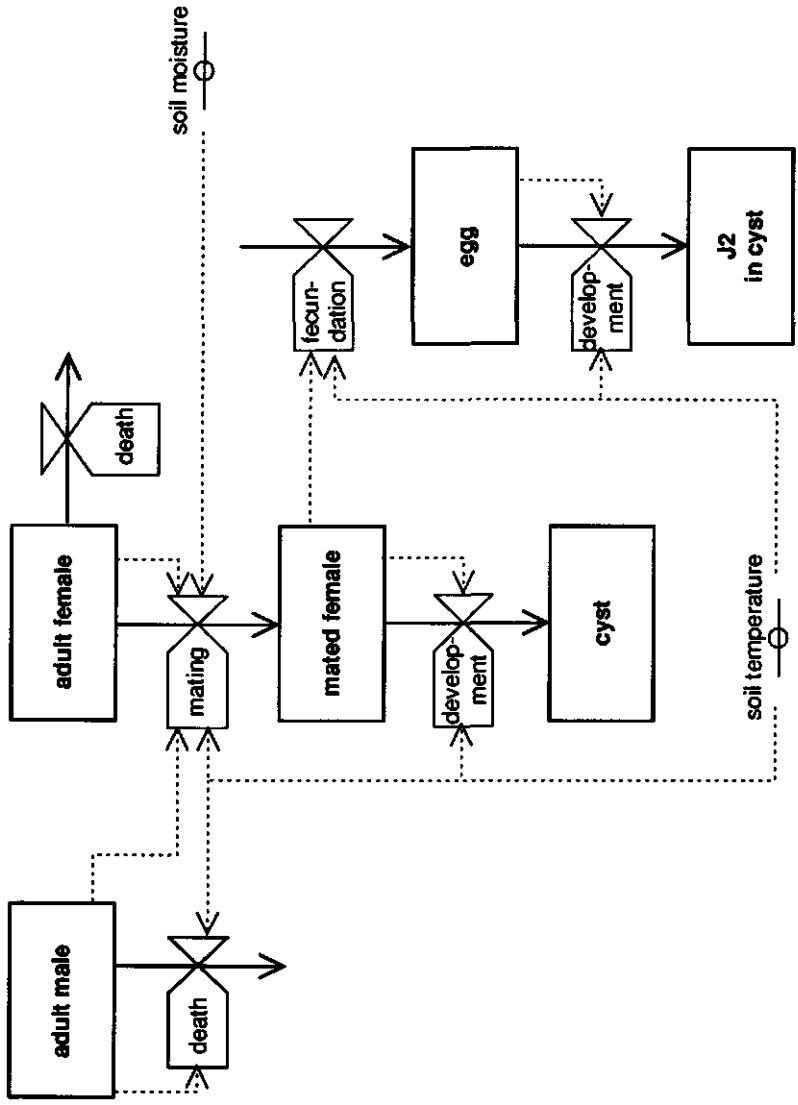


Figure 5.8. Relation diagram for mating and development of cysts and eggs of potato cyst nematodes.

Simulation results and discussion

The simulations are aimed at investigating PCN population dynamics and potato crop damage in relation to host-parasite interactions at the penetrated J2-stage, to species-specific temperature responses of hatching, penetration and juvenile development, and to cultivar-specific maturity class and root carrying capacity. Soil water content is set constant at field capacity and mass flow rate of soil water is zero. This implies, that distribution of PRD in soil is by diffusion only and effects of soil water potential on root growth, crop photosynthesis and movement and mortality of J2s and males in soil are absent. A regular distribution of PCN cysts in soil layers R1, R2, R3 and F3 is assumed (Figure 5.2); there are no PCN in deeper soil layers. The fraction of eggs, capable of hatching in water, is set at 0.3. The potato crop is a susceptible, late cultivar. Weather data from the Wageningen weather station (daily global radiation, maximum and minimum air temperature, maximum and minimum soil temperature at depth 5, 10 and 20 cm) are used. The time step of the rectangular (Euler) integration method used is one day for crop growth and 0.1 day for PCN population dynamics. Actual soil temperature at various depths during the day is calculated with a sinusoidal function of the tabulated maximum and minimum temperatures.

Effects of second stage juveniles in roots on photosynthesis

The reduction of photosynthesis by J2s is investigated, based on the following assumptions: (i) maximum photosynthesis rate at light saturation and initial light use efficiency are reduced due to the release of messenger substances in the xylem by root tips, in response to the formation of syncytia by J2s (as discussed in Chapter 2); and (ii) the duration of photosynthesis reduction is restricted to the period of syncytial initiation, which is 20 - 50 % of the total development period of J2s (estimated from Rice *et al.*, 1987). Here a value of 50 % is employed. Quantitative modeling of the effect of messenger substances associated with abiotic or biotic root stress on photosynthesis has not been done before, therefore two hypothetical mechanisms are here explored. According to the first hypothesis, the effect of messenger substances is governed by the stress level experienced by growing roots, irrespective of leaf area. This amount of stress is here related to the number of J2s initiating syncytia ('damaging J2s') per unit length of growing roots. The hypothesis is mathematically formulated as a linear

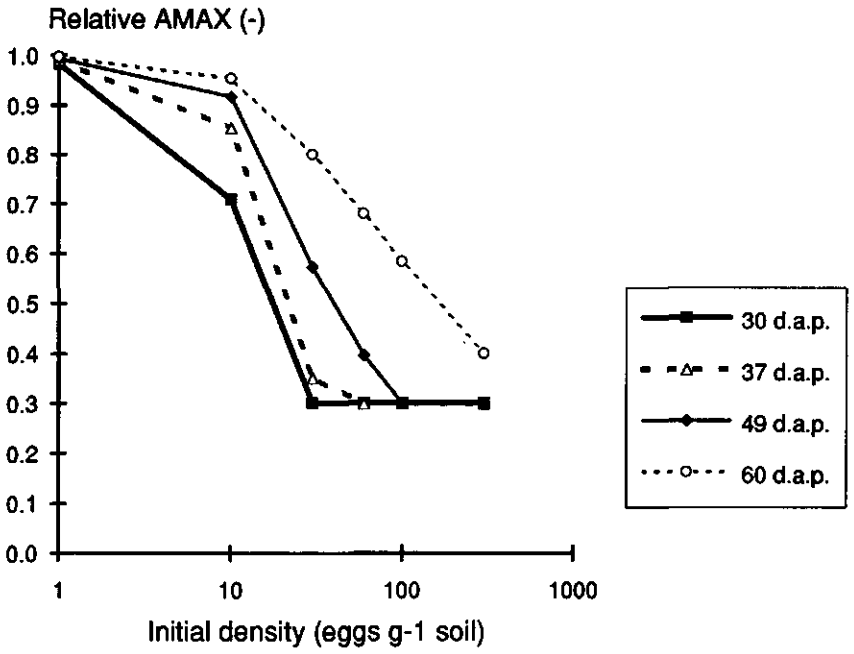


Figure 5.9. Simulated maximum assimilation rate at light saturation (AMAX) of potato at various initial densities of *G. pallida*, relative to the AMAX of the healthy crop, at various days after planting (d.a.p.), for the experimental conditions employed in Chapter 3.

relation between photosynthesis reduction and the density of 'damaging J2s', with a maximum photosynthesis reduction of 70 % (Chapter 3) occurring at 5 J2s cm^{-1} root (i.e. 50 % of the root carrying capacity) and higher densities. According to the second hypothesis, the effect of messenger substances is governed by their amount per unit leaf area, irrespective of the stress level per unit of growing roots. However, the amount of messenger substances is still related to the number of 'damaging J2s'. This hypothesis is formulated as a linear relation between photosynthesis reduction and the number of 'damaging J2s' per unit leaf area, with a maximum photosynthesis reduction of 70 % occurring at 5 J2s cm^{-2} leaf and higher densities.

These hypotheses were investigated with the experiment described in Chapter 3. The temperature data from the weather station were replaced by the data of the greenhouse temperature regime. Simulation started at July 7, with planting at 3 cm depth. The first hypothesis was rejected, because the simulated

density of 'damaging J2s' was almost constant between 30 and 60 days after planting at all initial densities, and could therefore not have caused the diminishment of photosynthesis rate reduction in this period. Apparently, the effect of messenger substances is not governed by their production rate. According to the second hypothesis, simulated reduction of maximum photosynthesis rate at light saturation at various days after planting for the various initial egg densities corresponded with experimental results (Figure 5.9 compared with Figure 3.2). The observed larger reduction of photosynthesis at 60 days after planting, compared with 49 days after planting, did not occur in simulation. This difference is difficult to interpret, because the experiment was carried out in the greenhouse with potted plants, while SUCROS was developed for field crops. However, the agreement between simulation and experiment is sufficiently close to conclude, that the effect of 'damaging J2s' on photosynthesis is inversely related to leaf area size rather than to root length.

Population dynamics and associated damage effects of *G.rostochiensis* and *G. pallida* in a warm and a cool season

Sub-models for various processes of PCN development from hatching to new cysts have been quantified *in vitro* and validated with independent data, as described above. Here, all sub-models are linked to investigate the effect of species-specific temperature responses of hatching and development rates on population increase and yield loss, in contrasting seasons. A cool and a warm season are simulated with Wageningen weather data for 1987 and 1990, respectively (Figure 5.10). Crop growth was initiated at April 28 in both years, with planting at 9 cm depth. The simulations were started at March 28, to allow for hatching in absence of PRD, and were finished at October 5.

The shape of the relation between final and initial population densities, averaged over the infested soil layers (Figure 5.11), agrees with experimental observations (e.g. Huijsman *et al.*, 1969; Seinhorst, 1986a). The maximum multiplication rate of about 50x at 1 egg g^{-1} soil, the equilibrium density of approximately 100 eggs g^{-1} soil and the maximum final density of 500-600 eggs g^{-1} soil at an initial 30 eggs g^{-1} soil have been measured in field experiments (Seinhorst, 1986a), indicating realistic simulation results. Both the seasonal temperature regimes and the species-specific responses to temperature caused variation of population increase. The final density of both species is slightly

higher in the warm season than in the cool season at intial densities below 60 eggs g^{-1} soil. At higher densities, the multiplication rate of *G. rostochiensis* is not affected by the season, while multiplication rate of *G. pallida* is lower in the warm season. The maximum difference in final density due to seasonal temperature differences was 95 eggs g^{-1} soil (22 %), for *G. pallida* at an initial 30 eggs g^{-1} soil. Differences in temperature response between the species also caused different rates of population increase.

Interaction with seasonal temperature regime occurred. In the warm season, multiplication of *G. rostochiensis* was lower than that of *G. pallida* at initial densities below 10 eggs g^{-1} soil, but at higher densities it was considerably higher, causing a maximum difference in final density of 180 eggs g^{-1} soil at an initial 60 eggs g^{-1} soil in the warm season. In the cool season, the difference between the species reversed again at 100 eggs g^{-1} soil. The equilibrium density and the initial density, giving the highest final density, are higher for *G. rostochiensis* than for *G. pallida*. The hypothesis, that multiplication of *G. rostochiensis* is larger than that of *G. pallida* in warm, and smaller in cool

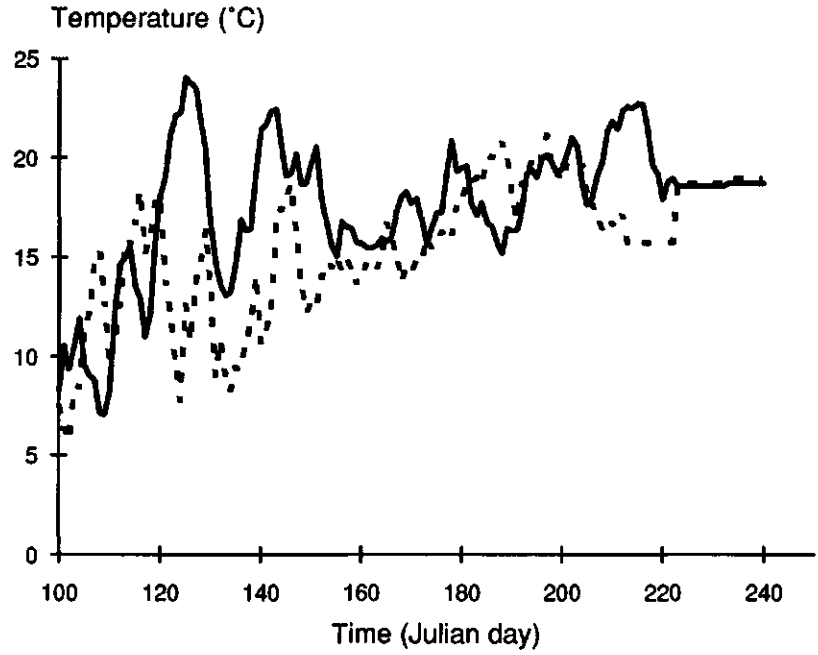


Figure 5.10. Average daily soil temperature at 5 cm depth in 1987 (- - - -) and 1990 (——), measured in Wageningen.

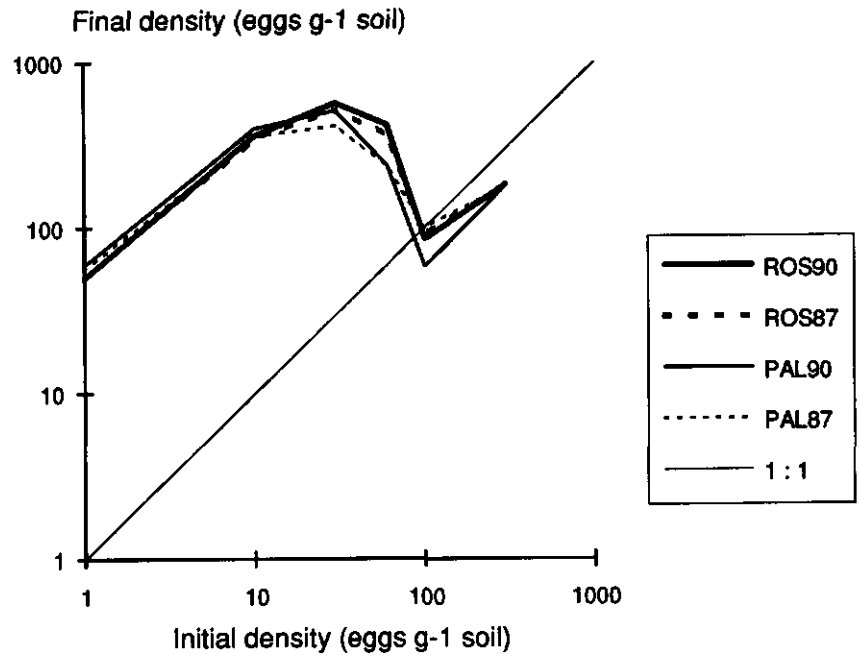


Figure 5.11. Simulated relation between initial and final density (eggs g^{-1} soil) of *G. rostochiensis* and *G. pallida* in 1990 (warm season) and 1987 (cool season) (ROS90, ROS87, PAL90 and PAL87).

climates (Foot, 1978; Franco, 1979; van Dongen, 1983), is not confirmed by simulation, because of interactions between seasonal temperature regime, initial density and multiplication rate. These interactions imply, that effects of competition between the species on population increase (den Nijs, 1992) cannot be simply predicted, but should be analyzed with dynamic simulation for a range of overall densities, proportional densities of the two species, and seasons.

Crop yield at various initial densities was affected by season and by PCN species (Figure 5.12). The shape of the curves and the simulated minimum yield agree with experimental observations (e.g Seinhorst, 1982a; Trudgill, Marshall & Phillips, 1990). In the cool season, a tolerance limit, i.e. the density below which no yield reduction occurs (Seinhorst, 1986b), was absent.

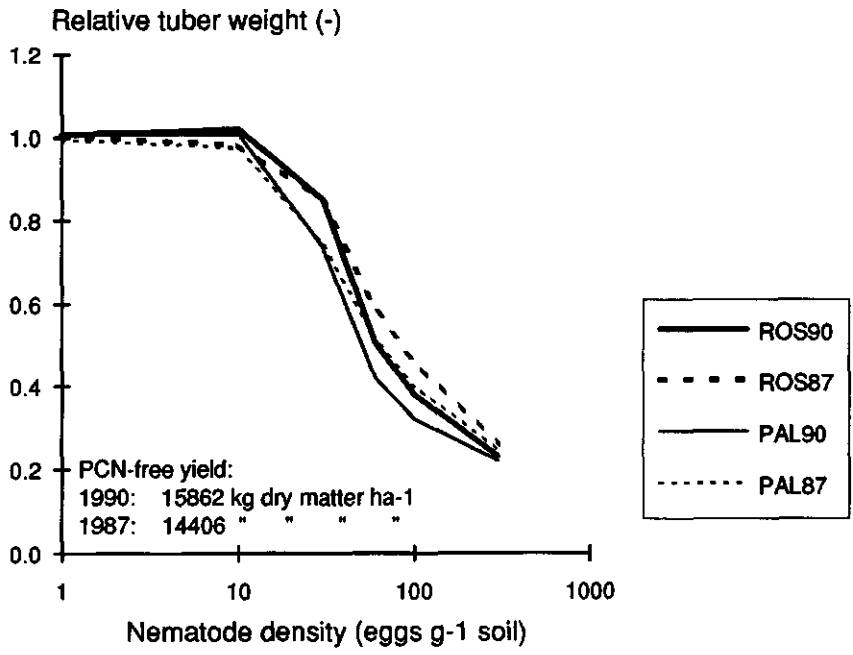


Figure 5.12. Simulated dry matter tuber yield of potato at various initial densities (eggs g^{-1} soil) of *G. rostochiensis* and *G. pallida*, relative to the yield of the PCN-free crop, for 1990 (warm season) and 1987 (cool season) (ROS90, ROS87, PAL90 and PAL87).

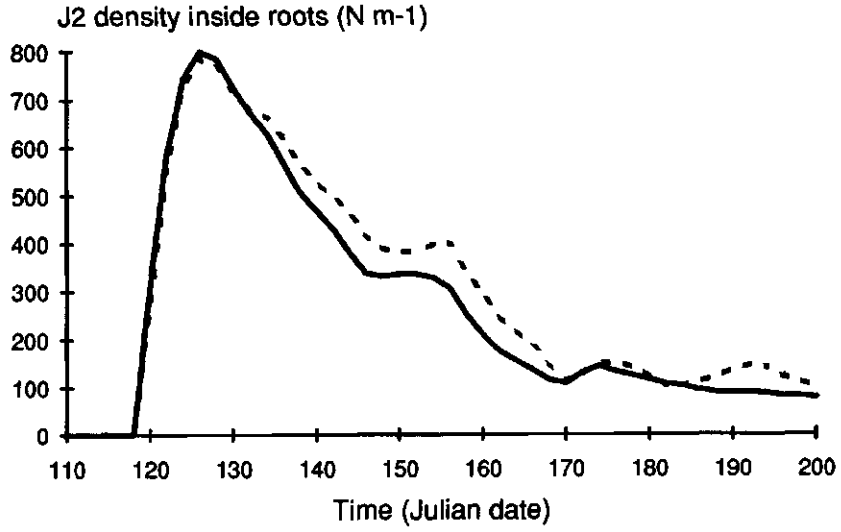


Figure 5.13. Simulated density of J2s, that initiate syncytia, of *G. rostochiensis* (——) and *G. pallida* (- - - -) inside potato roots (N m^{-1}), at 10 eggs g^{-1} soil. The results are for 1990.

In the warm season, damage is simulated above an initial 10 eggs g^{-1} soil only, which corresponds with experimental observations (Trudgill, Marshall & Phillips, 1990). Both seasonal temperature regime and species-specific responses to temperature caused variation in yield loss. Differences in the temperature regimes of 1987 and 1990 modified the relation between initial density of PCN and relative yield. For both species, relative yield in the warm season was higher than in the cool season at densities below 30 eggs g^{-1} soil, but the difference was reverse at higher densities.

For the warm season only, a small yield increase due to PCN infection at densities below 10 eggs g^{-1} soil was observed in simulation. This yield increase, measured sometimes in experiments as well (Chapter 3; Mulder *et al.*, 1991), was due to a larger net assimilation rate of the infected crop. Net assimilation rate, i.e. the difference between rates of gross photosynthesis and maintenance respiration of the standing biomass, was increased, because dry matter growth during syncytial initiation by J2s was more strongly reduced than the amount of intercepted light by the canopy. When the density of J2s in roots was decreasing again, and maximum assimilation rate and initial light use efficiency of the infected crop became similar to that of the healthy crop, gross photosynthesis was restored while maintenance respiration remained low. At the high irradiation levels of 1990, this resulted in higher yields at low PCN densities.

Yield loss due to *G. pallida* was larger than that due to *G. rostochiensis*, because the density of 'damaging J2s' in roots of *G. pallida* during the growing season remained higher (Figure 5.13). This was caused by prolonged hatching and smaller lipid depletion rates of *G. pallida*. Experiments, designed for comparison of yield loss due to *G. rostochiensis* and *G. pallida*, have not been done, but the simulation results are supported by Trudgill (cited in Evans & Haydock, 1990), who showed a consistently greater yield increase due to nematicides for sites, infested with *G. pallida* than for sites, infested with *G. rostochiensis*.

The simulation results for *G. rostochiensis* in 1990 were further analyzed with respect to root death, due to J2 densities exceeding the root 'carrying capacity' for syncytia, and the distributions of PCN multiplication rates, hatched fractions of the initial population and root length densities over soil layers. Total root death at 100 and 300 eggs g^{-1} soil was only about 15 % of the total root weight produced by a PCN-free crop. It resulted from a continuous mortality process over the period when J2s are present in excessive densities in the roots,

which might explain why this phenomenon, if existing, has gone unnoticed before. Its importance for PCN population dynamics however, requires detailed investigation of the relative importance of root growth rate reduction and root death rate for reduction of root weight of PCN-infected plants.

The PCN multiplication rates varied among soil layers (Table 5.2, for *G. rostochiensis* in 1990). Interactions between soil layer and initial population density occurred for the homogeneously distributed populations in these simulations, caused by dynamic interactions between root growth, hatching and root penetration. Consequently, the average population increase of non-homogeneously distributed PCN populations, as with natural infestations (Seinhorst, 1982b), cannot be easily predicted. This complicates the formulation

Table 5.2. Multiplication rate (Pf/Pi, i.e. final egg density / initial egg density), the unhatched fraction of the initial egg population, carried over to the next growing season, and the time to first root growth, expressed in days after planting (d.a.p.), in each of the soil layers under the ridge (R1-R5) and under the furrow (F3-F5), see Figure 5.2. Data are for *G. rostochiensis* in 1990, at 3 initial densities (eggs g^{-1} soil).

layer	Pf/Pi			carry-over			time to first root growth (d.a.p.)		
	initial density (eggs g^{-1} soil)			initial density (eggs g^{-1} soil)			initial density (eggs g^{-1} soil)		
	10	60	100	10	60	100	10	60	100
R1	44	10	0.51	0.34	0.45	0.51	0	0	0
R2	43	12	1.55	0.38	0.51	0.56	13	13	13
R3	32	5.6	0.61	0.50	0.59	0.61	20	20	21
R4	-	-	-	-	-	-	36	42	42
R5	-	-	-	-	-	-	44	52	54
F3	28	0.7	0.7	0.52	0.7	0.7	52	n.r.*	n.r.*
F4	-	-	-	-	-	-	54	n.r.*	n.r.*
F5	-	-	-	-	-	-	60	n.r.*	n.r.*

* n.r. : no root growth in this layer

of control measures for PCN.

The hatched fraction of the initial population decreased with depth of the soil layer and was higher under ridges than under furrows (Table 5.2). It decreased with increasing initial PCN density, due to reduced root growth and associated PRD production and distribution. The results are supported by Storey (1982a), who observed a 70 % carry-over for cysts in the furrows, indicating that transport mechanisms of PRD other than diffusion, e.g. mass flow of soil water, are insignificant. Rawsthorne and Brodie (1987) reported a concentration gradient of PRD in soil, as measured by egg hatch, that decreased with increasing lateral and vertical distance from the root zone which was restricted by nylon screens. However, this presumed PRD concentration gradient may have been in fact a root density gradient, since they report on some unplanned root growth occurring outside the restricted root zone.

Root length density is reduced with increasing initial PCN density in all soil layers including the PCN-free but, more importantly, the time at which root growth is initiated in layers below 35 cm depth is strongly increased by PCN (Table 5.2). This implies important interactions of PCN infection with water and nutrient deficiencies with respect to crop production, because reserves in deeper soil layers are not available for an infected crop. Simulation of these interactions are possible when the model is extended with effects of water and nutrient availability on crop growth. The open model structure of POTACYST allows for simple incorporation of water and nutrient balance routines and effects of water and nutrient shortage on crop growth and PCN population dynamics, when sufficient knowledge at process level is available.

Crop characteristics: tolerance and partial resistance

Potato cultivars differ in their level of tolerance for PCN infection. In Chapter 3 it was demonstrated, that tolerance differences among cultivars are not related to the level of photosynthesis reduction by PCN. The larger tolerance of cultivar Cara, compared with Pentland Dell, is largely explained by differetn dry matter distribution patterns between the cultivars, associated with the later maturity of Cara (Trudgill, 1987a). This effect of cultivar maturity class on crop yield at various densities of *G. pallida* was simulated with weather data for 1987, assuming that the relation between J2s in roots and photosynthesis rate is independent of maturity class (Figure 5.14). Reduction of tuber yield by PCN

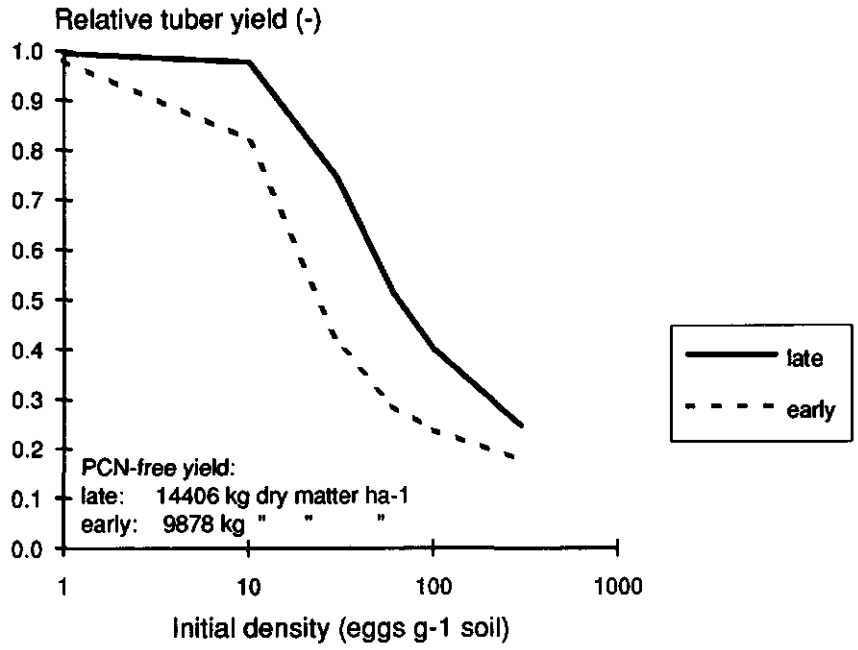


Figure 5.14. Simulated dry matter tuber yield at various initial densities (eggs g^{-1} soil) of *G. pallida*, relative to PCN-free yield, of a late (susceptible or resistant) and an early, susceptible cultivar, in 1987.

was larger for the early than for the late maturing cultivar, confirming the observations by Trudgill (1987a). Prolonged root growth, later tuber initiation and longer duration of tuber growth effectively reduced damage by PCN. However, these results do not exclude other possible mechanisms of PCN tolerance. For instance, reduction of root growth due to PCN, in interaction with drought spells during or after penetration of J2s in roots, may cause yield differences among cultivars differing in drought tolerance. Extension of the model with a soil water balance and effects of water shortage on crop photosynthesis and on dry matter distribution among plant organs, will allow investigation of these interactions.

Resistance to PCN of potato cultivars is based on various mechanisms, each affecting different stages of the PCN life cycle. Cultivars with partial resistance derived from *Solanum vernei*, which is the most important type of resistance to *G. pallida*, reduce PCN populations at initial densities above approximately 15 eggs g^{-1} soil, but increase populations at lower densities

(Mulder *et al.*, 1990). Yield losses of these cultivars are sometimes equal to those of susceptible cultivars (Velema & Boerma, 1987), indicating independence of resistance and tolerance. Turner and Stone (1984) observed, that penetration of J2s in roots and subsequent development to the J3 stage were not different among these partially resistant and susceptible cultivars. Appearance of later development stages was strongly reduced due to reduced development rates and increased mortality of J3 and later stages. Simulation of this mechanism for partial resistance confirmed the multiplication rates and yield losses observed in the field (Figure 5.15, for *G. pallida* in 1987). These results suggest, that all resistance mechanisms, acting upon PCN development after initiation of syncytia, are independent of cultivar tolerance. Consequently, resistance acting upon or before the stage of syncytial initiation, will enhance PCN tolerance of the cultivar. Support for this hypothesis is given by Trudgill & Cotes (1983a), who showed that cultivars most tolerant of *G. rostochiensis* all carry gene H1, which prevents initiation of syncytia by this species.

Concluding remarks

A mechanistic model for population dynamics of potato cyst nematodes and associated damage effects on potato plants has been developed. All relations and parameters are formulated in biologically and physically meaningful dimensions, implicating that all model elements can be quantified and tested experimentally. The stratification of the soil in layers under potato ridges and furrows and 'hatch compartments' within each layer, permits investigation of non-homogeneous PCN distributions. Two important interactions between the potato crop and the PCN population are modelled: hatching and crop photosynthesis. Hatching of the PCN egg population is simulated in dynamic interaction with spatial root growth dynamics, through the concentration of PRD in each seperate hatch compartment. Distribution of PRD through diffusion only, adequately explained hatching in various soil layers and at various initial population densities. Apparently, other distribution processes are not important for PCN population dynamics. Crop photosynthesis is simulated as a function of the density of J2s initiating syncytia in roots. It was demonstrated, that the number of J2s relative to leaf area, rather than infected root length, determined the level of photosynthesis reduction. The relation between PCN density and photosynthesis, observed in a greenhouse experiment, largely explained yield

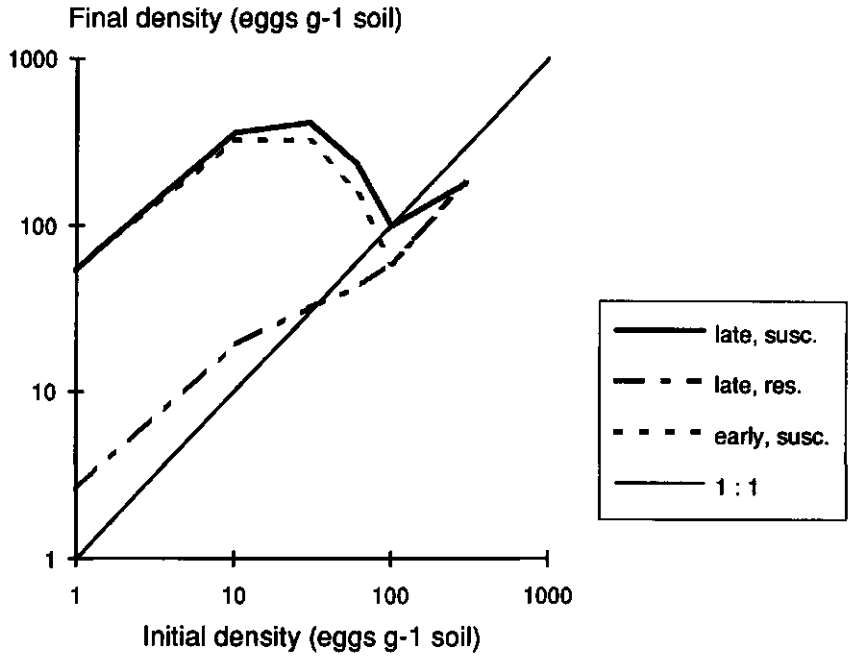


Figure 5.15. Simulated relation between initial and final density (eggs g^{-1} soil) of *G. pallida* on a late susceptible, a late resistant and an early susceptible potato cultivar, in 1987.

reduction of field crops. Apparently, the effect of syncytial initiation on photosynthesis is the major mechanism of yield loss due to PCN infection. This mechanism also explains effects of tolerance and resistance mechanisms, derived from independent experiments, on yield loss due to PCN.

Preliminary sensitivity analysis indicated, that simulated yield reduction is strongly affected by the parameter value for density-dependent photosynthesis reduction. This might indicate, that other factors than the number of 'damaging J2s' and leaf area contribute to photosynthesis reduction as well. A more stable formulation of the relation between 'damaging J2s' and photosynthesis reduction might be attained by including effects of root length. Further research on photosynthesis reduction at different shoot/root ratios and different leaf area sizes at the time of inoculation with hatched J2s, is required to improve understanding of the nature of this relation and the values of its parameters.

Possible interactions between plant processes and third and later nematode development stages may exist, e.g. withdrawal of plant substances

via syncytia (Müller *et al.*, 1981) and reduction of uptake rates of nutrients by plant roots (Trudgill, 1980). Growth conditions of the crop may also influence the development of PCN populations, e.g. diapause length of the next generation (Hominick, 1987) and offspring size (Franco & Evans, 1979). These host-parasite interactions are not investigated here, because the relations of whole - plant physiological processes with syncytial activity and with disturbance of mineral uptake rates are insufficiently understood for explanatory modeling.

Variation in temperature between two seasons caused variation of yield loss, probably in interaction with variation in radiation, and of PCN population increase. This illustrates, that the contribution of weather variability to overall variation of yield loss and population increase can be quantified by simulation with weather data for a range of years and locations. Knowledge of this uncontrollable background variation allows a more precise assessment of the performance of various PCN control measures in different environments and formulation of low-risk control strategies.

Chapter 6

General discussion

The need for dynamic, mechanistic modeling of potato - PCN interactions

Descriptive models for dose-response relations between initial and final densities of PCN (Seinhorst, 1967; 1986a) and between initial density and crop yield (Seinhorst, 1965; Oostenbrink, 1966; Brown, 1969; Elston *et al.*, 1991) have been developed. At best, parameters of these models summarize biological processes, but they vary greatly among experiments from different years or locations. Therefore, these models contribute little to understanding of the variable responses of population increase and crop yield to initial PCN density. They cannot be used for extrapolation and their practical application in breeding or agronomy remained limited. Understanding of the processes underlying population increase and yield loss, can improve insight in the observed variation. Much knowledge on these processes has been gained from experimental research. On the level of individual nematodes, processes of hatching, development and epigenic sex determination in relation to environmental factors have been examined (e.g. Perry, 1986; Mugniery, 1978; Mugniery & Fayet, 1984). For individual plants, effects of PCN on weight of seperate plant organs, on plant water relations and mineral nutrient contents have been found (e.g. Trudgill, 1980; Fatemy & Evans, 1986). On the cell level, many chemical and physiological aspects of hatching and syncytial activity (e.g. Atkinson & Fowler, 1990; Rice *et al.*, 1987) have been elucidated. However, conclusions on causal mechanisms of population increase and yield loss could not yet be formulated. For instance, decreased haulm growth of PCN-infected plants is attributed to chronic nutrient deficiency (Trudgill, 1986), but at high N, P and K application rates haulm and tuber weights of infected plants remained about 50 % reduced, at similar haulm nutrient contents of infected and control plants (Trudgill, 1980). Clearly, other factors than nutrients act in causal mechanisms of yield reduction.

Systematic analysis of the interactions between processes of PCN population development and crop growth is required, to gain insight in the causal mechanisms of yield loss and population dynamics, and their relative importance. In this thesis, such an analysis has been initiated by linking processes of hatching and nematode development to spatial distribution of root growth and crop photosynthesis, i.e. the primary growth process, using dynamic explanatory models (Rabbinge & de Wit, 1989). With this approach it was shown, that interacting processes of plant growth and nematode development, occurring between activation of J2s in cysts and appearance of the third juvenile stage, are of major importance for population increase and crop damage. The treatment of distinct processes in population dynamics and crop growth and their dynamic interactions, enables a systematic analysis of PCN tolerance and resistance components, and the formulation of a procedure for assessment of the required efficiency of PCN control measures and their performance in variable environments.

Development of second-stage juveniles determines population dynamics and damage

In this thesis, interactions between J2s and potato roots are identified as the main processes determining population increase and damage, on susceptible cultivars. Effects of competition among J2s for syncytial space on the fraction of developing females largely explain the density-dependent rate of population increase (chapter 5). Apparently these effects are dominant over competition effects on the number of eggs $cyst^{-1}$ (Seinhorst, 1986a) and soil moisture effects on movement of males (Wallace, 1960; for J2s), which were assumed absent here. Competition among J2s is influenced by root diameter and carrying capacity for syncytia. Both factors, which are probably correlated, have been assumed constant here, but vary greatly in real root systems (Vos & Groenwold, 1986; chapter 5). This variation, which is affected by soil temperature, soil compaction and cultivar (Ng & Loomis, 1984; Veen & Boone, 1990; Evans & Haydock, 1990), may contribute to variation of population increase within and among experiments. With the present model, combined effects of root diameter and density of J2s on the number of developing females can be analyzed, and the contribution of root diameter distribution to distribution of rates of population increase can be quantified. This will provide insight in the rates of population

increase, that are relevant for formulation of PCN control measures.

Reduction of photosynthesis during initial development of syncytia is the fundamental mechanism of damage, at optimal water and nutrient supply. However, other mechanisms could not be entirely excluded in this study, due to sensitivity of crop growth rate to the unknown duration of photosynthesis reduction by J2s. Possible secondary mechanisms of PCN might be: a reduction of potassium uptake rate (discussed in chapter 2); and effects of third and later juvenile stages on root growth and functioning (mentioned in chapter 5). Experimental research on the duration of photosynthesis reduction, by measuring leaf photosynthesis and transpiration rates, xylem sap constitution and nematode development at regular intervals after inoculation with hatched J2s, is most urgent for full understanding of damage and to decide for experiments on possible secondary mechanisms.

Photosynthesis reduction by J2s is assumed to be caused by stomatal closure, in response to messenger substances released by infected roots (chapter 2). This mechanism is similar to the initial plant reaction to abiotic stress on roots (chapter 2). This suggests interactions between effects of PCN and effects of the severity and period of drought stress on yield reduction. With severe early drought, hatching and penetration of J2s is inhibited and yield reduction is caused by drought only. With mild early drought, stomata are already closed at the time of syncytial formation and additional closure due to PCN infection is small, hence yield reduction due to PCN is less than at optimal water supply. With mild late drought, a positive interaction is expected: PCN-infected plants have smaller root systems than PCN-free plants due to photosynthesis reduction. When the upper soil layers are depleted of water, infected plants experience drought stress, while PCN-free plants still take up water from deeper layers. With severe late drought, additive effects of PCN and drought are expected: no water is available in the rooted soil volume, irrespective of root system size as influenced by PCN. Experimental data to test this hypothesis entirely are not available, although Haverkort *et al.* (1992) showed additive effects of severe drought stress and PCN on yield. The interactions might explain part of the variation in yield loss among years and locations. The effects of these interactions on crop yield, and criteria for the period and severity of drought stress, can be analyzed by linking the model presented here to a soil water balance model (e.g. Penning de Vries *et al.*, 1989). Effects of PCN and water shortage on canopy diffusion resistance should

be introduced in these models. This analysis may increase insight in the contribution of environmental variation to variation in yield loss by PCN.

Analysis of tolerance and resistance

Tolerance of potato cultivars to PCN is usually measured as yield loss of a test cultivar, relative to that of a standard cultivar, at the same initial density. Similarly, resistance is measured as PCN population increase on a test cultivar, relative to that on a standard cultivar. Tolerance and resistance levels vary greatly among experiments, but the ranking of cultivars is usually constant (Arntzen & Wouters, 1992; Phillips & Trudgill, 1985). Analysis of the causal mechanisms of tolerance and resistance might increase understanding of this variation and its implication for potato production. (Evans & Haydock, 1990; Dellaert & Meijer, 1986). The model presented here may facilitate interpretation of these mechanisms with respect to overall yield loss and population increase, by systematic analysis of processes of plant growth, nematode development and their interactions. This approach has been illustrated for tolerance differences based on cultivar maturity, and for partial resistance derived from *Solanum vernei* (chapter 5).

The major damage mechanism, i.e. photosynthesis reduction during syncytial development, is apparently not affected by tolerance and resistance characters of cultivars (chapter 3). At optimal water and nutrient supply, tolerance is therefore enhanced by any factor that prevents the establishment of syncytia (e.g. reduced hatching, gene H1 for damage by *G. rostochiensis*, increased emergence from roots of penetrated juveniles), and by any factor affecting the cumulative amount of assimilates partitioned to tubers (e.g. maturity class, time of canopy closure, green leaf area duration). When supply of water or nutrients is limiting crop growth, as often occurs temporarily, tolerance is enhanced by any factor contributing to root vigour, functioning and distribution. Tolerance based on these mechanisms is likely to be variable, because it depends on variable environmental conditions. A similar approach for analysis of resistance mechanisms has been used by Spitters & Ward (1988). With the present model, each mechanism can be evaluated in the context of interactions between potato, PCN and their environment, revealing the components of resistance and tolerance, most promising for practical implementation. The expression of resistance and tolerance mechanisms in biologically relevant

parameters enables experimental verification of the assumed mechanisms and might facilitate screening of clones.

PCN control in variable environments

Large variation of experimental results is a major problem encountered in research on PCN. In field experiments, this variation is partly due to sampling error, partly to biological variation and partly to environmental variation. Experiments on simulation models, which permit different population distributions and which explain changes in population and crop variables from underlying processes governed by the environment, eliminate variation due to sampling error and allow investigation of hypotheses on environmental and biological variation. This approach is illustrated in chapter 5, where the variation of population dynamics and yield loss due to differences in soil temperature between seasons and species-specific temperature responses of development was investigated.

Investigation of the other major factor of environmental variation, i.e. soil water content, requires further elaboration of the model sections on crop growth, spatial root distribution and hatching and movement of PCN. These aspects include the effects of drought stress on photosynthesis and assimilate partitioning (Penning de Vries *et al.*, 1989), effects of infiltration on dilution and distribution of potato root diffusate, effects of the narrower range of soil water contents permitting movement of second stage juveniles, compared with the range for potato root growth (Wallace, 1960, Penning de Vries *et al.*, 1989), and the effect of soil water content on PCN mating. Depending on rainfall intensity, upto 87 % of the precipitation infiltrates soil by stemflow (Jefferies & MacKerron, 1985), therefore soil water content in the vicinity of growing roots might allow movement and root penetration of nematodes, while the average soil water content of the field inhibits movement. This requires careful simulation of soil water content around growing roots, rather than the average soil water content.

Cropping techniques to reduce population densities of and yield loss by PCN (e.g. chemical and biological control, cultivar resistance and tolerance) can be formulated in terms of their effects on initial egg density, on development and mortality rates of the various stages in the PCN life cycle and on plant physiological processes affecting dry matter production and distribution. The modelling approach presented here, based on processes of crop growth and

population development governed by species- and cultivar-specific responses to environmental factors, can then be used to simulate the effects of these measures on yield loss and population increase over a range of years, locations, PCN populations and potato cultivars. In this way, effects of PCN control in variable environments are expressed as frequency distributions of yield loss and population increase, rather than just their maximum and minimum values. This will improve insight in the required efficiency of these cropping techniques for the attainment of desired yield levels and tolerable population densities, and the associated risks of these measures. This knowledge contributes to the development of a more sustainable agriculture, marked by optimum integration of economic and ecological goals (e.g. maximum farm profit and minimum environmental pollution). Each PCN control measure is characterized by a specific combination of inputs (e.g. labour, pesticides, biological control agents) and outputs (e.g. crop yield, pesticide emissions), and contributes differently to agricultural goals. With the present model, these input - output combinations can be quantified for a series of potato production systems ranging from low to high input, in region-specific environments. These production systems can be analyzed with multiple goal linear programming models, to find the optimum integration of agricultural goals (Schans, 1991). In this way PCN control strategies, most appropriate for sustainable agriculture in a certain region, are selected. With the development of a mechanistic model of interactions between potato crop growth and PCN population dynamics, this thesis has attempted to provide a basis for understanding of variation of PCN population increase and yield losses, and for development of sustainable PCN control strategies.

Summary

Potato cyst nematodes (PCN; *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* Stone) are highly persistent, soil-borne pests of potato, characterized by strong variations of population increase and yield reduction among years and locations. The population dynamics of PCN and their interactions with potato plants are insufficiently understood to explain this variation. In this thesis, dynamic models of potato crop growth from planting to harvest and PCN population development from hatching to formation of eggs in new cysts are linked, to gain more insight in the mechanisms of yield reduction and population increase. The links are established through effects of second stage juveniles in roots on photosynthesis, i.e. the principal process of plant growth, and through interactions among root growth, hatching and root invasion of second stage juveniles.

Reduction of photosynthesis by second stage juveniles, just after their penetration in roots, was experimentally investigated to explain yield loss from disturbance of underlying processes at the earliest possible stage (chapter 2). Simultaneous measurement of leaf photosynthesis and transpiration rates showed, that second stage juveniles reduce leaf gas exchange rates through reduction of stomatal opening. The significance of this physiological interaction for plant growth reduction, and its relation with tolerance differences among cultivars, were investigated by combining repeated leaf gas exchange measurements with growth analysis of plants, grown in soil infested with *G. pallida* (chapter 3). At 30 days after planting, when second and third stage juveniles were present in roots, both photosynthesis and transpiration rates were severely reduced by *G. pallida*. In the course of time these effects became less pronounced. There were no consistent differences among cultivars in the response of leaf gas exchange rates to nematode infection. Reduction of photosynthesis by *G. pallida* appeared additive to photosynthesis reduction due to leaf senescence. Reduction of total dry weight correlated with reduction of both leaf area and photosynthesis rate. The tolerance differences were not correlated with leaf photosynthesis and transpiration. Apparently these processes are not part of tolerance of plants.

The effects of temperature on the development rates of all stages in the PCN life cycle were experimentally quantified, to simulate PCN population

development as a function of soil temperature (chapter 4). Cumulative hatching *in vitro* was fitted to a negative exponential function. Its parameters, i.e. the time to first appearance of hatched juveniles, the maximum fraction of juveniles capable of hatching and the relative hatching rate, were all affected by temperature. The response to temperature of the maximum hatched fraction and the relative hatching rate were different between *G. rostochiensis* and *G. pallida*. The optimum temperature for hatching was lower for *G. pallida* than for *G. rostochiensis*. Development of juvenile stages, cysts and eggs of *G. rostochiensis* and *G. pallida* was studied in potato roots growing in Petri dishes. Development periods of juvenile stages were not different between species except at 24 °C, where second-stage juveniles of *G. pallida* developed slower than those of *G. rostochiensis*. The relative dispersion of the development to adult females was mainly caused during development of second-stage juveniles. Cyst maturation period was not different between species nor among temperatures. The reproduction rate (offspring female^{-1} day^{-1}) was similar for both species and it was lower at 15 °C than at higher temperatures. The maximum number of eggs cyst^{-1} was extremely variable and not different between species nor among temperatures.

Based on the bionomic data described above, a dynamic model of PCN population dynamics was developed and linked to a model of potato crop growth at optimal water and nutrient supply (Chapter 5). To simulate interactions between growing roots, hatching and root penetration, a model for the spatial distribution of root growth over soil layers was formulated, which calculated root length densities and diffusion of potato root diffusate per soil layer. Population increase was regulated by the density of second stage juveniles in roots, through effects on the sex ratio of the developing population, and by mortality of roots containing second-stage juveniles at densities exceeding the 'root carrying capacity'. Crop damage depended only on the relation between the number of juveniles initiating syncytia per unit leaf area and crop photosynthesis, as quantified in Chapters 2 and 3. The simulated relations between initial and final population density, and between initial density and tuber yield, agreed with experimental observations. Population increase and yield loss were different between *Globodera rostochiensis* and *G. pallida*, in interaction with initial density and temperature. Simulated effects of of resistance and tolerance mechanisms on yield loss and population increase agreed with independent experimental observations. Tolerance was enhanced by resistance, acting upon or before the

stage of syncytial initiation, and was apparently independent of resistance acting after this stage.

The explicit formulation of physiological processes in this model allows insight in population dynamics and yield loss, as affected by environment and crop characteristics at various spatial cyst distributions, and quantitative evaluation of PCN control strategies. Thus, more precise information on relations between inputs (e.g. labour, pesticides, biological control agents) and outputs (e.g. crop yield, pesticide emissions) of potato production becomes available, which contributes to the development of sustainable farming systems (Chapter 6).

Samenvatting

Populatiedynamiek van aardappelcysteaaltjes en de daarbij optredende schade bij aardappel

Aardappelcysteaaltjes (ACA; *Globodera rostochiensis* (Woll.) Skarbilovich en *G. pallida* Stone) zijn zeer persistente, bodemgebonden plagen van aardappel, gekenmerkt door grote variatie in populatietoename en opbrengstderving tussen jaren en plaatsen. De populatiedynamiek van ACA en hun interacties met aardappelplanten zijn onvoldoende bekend om deze variatie te verklaren. In dit proefschrift worden dynamische simulatiemodellen van aardappelgewasgroei en van ontwikkeling van ACA-populaties gekoppeld, teneinde meer inzicht te verwerven in de mechanismen van schade en populatietoename. De koppeling vindt plaats door effekten van tweede-stadium juvenielen in wortels op fotosynthese en door interacties tussen wortelgroei, wekking en invasie van wortels door tweede-stadium juvenielen.

Reduktie van de fotosynthese door tweede-stadium juvenielen, onmiddellijk na binnendringen in de wortels, werd experimenteel onderzocht om opbrengstderving te kunnen verklaren vanuit een verstoring van opbrengstbepalende processen in het vroegst mogelijke stadium (Hoofdstuk 2). Uit gelijktijdige metingen van fotosynthese- en transpiratiesnelheden bleek, dat tweede-stadium juvenielen de gasuitwisselingssnelheden van bladeren reduceren via een verandering van de stomatale opening. Het belang van deze fysiologische interaktie met plantegroei, en de relatie ervan met tolerantieverschillen tussen cultivars, werd onderzocht door herhaalde metingen van bladfotosynthese en -transpiratie te relateren aan de groei van planten in met *G. pallida* besmette grond (Hoofdstuk 3). Dertig dagen na het poten, toen tweede- en derde-stadium juvenielen aanwezig waren in de wortels, waren de fotosynthese- en transpiratiesnelheden sterk gereduceerd door G. *pallida.* Dit effekt werd in de loop der tijd zwakker. Er waren geen consistente verschillen tussen cultivars in de respons van fotosynthese- en transpiratiesnelheden op ACA-infectie. De reductie van de fotosynthese door het effect van *G. pallida* kwam bovenop de reductie ten gevolge van bladveroudering. De reduktie van het totale drooggewicht van de planten was gecorreleerd met reductie van bladopppervlak en fotosynthesesnelheid. Tolerantieverschillen tussen de

cultivars waren niet gecorreleerd met fotosynthese- en transpiratiesnelheden van bladeren. Blijkbaar maken deze processen geen deel uit van de tolerantie van planten.

Het effect van temperatuur op de ontwikkelingssnelheden van alle stadia in de levenscyclus van ACA werd experimenteel bepaald, teneinde de ontwikkeling van ACA populaties te simuleren als functie van de bodemtemperatuur (Hoofdstuk 4). De cumulatieve wekking *in vitro* werd beschreven met een negatief exponentiële funktie. De parameters ervan, te weten de tijd tot het eerste verschijnen van gewekte juvenielen, de maximale fractie juvenielen, die gewekt kan worden, en de relatieve weksnelheid, werden alle beïnvloed door de temperatuur. De reacties van de maximaal gewekte fractie en van de relatieve weksnelheid op temperatuur waren verschillend voor *G. rostochiensis* en *G. pallida*. De optimale temperatuur voor wekking was lager voor *G. pallida* dan voor *G. rostochiensis*. De ontwikkeling van juveniele stadia, cysten en eieren van *G. rostochiensis* en *G. pallida* werd onderzocht in aardappelwortels op Petrischalen. De tijd, benodigd voor ontwikkeling van de juveniele stadia, was gelijk voor beide soorten, behalve bij 24 °C, waar tweede-stadium juvenielen van *G. pallida* zich trager ontwikkelden dan die van *G. rostochiensis*. De relatieve spreiding in ontwikkeling tot volwassen stadia werd vooral veroorzaakt tijdens de ontwikkeling van tweede-stadium juvenielen. De periode, benodigd voor rijping van cysten, was gelijk voor beide soorten en werd niet beïnvloed door de temperatuur. De reproductiesnelheid (aantal nakomelingen vrouwtje^{-1} dag^{-1}) was gelijk voor beide soorten en lager bij 15 °C dan bij hogere temperaturen. Het maximale aantal eieren cyst^{-1} varieerde sterk en was gelijk voor beide soorten en de onderzochte temperaturen.

Op basis van deze resultaten werd een dynamisch simulatiemodel van de populatiedynamiek van ACA gekoppeld aan een model voor gewasgroei van aardappel bij optimale vocht- en nutriëntenvoorziening (Hoofdstuk 5). Met een model voor de ruimtelijke verdeling van wortels werden worteldichtheden en diffusie van wortelexudaten in verschillende bodemlagen berekend, teneinde de interakties te simuleren tussen groeiende wortels, wekking en penetratie van tweede-stadium juvenielen in wortels. De populatietoename was afhankelijk van de dichtheid van tweede-stadium juvenielen in de wortels, via effekten op de geslachtsverhouding van de zich ontwikkelende populatie, en van wortelsterfte bij dichtheden, groter dan het 'draagvermogen' van de wortels. Opbrengstvermindering was alleen afhankelijk van het verband tussen het aantal

juvenielen, dat syncytia initiëert, per eenheid bladoppervlak en de fotosynthesesnelheid van het gewas, zoals gemeten in Hoofdstukken 2 en 3. De gesimuleerde verbanden tussen begin- en einddichtheid van de populatie ACA, en tussen begindichtheid en knolopbrengst, kwamen overeen met experimenteel bepaalde relaties. De populatietoename en het opbrengstverlies waren verschillend voor *G.rostochiensis* en *G. pallida*, in wisselwerking met begindichtheid en temperatuur. De gesimuleerde effekten van resistentie- en tolerantiemechanismen kwamen overeen met resultaten van onafhankelijke experimenten. Tolerantie werd bevorderd door resistentiemechanismen, die aangrijpen op of vóór initiatie van syncytia, en was onafhankelijk van resistentiemechanismen die na dit stadium werken.

Door de mechanistische formulering van fysiologische processen in dit model wordt het inzicht in de populatiedynamiek van ACA en de daarmee gepaard gaande opbrengstverliezen, zoals beïnvloed door omgevingsfaktoren, gewaseigenschappen en ruimtelijke verdeling van de populatie, vergroot. Hiermee komt nauwkeuriger informatie beschikbaar over de relaties tussen inzet van produktiemiddelen (bijvoorbeeld arbeid, chemische en biologische bestrijdingsmiddelen), opbrengst en milieu-effekten. Deze kennis wordt gebruikt bij de ontwikkeling en evaluatie van bestrijdingsmethoden, die passen in duurzame teeltsystemen (Hoofdstuk 6).

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Curriculum vitae

Jan Schans werd geboren op 8 mei 1958 te Wageningen. Na het behalen van het diploma atheneum-B in 1976 aan het Thomas a Kempiscollege te Arnhem studeerde hij Planteziektenkunde aan de Landbouwhogeschool (nu: Landbouwuniversiteit) te Wageningen. Het doctoraalexamen werd behaald in 1983 en bestond uit de vakken Fytopathologie, Theoretische Teeltkunde, Algemene Agrarische Economie en Erfelijkheidsleer. Van januari 1984 tot november 1984 was hij in dienst van de Landbouwhogeschool bij de vakgroep Fytopathologie, waar hij assisteerde bij de voorbereiding van een onderwijselement 'Planteziektenkunde en Maatschappij'. Van november 1984 tot maart 1988 werd het in dit proefschrift beschreven onderzoek verricht bij de vakgroepen Nematologie en Theoretische Produktie-ecologie van de Landbouwuniversiteit. In deze periode initiëerde en coördineerde hij tevens een onderwijselement 'Groei- en opbrengstkortende factoren bij de teelt van gewassen'. Sinds 1988 is hij in dienst van het DLO-Centrum voor Agrobiologisch Onderzoek, waar hij modelmatig onderzoek verricht aan geïntegreerde produktiesystemen voor akkerbouw en de teelt van bloembolgewassen.