Quantitative studies on the management of potato cyst nematodes (*Globodera* spp) in The Netherlands

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Bibliographic abstract

This thesis describes research concerning the management of potato cyst nematodes in the Netherlands. Methods to automatically count larvae in suspensions using image analysis and to hatch larvae using new hatching devices are presented. The hatching process is modelled. Sub-sampling of soil samples after mixing was investigated including extraction errors. The effect of 1,3-dichloropropene on the hatchability of potato cyst nematodes is described in both laboratory and field experiments. A model describing the size and shape of infestation foci of potato cyst nematodes on marine, sandy and loamy soils is developed and used to evaluate existing soil sampling methods for detection and assessment of potato cyst nematode infestations. New sampling methods were developed for both seed and ware potato growers. A growth model is presented for plants affected by nematodes as well as an advisory system emphasizing the use of partially resistant potato cultivars in several cropping frequencies. The advisory system includes population dynamics, the relation between pre-plant density and relative yield, and the concept of relative susceptibility. It allows to calculate the probabilities of yield reduction in the field. The approach taken by the authors in nematological research is presented as the Seinhorst Research Program.

Key-words - advisory system, concentration-time products, models, detection probability, 1,3-dichloropropene, distribution patterns, dose/response relations, growth model, infestation foci, methyl-isothiocyanate, IPM, methodology, hatching curves, population dynamics, research program, sampling, sublethal dosages, yield reduction.

Stellingen behorende bij het proefschrift

'Quantitative studies on the management of potato cyst nematodes (*Globodera* spp) in The Netherlands'

Dit Proefschrift:

1. Zowel in de fysica (trillingsgolven) als in de biologie (distributiepatronen van organismen) kunnen 'ruis' en 'signaal' gelijkvormig zijn.
   Hoofdstuk 3.

2. In veel onderzoeksrapporten over het effect van grondontsmetting is het percentage gedode dieren overschat.
   Hoofdstuk 4.

3. Het effect van 1,3-dichloorpropeen beperkt zich niet tot het doden van nematoden.
   Hoofdstuk 5.

4. De verandering per eenheid afstand van aantallen cysten van aardappelcysteaaltjes in haarden is onafhankelijk van plaats en populatiedichtheid.
   Hoofdstuk 7.

5. Nematoden blijken beter voorspelbaar dan veel nematologen denken.
   Hoofdstukken 3, 4 en 9.

6. Nematodes make 'the same happen later'.
   Hoofdstuk 9 en Seinhorst, J.W. (1986). Effect of nematode attack an the growth and yield of crop plants. *In*: Lamberti, F. & Taylor, C.E. (Eds), *Cyst nematodes*. New York & London, Plenum Press: 191-210.

7. Integratie van wetenschappelijk onderzoek binnen en tussen vak disciplines is alleen succesvol als meetmethoden worden 'genormaliseerd'.
   O.a. hoofdstuk 11.

Overheid:

8. Phytosanitaire bemonsteringen uitgevoerd door Overheden moeten worden beschouwd als dure rituele handelingen.
   O.a. dit proefschrift, hoofdstuk 8.

9. Sociologisch onderzoek kan in dezelfde mate bijdragen aan de realisering van de MJGP-doelstellingen als biologisch onderzoek. Daarom zouden beide disciplines intensiever moeten samenwerken.
   J.S. Buurma, 1996. Farm management of fungicide use in tulips in The Netherlands. Acta Horticulture, no. 429, 89-95.
   J.D. van der Ploeg, 1997. Oorzaken van verschillen in middelenverbruik tussen bedrijven: schurftbestrijding in appelen. Publicatie Landbouw Economisch Instituut no. 4.143. 48 pp.

10. Vele procedures ter bestrijding van nematoden vertonen meer gelijkenis met rituelen uitgevoerd op instigatie van hogepriesters - zoals de bestrijding van roest in tarwe door de verbranding van dierlijke ingewanden in de Romeinse tijd - dan met rationale maatregelen die erop zijn gericht kosten van bestrijdingsmaatregelen af te wegen tegen werkelijke schade door nematoden.
    J.C. Zadoks, 1988. Van roesten, honden en sterren in de oudheid: een landbouwhistorische puzzel. Hermeneus 60: 1-8 en dit proefschrift.

11. Het belang om te investeren in genormaliseerde landbouwkundige meetmethoden wordt door beleidsmakers, landbouwwetenschap en landbouwpraktijk onderschat.

12. Bemonsteringsmethoden voorgeschreven door de European Plant Protection Organisation (EPPO) zijn niet bedoeld om risico's met betrekking tot plantenpathogenen te minimaliseren.

Wetenschap:

13. Consensus is de dood van de wetenschap.
    Ad Lagendijk in de column 'Interferentie': Klimaat en Popper van De Volkskrant op zaterdag 30 december 1995.

14. Het commercieel aanbieden van niet geijkte meetmethoden is moreel niet verantwoord.

15. De door Trudgill gesignaleerde export vanuit Nederland van overbodig geworden Rumptstad injecteurs naar Schotland is veelzeggend voor de voortgang van het nematologische onderzoek in beide landen.
    Openingsrede D.L. Trudgill ter gelegenheid van het "24th International Nematology Symposium" in Dundee, Schotland, 1998.

16. Oorzaken van biologische verschijnselen op ecosysteem niveau kunnen niet worden afgeleid uit resultaten van moleculair onderzoek.
    O.a. S.A.L.M. Kooijman, 1987, inaugurale rede "Theoretische biologie, een specialisatie in integratie' en vrij naar Anderson, P.W. Arrow, K.J. & Pines, D. (1988). *The economy as an evolving complex system* Proceedings of the evolutionary paths of the global economy workshop. September 1987, Santa Fe, New Mexico. Reading, UK, Addison-Westley, 317 p.

17. De controverse tussen Popper en Feyerabend over wetenschapsontwikkeling, zoals beschreven door Lakatos, vertoont gelijkenissen met tegenstellingen in kennisontwikkeling tussen 'onderzoek' en 'praktijk'.
    Lakatos, I. (1978). The methodology of scientific research programmes. *In*: Worrall, J. & Currie, G. (Eds). *Philosophical papers. Vol. 1.* Cambridge, U.K, Cambridge University Press, 250 p.

18. Biologen kunnen wel complexe expertsystemen ontwikkelen voor de beheersing van plantenpathogenen, maar de implementatie verloopt moeizaam. Een mogelijke oorzaak is dat sociaal-economische aspecten vaak onderbelicht blijven in deze expertsystemen.
    K.J. Blokker, 1984. '*Computergesteunde voorlichting: een decisiegericht voorlichtingskundig* onderzoek naar Epipre en andere geautomatiseerde informatiesystemen in de landbouwvoorlichting'. Ph.D. Thesis, Wageningen, The Netherlands, 389 p.
    C. Leeuwis, 1993. 'Of computers, myths and modelling: the social construction of diversity, knowledge, information, and communication technologies in Dutch horticulture and agricultural extension'. Ph.D. Thesis, Wageningen, The Netherlands, 469 p.

19. Duurzame oplossingen van landbouwkundige problemen bedreigen de continuïteit van verzelfstandigde landbouwkundige onderzoeksinstellingen. Het voldoen aan MJPG-doelstellingen voor nematiciden beëindigde de financiering van het onderzoek aan aardappelcysteaaltjes, waarvoor het merendeel van deze pesticiden werd ingezet.
    Eigen ervaring.

20. Volgens A. Mulder vraagt de praktijk om opbrengstreducties in tonnen in plaats van in procenten. Echter, op praktisch relevante beslismomenten zijn relatieve opbrengsten, anders dan absolute opbrengsten, goed voorspelbaar.
    A. Mulder, 1994. 'Tolerance of the potato to stress associated with potato cyst nematodes, drought and pH'. Ph.D. Thesis, Wageningen, The Netherlands, 190 p.

21. Volgens F.J. de Ruiter zijn late aardappelrassen toleranter voor aardappelcysteaaltjes dan vroege. Omdat deze bewering, evenals de onderliggende hypothese '..dat de knolgroei bij late rassen aanvankelijk achterblijft ten gunste van loof- en wortelgroei.', in strijd is met de waarnemingen (o.a. eigen onderzoek) kan hij worden ondergebracht in de categorie 'Myths and fairytales' (Seinhorst 1986).

F.J. de Ruiter, 1998. 'Potato crop growth as influenced by potato cyst nematodes (*Globodera pallida*) and abiotic factors'. Ph.D. Thesis, Wageningen, The Netherlands, 121 p.

Praktijk:

22. Grondontsmettingsapparatuur is een van de belangrijkste vectoren voor de verspreiding van het aardappelcysteaaltje.

Hofmeester, Y. (1991). *Soil adhering by machines*. Lelystad, The Netherlands, Annual Report 1990 of the Research Station for Arable Farming and Field Production (PAGV), publ. nr 54: 288-292 en Hoofdstuk 6 van dit proefschrift.

23. Tijdens de hoogtijdagen van de grondontsmetting werd de helft van het totale volume in de poot- en consumptieaardappelarealen toegepast. Meer dan 90% hiervan kwam op onbesmette grond terecht.

Hoofdstukken 6, 7 en 12 van dit proefschrift

24. Het gebruik van grondontsmettingsmiddelen in de teelt van poot- en consumptieaardappelen heeft de boeren geen cent opgeleverd.

Been T. H., Schomaker, C.H. & Molendijk, L. (1996) Adviezen naar aanleiding van uitslagen van de intensieve AM-bemonstering voor de poot- en consumptieaardappelteelt in gebieden waar besmettingen pleksgewijs voorkomen. Wageningen, The Netherlands. *IPO-DLO Rapport* 96-5, 40 p.

25. Boeren geven liever geld uit aan bestrijdingsmiddelen dan aan expertsystemen waarmee ze via scenarioberekeningen optimale bestrijdingsmethoden kunnen afleiden.

Eigen ervaring.

26. De meeste boeren zijn niet opgeleid om met complexe expertsystemen om te gaan. Helaas werken de simpele niet.

27. De resultaten van risicoschatting door waarschijnlijkheidsberekening zijn vaak contra-intuïtief. Onderzoek, voorlichting en praktijk zouden met dit fenomeen meer rekening moeten houden.

Simon Singh, 1997. Fermat's Last Theorem; The story of a riddle that confounded the world's greatest minds for 358 years. Fourth Estate, London: 362 p

Varia:

28. In werksituaties gaat het gezegde "men prefer blondes" in zijn oorspronkelijke betekenis niet op. Er is eerder sprake van "men prefer men".

Eigen ervaring.

29. Aanstaande ouders zouden eerst een hond moeten aanschaffen en de cursus 'De gehoorzame huishond' van de Dierenbescherming volgen. In deze cursus komen twee belangrijke elementen in de opvoeding van zowel hond als kind aan bod: Een goede onderlinge verstandhouding en consequent gedrag van de opvoeder.

T.H. Been and C.H. Schomaker, Indoornik, herfst 1998

Dedication

This PhD thesis results from 13 years of research concerning the problems of potato caused by potato cyst nematodes. It is dedicated to the memory of J.W. Seinhorst†, former head of the Nematology Department of the DLO-Institute for Plant Protection, IPO-DLO, Wageningen, The Netherlands, and to A. Oostrom†, our senior research assistant. Both men excelled in their own way, the former as a brilliant nematologist, the latter as a gifted methodologist. Both of them passed away too early to see this thesis completed.

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Contents

Chapter 1

General Introduction

1.1. Introduction

Potatoes are among the most profitable agricultural crops in arable farming in the Netherlands. Consequently, they are grown as frequently as conditions allow, especially in those areas, where farmers have almost no choice of other profitable crops. An average of 180,000 ha of potatoes is cropped annually of which about 120,000 ha consists of ware and seed potatoes and 60,000 ha of starch potatoes (Anonymous, 1991- 1997). Cropping frequency is limited by potato cyst nematode population build-up which, without control, leads to considerable crop losses. Control measures which reduce population densities of the potato cyst nematodes have been investigated starting right after the second world war (Oostenbrink, 1950) when the 'soil fatigue', from which potato crops suffered, was shown to be the result of a plant parasitic nematode. All kinds of solutions to the problem were discussed, even the use of small nuclear devices for full field treatment (Anonymous, 1956). Biological control, artificial hatching agents, organic amendments, soil inundation and catch crops were tested without much success. Finally, crop rotation, resistant potato varieties and soil fumigation became the building blocks of a widely used strategy to 'eradicate' the potato cyst nematode.

Potato varieties resistant against *Globodera rostochiensis* pathotype 1 were indeed successful. But early after their introduction reports were received on (partial) failure of resistant varieties caused by so called resistance-breaking biotypes (Dunnett, 1957; Jones, 1957; Laan & Huisman, 1957). More than a decade later a new species of the potato cyst nematode, *G. pallida,* was identified (Jones *et al,* 1970; Stone, 1972). Resistance to this species is believed to be based on multiple genes (Jones *et al,* 1981), contrary to the gene-for-gene relationship based on the H1-gene from *Solanum tuberosum* ssp. *andigena* which provides resistance to *G. rostochiensis* (Janssen, 1990). In 1977, Kort *et al.* defined an international pathotype scheme consisting of five pathotypes of *G. rostochiensis* and three of *G. pallida.* Resistant varieties in The Netherlands were required to possess resistance against all pathotypes of both potato cyst nematode species, which made breeding programs very difficult. As a result, *G. rostochiensis* populations, against which ample resistant potato cultivars were available, started to fade out while *G. pallida* began to spread all over the country. Frequently, 'resistant cultivars' were grown which were susceptible to the species present and population densities of potato cyst nematodes in the starch potato growing areas started to increase again.
At their introduction in the late sixties, nematicides were thought to be a temporary control measure, as the availability of other measures such as biological control and potato cultivars resistant to the, at that time, newly discovered pathotypes of *G. rostochiensis* 2 and 3 would make the use of nematicides obsolete. However, since the expectations as to effective biological control were not fulfilled and the new virulent pathotypes of *G. pallida* (pathotypes 2 and 3) emerged, the use of nematicides only

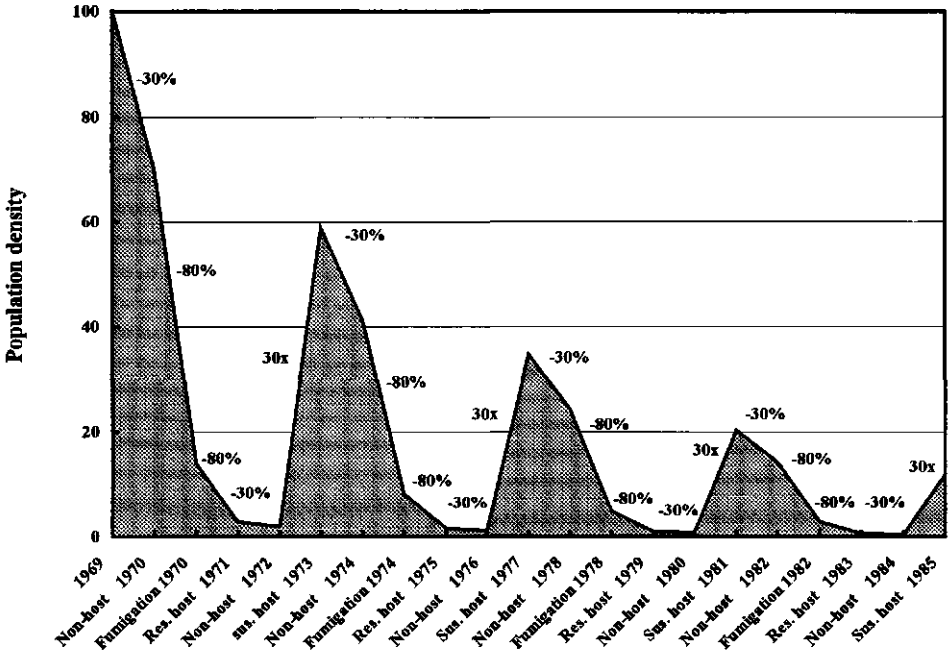


Figure 1.1. - Calculated development of population densities according to a scheme based on averages for multiplication of cyst nematodes on susceptible potato varieties (multiplication 30x), non-host crops (reduction 30%), resistant potato varieties (reduction 80%) and soil fumigation (reduction 80%).

increased.

Figure 1 shows the scheme used in the 1980s to advise the Dutch starch potato producing farmers on handling the potato cyst nematode (Mulder *et al*, 1981). It concerns the cropping of susceptible potato varieties (multiplication factor of the population density = 30), of non host crops (reduction of the population density by 30%), of resistant potato varieties (reduction 80%) and soil fumigation (reduction 80%). Resistant varieties had to be alternated with susceptible ones to slow down the selection of new virulent pathotypes. According to this scheme a gradual decline of population densities would occur until densities would become so low they could not be measured anymore.

All the values for multiplication and reduction of population densities of nematodes used in this advisory system were averages over large numbers of fields. The probability that such an average would apply to a certain field is quite small. No

farmer, however, would be so lucky that all these averages would occur in any of his fields at the same time. Moreover, these averages were valid only under rarely occurring conditions.

1. Multiplication rates, measured at low initial population densities, in fact varied between 3 to more than 80 on susceptible potato cultivars (Seinhorst, 1986*c*). Moreover, multiplication is density dependent; the lower the population density the higher the multiplication rate (Seinhorst, 1967, 1970, 1986*a*, 1993).
2. The use of resistant potato varieties will only be successful and reduce nematode populations by 80% if their resistance is 100% and growth of the host plant is not impeded. Although the juveniles cannot reproduce - syncytium formation is unsuccessful - juveniles do enter the root tips and plants will suffer damage (growth reduction at low and medium nematode densities). The higher the population density at planting, the smaller the plant and consequently the size of the root system which will result in a lower reduction of the population density in that part of the tilth that is normally in close contact with the root system (70%). In most cases potato cultivars are not 100% resistant, but partially resistant, and even when not damaged the estimated 80% reduction will not occur.
3. Soil fumigation was estimated to cause an 80% reduction of the population. However, this only occurred in favourable circumstances of soil structure, temperature and moisture, which are quite rare in the small time interval during autumn when fumigant nematicides are allowed be applied. Moreover, the micro-flora adapted quickly to the nematicides on both marine clay and sandy soils, so that sufficient mortality was only achieved around the injection layer.
4. Figures on natural decline of population densities when no host crops are grown were sparse and no information about the magnitude of field-to-field and year-to-year variation in the survival rate was known.

To make a long story short: the scheme did not reduce nematode populations or improve the financial returns of crops attacked by nematodes. In the mean time the first infestations became apparent in our latest and prime seed potato area, the Flevopolders. As export was endangered, legislation was harsh. Farmers, afraid of losing the possibility to grow seed potatoes, began to fumigate soils, as a precaution, on a large scale. During the second half of the 1980s the total volume of nematicides against potato cyst nematodes represented more than 60% of the total amount of pesticides used in agriculture in the Netherlands (Anonymous, 1991). The public and the government considered the intensive use of fumigants harmful to the environment in general and to the health of the farmers/applicators in particular. A significant decrease of the amount of the nematicide use was demanded.

1.2. Advisory systems

An alternative was required. Fundamentally, the use of averages had to be abandoned in favour of the stochasticity of the relevant parameters. This implies the need of stochastic models which in turn would supply the possibility to calculate cost-benefit predictions of control measures. As the advisory system was meant to be used by farmers and extension services, it should be straightforward and contain only a minimum number of parameters, but these should be biologically relevant. Therefore, the sub-models used are simplified models, derived from comprehensive ones. They do not apply to the complete range of situations in the field but only to those relevant to the potato grower. They are not just equations providing the best fit to experimental data but represent a vision concerning the mechanisms involved in plant nematode interactions and they have predictive value (see also Chapter 10). The advisory system, as it was planned at the beginning of this research, was designed as a stochastic simulation model consisting of 6 sub-models:

1. Effects of chemical treatments
2. Population dynamics
3. Relation between pre-planting density and relative yield
4. Partial resistance expressed as relative susceptibility
5. Survival rates during non-host crops
6. Spatial distribution patterns

In 1984, at the beginning of the research reported here, emphasis was placed on the efficiency of soil fumigation of heavy marine clay soils were the majority of the Dutch seed and consumption potatoes are grown. As a result, laboratory and field experiments were carried out to investigate the efficiency of 1,3-dichloropropene and metam-sodium. Leistra (1972) developed a theoretical model to calculate integrals of concentration time products (*CT*) at different depths in the soil after measuring concentrations at various times after application. If the relationship between *CT* and mortality of potato cyst nematodes were available, Leistra's model could be used to quickly evaluate new techniques of nematicide application, soil treatment before application, etc. Without such a model, patterns of *CT* estimates in different soil layers are compared without understanding their nematicidal significance. Also, a data set of nematicide effect throughout the infested tilth could be obtained for stochastic simulations and financial calculations.
However, the model proved to be difficult to apply because the *CT* products measured in the field differ significantly from those to which the nematodes are actually exposed to as a result of their incorporation in soil aggregates. Moreover, the poor results of fumigation on these clay soils confirmed the pessimistic expectations derived from previous research, when *CT* products in clay soils were compared to *CT* products in sandy soils (*e.g.* Rops and Smelt, 1985). Because of the negative cost benefit ratio of

soil fumigation, research on this subject was terminated.
One of the side effects of the field experiments was knowledge about the size of potato cyst nematode infestations on marine clay soils. Infestation foci covered only a small part of the area which is commonly fumigated. Most of the infested area had only low population densities and the total damage/ha was trivial. Research priority shifted towards spatial distribution patterns in the seed and ware potato areas in order to develop methods for the early detection of foci. High quality detection methods - detecting predetermined sizes of infestations with a predetermined detection probability - should render precautionary soil fumigation obsolete. The detection method might be used as a basis for an advisory system which emphasises control on indication. Control measures, if required, could be limited to the area actually infested. When detection could be followed by species identification, for instance by ELISA (Schots, 1988) the proper resistant cultivar could be applied. The only requirement would be a sound alternative for control by nematicides when no resistant varieties are present. The alternative presented itself as partial resistance of potato cultivars to cyst nematodes. The effect of partially resistant potatoes had been first published by Phillips (1984), Seinhorst (1984) and Seinhorst & Oostrom (1984), indicating lower multiplication rates and lower maximum population densities in the field even when partially resistant cultivars would be grown annually.
A similar scheme could be used for the starch potato areas. If sampling methods could be developed providing population density estimates with known variability, farmers could take control measures on indication and nematicide use could be restricted to those fields where the cost of soil fumigation could be paid by higher yields. Partially resistant potato cultivars could be used to keep low populations low, thereby avoiding the need for nematicide application.
The required relationships for population dynamics and yield reduction had been published by Seinhorst in the 1960's and 70's, their existence was taken for granted but never put to use in practice. One of the applications of Seinhorst's population dynamics model provided a basis for the objective measurement of the degree of partial resistance. Sampling methods for fields, however, were still in their infancy.

1.3. Goals and constraints

To produce such advisory systems a vast quantity of research had to be realised. Spatial distribution patterns at different scales of magnitude had to be established in both the seed and ware potato areas and the starch potato areas. The vertical distribution of cysts through the tilth over time had to be known to determine its effect on the accuracy of soil sampling methods. The decline of population densities in fields in different years should be assessed. Research to investigate partial resistance, the way it has to be measured, its variability, the method to test cultivars and its validation in the field had to be undertaken. And of course the different models had to be integrated

into an advisory system that can predict what will happen in the field within certain, useful, limits.
Besides pure scientific research, another hurdle had to be taken. An immense gap had grown between scientific research on the one hand and applied research and extension on the other. Materials and methods applied by the latter in the 1980s were out of date by almost two decades. Ideas concerning the interactions between nematodes and plants consisted of 'myths and fairy tales' (Seinhorst, pers. communication). A 'status quo' concerning some of the basic facts people believed in had to be shaken up. As scientific results had to be passed on to the farmers by extension workers, who claimed they could only spare one day per annum to update their knowledge concerning the potato cyst nematode problem, the difficulties encountered when transferring the principles and possibilities of a stochastic approach are imaginable. A tough opposition of old ideas had to be dealt with. The opposition was met by a process similar to erosion; by constantly ventilating the new concepts - sometimes in an extremely simplified fashion - searching for allies and relaxing only when the concepts came back to us, now brought forward by others.
Not all the research carried out in the course of this research effort could be incorporated in this thesis. Most of the investigations on partial resistance have been published elsewhere or will be published in the near future. The thesis will therefore 'sample' the research effort and present some parts such as the results on soil fumigation, soil sampling for detection, the effect of nematodes on the growth of their host, the use of partial resistance in an advisory system, and the methodology developed. All research was carried out respecting the principles laid down in the 'Seinhorst Research Program' (Chapter 11).

1.4. Summary

Laboratory and field experiments were carried out to investigate the efficiency of soil fumigation on heavy marine clay soils. This required the processing of thousands of larvae suspensions. In Chapter 2 an image analysis system is introduced to automate the counting of large numbers of larvae suspensions of *G. rostochiensis* and *G. pallida*. The result is a software program that can count up to 64 compartments with larvae suspensions without the aid of an operator. The time needed to count one compartment was reduced by 80% to one minute compared to 'manual' labour while the time for probe preparation remained the same. At least 95% of the larvae originating from hatching tests were recognized and counted.
Chapter 3 provides insight in errors in the estimation of cyst numbers due to sub-sampling and laboratory activities. Subsampling error is in accordance with a binomial error when soil is well mixed. Results show that mixing of sandy and clay soils posed no problem in either lab or field samples. However, large errors are introduced in about 10% of the samples by inadequate laboratory practices.

Nematicide trials require reliable results concerning the effect caused by the fumigant. Mortality is estimated by measuring hatchability of treated larvae compared to that of non-treated larvae. In Chapter 4, all sources of error contributing to the variability of hatching tests are identified. Solutions are presented to overcome these errors, caused by materials used and methodology applied. The variability of the hatching process could be minimized to less than 5%. The hatching process was modelled using a modified log-logistic equation. The model provides the possibility to check the progress of the hatching process and to shorten the time period required for hatching tests when laboratory reared cysts are used.

In Chapter 5 the results of testing the efficiency of 1,3-dichloropropene, which consists of two isomers, are presented. Only the (Z)-isomer actually killed nematodes when sufficiently high dosage were used. The (E)-isomer only inhibited hatching (decreasingly) for a period lesser than six month; usually the period between soil fumigation in autumn and planting of the potato crop in spring. Several different responses of the larvae to the fumigant affecting hatchability and survival could be distinguished.

Chapter 6 reports on field trials with standard application of 1,3-dichloropropene on marine clay soils. Mortalities of 50% in the upper 25 cm of the tilth were feasible. Accelerated breakdown of the active compound by microorganisms was measured in several fields. The use of an extra top soil treatment with metam sodium, immediately before winter ploughing, enhanced mortality significantly but is not a financially viable solution. Compared to the one ha which is fumigated commonly, only 5% of the area was actually infested which resulted in 95% of the active compound wasted on nematode free soil.

Research now was targeted at the development of new detection methods. First a general model for an infestation focus had to be established. In Chapter 7 a model is presented based on 40 data sets of densely mapped potato cyst nematode infestations originating from several cropping areas of ware and seed potatoes in The Netherlands. The model describes the size and shape of an infestation focus dependent on the population density at the focal centre. No differences between cropping areas could be found and the model therefore applies to all investigated areas.

With the use of this model and the knowledge available concerning the small scale distribution of potato nematode cysts, new sampling methods for detection can be developed. In Chapter 8 a computer program is described and applied to analyse the efficiency of existing Dutch sampling methods. As their performance was insufficient to satisfy the requirements postulated for a detection method to be used in an advisory system, several new, high performance, sampling methods for detection have been developed for both seed and ware potato growers.

Chapter 9 describes a growth model for potato plants attacked by nematodes. The relation between small and medium initial population densities and the relative total plant weight of potatoes can be derived from this model. To model tuber weight an extra modification was required as the retardation of tuber initiation is not of the same

magnitude as that of the total plant weight.
In Chapter 10 an advisory system is presented for the management of potato cyst nematodes emphasizing the use of partially resistant potato cultivars and providing the possibility of keeping population densities of potato cyst nematodes at a low level in short rotations. Stochastic models based on the population dynamics of potato cyst nematodes and the relation between pre-plant nematode densities and relative yield are combined. It enables farmers to evaluate risks and costs of different control measures in fixed rotations and to select a potato cultivar with optimum partial resistance to minimize yield reductions.
Finally, in Chapter 11 the 'Seinhorst Research Program' is postulated. It presents an effort to describe the empiric philosophy used and the methods applied in the nematological research at the IPO-DLO during the last 45 years. It consists of three hierarchic cycles concerning patterns derived from a continually growing data set called the 'empiric base' and theories developed to explain these patterns. Each time when data are added, patterns and theories are validated again. As theory building is only possible when patterns are derived from unbiased data sets the 'empiric base' has its own cycle of methodological patterns and theories to safeguard the quality of data collection.

Chapter 2

Using image analysis for counting larvae of potato cyst nematodes (*Globodera* spp.)

T.H. Been, E.M.J. Meijer, A.E. Beniers & J.W. Knol

2.1. Summary - A GOP-302 image analysis system - Context Vision, Sweden - was used to automate the counting of large numbers of larvae-suspensions of *Globodera rostochiensis* and *G. pallida*. These suspensions originated from hatching tests, which were conducted to estimate percentage mortality in field and lab experiments of nematodes exposed to nematicides. The result is called ANECS (Automatic NEmatode Counting System), a software program that can count up to 64 compartments with larvae suspensions successively without the aid of an operator. A special object carrier was developed. Images of up to eight object carriers (512 larvae suspensions) can be stored and image analysis can be suspended to off-office hours. The time needed to count one compartment was reduced by 80% to one minute compared to 'manual' labour while the time for probe preparation remained the same. The percentile error is highest at very low larvae densities (<20 per suspension) and is caused by pollution with small fibres carried by air during the handling of the larvae suspensions. This problem can be minimised by setting up clean-laboratory procedures. At least 95% of the larvae originating from hatching tests were recognized and counted. The program can and has been be adapted to count other nematode species or to suit more complicated problems like counting both larvae and eggs in one suspension.

2.2. Introduction

One of the most labourious and tedious occupations in nematological research is the counting of individuals of a pathogen. For instance, the number of cysts in debris, extracted from a soil sample or the number of eggs and/or larvae in a suspension. During 1985 till 1990 a substantial part of the research effort at the nematological department of IPO-DLO was directed towards the effectiveness of nematicides in the control of potato cyst nematodes. Dosage-response relationships of 1,3-dichloropropene (D-D), (Schomaker & Been, 1986) and methyl isothyiocianate (M.I.T.) were investigated in laboratory tests and the effectiveness of D-D was tested on fields with heavy marine clay soil (Been & Schomaker, 1987). In all these experiments percentage mortality of potato cyst nematodes was estimated.

Several methods to distinguish between living and dead potato cyst nematodes are available, ranging from "pricking" individual eggs (Fielding, 1951), the "kinked larvae" method (Staniland & Stone, 1953), bioassays (Hijink, 1971), colouring with various dyes e.g. new blue R (Sheperd, 1951), hatching tests (Fenwick, 1949, 1950*a*) and measuring the adenosine triphosphate (ATP) content of nematodes (Atkinson & Ballantyne, 1977 a, b). The last three methods are frequently used. Bunt & Van Eck (1978) compared these methods including a bioassay and a measurement of mortality of free living nematodes to estimate mortality after a soil fumigation and found hatch-

ing test to be one of the most reliable methods. Therefore, in all the experiments, percentage mortality was estimated by comparing the hatchability of treated larvae with that of untreated larvae.
The efficiency and reliability of hatching tests were increased by several methodological improvements which will be presented in another paper. The duration of the hatching tests varied from two weeks, with untreated nematodes, till six months with nematodes exposed to high dosages of nematicides (Schomaker & Been, 1986). A similar retardation of hatch, caused by nematodes was reported by Fenwick (1957) and Seinhorst & Den Ouden (1973). Every week the hatching solution had to be refreshed and hatched larvae counted to plot the sigmoidal hatching curve (Fenwick, 1951). The many different factors involved (fields, plots, soil layers, time after treatment and replicates) resulted in a considerable number of batches and thousands of larvae suspensions to be counted. 'Manually' counting one suspension takes five minutes on an average and only few people can perform this tedious microscopy work during a whole day. Moreover, the reliability of the 'manual' counts decreases as the duration of this work increases.
The possibility to automate this task was investigated using the at this time relatively new method of image analysis of digitized images using a computer. Several large systems were pretested in 1986/1987 on processing speed, image analysis software and user friendliness. Finally, a GOP-302 from Context Vision, Sweden was selected and equipped with a suitable selection of peripheral instruments. This paper presents the results of a three years' research effort into the composition of a suitable microscope configuration and the development of the required software.

2.3. Materials and methods

The System 's hardware configuration.
A GOP-302 image analysis system, manufactured by Context Vision, Sweden, was used to automatically estimate the number of nematode larvae (*Globodera Rostochiensis and G. Pallida*) in suspensions. An extra hard disk of 700 MB was added for the storage of large numbers of images. A Leitz Stabiplan microscope was selected as the most suitable for this application. It is an industrial microscope for automated applications. It consists of a solid granite base to obtain maximum stability during the scanning stage movements and a system adapter for a WILD M420 Macroscope or a binocular tube for micro objectives. For our purpose a binocular tube was used. The centre of the system adapter extends 26 cm, enough to equip it with a large scanning stage. On top of the binocular tube (1.25x) a phototube is attached with a projection lens (4x) which is connected to the camera with a C-mount adapter (0.1x). See Figure 2.1 for a display of the microscope set-up. In combination with a Leitz 1.6/.05 PLAN objective, a total magnification was obtained sufficient to display an image frame on the monitor covering exactly 1/4 of the total area of one compartment. The used lens

combination was the best compromise between a large magnification needed for sufficient contrast and a small magnification to obtain a large scanning area. A 8" Märzhauser scanning stage was used, with a maximum speed of 42 mm/sec and an accuracy of 2.5 µm. It is mounted to position the perspex 64 compartment object carriers, specially developed for this purpose by Hasselblad, Sweden. The scanning stage (SSCO2) is controlled by the computer using a stage and focus controller developed by IDUNA, The Netherlands. For optimal illumination of the objects an electronically stabilized Novalux A4EIR light source was used, emitting homogeneous light in an A4 area.

Object carrier

A special object carrier with 64 compartments had to be developed fitting the 8" Märzhauser scanning stage. Several prototypes of different construction were developed and tested. The first consisted of one layer of plastic (PVC). The object compartments were formed by vacuum suction of the material after heating. In this way compartments had slanted sidewalls, resulting in no visible connections of larvae to the walls of the compartment after segmentation. However, during the cooling period following manufacturing, the object carriers slightly deformed, making them not completely level with the surface of the scanning stage. As a result the bottom of the compartments drifted out of focus during movement of the scanning stage. Auto focussing had to be added to solve this problem but scanning time was increased considerably, as each final image of a compartment was the result of images of all four quadrants of the compartment. Moreover, auto focussing at this low magnification tended to fail.

A second prototype, also with slanted walls, consisted of two perspex layers glued together, the lower one solid, the upper one with compartments cut out by laser.

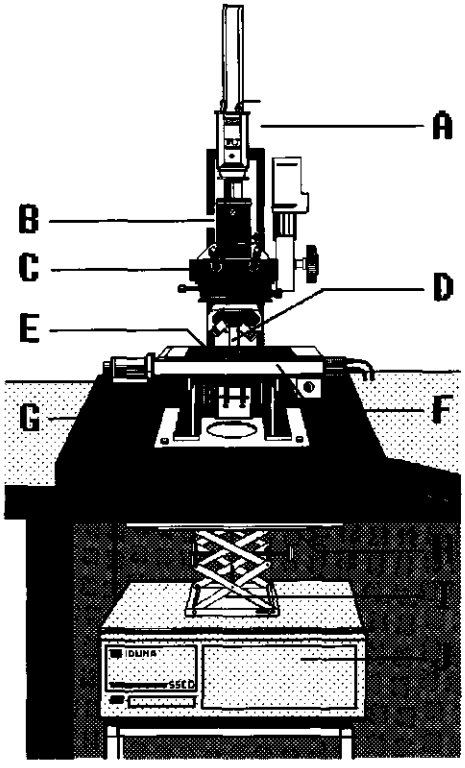


Figure 2.1. - Overview of the microscope setup. A: 2/3" B/W CCD camera. B: 0.4x photo tube. C: Leitz Stabiplan microscope. D: 1.6/0.05 P objective Leitz. E: multiple compartments object carrier. F: 8" Scan stage. G: Granite base. H: Electronically controlled light source. I: Labo lift. J: Stage and focus controller.

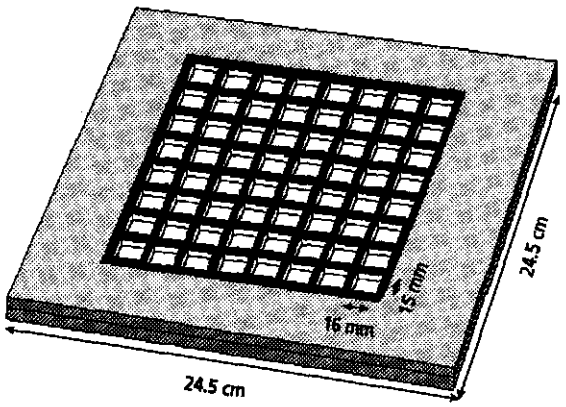


Figure 2.2. - Object carrier with 64 compartments. The whole object carrier measures 24,5 by 24.5 cm. The compartments are centred on the carrier in a 8 * 8 square of 15,6 by 15,6 cm. Each compartment measures 15 by 16 mm and has a volume of approximately 1.2 m.

These object carriers were level by definition, therefore auto focussing became obsolete. However, after gluing, the connection of the slanted compartment wall with the bottom perspex plate produced clearly visible lines in the scanned images. Image analysis also suffered from glue stains on the bottom of the compartments. Both artifacts tended to connect with larvae during segmentation, causing serious problems of these larvae to be detected and counted as one. Moreover, the glue slowly dissolved after the killing/staining solution was added to the larvae suspensions. After a couple of runs the object carriers started leaking between plates and were useless.

The final development (Figure 2.2) also consisted of two perspex plates. A new glue was used which was sprayed over the bottom of the upper perspex plate after which both plates were combined by heat and pressure. Glue artifacts were almost absent. To enable straightforward segmentation the object compartments now had vertical walls and the upper surface of the multiple compartment object carrier was painted black to obtain maximum contrast with the bottom plate. During image analysis the black edge of the upper perspex plate around the compartment can be subtracted from the image and connecting larvae dissociated easily. The dimensions of a single compartment is 15 by 16 mm, each with a volume of approximately 1.2 ml. The whole object carrier measures 24.5 by 24.5 cm.

Biological specimen

Cysts of *Globodera rostochiensis* and *G. pallida* were soaked in water and crushed in suspension using a plunger (Seinhorst & Den Ouden, 1966). Eggs were retrieved by sieving out cyst walls with a 250 µm sieve. Eggs were counted in a 1 ml suspension (Seinhorst & Den Ouden, 1973) to estimate those present in the stock solution. A volume containing the desired number of eggs was pipetted into a glass tube (1000 eggs for untreated batches) sealed at the bottom by a gauze (22.4 µm, Monadur), which withholds eggs but not active larvae. The tubes were then placed into glass cups containing 1 ml of the natural hatching agent of potato plants. When necessary, tubes were placed in new cups containing fresh hatching agent, while cups, containing hatched larvae were stored in the refrigerator at 4 °C. Storage time never exceeded four

weeks as after such a period of time the food resources of the nematodes decrease and starch globules diminish in size and finally become completely absent. Then, staining of the larvae for image analysis becomes impossible and the recognition of objects unreliable. The whole cup, containing slightly less than one ml of larvae suspension, was emptied and rinsed into the selected object compartment. Before counting, 20 µl of a saturated iodine solution (iodine dissolved in 96% alcohol) was added, which caused larvae do die instantaneously, to stretch and to stain. If necessary, a small amount of bidestillated water was added to obtain a level surface of the fluid. Image acquisition (scanning), was performed after a pause of 5 minutes, needed for the larvae to gain maximal contrast, but within one hour as the larvae loose their stain after that period. Moreover, waiting for too long causes evaporation of the sample fluid resulting in a meniscus that blurs the image.

Larvae from these hatching tests were used to investigate which features, e.g. length, area, compactness, can be used to distinguish them from other objects by image analysis algorithms. After program building hatching tests were analyzed both by the automated system and by 'manual' counting to establish the efficiency and reliability of the developed algorithm.

2.4. Results

Discriminating features

Segmentation of objects from a background can be achieved by thresholding a grey value image, which consists of a number of picture elements (pixels), each having a grey value between 0 and 255. When thresholding the image, all pixels with a grey value beneath a certain threshold value ($0 \leq$ value ≤ 255) will be adjusted to zero; all other will be adjusted to one. Thus, a binary image is created consisting of a background (value = zero) with one or more objects, consisting of one or more pixels with the value one. These objects can be submitted to calculations. One of the simplest calculations is counting the number of pixels that form an object as a measure of its area (see Rosenfeld & Kak, 1976 for a general introduction in image analysis). The GOP-302 can measure up to 17 different features of each object almost instantaneously. Using the values obtained for a number of chosen features of the object, decisions can be made whether these values are within the accepted range for each feature and therefore, weather the object can be classified as a larvae or not (e.g. egg or artifact caused by contamination of the suspension). Therefore, the first step in building the algorithm is to investigate which of the features or combination of features allows reliable discrimination between larvae and other objects.

As suspensions can contain several hundred larvae per ml, many larvae will connect into clusters after thresholding, thus forming a single object. Therefore the algorithm finally had to distinguish between single larvae, double larvae and clusters of more than two larvae.

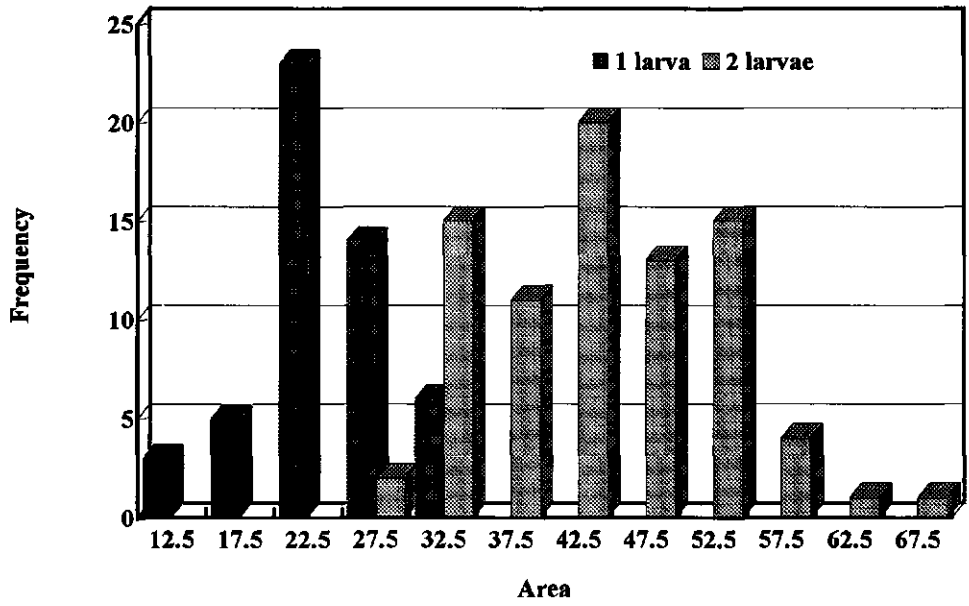


Figure 2.3. - Frequency distribution of **area** of single larvae and clusters of two larvae. Area is used as a classification feature. Although a slight overlap does occur, the resulting error is negligible.

A combination of three features proved to be the most suitable to achieve the desired purpose. For single larvae: **area** is used to find all objects having an area which falls into a range of area measurements likely to originate from a single larva. **Compactness** is employed as a qualifier to remove all objects which have the area of a single larva but not its shape. **Compactness** is a measurement of shape; circular objects will get the value 1, while the more unlikely objects resemble a circle, the higher the value for **compactness** will be. Finally **ellipse compactness**, also a shape measurement, is applied as a third qualifier. See GOP-302 reference manual (Context Vision, 1987) for an explanation of the quoted features.

For pairs of larvae and clusters the same approach is used with adapted values for the different features. In Figure 2.3 the frequency distribution of several **area** measurements are displayed of single and paired larvae. A slight overlap occurs. However, the resulting error is negligible as two more qualifiers are used for the final decision. After classification a C-program is invoked to calculate the average area of a single larva using the **area** measurements of all recognized single and double larvae. The number of larvae in clusters is then calculated by dividing the cluster area by the average single

nematode area. This procedure is repeated for every compartment. See for a full description of the algorithm used the ANECS user manual (Been *et al.*, 1995).

Program performance

After program development, debugging and evolution the final program was tested for its performance by applying it on several samples of nematode larvae originating from various hatching tests. In Figure 2.4. automated counts are expressed as percentages of 'manual' counts. There is an apparent tendency to overestimate the number of larvae at low numbers, especially those below 20 larvae per suspension. Overestimation is caused by pollution of the larvae suspension. As the image enhancement algorithm (see Been *et al.*, 1995) takes care of most objects that do not have the shape of a larva, only those that resemble larvae cause errors. The pollution of the larvae suspension, mostly little fibre particles originating from clothing, is carried by air and takes place when vials are handled and the compartments of the object carrier are loaded. Clean-laboratory procedures and air handling could reduce these errors drastically.

At higher nematode numbers at least 90% of the larvae are detected as such. The underestimation was caused by a classification error in the algorithm. When suspensions with very high nematode numbers were analysed, large clusters of larvae can be

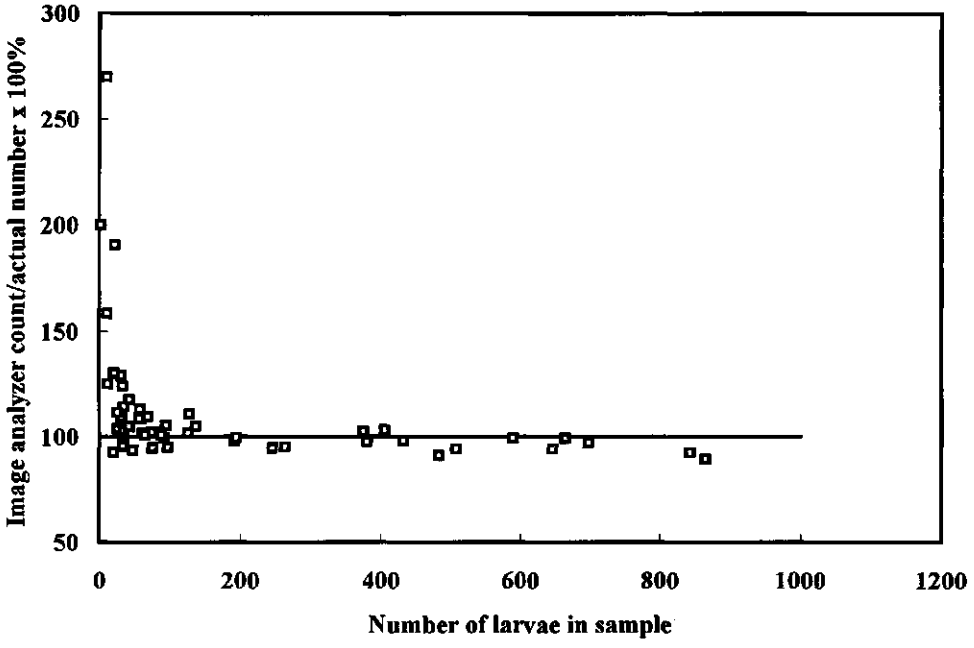


Figure 2.4. - The percentile count obtained by computer analyses compared to the actual number of larvae present in the sample. The highest errors occur with low nematode numbers (<20). They mostly are the result of small pollutions in the liquid (e.g. tissue fibres transferred by air.

formed. These were sometimes ignored by the program as they were classified as a large object (such as a piece of cyst shell) polluting the suspension. Moreover, clusters with an area of more than 50 single larvae are automatically rejected as these objects are regarded as being pollutions by definition. This rule was implemented as a consequence of results of previous testings.

However, a large percentile error at low numbers of nematodes, causes no large errors at the end of a hatching test provided that also suspensions with larger numbers of larvae are counted as is the case with hatching tests. Figure 2.5 displays three examples of complete hatching tests with variable cumulative numbers of larvae at the end of the test where automated counts are compared with 'manual' counts. The smaller the final number of larvae, the smaller the errors made by the program. However, in all tests at least 95% of the larvae present were detected as such. The increasing error in nematode counts at higher total cumulative counts in Figure 2.5 is again caused by the presence of very large clusters of larvae which were classified as a pollution of the suspension and not as nematodes. At higher larvae numbers these clusters will occur more frequently.

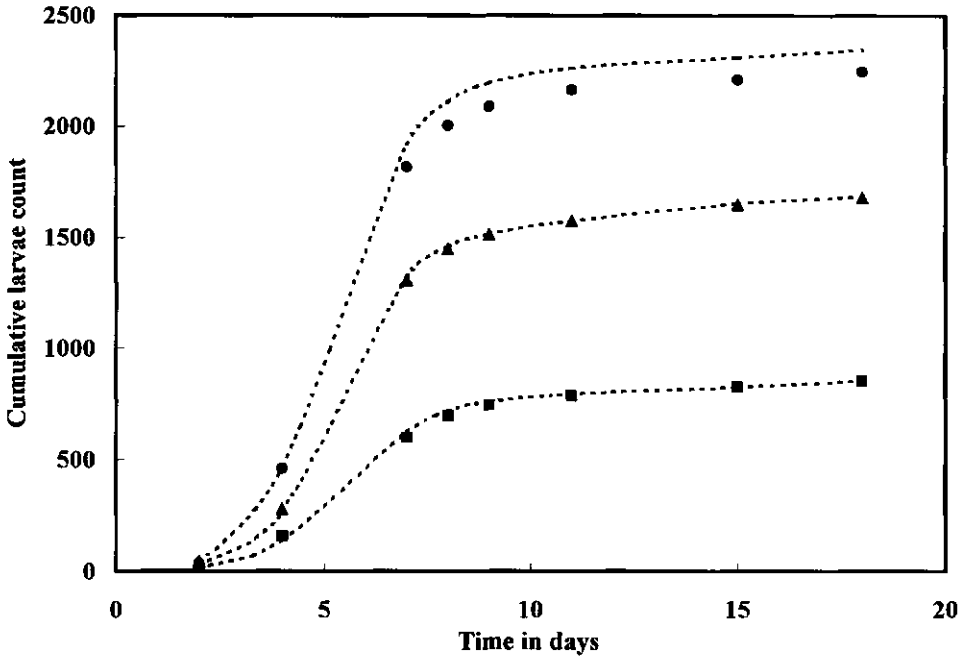


Figure 2.5. - Comparison between manual count and computer analysis of three hatching tests with different numbers of eggs. Lines: cumulative manual counts. Dots: Cumulative counts of image analysis system. (■) 99% of larvae detected, (▲): 97% of larvae detected, (●): 95% of larvae detected.

Although a minimum of 95% of the hatched larvae were detected as such, an attempt was made as to solve the problem of unwanted rejections of large nematode clusters. Finally, the use of an **area/length** index for clusters proved to be very promising. Figure 2.6 demonstrates the linear relationship between area and length of larvae clusters. The calculated index is almost the same at any number of nematodes. However, large pollutions with an area above 100 pixels can be detected because this feature is changed. Rejection of these cluster, having an **area/length** index above 2.3 and below 1.4, and acceptance of those within this range can decrease the number of erroneous rejections. Therefore, this index will be incorporated in the next update of the program.

Three batches of *Globodera pallida* and two of *Globodera rostochiensis* were investigated with regard to differences in size expressed as **area,** which is the most important classification feature of the program. As all populations were multiplied in previous years on the susceptible cultivar Irene the differences between batches from each species were age differences; Rol' 86, Rol' 88, Pa3 '84, Pa3 '86 and Pa3 '88. ANOVA distinguished two groups. One consisting of Rol '86, Rol '88 and Pa3 '84 and a second one consisting of Pa3 '86 and Pa3 '88 with an average area of 24 and 26 pixels respectively. Frequency distributions of single batches were consistent with a normal distribution. Figure 2.7 illustrates the frequency distribution of these area measurements of

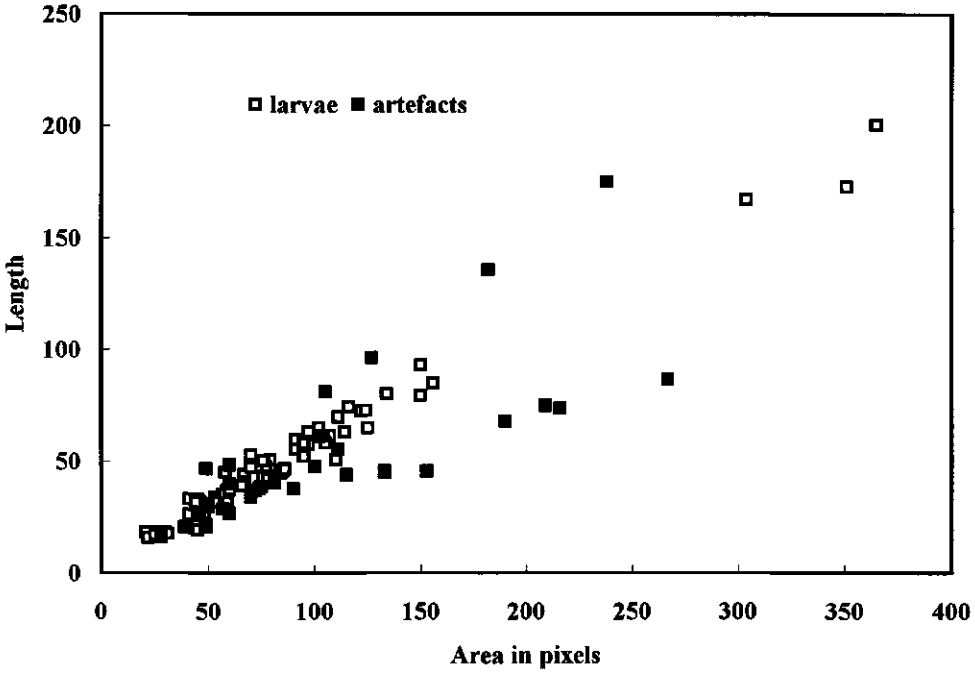


Figure 2.6. - Relation between **area** and **length** of clusters of more then two larvae; clusters of larvae (□) and pollutions in suspension (■).

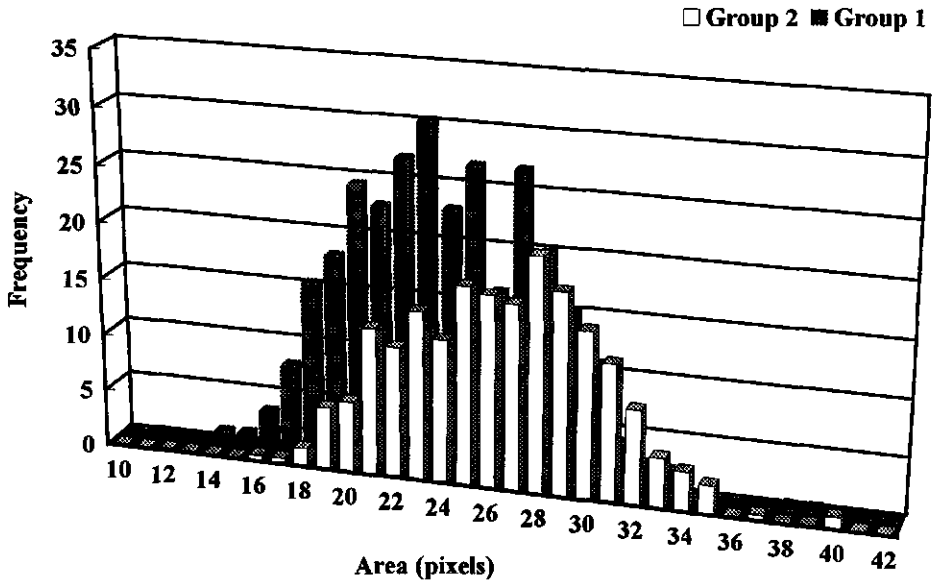


Figure 2.7. - Area measurements of single larvae of two different groups of nematodes originating from three batches of *Globodera pallida* and two batches of *Globodera rostochiensis*. Group 1 consists of Ro1'86, Ro1'88 and Pa3'84, group 2 of Pa3'86 and Pa3'88.

both groups. Although there is a significant difference between the average **area** of both groups, both 95% probability intervals, 16 to 32 for group one and 18 to 34 pixels for group two, are within the range of the classification interval of **area** for one larva (10 to 35 pixels) used by ANECS. Therefore, the differences found in **area** between the two groups have no influence on the performance of the program. It can be assumed that the program will perform equally well when other batches of potato cyst nematodes are counted. This has been proven during several years of operation.

2.5. Discussion

The result of this research has been a software application that can count up to 64 compartments with nematode suspensions without the aid of an operator. As scanning of the compartments is separated from the analysing part of the program, analysis of images (counting of the larvae) can be suspended to night hours to save operation time of the system during the day. After analysis, ANECS provides the possibility to inspect the results by displaying the original image together with an coloured overlay representing the detected and counted larvae (Figure 2.8). Corrections can then be made in case a very high precision is needed. For a full description of the program options see

the ANECS user manual (Been *et al.*, 1995).
Special attention was payed to the implementation of laboratory procedures to obtain clean and unpolluted larvae suspensions. Best results were obtained when hatching cups were machine-washed, dried by filtered air and stored in air tight boxes. Cups with suspension waiting to be counted were stored in plastic boxes at 4 °C. As the kitchen equipment needed for washing and drying is standard in modern laboratories, these precautions do not take extra time. The time required to prepare the probes is the same or even less, as 64 probes are prepared successively.
As displayed in Figure 2.4. the analysing process still suffers from errors caused by small pollutions of fibres. Improvements could be made by avoiding draft and using fibre-filtrated air and laboratory clothing at the location of the image analysis system. However, as can be concluded from Figure 2.5. the error made by the program at the end of a hatching tests with several cumulative counts is low. At least 95% of all larvae of one sample were recognized and counted. This percentage can still be improved by adding the length/area index to the calculating algorithm of the program.
Whereas the time required for the 'manual' count of one suspension averages five minutes, for the same job the image analysis system only requires an average of one minute, by this reducing counting time by 80%. Running the image analysis during night hours saves even more time. Another improvement is the absence of bias occurring when counts are made by different individuals and the increasing errors with time caused by fatigue when submitted to this labour for longer periods.
The program is now in operation for several years and satisfies the goals set. The program can and has been adapted for counting other nematode species. For instance to count beet cyst nematode larvae (*Heterodera schachtii*), only the upper and lower margins of the discriminating features used in the classification process had to be adapted. Juveniles and eggs of *Meloidogyne hapla* and *M. chitwoodi* have been counted by Van der Beek *et al.* (1996). Adding egg recognition to the program proved to be quite simple. A filter operation is used to erase all thin objects, such as larvae, but not the eggs. Thresholding this image and classifying these objects renders a fair approximation of egg numbers.
The program is not suited to distinguish between different species and therefore to count the numbers of different species in one suspension. This kind of image analysis would require texture analysis at very high magnifications, which implies constant autofocusing and movement of the scan stage as well as the use of stored recognition patterns to identify objects. This is theoretically possible for those species which do not have to be manipulated under the microscope to distinguish certain characteristics. However, even at the present state of the art, computers would consume too much time to equal or even improve on 'manual' labour.
However, image analysis will prove to be of increasing value for nematological research. Heinicke & Schultz (1994) recently succeeded in solving another labourious task; counting eggs and larvae in unfiltrated suspensions of crushed cysts of *Globodera rostochiensis* and *Heterodera schachtii* originating from soil samples. Their method is

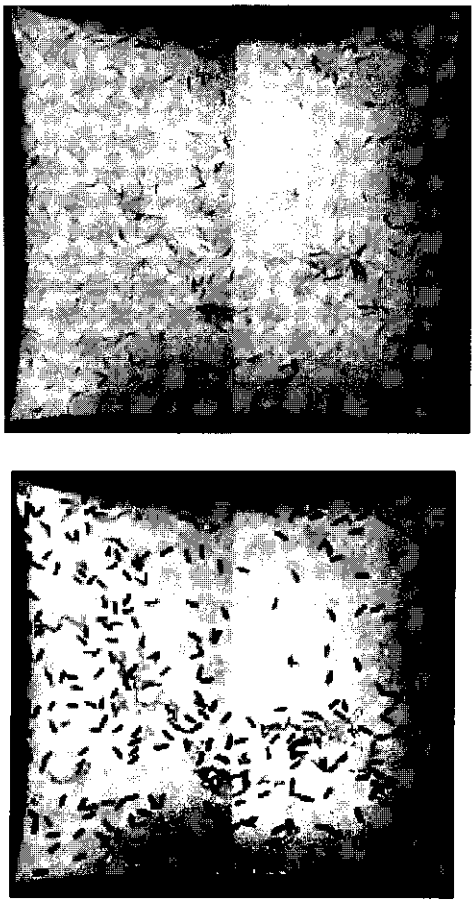


Figure 2.8. - A: The original image after a C-subroutine called *grab.c* took four pictures of each quadrant of the selected compartment, reduced these images in size and composed one new image presenting the whole compartment. B: Original image with overlays (coloured on screen) displaying all objects, which have been recognized as being nematode larvae (single larvae in black, pairs in grey and clusters outlined). The total sum of counted larvae is displayed on the top line of the image (not displayed here). Compartment number, number of single larvae, pairs, clusters and total number are stored in a data file.

intended for the routine estimation of the population densities on farmers fields as a basis for nematode control. The time required for one probe varies between 1 to 10 minutes per probe, dependent on nematode species and numbers of nematodes in the suspension. The counting process is terminated when the variance is stable. Another possibility of using image analysis is demonstrated by Hendriks *et al.* (1994), who

used the GOP-302 system to measure the lipid concentration in larvae of potato cyst nematode.

The GOP-302 image analysis system is now outdated. ANECS is currently converted to run under X-Windows which makes the program independent of the platform on which it is used, provided the UNIX operating system is available. Detailed information on the hardware used and acquisition of the software can be obtained by writing to IPO-DLO.

Chapter 3

Errors due to subsampling of soil samples with *Globodera rostochiensis* or *G. pallida* and to other laboratory procedures

C.H. Schomaker & T.H. Been

3.1. Summary - Several fields, infested with potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) were sampled by collecting bulk samples of approximately 70 cores amounting to 1.8 or 2.5 kg soil from a number of square metre plots located in a regular grid pattern over a 0.33 ha area. Bulk samples from five fields, I-V, were thoroughly mixed and from one field, VI, lightly mixed, and subsequently divided into three subsamples of approximately equal weights. Two, sometimes three, subsamples were elutriated separately. Cysts were elutriated by two commercial laboratories, 1 and 2, and separated from the debris and counted at two research laboratories, 0 and 3. Random bulk samples from five fields, I-V, were divided into three portion after thoroughly mixing and taken to Laboratory 0, to compare elutriation precision and accuracy of commercial and scientific laboratories and to check the quality of mixing. To this purpose, pairs or triples were divided into classes. The expected value of the variance within pairs was estimated per class and could be described by a distribution function analogue to a negative binomial distribution, but with three in stead of two parameters. Cysts appeared to be randomly distributed in the well mixed samples, resulting in a binomial or trinomial distribution between pairs or triples. The expected values of the coefficient of variation associated with elutriation were 3.6, 9.6 and 5.5% in the Laboratories 0, 1 and 2, respectively. The upper 95% confidence limit, $\delta_{0.95}$, of coefficients of variation associated with elutriation in Laboratories 1 and 2, were estimated by the differences in 95% upper limits of coefficients of variation between the Laboratories 1 and 2 on the one hand and Laboratory 0 conversely. This difference, $\delta_{0.95}$, ranged from 73% to 42% for Laboratory 1 and from 43% to 19% for Laboratory 2 if 10 to 100 cysts were counted in samples. The consequences of these laboratory errors for the accuracy of sampling methods for both research and extension purposes are discussed.

3.2. Introduction

In many nematological studies, in which soil sampling is an important step in data collection, it is common practice to collect a bulk sample but to investigate only a part, taken from the bulk sample after mixing. The size of such a subsample is mostly small, but never infinitely small relative to the bulk sample. Inspection of a volume of, for instance, The Journal of Nematology rouses the suspicion that the relative size of subsamples is often arbitrary. Johnson *et al.* (1995), studying effects of coastal bermuda grass sod rotation and fallow on population dynamics of *M. incognita* collected a bulk sample of about 2500 cm^3 and assayed nematodes from a subsample of 150 cm^3. Todd *et al.* (1995), investigating field responses of soybean to *Heterodera glycines,* estimated population densities of the nematodes in 100-cm^3 subsamples from

4712 cm^3 bulk samples. Weaver *et al.* (1995) compared effects of Sorghum-Sudangrass hybrids with fallow and monoculture soybean on crop yield and nematode population densities of *Meloidogyne* spp. and *H. glycines*, taking bulk samples of approximately 2160 cm^3 and investigating subsamples of 100 cm^3. Mc Sorley & Gallaher (1995), determining effects of yard-waste compost amendments on densities of plant-parasitic nematodes in several vegatable crops, collected 589 cm^3 bulk samples, but nematodes were only extracted from a 100-cm^3 subsample. MacGuidwin & Layne (1995) investigating the respons of nematode communities to Sudangrass and Sorghum-Sudangrass hybrids grown as Green Manure Crops, also collected bulk samples of 589 cm^3 but investigated 200-cm^3 subsamples. From this enumeration a slight preference for 100-cm^3 subsamples can be registred, but neither author documented the choice of bulk sample and subsample size, for instance in relation to the expected population densities of the nematodes and the maximum coefficient of variation to be allowed for the purpose of investigation.
Others, for instance Jones (1945), Anscombe (1950), Moriarty (1960), Müller (1988) and Seinhorst (1988) paid more attention to the statistical aspects of methodology. Jones and Anscombe supposed that the expected error made by subsampling follows a Poisson distribution, provided that the bulk sample be well mixed. Müller (1988) assumed that, if large subsamples are taken, the subsampling error may be smaller than according to a Poisson distribution but does not mention the size of subsample where a significant deviation from the Poisson distribution would become manifest and gives no theoretical explanation of the deviation. Moriarty (1960) found variance due to subsampling to be proportional to a value between the mean cyst counts and the square of this mean, but nearer to the latter. Seinhorst (1988) found empirically that, if subsamples are taken from well mixed bulk samples, the additional error to the (negative binomial) sampling error is overestimated by the Poisson distribution and better described by a binomial distribution, but he could not explain the phenomenon.
In this paper we will try to develop a general theory about the relation between cyst counts and subsampling error and about the influence of subsampling errors and errors due to extraction, on total sampling error. This theory may contribute to a better discrimination between 'signal', either strong (infestation foci) or weak (small scale distribution patterns), and 'noise' caused by measurement methods.

3.3. Materials and methods

Experimental procedures
- Data collection
Infested fields in Drente and the Flevopolders, all about 1/3 ha, were intensively mapped to establish the spatial distribution patterns of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. Seinhorst (1982, 1988) assumed a negative binomial distribution with an aggregation factor, k, of 70 for soil samples of 1.5 kg

from 1 m^2 and recommended to count at least 200 cysts per sample to keep sampling errors, expressed as coefficients of variation, under 14%. To obtain the same cyst count, and therefore the same sampling error, at any population density, more soil must be collected at small nematode densities than at higher densities. As expected densities in most fields were unknown, bulk samples of 1.8-2.5 kg per m^2 area were collected and, after thorough mixing, divided in three subsamples, A, B and C, of about the same size. Subsample A was elutriated first and the cysts in it were separated from the debris and counted. If the cyst count was smaller than 200, subsample B followed the same procedure. The cysts in all three subsamples A, B and C, were counted if the cumulative count from the subsamples A and B was less than 200. In some cases, when it was known beforehand that population densities would be small, cysts from two or three elutriated subsamples were separated and counted together. These counts were of no use in this study. Only the results of (parts of) six fields, where two or three subsamples per bulk sample had been investigated separately, for convenience further indicated as 'pairs', were analysed.

- Fields

Fields I-V and field VI, located in Drente and Flevoland, The Netherlands, respectively, were sampled in a stratified manner by taking, in a regular pattern, bulk samples from square metre plots (1.33 x 0.75 m^2). Each bulk sample consisted of 70 cores and had an average weight of approximately 1.8 kg (field I-V) or 2.5 kg (field VI). The number of bulk samples taken amounted to 333-350 for fields I -V and to 150 for field VI. Cyst distribution in fields I-IV was more or less uniform, but fields V and VI contained a large infestation focus.

Before extraction, when the soil was still moderately moist, each bulk sample from fields I-V was thoroughly mixed in a metal dish of about 50 cm diameter, and subdivided in three subsamples, each of about 600 g (A, B and C). All subsamples, with an exception of triples from 5 randomly chosen bulk samples per field, were sent to a commercial soil sampling agency, further referred to as Laboratory 1, for elutriation. This task was performed with a Schuiling centrifuge (Folkerts, Pancreas, The Netherlands).

The subsamples from the randomly chosen bulk samples from field I-V, usually five per field and twenty-nine in total, were taken to the IPO-DLO laboratory, further referred to as Laboratory 0, to investigate the quality of mixing and to compare the precision and accuracy of the elutriation methods in the two laboratories. Cysts from these subsamples were extracted with the Seinhorst elutriator (Seinhorst, 1964), separated from the debris with Seinhorst's mini-Fenwick can (Seinhorst, 1974) and counted.

Bulk samples from field VI were only lightly mixed as it was the intention not to investigate the subsamples separately but to estimate the cysts in the whole bulk sample. The reason why the bulk sample was divided into three portions of about equal weight (average 860 g) was that the elutriator, a carousel (Pollähne, Hannover, Germany), of the commercial soil sampling agency (further referred to as Laboratory 2), charged with the elutriation, could not handle soil samples larger than 1 kg.

A, B, C	-	Cyst numbers in the three subsamples of approximately equal weights
log A, B, C	-	^{10}log-transformed cyst numbers in the three subsamples
'Binomial' distrib.	-	Binomial or trinomial distribution
c_j	-	constant between 0 and 0.02 chosen per class j and added to s^2_j during ^{10}log-transformation to reduce skewness of the distribution of s^2_j
CV_{ij}	-	Coefficient of variation of the i-th untransformed 'pair'in the j-th class
CV_j	-	Ratio of S_j and M_j, estimate of the average of coefficients of variation CV_{ij} in the j-th class with n_i 'pairs'
$CV_{ij,\,'bin,'}$	-	Coefficient of variation per pair ij if cyst numbers in pairs had been 'binomial' distributed variables
$CV_{j,\,'bin'}$	-	Coefficient of variation per class j if cyst numbers in pairs had been 'binomial' distributed variables
D_{ij}	-	Difference between CV^2_{ij} and $CV^2_{ij,\,'bin'}$ per pair ij
D_j	-	Difference between CV^2_j and $CV^2_{j,\,'bin'}$ per class j
δ	-	The difference in CV^2_j between the random samples from the reference data set '*reference*' on the one hand and the data sets *'field I-IV'*, *'field V'* and *'field VI'* conversely
$\varepsilon\delta$	-	Expected value of δ
i	-	Discrete variable between 1 and n_j, identifier for individual 'pairs' per class j
j	-	Discrete variable between 1 and t, identifier for individual classes j per data set
k_1	-	Constant, equalling to q in perfectly mixed subsamples.
εk_1	-	Expected value of k_1
k_2	-	Constant, analogue of the coefficient of aggregation in the negative binomial distribution. If $k_1 = q$, then $k_2^{-0.5}$ represents the coefficient of variation due to laboratory procedures other than subsampling
εk_2	-	Expected value of k_2
k_{2*}	-	Adaptation of k_2, including only errors due to counting and subsampling with imperfect mixing
k_3	-	Coefficient of aggregation in the negative binomial distribution describing small scale distribution patterns of nematodes
εk_3	-	Expected value of k_3
k'_3	-	Coefficient of aggregation in the negative binomial distribution describing an aggregated distribution of cysts in subsamples due to imperfect mixing.
k_{ex}	-	Coefficient with respect to extraction errors
k_c	-	Coefficient with respect to counting errors
λ_{ij}	-	Variable per pair ij, equalling $^{10}\log(s^2_{ij}+c_j)$
λ_j	-	The arithmetic mean of λ_{ij} per class j

m_{ij}	-	Arithmetic mean of the *i*-th log-transformed 'pair' in the *j*-th class
m_j	-	Arithmetic mean of m_{ij} in a class with n_j 'pairs'
m_j-$2s_j$ to m_j+$2s_j$	-	Confidence interval comprising approximately 95% of the log-transformed cyst numbers
10^{m-2s} to 10^{m+2s}	-	Confidence interval comprising approximately 95% of the untransformed cyst numbers
M_{ij}	-	Arithmetic mean of the *i*-th untransformed 'pair' in the *j*-th class
M_j	-	Back-transformed mean m_j as estimate of the average of M_{ij} in the *j*-th class with n_j 'pairs'
n_j	-	Discrete variable representing the number of 'pairs' per class *j*
'Pair'	-	Two or three investigated subsamples each representing one-third of a bulk sample
p	-	Parameter in the 'binomial' distribution. Investigated proportion of a bulk sample. In the present data sets p=0.33
q	-	Parameter in the 'binomial' distribution. Not-investigated proportion of a bulk sample. In the present data sets q=0.67
S^2_{ij}	-	Variance of the *i*-th untransformed 'pair' in the *j*-th class
S_j	-	Back-transformed expected value s_j as estimate of the average standard deviations S_{ij} in the *j*-th class with n_j 'pairs'
s^2_{ij}	-	Variance of the *i*-th log-transformed 'pair' in the *j*-th class
s^2_j	-	Expected value of s^2_{ij} in a class with n_j 'pairs. s^2_j equals the arithmetic mean of s^2_{ij} if the distribution of s^2_{ij} tends to a normal and to antilog $(\lambda_j+0.5\sigma^2_{j,\lambda})$-$c_i$ if s^2_{ij} is approximately log-normally distributed
$\sigma_{j,D}$,	-	Standard deviation per class *j* of D_{ij}
$\sigma^2_{j,\lambda}$	-	Variance of λ_{ij} per class *j*
t	-	Discrete variable representing the number of classes per data set
T_{ij}	-	Total number of cysts per bulk sample per 'pair' *ij*
z_x	-	'Critical point' in a standard normal distribution, leaving 1-x probability in the upper tail (Neave, 1978)

Notes

1. Parameters or variables α, per pair *ij*, are indicated by a subscript *'ij'* and represented as α_{ij}
2. The expected value of a given parameter or variable, α_{ij}, per class *j*, is indicated by the subscript *'j'* and represented as α_j
3. Parameters or variables, α_{ij} or α_j per data set *Y* are indicated as $\alpha_{ij,Y}$ or $\alpha_{j,Y}$ respectively
4. The expected value of a given parameter or variable, α, per data set, *Y*, is represented as $\varepsilon\alpha_Y$
5. The estimate of a given parameter *x*, per data set *Y*, with respect to a confidence limit, *x*, is indicated by the subscript *'Y,x'* and represented as $\alpha_{Y,x}$
6. Standard deviations of log-transformed cyst numbers per 'pair', *ij*, or per class, *j*, are indicated with *'s'*, with subscript *'ij'* and *'j'* respectively
7. Standard deviations of untransformed cyst numbers per 'pair', *ij*, or estimates per class, *j*, are indicated with *'S'*, with subscripts *'ij'* and *'j'* respectively
8. Standard deviations of another variable, *v*, than of cyst numbers per 'pair', *ij*, or per classes, *j*, are indicated as *'σ'*, with subscript *'v'*.

Definitions, continued

Mc Cullagh *et al.* (1992) explain that the binomial distribution arises in a situation that Y_1 and Y_2 are independent Poisson random variables with means μ_1 and μ_2. The sum, m, of Y_1 and Y_2 has also a Poisson distribution with mean $\mu_1+\mu_2$.

$$Pr[Y_1=y|Y_1+Y_2=m]=(\frac{m}{y})[\pi]^y[1-\pi]^{m-y} \quad (1)$$

Then the conditional distribution of Y_1, given that $Y_1+Y_2=m$, is described by the binomial distribution function (equation (1)), where $\pi = \mu_1/(\mu_1+\mu_2)$ and y is a random variable > 0. If Y_1 represents the number of cysts in the investigated part of the bulk sample and Y_2 is number of cysts in the not-investigated part of the bulk sample then equation (1) depends on the investigated proportion, π, of the bulk sample and on m, the sum of Y_1 and Y_2.

Explanatory note 1

After elutriation, the subsamples from all fields were sent back to Laboratory 0 where cysts were separated from the debris with Seinhorst's mini-Fenwick can and counted. Exceptions were cysts in the B-subsamples of fields I, II and IV which were separated from the debris by Laboratory 1 with a 'Schuiling' separator and counted by Laboratory 3.

- Data sets

The results were placed in four data sets, named '*reference*', '*fields I-IV*', '*field V*' and '*field VI*'. In subscripts these names are shortened to '*ref*', '*I-IV*', '*V*' and '*VI*' respectively. Data set '*reference*' contained the cyst numbers in the three subsamples of the random bulk samples from fields I-V, obtained after extraction and counting of cysts by Laboratory 0, while numbers of cysts in subsamples from fields V and VI, extracted by Laboratories 1 and 2, respectively, and counted by Laboratory 0, were placed in the data sets '*field V*' and '*field VI*'. Numbers of cysts in the two subsamples from fields I-IV, extracted by Laboratory 1 and counted by Laboratories 0 and 3, were analysed in one data set named '*fields I-IV*', because cyst ranges of the separate fields were too narrow to model the patterns of variation. A summary of the fields, the number of bulk samples, subsamples and the tasks performed by the various laboratories is given in Table 3.1.

Theoretical considerations

If a soil sample is perfectly mixed, the original negative binomial distribution of cyst numbers per unit weight is wiped out and the cysts are randomly distributed in the soil (Seinhorst, 1988). If infinitely small subsamples were taken, the distribution of the cysts between the subsamples would follow a Poisson distribution. However, in nematological practice subsamples are usually not so small in comparison with bulk soil samples and therefore the cysts in the subsample and in the remainder of the bulk sample do not follow a Poisson distribution, as might be assumed intuitively, but rather

a binomial distribution (Seinhorst 1988). Seinhorst's conclusions are theoretically supported by McCullagh *et al.*, 1992, whose explanation is summarized in *Explanatory note* 1.

To visualize the mathematical problem, coefficients of variation of cyst counts in subsamples were calculated in a model study for bulk samples with 100 cysts, assuming a binomial or a Poisson distribution of cyst counts between subsamples. The investigated proportion p of the bulk sample varied from 0.1 to 1. The two functions of the coefficients of variation are graphically compared in Figure 3.1. Two conclusions can be drawn. First, if the investigated proportion of the bulk sample is relative small, the coefficients of variation calculated with the binomial and Poisson distributions are approximately similar. Second, the assumption that cyst numbers between subsamples are Poisson distributed is basically wrong because this distribution misses a correction for finiteness and its application to subsampling of soil samples would imply a considerable coefficient of variation even if the subsample represents a proportion equal to 1 of the bulk sample.

Basic mathematical analysis

The purpose of the mathematical analysis was to characterize the distribution of cyst numbers within 'pairs' by placing 'pairs' with similar means in the same class and regarding the variance in cyst numbers of 'pairs' within one class as replications. The calculations made per data set were divided into 8 steps to make the mathematical analysis more accessible.

Table 3.1. - Summary of fields investigated, number of bulk- and subsamples used for analysis, organic dry matter and assignment of tasks to different laboratories: L0 - IPO-DLO, L1 - commercial laboratory 1, L2 - commercial laboratory 2, L3 - commercial laboratory 3. Samples (-) were not investigated.

Field nr.	% org. matter	Number of samples		Mixing and sub-sampling	Extraction			Counting		
		bulk	sub		A	B	C	A	B	C
I	4.7	36	2	L0	L1	L1	-	L0	L3	-
II	4.1	103	2	L0	L1	L1	-	L0	L3	-
III	7.1	72	2	L0	L1	L1	-	L0	L3	-
IV	6.1	108	2	L0	L1	L1	-	L0	L3	-
V	4.4	335	2	L0	L1	L1	-	L0	L0	-
VI	2.5	147	3	L0	L2	L2	-	L0	L0	L0
random I-V	4.1-7.1	29	3	L0	L0	L0	L0	L0	L0	L0

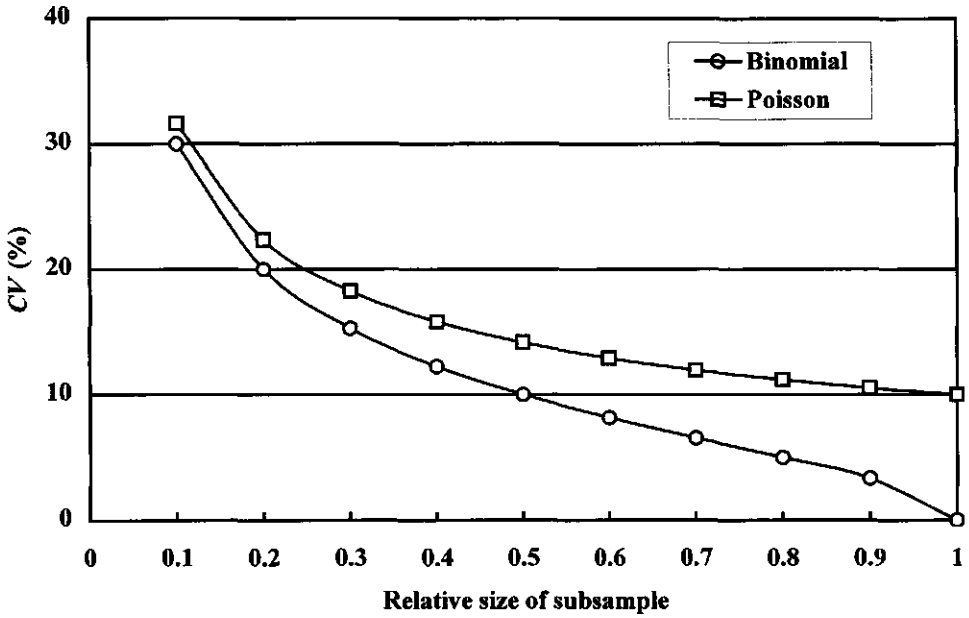


Figure 3.1. - Results from a model study, showing the relation between relative size of one subsample from a bulk sample, and coefficient of variation, *CV*, in percent, according to a binomial (○) or a Poisson (□) distribution of cysts between the subsample and the remaining part of the bulk sample. The total number of cysts in the bulk sample was set at 100 cysts.

Step 1: Conversion of cyst counts

Cyst counts were converted to cyst numbers per average weight of the subsamples, which was 600 g for fields I-V and 870 g for field VI. Conversion does not influence the coefficients of variation between the subsamples (as the standard deviation and the mean cyst number of 'pairs' are multiplied by the same factor), but it may influence the parameters of the models. Therefore, the converted weights were kept as close to the original weights as possible. Per 'pair' *ij* the mean M_{ij}, the variance S^2_{ij} and the coefficient of variation CV_{ij} were calculated.

Step 2: log-transformation of cyst numbers

Cyst numbers of 'pairs' *ij* were ^{10}log-transformed so that they could be divided into classes with approximately normally distributed means of 'pairs', and with a sufficiently stable variance within 'pairs' to assume its expected value to be constant over given class intervals of cyst numbers. As base of the logarithm a rational number was preferred over an irrational number, but otherwise the choice of the number 10 was arbitrary.

Step 3: Division into classes

Cyst numbers of 'pairs' were divided into j classes with each n_j pairs. Class width was chosen so that the ratio between the largest and the smallest values of the means of the untransformed 'pairs' would be ≤ 2. After a first run of the basic calculations, to determine the class borders, two situations gave cause to combine classes. First, if individual pairs had small means and large coefficients of variation, overlap among neighbouring classes could be avoided by combining such classes. Second, if two neighbouring classes j contained only small numbers, n_j, of 'pairs' and had small expected values, s^2_j, of the variance s^2_{ij}, these classes were combined so that n_j was sufficiently large to estimate s^2_j more accurately.

Step 4: Calculation of m_{ij}, s^2_{ij}, m_j and s^2_j

First the arithmetic mean m_{ij} and the variance s^2_{ij} were calculated per log-transformed 'pair' ij. Subsequently, the expected values m_j of m_{ij} and s^2_j of s^2_{ij} per class j were computed. If the distributions of s^2_{ij} and m_{ij} approached normality the arithmetic means of log-numbers were taken as their expected values. The maximum and minimum values of m_{ij} per class were deliberately chosen so that in most cases the arithmetic mean could be taken as centrality parameter. However, in *'field I-IV'*, *'field V'* and *'field VI'* the distributions of s^2_{ij} within classes were negatively skewed. Taking the arithmetic means as expected values would be misleading as these means would be disproportionally influenced by a small category of large values. Simple log-transformation did not solve the problem. Therefore, a constant c was added to values of s^2_{ij} and their sum was ^{10}log-transformed. As skewness varied between classes a constant, c_j, per class j was chosen so that skewness, calculated from the second and third central moment (Mood *et al.*, 1986), would be smaller than 0.01. Subsequently, the expected values s^2_j per class j were calculated from the arithmetic means and the variance of $^{10}\log(s^2_{ij}+c_j)$ applying equation (2) (Mood *et al.*, 1986). In equation (2), $^{10}\log(s^2_{ij}+c_j)$ is referred to as λ_{ij}, the expected value of λ_{ij} per class j as λ_j and the variance of λ_{ij} per class j as $\sigma^2_{j,\lambda}$.

$$s^2_j = antilog(\lambda_j + 0.5\sigma^2_{j,\lambda}) - c_j \qquad (2)$$

Step 5: Back-transformation of m_j and s_j to M_j and S_j

Per class, m_j and s_j of log-transformed pairs were back-transformed to the estimates, M_j and S_j, of the expected values of M_{ij} and S_{ij}, respectively, of the untransformed 'pairs'. If it was assumed that the difference between the ratio's of minimum and maximum values of untransformed and log-transformed cyst numbers within pairs, is negligible at relative small coefficients of variation ($\leq$20%) within pairs, (Slob, 1986), then per class j the 95% confidence intervals of log-transformed and untransformed cyst numbers could be approached by $m_j \pm 2s_j$ and $10^{mj \pm 2sj}$ respectively. The assumption was checked in the Results. Consequently, the back-transformed

expected values M_j were estimated by the sum of the lower and upper limit of the 95%-interval of M_{ij}, divided by two (equation 3).

$$M_j=(10^{m_j+2s_j}+10^{m_j-2s_j})/2 \tag{3}$$

Similarly, the back-transformed expected values S_j were estimated by the difference between the upper and the lower limit of the 95%-interval of M_{ij}, divided by four (equation 4).

$$S_j=(10^{m_j+2s_j}-10^{m_j-2s_j})/4 \tag{4}$$

The expected value, CV_j, per class j, of the coefficients of variation, CV_{ij}, of the back-transformed 'pairs' is described by equation (5), representing the ratio of equations (4) and (3) in a reduced form.

$$CV_j=\frac{10^{4s_j}-1}{2(10^{4s_j}+1)} \tag{5}$$

Step 6: Calculation of D_{ij} and D_j

The square of the coefficient of variation per 'pair' according to a 'binomial' distribution is given by equation (6) (Mood *et al.*, 1986)

$$CV^2_{ij,'bin'}=\frac{S^2_{ij,'bin'}}{M^2_{ij,'bin'}}=\frac{p\cdot q\cdot T_{ij}}{p^2\cdot T^2_{ij}}=\frac{q}{M_{ij}} \tag{6}$$

where p is the investigated proportion of the bulk sample, q = 1- p and $p\cdot T_{ij} = M_{ij}$, the arithmetic mean of the untransformed 'pairs'. Similarly, the square of the coefficient of variation, $CV^2_{j,'bin'}$, per class, j, according to the 'binomial' distribution is described by equation (7)

$$CV^2_{j,'bin'}=\frac{q}{M_j} \tag{7}$$

D_{ij}, the difference between squares of the coefficients of variation CV_{ij} and $CV_{ij,'bin'}$ per 'pair' ij, (equation (8)) and D_j, the difference between squares of the coefficients of variation CV_j and $CV_{j,'bin'}$ per class j (equation (9)) were calculated with the Taylor Series (Mood *et al.*, 1986).

$$D_{ij}=CV_{ij}^2-CV^2_{ij,'bin'} \tag{8}$$

$$D_j=CV_j^2-CV^2_{j,'bin'} \tag{9}$$

Step 7: Estimation of the confidence limits of D_{ij} and CV_{ij}.

a. If values D_{ij} were normally distributed the confidence limits of D_{ij} and CV_j could be calculated as follows. First, per class j the standard deviation $\sigma_{j,D}$ of D_{ij} is calculated with equation (10).

$$\sigma_{j,D}=\sqrt{\sum \frac{(D_{ij}-D_j)^2}{n_j-1}} \tag{10}$$

Second, the normality of the distribution of D_{ij} around D_j was checked by comparing the observed percentages D_{ij} of 'pairs' within the boundaries of $D_j \pm 3 \cdot \sigma_{j,D}$ with the expected percentages according to a normal distribution. Third, the x% upper and lower limits of D_{ij} were approximated with D_j, plus and minus z_{1-x} times the standard deviation, $\sigma_{j,D}$. The confidence limits of CV^2_j were estimated by the upper and lower limits of D_j and D_{ij}, plus the square of the 'binomial' coefficient of variation, $CV_{j,bin}$, per class j and $CV_{ij,bin}$ per 'pair' ij, respectively.

b. If values D_{ij} were not normally distributed in all classes, but at least in a few classes with large values of M_j, then a x% upper confidence was estimated as follows. Per class j where D_{ij} was close to normally distributed the standard deviation $\sigma_{j,D}$ of D_{ij} was calculated with equation (10). The x% upper limit of these classes was estimated by $D_j+z_x \cdot \sigma_j$. Subsequently, by trial and error, for all classes a x% upper confidence limit was constructed, using equation (13), so that it fitted the estimates of the x%-upper limits in classes with normal distributions of D_{ij} and comprised x% of all values of D_{ij}. Such a method of estimation is only suitable for large data sets.

c. For 'true' pairs, consisting of cyst numbers from two subsamples, any confidence limit $S^2_{ij,x}$, and therefrom $CV_{ij,x}$ and $D_{ij,x}$, can be calculated directly from the confidence limits of the cyst numbers A and B as follows. If 1-x to x % (=2x-1%) of all numbers A and B are between $M_j \pm z_x \cdot S_j$, where $\pm z_x$ are 'critical points' in a standard normal distribution leaving 2x probabilities in the upper and lower tails, then the same 2x-1% of all deviations A-M_{ij} and B-M_{ij}, which are equal but opposed in sign for a given pair, are between $\pm z_x \cdot S_j$. For 'true' pairs the squares of these deviations equal $0.5 \cdot S^2_{ij}$. Hence, for a given confidence interval 2x-1 of pairs A an B, S^2_{ij} equals equation (11) or is smaller.

$$S^2_{ij,2x-1}=2 \cdot z_x^2 (A-M_{ij})^2=2 \cdot z_x^2 \cdot S_j^2 \tag{11}$$

S^2_j is described in equation (12) of Results and Conclusions. It should be noted that the values S^2_{ij} are positive and that their number is half the number of the deviations. For instance, the 95% *upper confidence limit* of S^2_{ij} is obtained by using the absolute value of z_x, appropriate for the 95% *confidence interval* of A and B and associated with the 0.025 and 0.975 limits.

Step 8: Visualisation, pattern analysis and theory building

Relations between M_j and S_j^2; M_j and CV_j; and M_j and D_j were visualized in graphs and general patterns were described mathematically. For theory building, the equations were chosen so that they were related with known statistical distributions, and their parameters had a biological meaning. For details about the philosophy behind pattern analysis and theory building in this and other papers by the authors, the reader is referred to Schomaker & Been (1998*c*).

The equations were fitted to the data by trial and error, using least squares with two weights. The first weight, chosen because of a varying number, n_j, of 'pairs' per class j, was the ratio of the number of pairs per class, n_j, and total number of 'pairs' in the data set, times the number of classes, t. The second weight was the ratio $2/10^{4s_j}$, used in classes where $10^{4s_j} \leq 2$. The particulars of the data sets and the results of the basic calculations are summarized in Table 3.2.

3.4. Results and conclusions

General patterns

- The relation between M_j and S_j^2

In all data sets, the expected value S_j^2 per class j of the variance S_{ij}^2 per 'pair' ij was well described by equation (12), which closely resembles or equals (for k_1=1) the equation for the variance of negative binomial distributed variables (Bliss & Fisher, 1953). M_j represents the expected value of the averages M_{ij} of 'pairs' ij in class j, and εk_1 and εk_2 are expected values of the constants k_1 and k_2. The constant k_1 is an analogue of the coefficient of aggregation in the negative binomial distribution.

$$S_j^2 = \varepsilon k_1 \cdot M_j + \frac{M_j^2}{\varepsilon k_2} \tag{12}$$

- The relation between M_j, CV_j and CV_{conf}

The relation between the class mean of averages of pairs M_j and the expected value, CV_j, per class j of the coefficients of variation of 'pairs' ij, CV_{ij}, was obtained by dividing S^2_j in equation (12) by M^2_j and was described in equation (13).

$$CV_j = \sqrt{\frac{\varepsilon k_1}{M_j} + \frac{1}{\varepsilon k_2}} \tag{13}$$

Similarly, the relation between M_j and any confidence limit, $CV_{ij,x}$, of CV_{ij} is described by equation (14).

$$CV_{ij,x} = \sqrt{\frac{k_1}{M_j} + \frac{1}{k_2}} \tag{14}$$

Equations (13) and (14) equal the equation for the coefficient of variation of negative binomial variables for k_1=1. The first terms of equations (13) and (14) represent the variance and coefficient of variation, respectively, according to a 'binomial' distribution if k_1=q.

- The relation between M_j,D_j and $D_{ij,x}$

Expanding equation (9) for D_j with equation (7) for $CV^2_{j,'bin'}$ and the square of equation (13) for CV_j results in equation (15) for D_j.

$$D_j = \frac{\varepsilon k_1 - q}{M_j} + \frac{1}{\varepsilon k_2} \tag{15}$$

The same procedure, expanding equation (8) for D_{ij} with equation (7) for $CV^2_{j,'bin'}$ and the square of equation (14) for $CV_{ij,x}$ gives equation (16) for $D_{ij,x}$

Table 3.2. - Particulars of data sets and results of the basic analysis.

Data set	*Reference*	*field I-IV*	*field V*	*field VI*
subsamples per bulk sample	3	3	3	3
investigated subsamples per bulk sample	3	2	2	3
number of 'pairs' per data set	29	315	335	147
number, n_j of 'pairs' per class j	4-6	26-144	14-64	22-42
mean weight of subsamples	600 g	600 g	600 g	870 g
number of classes t	6	4	9	5
range of cyst counts per 'pair'	23-1254	32-824	3-700	3-700
εk_1	0.67	0.67	0.67	0.9
εk_2	753	55	108	330
95% upper limit k_1	1.27	5.4	5.4	3
95% upper limit k_2	330	7	14	45
% CV_{ij} of individual 'pairs' ij	0.2-12	0.1-103	0.2-121	0.2-81
% CV_j per class j	3.5-9.3	12.5-18.5	10.5-34.3	10-38

$$D_{ij,x} = \frac{k_1 - q}{M_j} + \frac{1}{k_2} \qquad (16)$$

If the distribution of the cysts in the subsamples is perfectly randomized, εk_1 equals q and D_j, the coefficient of the remaining variation associated with laboratory errors other than subsampling (cyst extraction from the soil and cyst counting), is independent of M_j and equals the constant εk_2^{-1}. If $\varepsilon k_1 > q$, cysts are not perfectly randomized in the subsamples and possible are aggregated. In such a situation εk_2^{-1} represents not only the coefficient of variation due to other laboratory errors than subsampling, but also a coefficient of aggregation, due to a clustered distribution of cysts in the soil.

'Reference' data set

- Details of the data set

All three subsamples ('pairs', here actually triples) per bulk sample, were investigated. Twenty-nine 'pairs' with an average weight of 600 g were divided into 6 classes and the number of 'pairs' n_j per class j ranged from four to six. Mean cyst numbers per 'pair', M_{ij}, ranged from to 23 to 1254. The mean and variance of the log-transformed 'pairs', m_{ij} and s^2_{ij} respectively, were so homogeneously distributed over the range of results per class j that the arithmetic class means, m_j and s^2_j, respectively, could be considered as suitable estimates of the expected values. The coefficients of variation per 'pair', CV_{ij}, varied from 0.2 to 12%. The expected value CV_j of CV_{ij} per class j ranged between 3.5% in class 6 with the highest cyst numbers (M_j=900) and 9.3% in class 1 with the smallest cyst numbers (M_j=36).

- Models and parameter estimation

The relations between M_j, as the independent variable, and S_j^2, CV_j and D_j as dependent variables, and the mathematical equations (12), (13) and (15) describing these relations are visualized in Figure 3.2A,B and C, respectively, against the back-ground of M_{ij} plotted against S_{ij}^2, CV_{ij} and D_{ij}, respectively. The estimates of εk_1 and εk_2 in the equations (12), (13) and (15) were 0.67 and 753, respectively. In class 1, the estimate of CV_j (0.093) deviated strongly from its expected value (0.143) according equation (13) with εk_1=0.67, and was better described by equation (13) with εk_1=0.22. No theoretical explanation of this deviation could be given. It cannot have been caused by a more accurate counting of small than of large numbers as the value of CV_j remains practically the same (0.099 instead of 0.093) when in class 1 an extraction and counting error of zero should be assumed so that $k_2 \to \infty$ and $1/k_2 \to 0$. For lack of a more satisfactory explanation the deviation of CV_j in class 1 is considered a random aberration.

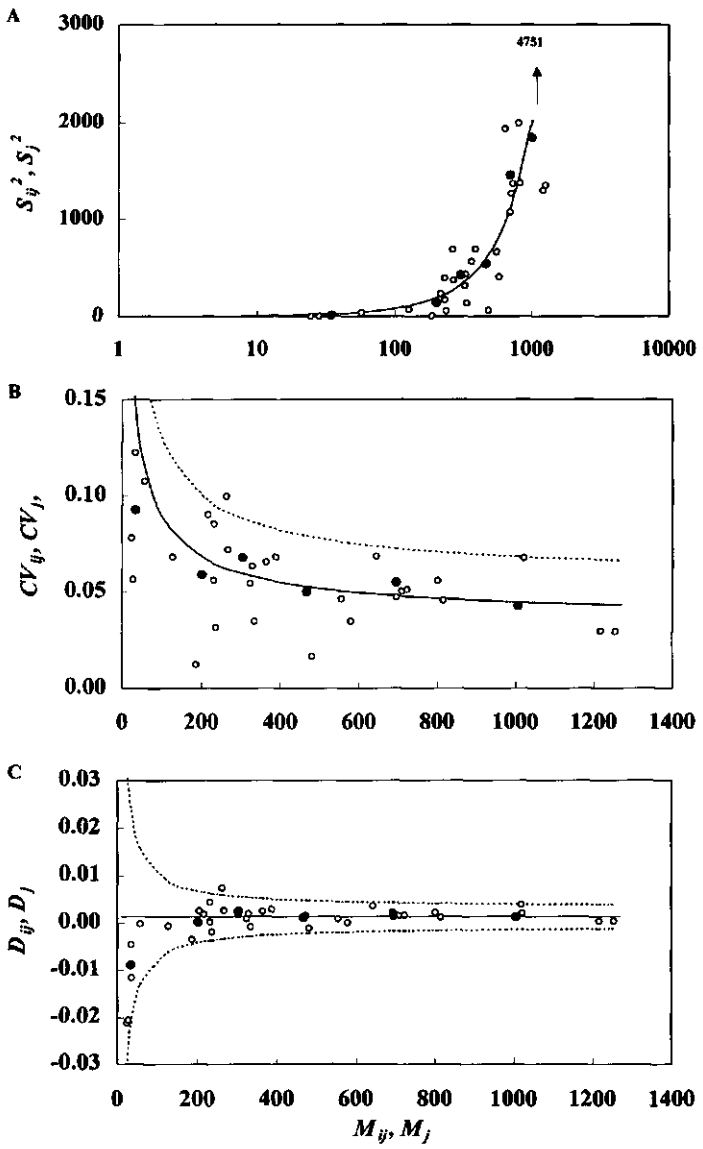


Figure 3.2. - Data set '*reference*'.The relation between the back-transformed class-mean, M_j, and A: the expected value, S_j^2, per class j of the variance of 'pairs', S^2_{ij}. B: the expected value, CV_j, per class j of coefficients of variation of 'pairs', CV_{ij}, the 95% upper confidence limit of CV_{ij}. C: the difference, D_j, between CV^2_j and $CV^2_{j,bin}$, and the 2.5% and 97.5% confidence limits of D_{ij}. In the back-ground of A, B, and C, respectively, S^2_{ij}, CV_{ij} and D_{ij} are plotted against M_{ij}. Expected values of variables per class j are indicated as black dots (●), variables per 'pair' ij as open dots (○), fitted models of expected values as solid lines (—) and confidence limits as broken lines (----).

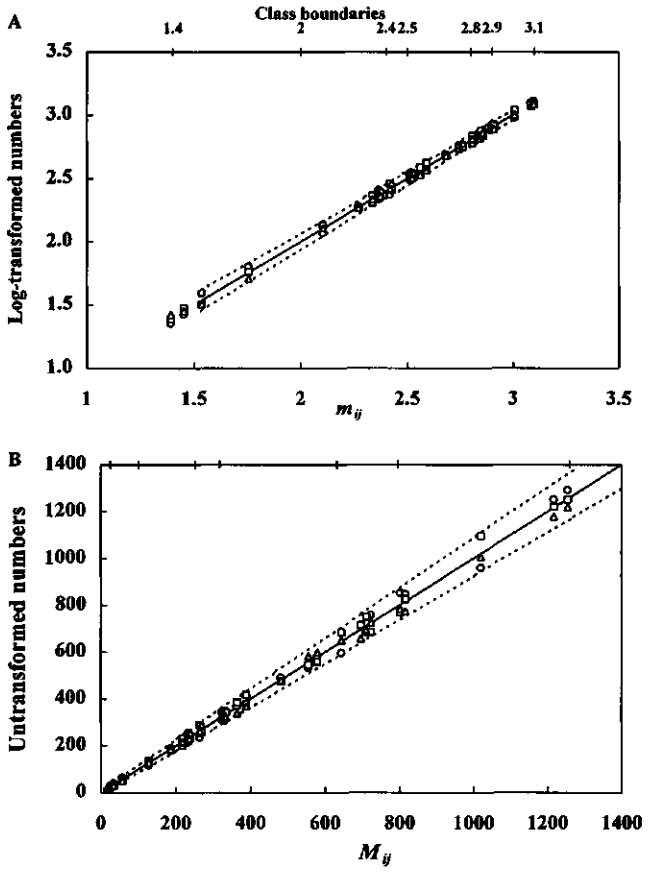


Figure 3.3. - Data set '*reference*'. A: ^{10}log-transformed cysts numbers, logA, logB and logC, in 'pairs', plotted against their means, m_{ij}. The expected value of m_{ij} per class j, m_j, is indicated as a solid line (—), 95% confidence interval, $m_j \pm 2s_j$, is indicated between broken lines (----) . B: Untransformed cysts numbers, A, B, and C, in 'pairs', plotted against their mean, M_{ij}. The back-transformed expected value M_j, of M_{ij} per class j, is indicated as a solid line (—). The 95% confidence interval, $M_j \pm 2S_j$ is indicated between broken lines (----). Class boundaries are indicated at the top of each graph.

In Figures 3.2B and C, the boundaries $CV_j \pm 2 \cdot \sigma_{j.D}$ were drawn. Table 3.3, where percentages D_{ij} of 'pairs' per class j within the boundaries of $D_j + 3 \cdot \sigma_{j.D}$ were compared with the expected percentages if values of D_{ij} had been distributed normally, shows that, although values of D_{ij} per pair ij were distributed close to normal around D_j, the distribution was slightly leptokurtic and, due to class 1, somewhat negatively skewed, illustrating that the distribution is composed of two superimposed distributions, one for

counting and one for extraction errors. Nevertheless, the 95% confidence limits of D_{ij}^2 could be approximated fairly with D_j plus and minus twice the standard deviation $\sigma_{j,D}$ and be described by equation (15) with q-k_1=±0.7 and k_2 between 303 and -1,471 (1/(0.0013±0.00199) for the upper and the lower limit respectively. Consequently, the 97.5% upper and 2.5% lower limits of CV_{ij} could be described by equation (13) with k_1=q±0.7 and k_2 between 303 and -1,471. The 95% upper limit of CV_{ij} was described by equation (14) with k_1= 1.27 and k_2=330.

Figure 3.3A shows the log-transformed cyst numbers of 'pairs', actually here triples, plotted against their means, m_{ij}, the class mean, m_j, and the 95% confidence interval m_j±2s_j. Figure 3.3B gives the same information as Figure 3.3A for the untransformed cyst numbers of 'pairs', their means M_{ij}, the back-transformed expected values, M_j per class j and the 95% confidence interval M_j±2S_j. All cyst numbers lay within the estimated 95% confidence interval.

- Conclusions

The main conclusion from the mathematical analysis is that, on the average, the quality of mixing, expressed by the parameter εk_1, is perfect, because εk_1 equals q. Consequently, the first term, $(\varepsilon k_1 - q)/M_j$ of equation (15) is zero and D_j, the difference between the expected value, CV^2_j, per class j of squares of coefficients of variation per pair, CV^2_{ij}, and $CV^2_{j,bin}$, is independent of M_j and equals $1/\varepsilon k_2$. Therefore $\varepsilon k_2^{-0.5}$ can be interpreted as the average coefficient of variation associated with cyst extraction and counting in Laboratory 0. It is estimated as 3.6% (=$753^{-0.5}$·100%). Values of $1/k_2$ of individual pairs had a close to normal distribution. The 97.5% upper limit of CV_{ij}, and the expected value, CV_j, of CV_{ij} did not exceed 10% and 7%, respectively, if more than 200 cysts were counted.

Table 3.3. - Check for normality of D_{ij} in the '*reference*' data set.

	Distribution of D_{ij} over classes $\sigma_{j,D}$ as observed (upper row) and as expected (lower row) according to a normal distribution					
	D_j -2$\sigma_{j,D}$ to D_j -3$\sigma_{j,D}$	D_j -$\sigma_{j,D}$ to D_j -2$\sigma_{j,D}$	D_j to D_j -$\sigma_{j,D}$	D_j to D_j +$\sigma_{j,D}$	D_j +$\sigma_{j,D}$ to D_j +2$\sigma_{j,D}$	D_j +2$\sigma_{j,D}$ to D_j +3$\sigma_{j,D}$
D_{ij}= (CV_{ij}^2-CV_{bin}^2)	0	15	37	33	11	4
Normal Distrib.	3	13	34	34	13	3

'Field V'

- Details of the data set

The bulk samples from *'field V'* were divided into three subsamples with an average weight of 600 g each, but only two subsamples ('pairs') were investigated. The infestation consisted of a large focus and consequently there was a wide range, from 3 to 700, of mean cyst numbers, M_{ij}, per 'pair'. The 335 pairs were divided into nine classes and the number, n_j, of 'pairs' per class varied from 14 to 37 in the classes, with j = 1, 2, 3, 7, 8 and 9 and from 52 to 64 in the classes with j = 4, 5 and 6. The distributions of s^2_{ij} within classes were negatively skewed and therefore the arithmetic means of s_{ij} were not taken as expected values, s^2_j, per class, but the back-transformed arithmetic means (minus c_j) of $\lambda_{ij}={}^{10}\log(s^2_{ij}+c_j)$ (see step 4 in 'Mathematical analysis'). The coefficients of variation per 'pair', CV_{ij}, varied from 0.2 to 121%. The expected values of the coefficients of variation per class, CV_j, from 10.5% in class 9 with the highest cyst number (M_j= 482.2) to 34.2% in the class 1 with the smallest cyst number (M_j=8.4).

- Models and parameter estimation

The relations between M_j, as the independent variable, and S^2_j, CV_j and D_j as dependent variables, and the models describing these relations according to equations (12), (13) and (15) are shown in Figure 3.4.A,B and C, respectively, against the back-ground of S^2_{ij}, CV^2_{ij}, CV_{ij} and D_{ij}, respectively, plotted versus M_{ij}. The estimates of εk_1 and εk_2 in the equations (12), (13) and (15) were 0.67 and 108, respectively.

The 95% upper confidence limit of S^2_{ij} was estimated as described in step 7c of 'Mathematical Analysis'. The estimates of k_1 and k_2 for this 95% upper limit were 5.4 and 14 respectively. The 95% upper limits of CV_{ij} and D_{ij}, as described by equations (14) and (16) were drawn in Figure 3.4.B and C.

Figure 3.5A shows the log-transformed cyst numbers of 'pairs' plotted against their means, m_{ij}, the class mean, m_j, and the 95% confidence interval $m_j \pm 2s_j$. Figure 3.5B gives the same information as Figure 3.5A for the untransformed cyst numbers of 'pairs', their means, M_{ij}, the back-transformed expected values, M_j per class j and the 95% confidence interval $M_j \pm 2S_j$. Actually 95.5% of all cyst numbers lay within the estimated 95% confidence interval.

- Conclusions

Data set *'field V'* demonstrated, as did the *'reference'* data set, that on average, cysts were perfectly randomized in the subsamples and therefore binomially distributed between subsamples. This was expected, as bulk samples in both data sets were submitted to the same method of soil mixing. The expected value of the coefficient of variation, associated with cyst extraction (in Laboratory 1) and counting (in Laboratory 0), $\varepsilon k^{-0.5}$, was higher than in the *'reference'* data set, namely 0.096. The most important distinction between the two data sets, *'reference'* and *'field V'*, is the difference in the

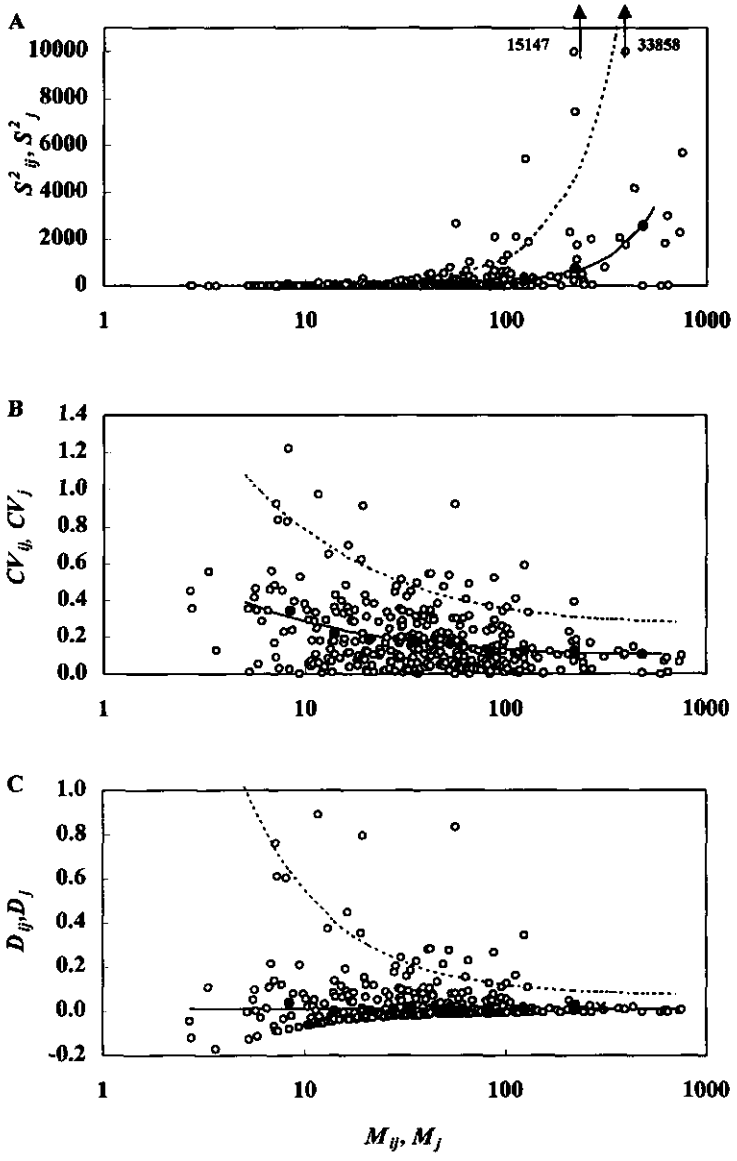


Figure 3.4. - Data set '*field V*'. The relation between the back-transformed class-mean, M_j, and A: the expected value, S^2_j, per class j of the variance of 'pairs', S^2_{ij}, and equation (12) describing S^2_j. B: the expected value, CV_j, per class j of coefficients of variation of 'pairs', CV_{ij}, the 95%-confidence limit of CV_{ij} and equation (13) and (14) describing CV_j and the confidence limit, respectively. C: the difference, D_j, between CV^2_j and $CV^2_{j,'bin'}$ and the 95% confidence limit of D_{ij}. In the back-ground of A, B and C, respectively, S^2_{ij}, CV_{ij} and D_{ij} are plotted against M_{ij}. Expected values of variables per class j are indicated as black dots (●), variables per 'pair' ij as open dots (○), fitted models of expected values as solid lines (—) and confidence limits as broken lines (----).

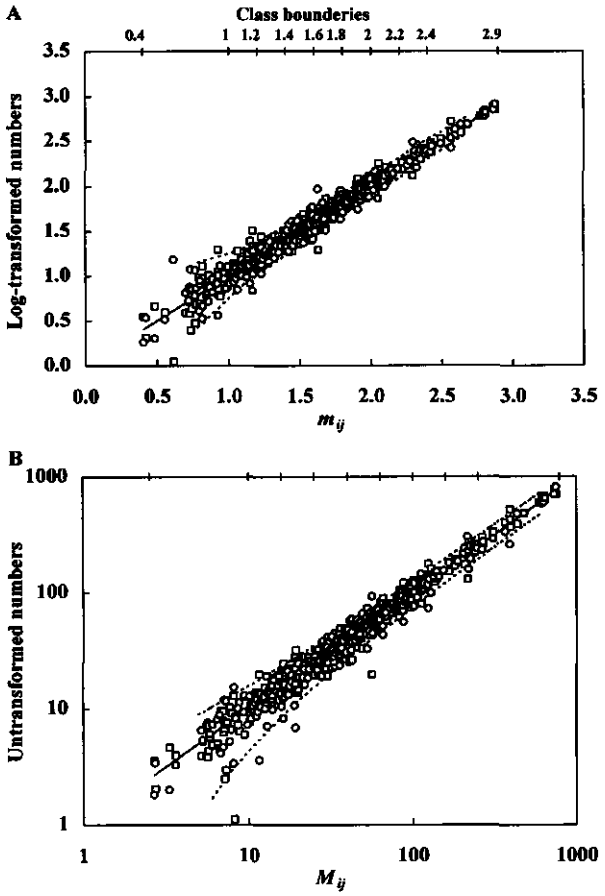


Figure 3.5. - Data set '*field V*'. A: ^{10}log-transformed cyst numbers, logA and logB, in 'pairs', plotted against their mean, m_{ij}. The expected value of m_{ij} per class j, m_j, is indicated as a solid line (—). The 95% confidence interval, $m_j \pm 2s_j$, is indicated between broken lines (----). B: Untransformed cyst numbers, A and B, in 'pairs', plotted against their mean, M_{ij}. The expected value of M_{ij} per class j, M_j, is indicated as a solid line (—). The 95% confidence interval, $M_j \pm 2S_j$, is indicated between broken lines (----). Class boundaries are indicated at the top of each graph.

95% upper confidence limits of CV_{ij} and D_{ij}, described by equations (14) and (16). For the '*reference*' and '*field V*' data sets the estimates of k_1, for the 95% confidence limits were 1.27 and 5.4, respectively, and of k_2 330 and 14.

'Field VI'

- Details of the data set

All three subsamples ('pairs', here triples) of *field VI* were investigated. The mean cyst number, m_{ij}, per 'pair' i ranged from 3 to 700 cysts (per 870 g). The 147 'pairs' were divided into five classes and the basic statistics were calculated as described above. The number of counts n_j per class ranged from 22 to 42. The distributions of s^2_{ij} within classes were negatively skewed and therefore the arithmetic means of s_{ij} were not taken as expected values, s^2_j, per class, but the back-transformed arithmetic means (minus c_j) of $\lambda_{ij}={}^{10}\log(s^2_{ij}+c_j)$ (see step 4 in 'Mathematical analysis').

The coefficients of variation CV_{ij} per 'pair' varied from 0.2 to 81%; the average coefficients of variation per class, CV_j, varied from 10% in class 5 with the highest class means (M_j=152.2) to 38% in the class 1 with the smallest class means (M_j=8.6).

- Models and parameter estimation

The relations between M_j, as the independent variable, and S^2_j and CV_j as dependent variables, and the equations describing these relations, (12) and (13), are shown in Figure 3.6A and B where S_{ij} and CV_{ij} are plotted against M_{ij} in the back-ground. The estimates of εk_1 and εk_2 in the equations (12) and (13) were 0.9 and 330, respectively. The expected value, εk_1, of k_1, was larger than q, implying that cysts were not distributed perfectly in the subsamples, which coincides with the statement in Materials and Methods that bulk samples of *'field VI'* were only superficially mixed. Calculating D_{ij} and its 95% upper limit would therefore be meaningless. Instead, the relation between M_j and $1/k_2$ and its 95% upper limit were calculated and depicted in Fig.3.6.C, against the back-ground of $1/k_2$ versus M_{ij}. As 'pairs' in this data set were actually triples and $1/k_2$ was not normally distributed in all classes, step 7b of the 'Mathematical analysis' was followed. The classes 4 and 5 were sufficient homogeneously distributed to estimate standard deviations, $\sigma_{j,k2}$ with equation (10) and the number of 'pairs' is sufficient large (147) to estimate the 95% confidence limits by trial and error for the other classes. The estimates of k_1-εk_1 and k_2 for the 95% upper limit were 2.1 and 45 respectively. The 95% upper limits of CV_{ij} could be constructed with equation (14) and the estimates, 3 and 45 of k_1 and k_2, respectively, found for the 95% upper limit of $1/k_2$. The 95% upper limits of CV_{ij} and $1/k_2$ were drawn in Figure 3.6.B and C.

Figure 3.7A shows the log-transformed cyst numbers of 'pairs', actually here triples, plotted against their means, m_{ij}, the class mean, m_j, and the 95% confidence interval $m_j \pm 2s_j$. Figure 3.7B gives the same information as Figure 3.7A for the untransformed cyst numbers of 'pairs', their means M_{ij}, the back-transformed expected values, M_j per class j and the 95% confidence interval $M_j \pm 2S_j$. Actually 98% of all cyst numbers lay within the estimated 95% confidence interval.

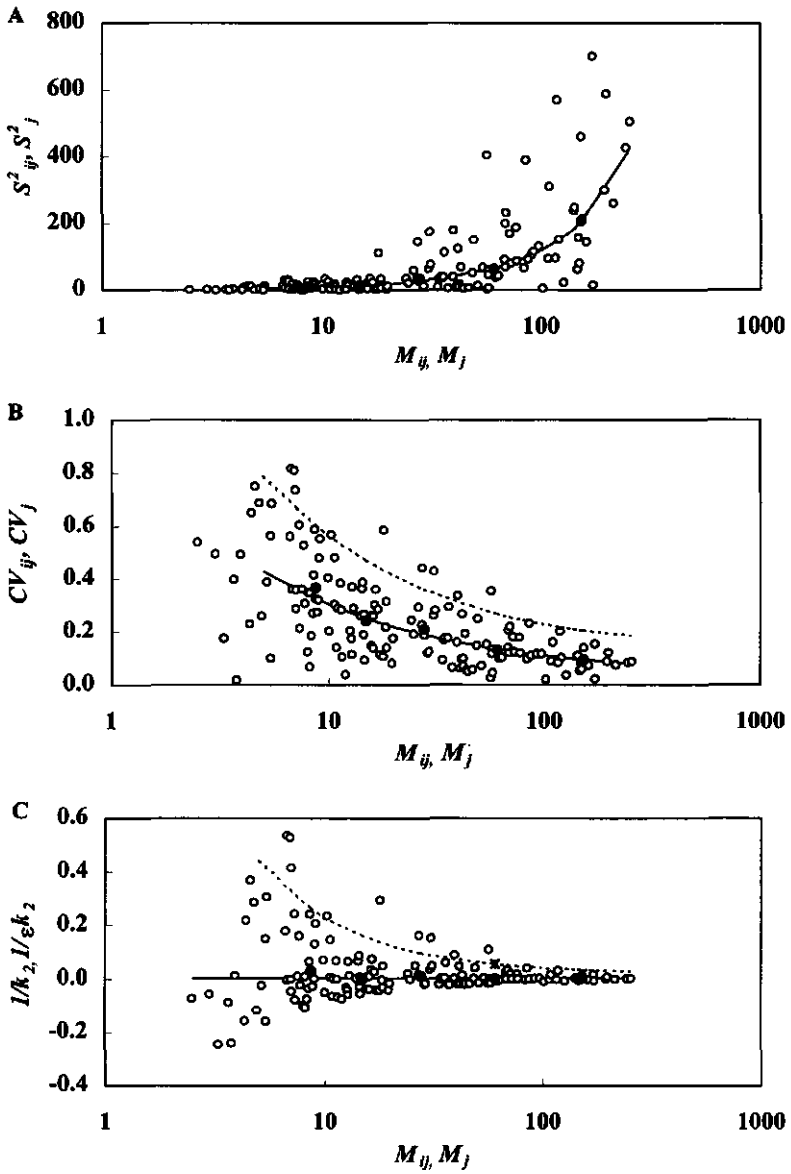


Figure 3.6. - Data set '*field VT*'. The relation between the back-transformed class mean, M_j, and A: the expected value, S^2_j, per class j of the variance of 'pairs', S^2_{ij}. B: the expected value, CV_j, per class j of coefficients of variation of 'pairs', CV_{ij} and the 95% confidence limit of CV_{ij}. C: The expected value, $1/\varepsilon k_2$, and the 95% confidence limit of $1/k_2$. In the back-ground of A, B, and C, respectively, S^2_{ij}, CV_{ij} and $1/k_2$ are plotted against M_{ij}. Expected values of variables per class j are indicated as black dots (●), variables per 'pair' ij as open dots (○), fitted equations of expected values as solid lines (—) and confidence limits as broken lines (- - -)

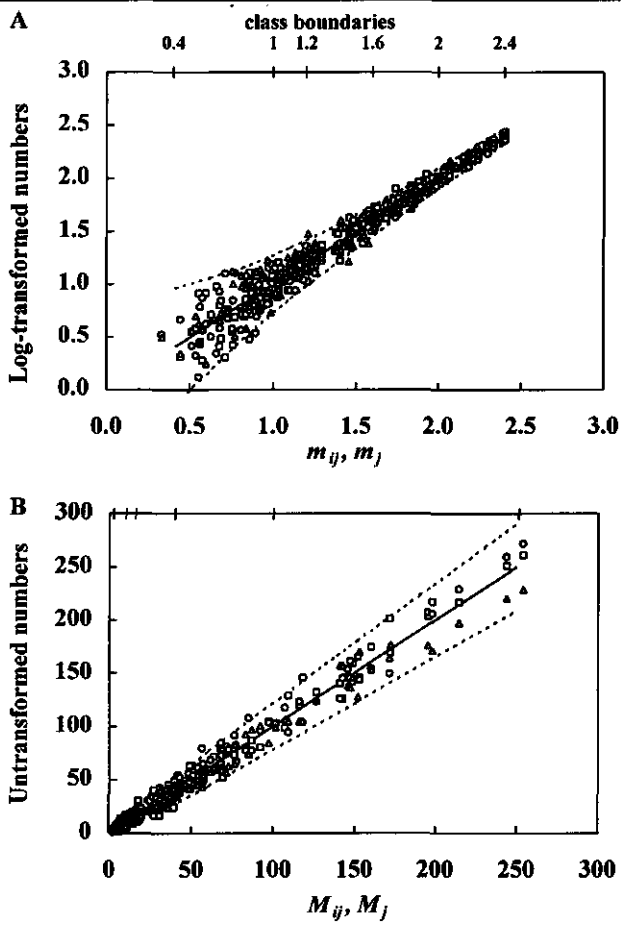


Figure 3.7. - Data set *'field VI'*. A: log-transformed cysts numbers, logA, logB and logC, in 'pairs', plotted against their mean, m_{ij}. The expected value of m_{ij} per class j, m_j, is indicated as a solid line (—). The 95% confidence limits, $m_j \pm 2s_j$ are indicated as broken lines (----). B: Untransformed cysts numbers, A, B, and C, in 'pairs', plotted against their mean, M_{ij}. The expected value of M_{ij} per class j, M_j, is indicated as a solid line (—). The 95% confidence limits, $M_j \pm 2S_j$ are indicated as broken lines (----). Class boundaries are indicated at the top of each graph.

- Conclusions

The deviation of εk_l from q (=0.67) indicates that cysts were not perfectly random distributed in the subsamples. The estimate of εk_l for *'Field VI'* differs from the estimates for the other data sets. Such a deviation could be expected, since bulk samples of *'field VI'*, contrary to bulk samples of other fields, were only superficially mixed. As εk_l=0.9, D_j, the difference between the expected value, CV^2_j per class j of squares of coefficients of variation per pair, CV^2_{ij}, and $CV^2_{j,'bin'}$ still depends on M_j and

equals $0.23/M_j+1/\varepsilon k_2$. Theoretically $\varepsilon k_2^{-0.5}$ (= 0.055) must be interpreted as the average coefficient of variation associated with cyst extraction in Laboratory 2 and cyst counting in Laboratory 0, added to the square root of the reciproque of a coefficient of aggregation, $\varepsilon k_3^{-0.5}$, due to imperfect mixing. This coefficient of aggregation, εk_3 must be large, implying that the coefficient of variation connected with it is small, as the estimates of $\varepsilon k_2^{-0.5}$ of the 'reference' data set (0.036) and *'field VI'* (0.055) are similar. If a coefficient of variation due to extraction and counting should be assumed to equal that of Laboratory 0, the coefficient of aggregation in the subsamples due to imperfect mixing is 587 $(1/330\text{-}1/753)^{-1}$.

'Field I-IV'

- Details of the data set

The bulk samples from *'field I-IV'* were divided into three subsamples with an average weight of 600 g each, but only two subsamples ('pairs') were investigated. The infestations on all four fields were more or less uniform with 95% of the mean cyst numbers, M_{ij}, per 'pair' in a narrow range, between 100 and 250. The 315 'pairs' were divided into four classes. The number, n_j, of 'pairs' per class varied from 26 to 144. The distributions of s^2_{ij} within classes were negatively skewed and therefore the arithmetic means of s_{ij} were not taken as expected values, s^2_j, per class, but the back-transformed arithmetic means (minus c_j) of $\lambda_{ij}={}^{10}\log(s^2_{ij}+c_j)$ (see step 4 in 'Mathematical analysis').

The coefficients of variation per 'pair', CV_{ij}, varied from 0.1 to 103%, while the expected values of the coefficients of variation per class, CV_j, ranged from 12.5% in class 9 with the highest cyst number (M_j= 415) to 18.5% in the class 1 with the smallest cyst number (M_j=77).

- Models and parameter estimation

The relation between M_j, as independent variable, and CV_j and D_j, as dependent variables, and their descriptions by equations (13) and (15), respectively, are shown in Figure 3.8.A and B. In the back-ground of Figure 3.8.A and B, CV_{ij} and D_{ij}, respectively, are plotted versus M_{ij}. The estimates of εk_1 and εk_2 in equations (13) and (15) were 0.67 and 55, respectively. The 95% confidence limit of $S^2_{ij,I\text{-}IV}$, and was estimated as described in step 7c of 'Mathematical analysis'. The estimates of k_1 and k_2 for this 95% confidence limit were of $CV_{ij,I\text{-}IV}$, and $D_{ij,I\text{-}IV}$, were 5.4 and 7 respectively. The 95% upper limits of CV_{ij} and D_{ij} were drawn in Figure 3.8.A and B.

Figure 3.9A shows the log-transformed cyst numbers of 'pairs' plotted against their means, m_{ij}, the class mean, m_j, and the 95% confidence interval $m_j \pm 2s_j$. Figure 3.9B gives the same information as Figure 3.9A for the untransformed cyst numbers of 'pairs', their means M_{ij}, the back-transformed expected values, M_j per class j and the 95% confidence interval $M_j \pm 2S_j$. Actually 96% of all cyst numbers lay within the estimated 95% confidence interval.

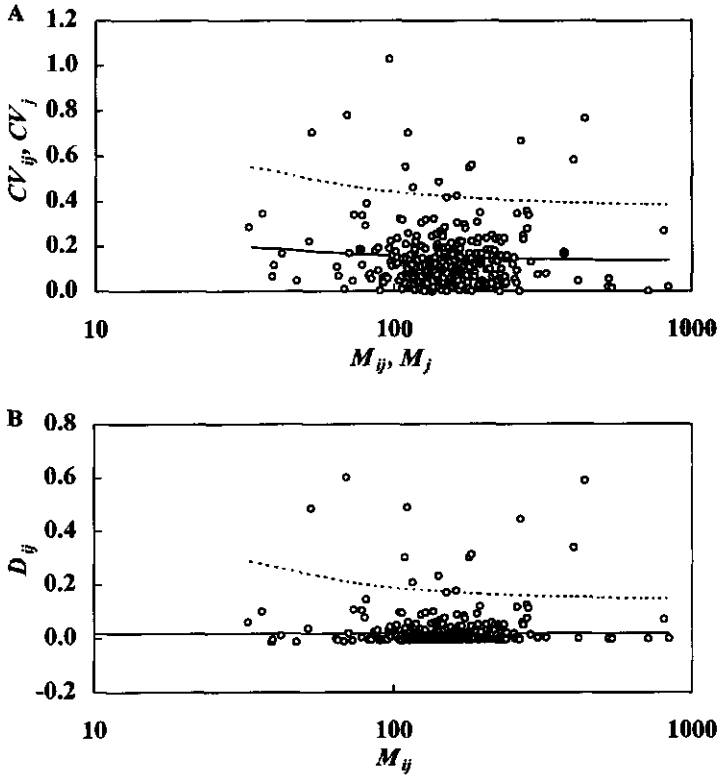


Figure 3.8. - Data set *'field I-IV'*. The relation between the back-transformed class mean, M_j, and A: the expected value, CV_j, per class j of coefficients of variation of 'pairs', CV_{ij} and the 95% confidence limit of CV_{ij}. B: the difference, D_j, between CV^2_j and $CV^2_{j,bin}$ and the 95% confidence limit of D_{ij}. In the back-ground of A, and B, respectively, CV_{ij} and D_{ij} are plotted against M_{ij}. Expected values of variables per class j are indicated as black dots (●), variables per 'pair' ij as open dots (○), fitted models of expected values as solid lines (—) and confidence limits as broken lines (----).

- Conclusions

Although modelling and parameter estimation in data set *'field I-IV'* were hampered by the narrow range of cyst numbers, the conclusion from the '*reference*' data set and data set *'field V'* that on average, cysts were perfectly randomized in the subsamples and therefore, binomially distributed, were not contradicted. This result was expectable as the bulk samples in the three data sets were submitted to the same method of mixing.

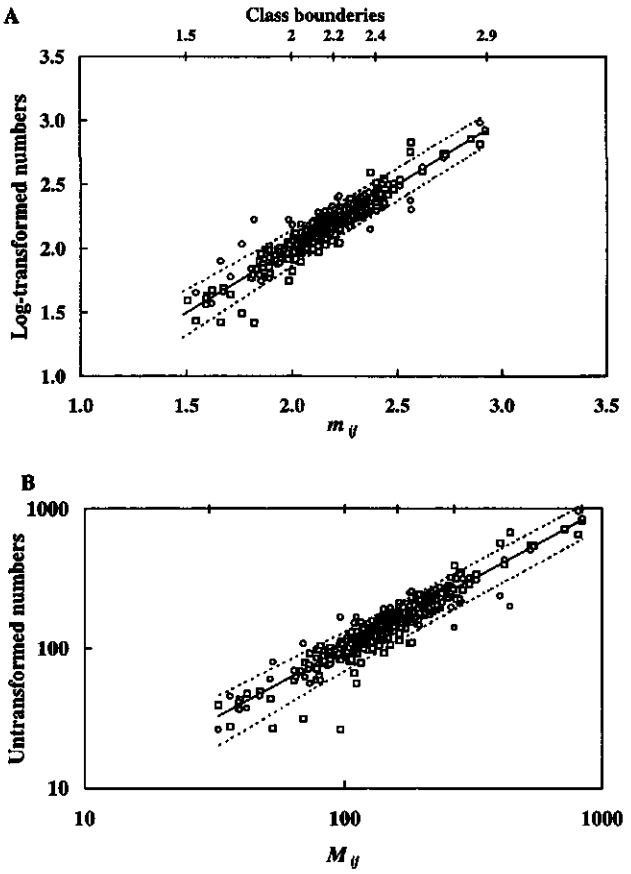


Figure 3.9. - Data set '*field I-IV*'. A: ^{10}log-transformed cysts numbers, logA, logB and logC, in 'pairs', plotted against their mean, m_{ij}. The expected value of m_{ij} per class j, m_j, is indicated as a solid line (—). The 95% confidence limits, $m_j \pm 2s_j$ are indicated as broken lines (----). B: Untransformed cysts numbers, A, B, and C, in 'pairs', plotted against their mean, M_{ij}. The expected value of M_{ij} per class j, M_j, is indicated as a solid line (—). The 95%-confidence limits, $M_j \pm 2S_j$ are indicated as broken lines (----). Class boundaries are indicated at the top of each graph.

However, the expected value of the coefficient of variation, associated with cyst extraction and counting, the square root of $1/\varepsilon k_2$, was higher than in the other data sets, namely 0.135 (Table 3.2), as was the 95% upper limit, the square root of $1/k_2$ (0.33), probably because the cysts in the subsamples A and B were counted in two different laboratories (Table 3.1).

3.5. Discussion

In the Results, the sensitivity of the measurement 'equipment', (mixing with subsampling, extraction and counting) used by nematologists to estimate relevant signals in the field, e.g. the estimation of population densities, were quantified for three laboratories, using data sets from several fields. We can now evaluate the suitability of this 'equipment' to measure three signals already quantified, namely the coefficient of aggregation of small scale distribution patterns of nematodes (Seinhorst, 1988) and a sampling method, AMI-100, for the detection of small infestation foci, applied by two commercial laboratories, Laboratories 1 and 2 (Chapter 8). As subsampling is not common practice for detection, first an effort is made to separate the extraction error from the subsampling and counting errors. This is done by comparison of the data sets *'field V'* , *'field VI'* and *'field I-IV'* with the *'reference'* data set.

Comparison of the data sets

- The results of *'reference'* and *'field V'*

In the *'reference'* data set the average coefficient of variation, CV_j, at large cyst numbers is 3.6%, the smallest of all data sets (Table 3.2). Therefore, the results in Laboratory 0 were used as a standard to estimate extraction errors in data set *'field V'* that must have the same variation due to subsampling and counting, as these tasks for both data sets were exclusively performed by Laboratory 0. Consequently, as is shown in equation (17), the data sets *'reference'* and *'field V'* differ only in that extraction in the former case is done by Laboratory 0 and in the latter by Laboratory 1.

$$CV^2_{total}=CV^2_{subsampling}+CV^2_{extraction}+CV^2_{counting} \tag{17}$$

As the expected value of k_1 for both data sets equals the proportion q of the bulk sample that was not investigated, so that the first term of equation (17) equals the square of $CV_{'bin'}$, the coefficient of variation according to a 'binomial' distribution, it seems safe to make two conclusions. First, that the expected value of the coefficient of variation associated with subsampling is well described by a 'binomial' distribution. Second, that the expected value of $1/k_{2,ref}$, which stands for $1/k_2$ for the *'reference'* data set, can be attributed to counting and extraction by Laboratory 0.

For the expected values of the squares of coefficients of variation, equation (17) can be rewritten as:

$$\varepsilon CV^2_{total}=\varepsilon CV^2_{'binomial'}+\varepsilon CV^2_{extraction}+\varepsilon CV^2_{counting} \tag{18}$$

As for all data sets counting was done in Laboratory 0, and we know that for Laboratory 0 the sum of both errors was 3.6% (Table 3.2.), we can safely assume that both errors were small with coefficients of variation between 1% and 3% each. However, with the estimation of confidence limits of extraction errors a difficulty

arises. The parameter k_2 in the upper limit of CV_j consists of two components, one due to extraction and counting errors, and a 'true' coefficient of aggregation, k'_3, associated with a clumped distribution of cysts in the subsamples due to imperfect mixing. In the present data sets these two types of variation cannot be separated. For convenience, in the following calculations extraction errors in Laboratory 0 were assumed negligible and both the deviations of k_2 from εk_2 in the '*reference*' data set were entirely attributed to subsampling and counting.

Then, equation (19) applies for all other probabilities, x, than the expected value, CV_j of the total coefficient of variation CV_{ij}.

$$CV_{ij,x,ref}=\sqrt{\frac{k_{1,ref}}{M_j}+\frac{1}{k_{ex,ref}}+\frac{1}{k_{2*,ref}}}\approx\sqrt{\frac{k_{1,ref}}{M_j}+\frac{1}{k_{2*,ref}}} \tag{19}$$

In equation (19) $1/k_{2*,ref}$ equals $1/k_{c,ref}+1/k'_{3,ref}$. The parameter $k_{ex,ref}$ is a coefficient associated with extraction in the '*reference*' data set and the parameter $k_{2*,ref}$ incorporates a 'true' coefficient of aggregation, $k'_{3,ref}$, and a coefficient associated with counting, $k_{c,ref}$, in the '*reference*' data set.

The difference between the squares of the coefficients of variation of '*field V*' and the '*reference*' data set, indicated as, δ_V, serves as an estimate of the extraction error made by Laboratory 1. The expected value, $\varepsilon\delta_V$, of this difference δ_V is described by equation (20) and equals 0.089, which is only slightly smaller than the square root of $1/\varepsilon k_{2,V}$ (0.096).

$$\varepsilon\delta_V=CV^2_{j,V}-CV^2_{j,ref}=\frac{1}{\varepsilon k_{2,V}}-\frac{1}{\varepsilon k_{2*,ref}}=\frac{1}{108}-\frac{1}{753}=\frac{1}{126} \tag{20}$$

The difference, $\delta_{0.95}$, between the squares of the 95% confidence limits of '*field V*' and the '*reference*' data set is described by equation (21). The subscripts '*V*', and '*ref*' refer to accompanying variables or parameters with respect to the data sets '*field V*' and '*reference*' respectively, the subscript '0.95' refers to accompanying variables and parameters for the 95% confidence limit

$$\delta_{0.95}=CV^2_{0.95}-CV^2_{0.95,ref}=\frac{k_1-k_{1,ref}}{M_j}+\frac{1}{k_2}-\frac{1}{k_{2*,ref}} \tag{21}$$

The 95% upper confidence limit of extraction error, $\delta_{0.95}$, of Laboratory 1 was calculated by subtracting the 95% upper limit of $CV^2_{j,ref}$ from $CV^2_{j,V,0.95}$ Then, equation (21) can be rewritten as

$$\delta_{0.95,V}=\frac{5.4-1.27}{M_j}+\frac{1}{14}-\frac{1}{330}=\frac{4.1}{M_j}+\frac{1}{15} \tag{22}$$

The relations between M_j as independent variable and $\varepsilon\delta$ and $\delta_{0.95}$, as dependent variables, depicting the expected value, and the 95% confidence limit of the

coefficient of variation associated with extraction in Laboratory 1, are visualized in Figure 3.10.

- The results of the data sets '*reference*' and '*field VI*'

For '*field VI*' $\varepsilon\delta$ and $\delta_{0.95}$ were calculated much in the same way as for '*field V*' with equations (20) and (21), except that equation (20) for the expected value, $\varepsilon\delta$, of the extraction error made by Laboratory 2 was slightly adapted to equation (23) because of light mixing only of bulk samples before subsampling.

$$\varepsilon\delta_{VI}=CV^2_{j,VI}-CV^2_{j,ref}=\frac{\varepsilon k_{1,VI}-\varepsilon k_{1,ref}}{M_j}+\frac{1}{\varepsilon k_{2,VI}}-\frac{1}{\varepsilon k_{2^*,ref}}=\frac{0.23}{M}+\frac{1}{587} \tag{23}$$

For the 95% upper confidence limit of extraction error of Laboratory 2 an average value was calculated by following the same procedure as for '*field V*'. The results of these calculations are not quite comparable with those for '*field V*', as εk_1 for '*field VI*' was larger than the parameter q of the 'binomial' distribution, due to light mixing only of the bulk sample before subsampling. Nevertheless, for $\delta_{0.95,VI}$ of '*field VI*', equation (20) was rewritten as

$$\delta_{0.95,VI}=\frac{3-1.27}{M_j}+\frac{1}{45}-\frac{1}{330}=\frac{1.73}{M_j}+\frac{1}{52} \tag{24}$$

- The results of the data sets *'field V'* and *'field I-IV'*

Data sets *'field V'* and *'field I-IV'* differ only in that cyst counts of subsamples A and B of the former data set were made exclusively in Laboratory 0 but subsamples of the latter were counted by Laboratories 0 and 3, respectively. Therefore, *'field I-IV'* is compared with *'field V'* instead of the *'reference'* data set.

The expected value of the coefficient of variation, associated with cyst extraction (in Laboratory 1) and counting (in Laboratories 0 and 3), $\varepsilon k_{2,I\text{-}IV}^{-0.5}$, was 0.135, respectively 0.13, 0.095 and 0.12 higher than in the data sets *'reference'* (with $\varepsilon k_{2,ref}^{-0.5}=0.036$), *'field V'* (with $\varepsilon k_{2,V}^{-0.5}=0.096$) and *'field VI'* (with $\varepsilon k_{2,VI}^{-0.5}=0.055$). Analogue differences for the 95% confidence limits between data set *'field I-IV'* on the one hand and the data sets *'reference'*, *'field V'* and *'field VI'* on the other hand were 0.37, 0.27 and 0.35, respectively.

We know from equation (19) that the expected value, $\varepsilon\delta_V$ of the extraction error for data set *'field V'* is 0.089. If it is assumed that the expected values of the extraction error for data sets *'field V'* and *'field I-IV'* were the same, the expected value of the counting error in data set *'field I-IV'* can be obtained by extracting $\varepsilon\delta_V$ from $\varepsilon k_{1,I\text{-}IV}$ and taking the square root. It equals 0.11. It can be concluded that counting errors affect mainly the parameter k_2 and not or hardly k_1 and thus are independent of cyst counts.

- The results of '*field V*' and '*field VI*'

If the quality of the two laboratories, 1 and 2, are compared, the quality of Laboratory 2 surpasses that of Laboratory 1. Both the expected values of coefficients of variation

and the 95% upper limit were smaller for Laboratory 2 than for Laboratory 1 in spite of the fact that Laboratory 2 was slightly handicapped because of the imperfect mixing of subsamples. So-called 'ring investigations', in which both laboratories participated for years, did not quantify these differences in extraction quality.
The afore-said estimates of the expected values and 95% confidence limits of coefficients of variation, associated with subsampling, extraction and counting in the various laboratories, are summarized in Table 3.4. In the following the consequences of these estimates for the estimation of the small scale distribution and the detection probability of small foci will be discussed.

Consequences of the 'noise' estimator CV_j
- For the estimate of εk_3 in $CV_{distrib}$
One of the signals, which we intended to pick up, was the small scale distribution pattern of potato cyst nematodes. On the base of data published so far, a pattern to be expected is that of a negative binomial distribution, with an average coefficient of aggregation, εk_3, of 70 (Seinhorst, 1988). The coefficient of variation associated with the small scale distribution of cysts, $CV_{distrib}$, is described by equation (25)

$$CV_{distrib} = \sqrt{\frac{1}{T_j} + \frac{1}{\varepsilon k_3}} \qquad (25)$$

Table 3.4. - Summary estimates of k_1 and k_2 associated with the expected values and 0.95 confidence limits of coefficients of variation associated with subsampling, extraction and counting, and various laboratories.

Type of error	Mixing and subsampling		Extraction		Counting
Laboratory	exp. value	95% limit	Exp. value	95% limit	Exp. value
L 0	$k_1 = 0.67$ $k_2 = 0$	$k_1 < 1.27$ $k_2 = 330$	nill	nill	$k_1 = 0$ $k_2 < 1000$
L 1	-	-	$k_1 = 0$ $k_2 = 126$	$k_1 = 4$ $k_2 = 15$	-
L 2	-	-	$k_1 = 0$ $k_2 = 587$	$k_1 = 1.7$ $k_2 = 52$	-
L 3	-	-	-	-	$k_1 = 0$ $k_2 = 98$

- No information available

The parameter εk_3 represents the expected value of the coefficient of aggregation and equals 70 for bulk samples of 1.5 kg (Seinhorst, 1982). The parameter T_j represents the number of cysts counted in a sample and equals M_j. If a subsample is taken, after thoroughly mixing of the bulk sample, the total coefficient of variation due to sampling and subsampling (if cysts were perfectly random distributed in samples) equals equation (26), adapted from Seinhorst (1988).

$$CV^2_{total}=CV^2_{sample}+CV^2_{subsample}=\frac{1}{T_j}+\frac{1}{k_3}+\frac{q}{p\cdot T_j}=\frac{p}{p\cdot T_j}+\frac{1}{k_3}+\frac{q}{p\cdot T_j}=\frac{1}{p\cdot T_j}+\frac{1}{k_3} \qquad (26)$$

The parameter p is the proportion of the investigated bulk sample. Then, $p\cdot T_j$ represents the number of cysts counted in a subsample. In Fig.3.10, the average coefficient of variation, $CV_{distrib}$, according to a negative binomial distribution with $k_3 = 70$ is shown as a bold solid line. $CV_{distrib}$, increased by $\varepsilon k_{1,I\text{-}IV}$, the expected value of extraction and counting errors made by Laboratories 1 and 3, is shown as a thin solid line. $CV_{distrib} + k_{1,I\text{-}IV}$, increased by an error due to subsampling if subsample size was 10% of the bulk sample, is given as a broken line in Fig.3.10. Increase of $CV_{distrib}$, with $\varepsilon k_{1,ref}$, the counting and extraction error made by Laboratory 0, is depicted in Fig. 3.10 as grey dots. Three conclusions can be drawn: First, that subsampling does not influence the total coefficient of variation, irrespective the relative size of the subsample, provided that sufficient entities are counted, for potato cysts nematodes 250 cysts or more. Second, that the estimate of k_3 is considerably reduced by the addition of seemingly moderate extraction and counting errors made by Laboratories 1 and 3, respectively, and equals 30 instead of the expected 70. Third, the extraction and counting errors made by Laboratory 0 hardly influence the estimate of k_3.

To adjust nematode counts to the coefficient of variation necessary for the purpose of investigation, sufficiently large bulk samples should be taken. These large bulk samples have large coefficients of aggregation, provided that the area from which the bulk samples are taken is a sufficiently homogenous area with the same average nematode density. Although the 'true' coefficient of aggregation, k_3, is proportional to the weight of the sample (Seinhorst, 1988), it is not reduced by subsampling of bulk samples. These bulk samples must be well mixed and divided into a number of equal portions which could be investigated sequentially.

However, for at least 10% of all investigated 'pairs' in *'field V'* and *'field I-IV'* the extraction error was not constant, but depended on the number of cysts in the subsamples and was estimated by $\delta_{0.95,V}$. If the coefficient of variation, $CV_{distrib}$, is increased with both the extraction error, $\delta_{0.95,V}$, and the counting error the estimate of k_3 also becomes dependent on cyst counts and varies from 0.33 (if 1 cyst is counted) to 12 (if more than 1,000 cysts are counted). This phenomenon is shown in Fig. 3.10 by the upper broken line, where the estimates of k_3 are printed on the right side of the calculated data points (▲).

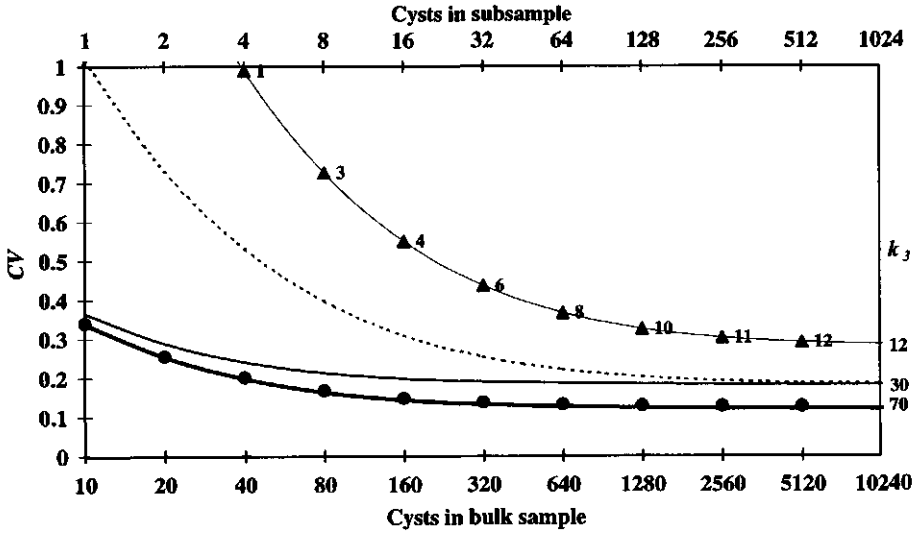


Figure 3.10. - The relation between cyst numbers, either in the bulk sample or in a subsample with relative size of 10% of the bulk sample, and i) the coefficient of variation $CV_{distrib}$, the negative binomial distribution with coefficient of aggregation, k_3, of 70, indicated as a bold solid line (—); ii) the sum, Σ_1, of $CV_{distrib}$ and the expected value of the coefficient of variation, $\varepsilon k_{1,1\text{-}IV}$, associated with extraction in Laboratory 1 and counting in laboratory 3, depicted as a thin solid line (—); iii) the sum Σ_2, of Σ_1 and the coefficient of variation, CV_{bin}, due to subsampling, indicated as a broken line (----); iv) the sum Σ_3 of $CV_{distrib}$, the counting error by Laboratory 3 ($\varepsilon k_{1,1\text{-}IV}$-$\varepsilon\delta_V$), and the 95% confidence limit of the extraction error, $\delta_{0.95,V}$, made in Laboratory 1, given as a thin solid line with black triangles(▲—▲); v) the sum Σ_4 of $CV_{distrib}$ and the expected value of the coefficient of variation,$\varepsilon k_{1,ref}$, due to extraction and counting errors in Laboratory 0, represented as black dots (● ●).

These findings have consequences for the estimates of k_3 from regression analysis of foci in Chapter 7, where cyst extraction was in most cases done by the commercial Laboratories 1 and 2. First, the assumption that values of k_3 are constant within a focus is violated. Second, most estimates of k_3 from regression analysis, will be underestimated by various, but unknown degrees. Fortunately, the estimated gradients were not biassed by extraction and counting errors.

A feature, also to be deduced from the results, is that, if subsamples are taken and/or laboratory errors are dependent of cyst numbers in samples, 'noise' and 'signal' are congruent. This congruence may be deceitful. In many biological reports distribution patterns of nematodes, but also of other soil-borne organisms, are often described by a negative binomial function, mostly with small estimates for the coefficient of aggregation k_3. It cannot be excluded that such a distribution function and coefficient of aggregation may indicate the 'noise' produced by the investigator rather than the

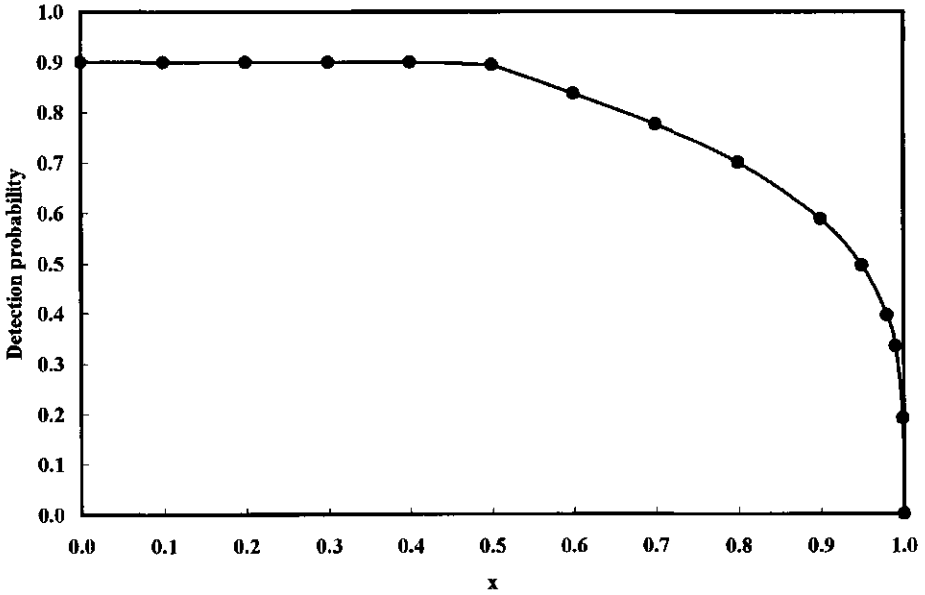


Figure 3.11. - The relation between the average detection probability of a standard infestation focus with central density of 100 cysts per kg of soil, and upper limits, x, associated with extraction and counting errors made by commercial laboratories. The resulting average detection probability is represented by the surface under the solid, dotted line.

distribution pattern of the organisms studied, especially in cases that the quantitative consequences of sampling and laboratory procedures are poorly documented.

- For extension purposes

Coefficients of variation associated with extraction, especially when larger than or equal to the coefficients of variation associated with sampling methods, will influence the accuracy and precision of sampling methods. We will discuss probabilities of biasses by laboratory methods for the AMI 100 method, described by Been & Schomaker (1998b), prescribing 3.6 kg of soil per one third of a hectare, taken in a grid pattern of 5 x 5 m^2, to detect foci with a central density of 100 cysts per kg of soil with an average probability of 90%. A model study, consisting of 500,000 Monte Carlo simulations, was made, using the simulation model, SAMPLE, written by Been & Schomaker (Chapter 8) which provides the sampling results of the described infestation focus, assuming a negative binomial small scale distribution of cysts with a coefficient of aggregation, k_3, of 70 for soil samples of 1.5 kg (Seinhorst, 1988). The result of this model study was a negative binomial distribution with mean 2.9 and a coefficient of aggregation of 4.13. The 95% interval comprised cyst counts between 0

and 10. The expected value of the extraction error (0.9), made by Laboratory 1, if added to the coefficient of variation, associated with the number of cysts per sample, changed the average detection probability only slightly.
However, extraction errors, associated with probabilities ranging from the expected value to the 0.0005 level (calculated following step 7c of mathematical analysis), resulting in values of $k_{l,I\text{-}IV,x}$-$k_{l,ref,x}$ larger than 1 decreased the average detection probability from 90% to 80%. This changed detection probability was estimated by calculating, for each possible cyst count in the above mentioned frequency distribution, the probability at zero cysts, given the coefficients of variation due to extraction at the various probability levels and by adding these probabilities to the probability of zero cysts in the negative binomial distribution, associated with field sampling and equalling 0.1. The results of these calculations are visualised in Fig. 3.11.
Resuming, we can conclude that increase of the parameter k_l for extraction and other laboratory errors to values larger than zero results in a decrease of the average detection probability of small infestation foci. Results from Laboratory 1 give a higher probability at such biasses than results from Laboratory 2. Variation in k_2, as observed in the present data sets, is of minor consequence to detection probability for extension purposes.

In the so-called 'ring-investigations' much attention is paid to extraction efficiency and little to the variation of extraction efficiency. However, a reduced extraction efficiency can easily be compensated by taking a slightly larger soil sample, provided that it is accompanied by a small coefficient of variation. It would be useful for both commercial and research laboratories to investigate the source(s) of the variation in extraction efficiency, especially with respect to the parameter k_l, operating at small cyst counts. To this purpose, the effect of all laboratory errors, elutriation, separation and counting, on the parameter k_l must be separately investigated at sufficiently wide ranges of cyst counts (for instance, by investigating samples from large infestation foci) and be related to both technical and human factors.

Chapter 4

Ways to improve the accuracy of hatching tests for *Globodera* spp. with special emphasis on nematicide trials

T.H. Been & C.H. Schomaker

Summary 4.1. - Hatching tests using potato root diffusate are labourious and yield quite variable results. Sources of variability were identified and analysed, and solutions were presented. A method was developed to conduct hatching tests using inert materials so that the total variation at the end of the test is minimized. A number of hatching tests was carried out to increase reliability, optimize the method and limit the amount of work. Thus, it was possible to obtain a coefficient of variation (*cv*) of the hatching process which is in accordance with the combined errors expected when a certain number of cysts is treated and eggs are used in a hatching test. An Appendix is provided listing the different errors and ways to calculate and cope with them. The results indicate that the hatching process is no longer an important source of variation for the end result. All variation higher than expected could be explained by variation between replications of batches with the same treatment, indicating that small differences in nematicide application cause major differences in the end result. The treatment effect was more important in field experiments than in laboratory experiments.

The hatching curve could be described adequately by a log-logistic curve with 3 parameters (λ final number of hatched larvae, α time, β slope parameter). Addition of a fourth parameter (γ, incubation time) improved the fit of the hatching curve significantly. Using the log-logistic model, final hatch can be predicted with a certain error before the actual hatching test ended, but in general final hatch is underestimated. When an error of 5% is accepted, the length of time required to perform a hatching test of a laboratory experiment can be reduced by 80% for untreated batches and by 40 to 80% for batches treated with nematicides. Acceptable reduction is negatively correlated with the concentration of the fumigant used.

Hatching tests with cysts originating from field experiments are unsuitable for prediction using a time limited data set. In cyst batches from the field compound hatching curves could be distinguished in 4 out of 6 fields, indicating that the soil samples contained at least two fractions of cysts with different hatching responses. Prediction would cause a significant underestimation of final hatch and consequently an overestimation of mortality.

4.2. Introduction

Several methods have been developed to distinguish between living and dead potato cyst nematodes, such as 'pricking' individual eggs (Fielding, 1951), the 'kinked larvae' method (Staniland & Stone, 1953), colouring with various dyes (e.g. Boyd, 1941; Kämpfe, 1956; Fenner, 1962; Shepherd, 1962), bioassays (Hijink, 1971), hatching tests (Fenwick, 1949, 1950a) and measuring the adenosine triphosphate (ATP) content of nematodes (Atkinson & Ballantyne, 1977*a,b*).

Most of these methods have severe disadvantages especially when used to evaluate nematicide trials. E.g. pricking the egg shell and observing the way the larva emerges is too labourious to use for experiments where tens of thousand individuals have to be screened. Bioassays, which measure the multiplication of cysts after a nematicide treatment are biassed as they ignore the density dependent multiplication rate of nematodes (Seinhorst, 1993), which will obscure the true result of the fumigant application.

Three methods are used frequently, 1) staining with New Blue R, 2) ATP-analysis and 3) hatching tests. The simplest is to stain dead eggs and larvae with New Blue R. An immersion period of one week in a 0.05% solution of the stain, after soaking in water for a week, was claimed sufficient and the proportion of 'doubtfuls' rarely exceeded 2-3% (Shepherd, 1962). The method has been used in The Netherlands, but it had to be adapted in several ways. The concentration of the New Blue R was doubled and the period used to stain the cyst contents extended to two weeks. An immersion period of one day in clear water had to be added to discolour eggs-shells. A period of six months was introduced between nematicide treatment and testing, as the cyst content of young cysts always coloured during this period regardless of being dead or alive (Van Eck, 1978) and not all dead eggs of older cysts stained in that period (Hijink, 1971). The percentage 'doubtfuls' was higher than found by Shepherd (Van Eck, 1978). Notwithstanding numerous improvements, the method was found to be too variable for use in scientific experiments, so that it can only be applied as a kind of quick-and-dirty method to take 'a first glance'.

The second method, ATP analysis, although elegant and fast, has its own pitfalls as bacterial ATP is measured too. The method was investigated in The Netherlands by Van Eck (1978) but results were inexplicable and coefficients of variation (*cv*) were high (58%). The method is still being pursued because of its potential to save time. Recently, Huijbregts *et al* (1996) estimated viability of *Heterodera schachtii* populations by measuring ATP, ADP and AMP contents directly using HPCL.

The third method, the hatching of potato cyst nematodes using the natural hatching agent of the potato plant, was originally developed by Fenwick (1949, 1950a,b, 1951a,b). He elegantly described the hatching process of cyst nematodes and the influence of nematicide treatments and physical conditions on the hatching process. The hatching process needed an increasing time period with increasing doses of nematicide applied and could last for more than three months. Although Fenwick (1951b) also provided a faster method which saves considerable time his methods are rarely used. Other researchers employing hatching tests reported high variability.

Bunt & Van Eck (1978) compared the three methods and found hatching tests with natural and artificial compounds to be the most reliable method for measurement of mortality. Still, a *cv* of 25% for the hatching tests was reported.

However, sources of error contributing to the variability of hatching tests can be identified (see next section) and it should be possible to redesign hatching tests in such

a way that their accuracy is increased. Several experiments were undertaken between 1985 and 1988 to quantify the effect of fumigants on the mortality of *Globodera* spp. Two laboratory experiments, one with 1,3-dichloropropene (Schomaker & Been, 1998*a*) and one with methyl-iso-thiocyanate (MIT) (unpublished), each performed at three different dates after treatment, and two field experiments, each in three fields on marine-clay soils (Been & Schomaker, 1998; unpublished data), were used to analyse and improve hatching tests. The results are presented in this paper.

4.3. Problems

Several sources of error increase the variability of hatching tests, especially when conducted to estimate mortality after a nematicide treatment.

I. Hatching device. Hatching devices were mostly designed of materials such as plastic, using glues for construction. These materials were not inert and the chemicals evaporating from these devices influenced hatching tests and added to their variability (Den Ouden, personal communication). Equipment, therefore, should be made of inert materials, preferably glass.

II. Test unit. Almost always a certain predetermined quantity, be it number (Bunt & Van Eck, 1978), volume or weight (Arntzen, 1993), of cysts is used in hatching tests. However, cysts vary in size and in number of eggs/cyst, even with cysts of the same size. If populations contain cysts of different age classes (field populations), even empty cysts will be included and variability will be high. If these field populations have been treated with nematicides variability will be even higher.
Some authors reported a linear relationship between a volume or weight of cysts in a sample or batch and the corresponding number of eggs in that sample (Arntzen, 1993). There is no doubt about such a correlation but its application can be questioned. Keeping in mind that the parameters of this relationship only apply to the population tested (origin and age being crucial factors for every population and therefore by no means transferable to any other batch), the confidence intervals of the regression parameters are unacceptable large. Other researchers tried to reduce the variability of cyst contents by grading them and using a certain fraction which had the lowest variability (Twomey *et al*, 1995).
The use of laboratory reared populations, containing only healthy cysts with a complete cyst content, can decrease variability of the cyst content but proved to be insufficient (see below). Fenwick (1950a) reported a *cv* ranging from 7 to 21% for hatched untreated larvae when batches of 100 cysts were used. Been & Schomaker (1998) and Schomaker & Been (1998*a*) used batches of counted cysts, with numbers up to more than 3000 cysts/batch, for their nematicide trials. Of all these batches the total egg content was estimated. Coefficients of variation of these batches are displayed in Figure

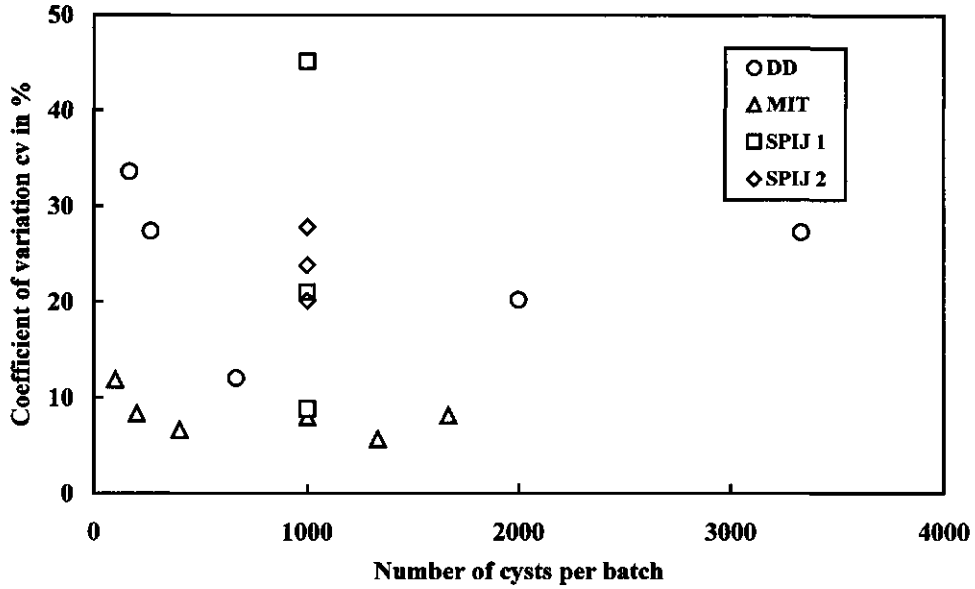


Figure 4.1. - Coefficient of variation in per cent of the number eggs and larvae per batch of cysts (*Globodera* spp, see Table 4.1.), with 15 to 120 replications per batch and batch sizes ranging from 166 up to 3300 cysts/per batch (manually counted), originating from two experiments with laboratory reared populations (DD, MIT) and two field experiments (SPIJ1, SPIJ2), each with three field populations (see also Table 4.1.).

4.1. (○ and △). Even these one year old laboratory reared populations, grown on potatoes in pathogen-free artificial soil, displayed a *cv* as large as 33%. Six field populations (□, ◇) had a maximum *cv* of 45% at 1000 cysts/batch. On the other hand, the population used in the laboratory experiment with MIT had a *cv* less then 10%, like one of the field populations in Figure 4.1. When hatching tests are carried out (especially in comparative experiments) the use of cysts as inoculum results in a large source of variation with unknown magnitude as to the number of hatched larvae.
Treatment error and experimental design error will be added. In nematicide tests, where relative hatchability is calculated using the number of untreated hatched larvae as a reference, the cyst error is included twice and can it be so large that any resulting survival ratio will be meaningless. The only possible solution for this problem is to eliminate the variability of cyst contents by substituting cysts as a primary test unit by eggs.

III. Recovery. Hatching tests with nematicides were frequently conducted too soon after application of a nematicide treatment. Then, hatching may be completely inhibited. Fenwick (1950a) called this effect a 'prolonged delay in hatching'. Been &

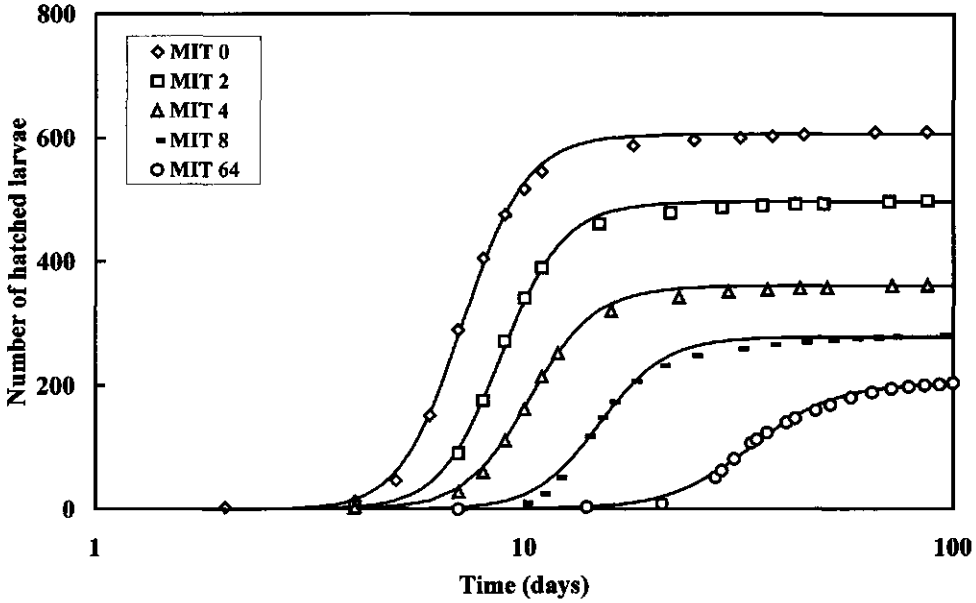


Figure 4.2. - Hatching curves of batches of larvae of potato cyst nematodes (*Globodera rostochiensis*) exposed to different dosages of methyl-iso-thiocyanate (MIT 2, 4, 8, 64 -µg/ml) and a untreated control (MIT 0), unpublished results. A hatching curve shows the cumulated total number of hatched larvae plotted against log time in days.

Schomaker (1987) and Schomaker & Been (1998*a*) reported that the Z-isomer of 1,3-dichloropropene inhibited hatching completely during 6 weeks after application. The effect decreased gradually afterwards and 7 months after application all surviving larvae had recovered from treatment and were hatchable. Larvae at low dosages of the E-isomer of 1,3-dichloropropene also recovered partly in the course of time. Hatching tests, therefore, have to be delayed either until after any temporary effect has vanished or a biologically meaningfull time period has passed, e.g. the period between soil fumigation in autumn and potato planting in spring.

IV. Duration. Most hatching test were carried out during a fixed time period. Fenwick (1957) already described that the hatching process was retarded by nematicides, independent of the date of the hatching test. The retardation was proportional to the dosage applied. Seinhorst & Den Ouden (1973) and Schomaker & Been (1987, 1998*a*) reported the same effect for 1,3-dichloropropene (both isomers) and Schomaker & Been (unpublished data) for MIT (Figure 4.2.). Hatching tests can last up to three or four months, especially when, in field experiments, cysts from different depths of the tilth are exposed to a different dosage. In The Netherlands, all hatching tests for the efficiency of fumigant nematicides in field experiments lasted only 6 weeks. As a

result mortalities were overestimated since, at the time of termination of the experiments, hatch was still in the 'near linear' part around the point of inflection of the log-logistic hatching curve (Figure 4.2.). Figure 4.3. gives an example from a hatching test with a fixed time period of 98 days using cysts from a farmer's field, treated with 1,3-dichloropropene in the laboratory. As only 40 cysts per replication were used it became difficult to fit the hatching curve and to estimate the final numbers hatched. It is obvious that even 98 days were too short a period to estimate mortality accurately.

V. Biotic constraints. Because of the long duration of hatching tests the risk of bacterial or fungal growth interfering with the hatching process is considerable. When potato cyst nematodes emerge from their eggs, the egg content surrounding the larvae will spill into the hatching fluid, adding trehalose. This sugar, which normally protects the larvae within the egg against environmental factors (desiccation, frost), supplies nutrients for bacteroids and fungi. When field populations are used, microorganisms are automatically introduced into the test cups.
Adding Streptomycin helps to suppress bacterial growth without interfering with the hatching test. Periodical renewal of the hatching agent, which decreases in activity, will also remove the released trehalose. Therefore, the hatching device should consist of two compartments, one containing the hatching agent, the other one containing potato cyst nematodes, separated from each other by a material permeable for the hatching agent and the hatched larvae.

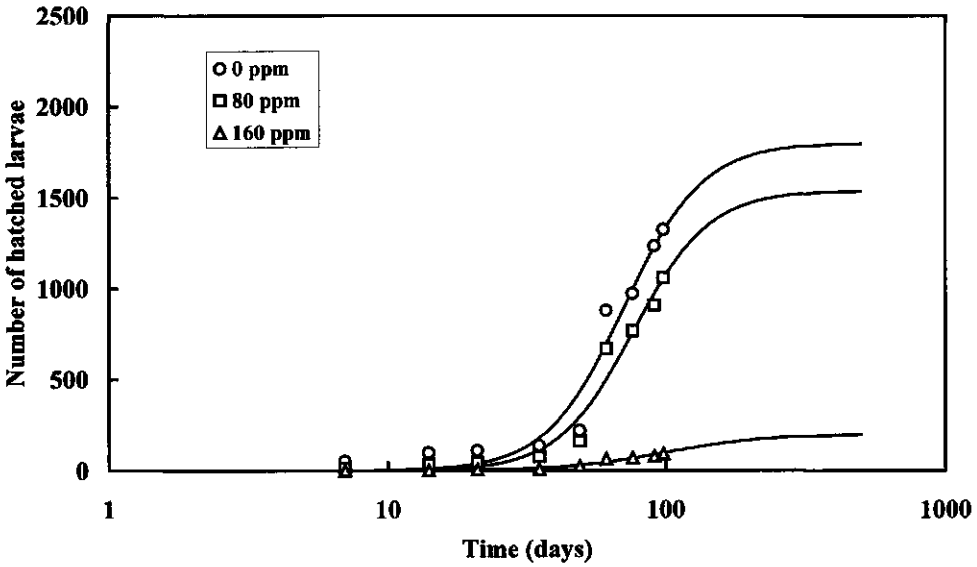


Figure 4.3. - Hatching curves of larvae of potato cyst nematodes (*Globodera pallida*) treated with 1,3-dichloropropene at two concentrations and of an untreated control. Data obtained from a hatching test with a duration fixed at 98 days. Solid lines show the expected hatching curve and final hatch, using a GENSTAT V program for fitting the log-logistic hatching curve.

4.4. Materials and Methods

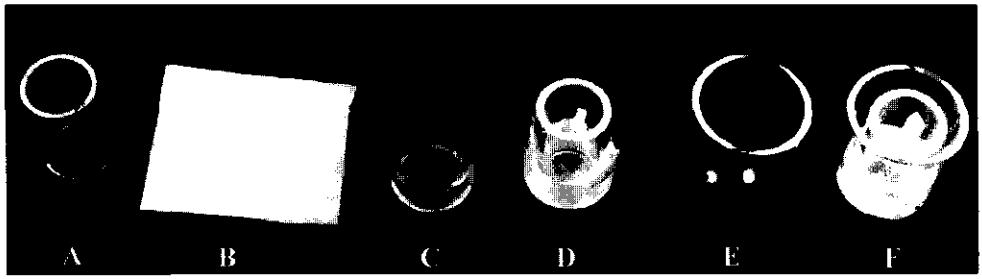
I. Hatching device

The hatching device described is a modification of the hatching device (30µ aperture sieve placed in tubes with the hatching agent) used by Seinhorst & Den Ouden (1973). It consists of a glass tube (14 mm diam, 20 mm length, Figure 4.4.a) with both cut ends rounded. One end, now the bottom end, will be covered by a gauze (22.4 µm, Monadur; 30 by 30 mm, Figure 4.4.b), attached to the glass tube by a 0.5 cm piece of inert medical tubing (Nalgene 8000, NON-TOXIC autoclavable Lab/Food, Grade tubing 7/16" IDx 1/16" Wall, Figure 4.4.c). For convenience, the whole is called test tube (Figure 4.4.d). Specimens will be pipetted onto the gauze inside the test tube. The stated mash size will retain eggs and, if still present, cyst walls but cannot withhold active larvae. The test tube is placed in a glass cup (Figure 4.4.e) with only one side open and the other side closed and concave (20 mm diam, length 28 mm). Together they form a hatching device (Figure 4.4.f). By using a concave bottom, the outside tube permits the test tube to be submerged into the hatching fluid but not to rest on its bottom. Only one ml of hatching agent is required to suspend all eggs. Refreshment of the hatching agent is easily performed by placing the test tube in a new cup containing fresh hatching agent. The old cup can be stored in at 4°C in a refrigerator before counting. Up to 25 hatching devices can be placed in a specially designed perspex carrier (dimensions 12.7 * 16.2 cm), which in turn is placed inside a plastic box (Figure 4.4.g) which only has a few small openings, to prevent fast evaporation of the hatching agent. Plastic boxes can be stacked and placed inside a incubator, in the dark, at the optimal hatching temperature of 20°C (Fenwick, 1951a). Extra dishes with water are placed inside the incubator to increase humidity and to prevent evaporation from the hatching devices. As the hatching process is optimal in the dark (light inhibits hatching), the incubator is quite handy.

II. Test unit

Hatching tests should be conducted with known numbers of individuals so that variability is minimized. Thus, batches with known numbers of eggs are used instead of known numbers of cysts. Batches of at most 1000 cysts are presoaked in 1,5 ml water in a glass vial (15,5 mm inside diameter and 75 mm high), for at least 24 hours before being crushed in suspension. Cysts are crushed inside the glass vials using a plunger (Seinhorst & Den Ouden, 1966) to free eggs and larvae, approximately 95 and 5% respectively, without damaging them. At higher cyst numbers (>1000) not all cysts will be crushed and batches have to be split up before crushing. Eggs and larvae are retrieved by sieving out cyst walls using a 250 µm sieve. Prior to the density estimation, the resulting suspension is diluted to obtain approximately 1000 eggs and larvae per ml by presuming an average cyst content of 200 eggs per cyst and adding the required amount of water.

Eggs and larvae sink to the bottom of the measuring glass because of their specific



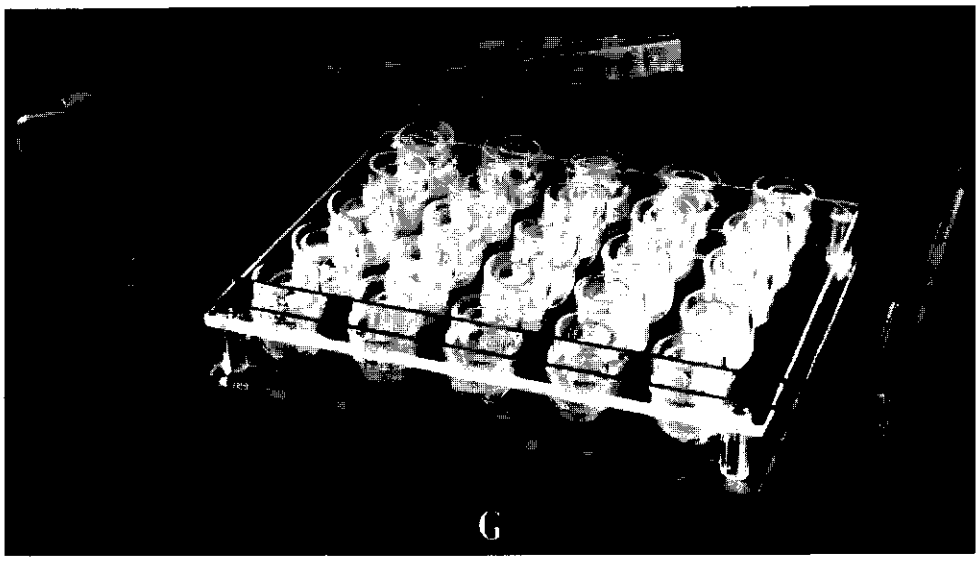


Figure 4.4. - Material used for the hatching tests: A: small glass tube (14 mm diam., 20 mm length) both openings rounded. B: A piece of gauze (3 by 3 cm), 22,4 µ mesh. C: A piece of medical tubing (diameter 7/16", 0.5 cm long). D: Outer glass cup; Duran glass (20 mm diam. 28 mm length). E: perspex carrier; two perspex plates 12.7 * 16.2 cm, the upper one with holes (diameter 21 mm) for inserting hatching vials. F: Complete hatching device. G: Plastic box 22 * 14 * 5 cm with perspex carrier holding 25 hatching devices.

weight. Before pipetting a certain amount into a counting chamber or onto the gauze inside the test tube, the eggs and larvae have to be brought in suspension again. Re-suspending has to be done by random movements rather than by stirring as the latter will cause the larvae to rotate alongside to the walls of the measuring glass. If the procedure is followed scrupulously, the pipetting error will follow a Poisson distribution. Of the suspension, a volume of at least 0.5 ml is counted to estimate the real density in the stock solution. If less than 300 eggs were counted another 0.5 ml was

counted to stabilize estimation error. A minimum of 300 eggs counted yields a *cv* of 5.8% (STEP 2, Appendix A). Next, the required number of eggs was pipetted into the test tube by using an adjusted volume based on the density estimation. As only the volume pipetted is adjusted, the actual error (expressed as *cv*) made when pipetting the required number of eggs will be the same as that made when the density of the stock solution is estimated.
Because it is sometimes impossible and most of the times inconvenient to try and pipet the exact volume needed to obtain the desired number of larvae, the actual volume pipetted is adjusted upwards (e.g. 1,1567 ml will be set to 1,2 ml) and a correction factor is calculated to transform all future hatching data from that hatching device.

III. Considerations about nematicide trials
Field trials. In field trials with nematicides cysts will be exposed to a certain dosage of the nematicide. All eggs within one cyst will have been exposed to the same dosage and their response (susceptibility) to the chemical will be more or less identical. Different cysts, however, will be exposed to different dosages, either because of the vertical location of cysts in the tilth or because of local variations in soil structure. In marine clay soils, cysts can be incorporated in clods and thus be out of reach of the chemical. - Thus, even though there might be differences in sensitivity to the nematicide between eggs within a cyst, mortality will vary more between cysts. Therefore, the assumption is made that, according to the dosage applied, the cyst content is either completely killed or completely alive. When a soil sample is collected and a certain number of cysts is extracted, a binomial error for the number of live cysts extracted from the soil sample is added. When, for instance, 70% of 1000 cysts collected are dead a *cv* of 4.8% is introduced for the number of live cysts (STEP 1, Appendix A).
Laboratory trials. The rigid assumption that cysts exposed to nematicides are completely killed or completely alive when cysts are exposed in the laboratory (Schomaker & Been, 1998*a*), is questionable because under laboratory conditions no physical barriers to the chemical are present. In that case, the number of cysts needed for an experiment can be drastically reduced as the egg will be the primary unit affected by the nematicide so that only the number of eggs exposed to the nematicide will require a certain minimum.
General. Because cysts are easily reared, cysts were regarded as the primary unit affected by the nematicide in both laboratory trials discussed in this paper. After preparation of the stock solution a certain number of eggs is pipetted into the test tube. This fraction consists of dead and alive eggs. Again, when drawing a sample from that stock solution a binomial error is added, identical to STEP 1 (STEP 4, Appendix A).
Both binomial errors can be minimized by adapting the number of cysts and the number of eggs per test tube to the mortality expected, as has been done in the second laboratory and the second field experiment.

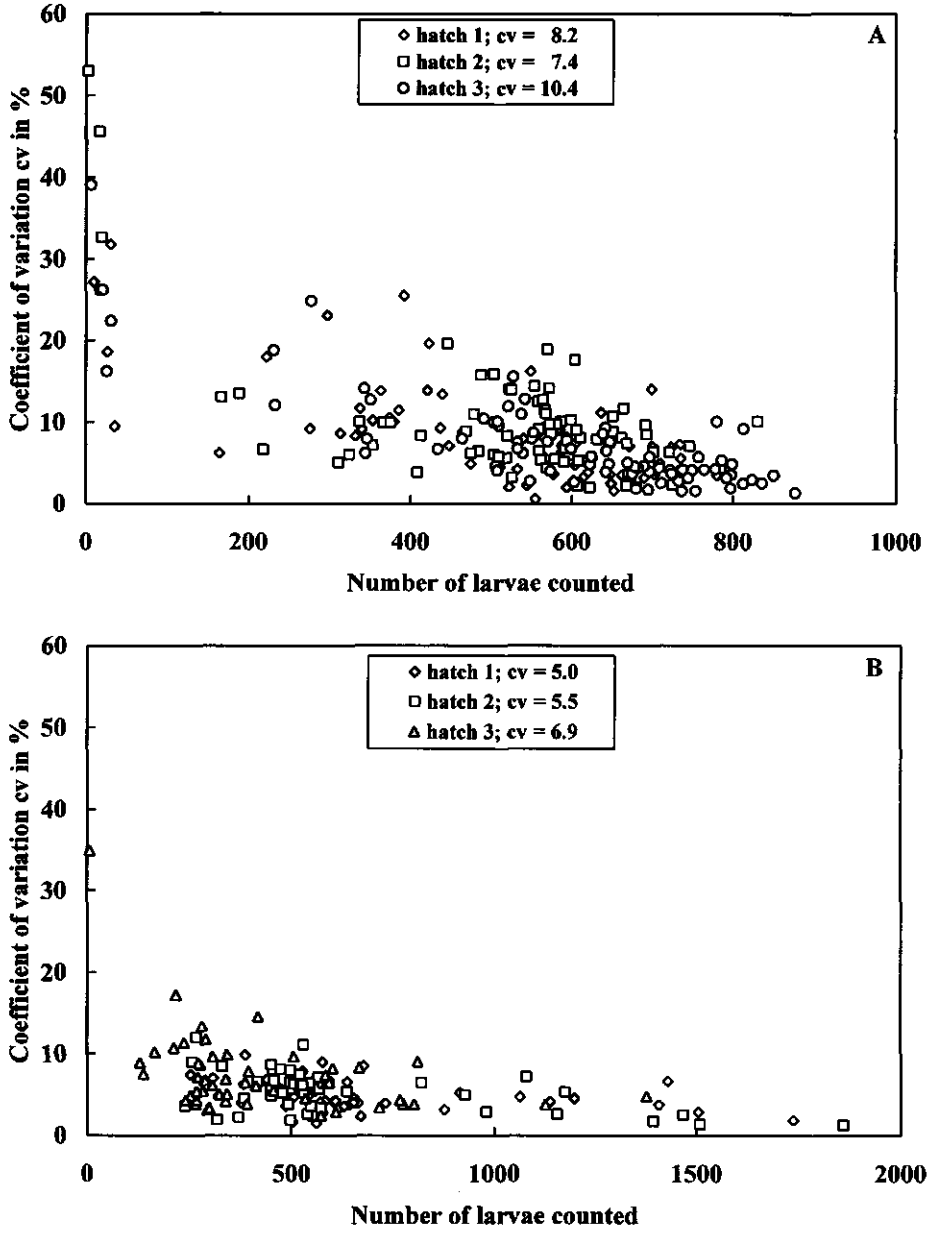


Figure 4.6. - A: Coefficient of variation in per cent of the replicates per hatch, used to measure variability of the hatching process in lab experiment DD, plotted against number of larvae counted. Hatching tests were repeated in time (Table 4.1.). B: Coefficient of variation in per cent of the replicates per hatch, used to measure variability of the hatching process in lab experiment MIT, plotted against number of larvae counted. Hatching tests were repeated in time (Table 4.1.).

Per hatch, mean *cv* ranged from 7 up to 10%. In the second laboratory experiment, with MIT, the number of eggs pipetted per test tube was adapted to the expected mortality and had a maximum of 4000 eggs/test tube at the highest density. Now, sufficient numbers of hatched larvae could be counted at higher mortalities too, and the mean *cv* per hatch was between 5 and 7% (Figure 4.6.B.). Mean *cv*'s (Table 4.1., last column) were highest in the first field experiment (SPIJ1, 2000 larvae pipetted), especially in field A with a mean *cv* of 15.8%, but were of the same order as for all other experiments due to the adaptation of the number of larvae per hatching device to the expected mortality.

In Table 4.1., mean *cv*'s of the untreated batches are provided for all four experiments and sub-experiments analysed. The minimum *cv* expected was calculated using the equations in Appendix A and is presented as 'expected'. There are no significant differences between expected and observed *cv*'s in all experiments except in field A. More detailed investigation of the data from field A revealed some deviant replications yielding differences of more than 100% of hatched larvae although sufficient numbers could be counted. This exceptional result, among hundreds of untreated and treated batches investigated, is considered to be caused by an inoculation error rather than actual variability.

Using Appendix A, the expected minimum *cv* for treated batches was calculated. To be brief, only the relation between observed and mean expected *cv*'s per replicate of the hatching test for MIT is presented graphically in Figure 4.7.A. For all three hatching dates the expected *cv*'s, calculated using assumption t3 (Appendix A), were higher than the observed *cv*'s. In Figure 4.7.B the observed mean *cv*'s per treatment are related to those expected using assumptions t3 and t4 (Appendix A), which only differ in the unit the nematicide acts upon (cysts versus larvae). In Table 4.3. this comparison is presented for all sub-experiments with a + indicating the best fit. Overall, there is no way to decide which assumption is more probable, the main reason being the fact that the number of cysts involved in all experiments was so large (to accommodate for the error according to STEP 1, Appendix A) that the error according STEP 1 was small anyhow.

II. Treatment error

Analysis of error terms showed that variation between replications of the treatments was much higher than the variation between replications of the hatching process. In Figure 4.8. the relation between the two errors is presented for all treatments of the 12 sub-experiments. Treatment error was larger than hatching process error, 4 to 5 times in the lab experiments and over 10 times in the field experiments.

III. Modelling the hatching curve

Table 4.4. gives the goodness of fit of the log-logistic model, expressed as the variance accounted for, R^2, in four experiments and sub-experiments. For comparison the hatching process was also fitted to a probit curve. For all twelve sub-experiments the

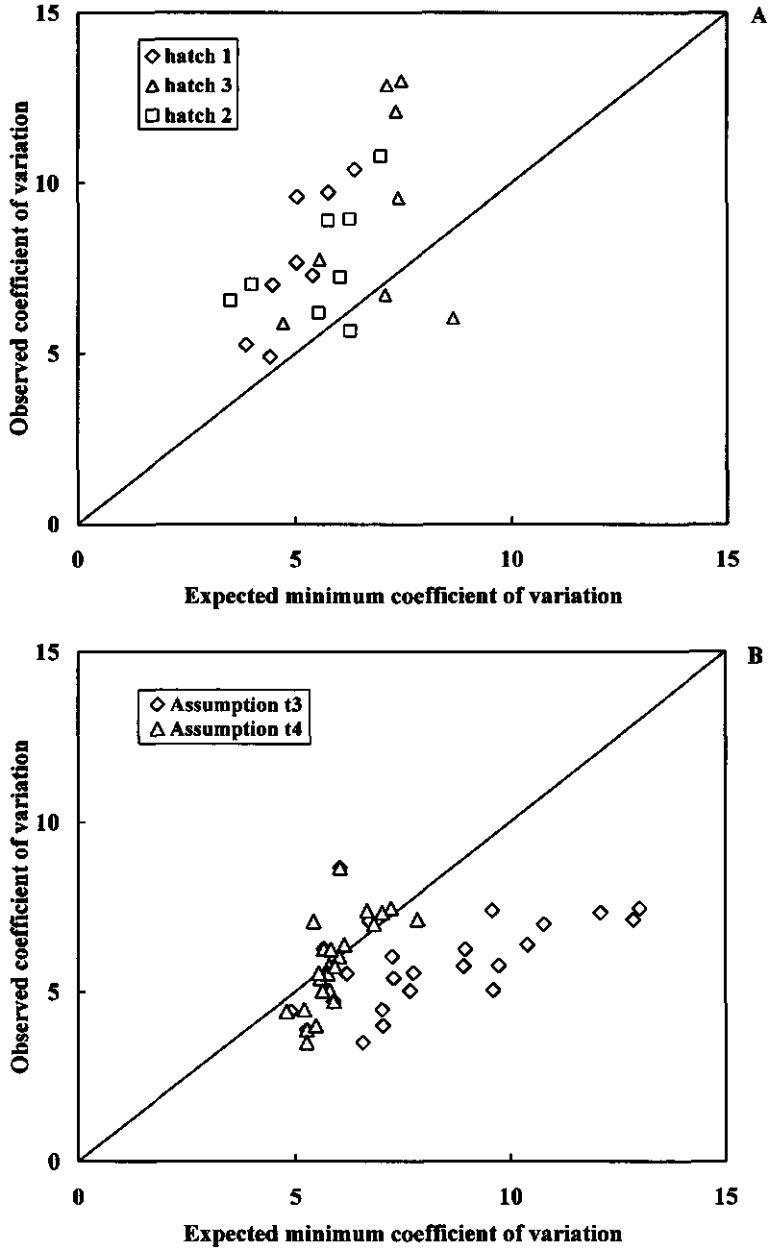


Figure 4.7. - A. Relation between expected minimum *cv* and observed *cv*, both in per cent, for the replications of nematicide treatment (control and 7 dosages) of MIT, according to Appendix A, at all three hatching dates. B. Relation between expected minimum *cv* according to assumption t3 and t4 (Appendix A), concerning the primary unit affected by the nematicide, respectively, cysts and eggs, and observed *cv*, both in per cent for all treatments and hatching dates.

Table 4.3. - Expected minimum error according to assumption t3 (cysts as the primary units) and t4 (eggs as the primary units, see Appendix A) compared to observed error for all twelve sub-experiments. '+' indicates best fit to observed error, '*' indicates that the best fit is significantly better than the fit according to other assumption.

Experiment	Sub-experiment	Assumption t3	Assumption t4
I. DD	hatch 1	+*	
	hatch 2		+
	hatch 3	+	
II. MIT	hatch 1		+*
	hatch 2		+*
	hatch 3		+*
III. SPIJ 1	field A	+*	
	field B		+*
	field C	+*	
IV. SPIJ 2	field H		+
	field K		+
	field V		+

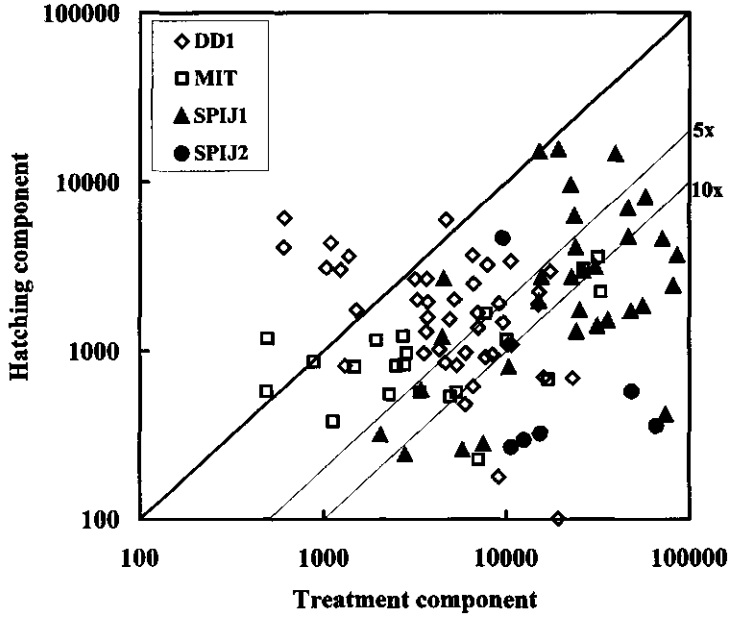


Figure 4.8. - Components of variability (hatching component versus treatment component) of all treatments for all sub-experiments of Table 4.1. Generally, treatment error is 5 times and 10 times higher than hatching error for lab (DD, MIT) and field (SPIJ1, SPIJ2) experiments, respectively.

log-logistic curve provided better fits. Although the improvement is only small, it was significant because of the large number of lines fitted. The under and over estimations of fitted relative to actual values, listed in the last three columns of Table 4.4. as 'Absolute error (%)', remains low for the two lab experiments, but is higher for the field experiments.

When hatching curves were examined closer, a systematic deviation of the calculated curves from the real data was observed, notwithstanding the high fit already obtained. The log-logistic model overestimates actual cumulative counts in both the concave and convex part of the sigmoidal hatching curve producing a systematic error (Figure 4.3.). The deviation is primarily caused by the assumption that hatching starts at $t = 0$, immediately after the eggs are submerged in the hatching agent. The introduction of a delay factor - an 'incubation time'- as an extra parameter in the log-logistic equation improved the fit between observed and calculated values (Table 4.4.), with nearly 100% of the variance accounted for in individual curves. The delay factor spans the period from the first submerging of the eggs in the hatching agent over the time required for the hatching agent to reach the nematode till the moment the first nematode emerges from the egg. The equation is altered as follows:

$$F(t)=\frac{\lambda}{1+\exp^{-\beta*\log(t-\gamma)-\alpha}} \qquad \text{For } t > \gamma \qquad (2)$$

and

$$F(t)=0 \qquad \text{For } t \leq \gamma$$

γ: Time span between submerging the eggs in the hatching agent over the time required for the hatching agent to reach the nematode till the moment the first nematode emerges from the egg (in days).

In some of the field sub-experiments the error became quite high (Table 4.4.). In those cases, closer inspection of the relevant hatching curves indicated that two or more curves were superimposed. The seemingly good fit for field A, with a small error, consisted also of more than one curve. Figure 4.9. shows hatching curves from fields A and C of the SPIJ1 experiment. Original data, overall fit, fit of separate curves and a compound fit of larvae from one test tube are presented. Figure 4.9. suggests that the soil samples contained two and three fractions of cysts, respectively, with different hatching curves. The same effect was found in the hatching curves of fields H and K of the SPIJ2 experiment, but was absent in fields B and V or any of the laboratory tests.

IV. Prediction

The log-logistic curve (with and without delay) was used to test the possibility to predict the final hatch using a data set reduced in time. By iteratively omitting the last data point in time and performing new analyses both R^2 and final hatch (λ) were estimated. One lab experiment, MIT1, and one field experiment, SPIJ1, were used.

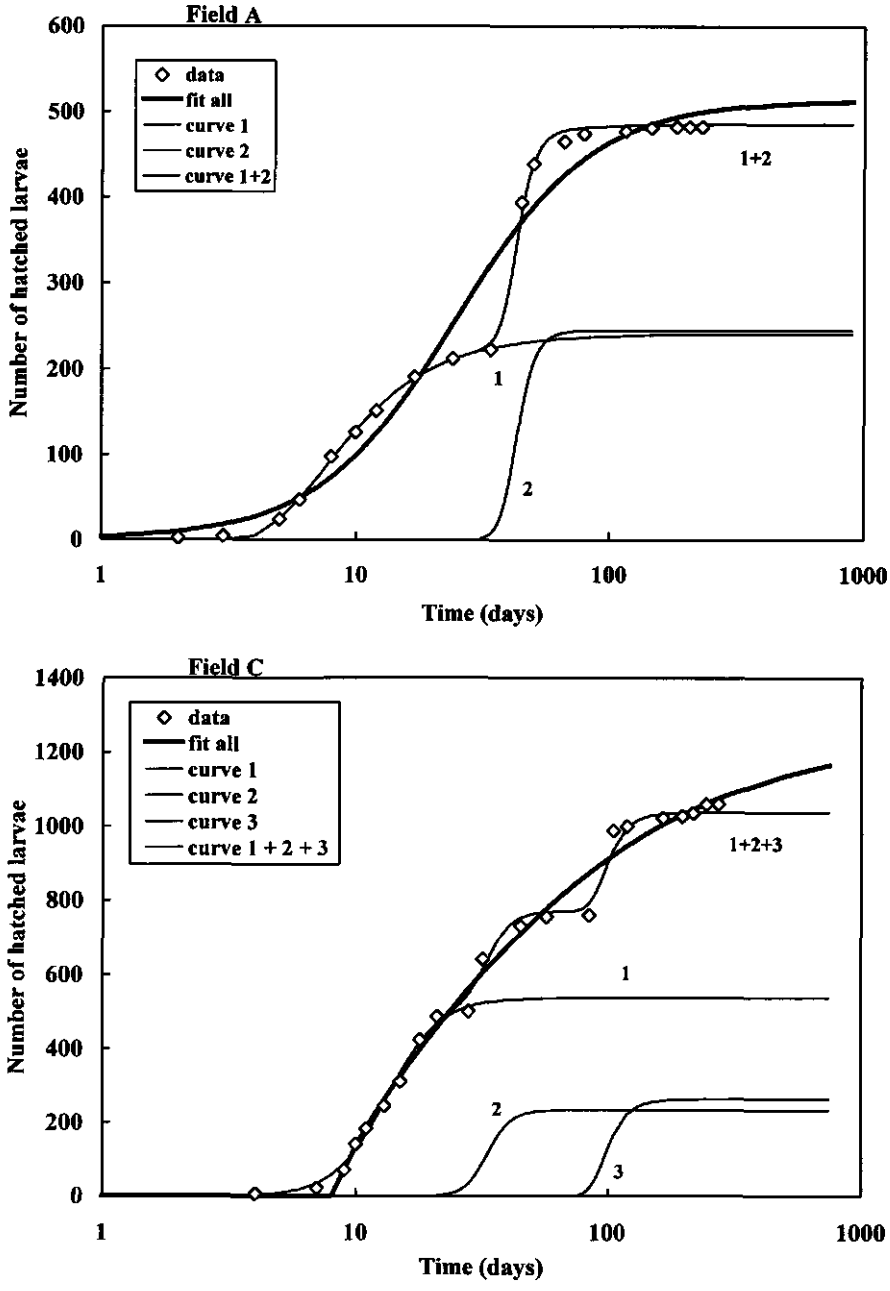


Figure 4.9. - Compound hatching curves originating from fields A and C of the SPIJ1 experiment (Table 4.1.) depicting two and three superimposed hatching curves, respectively. Original observations (◆), overall curves fitted according to eq. 2 using all data, separately fitted curves and a compound curve using two, respectively, three log-logistic equations.

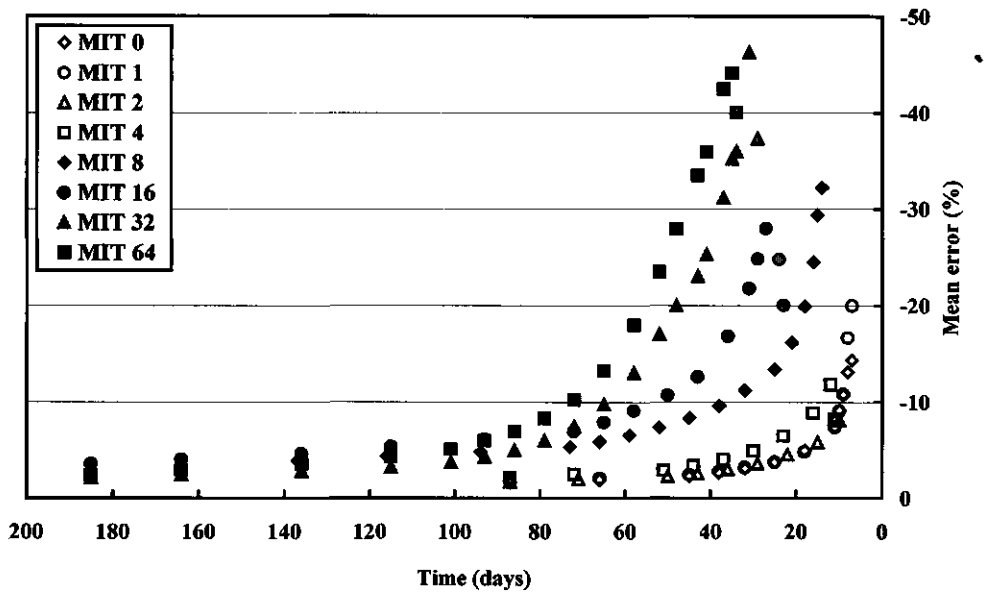


Figure 4.11. - Mean error (in per cent) of final hatch of 30 hatching curves per treatment of the MIT lab experiment, first hatching date, versus time of duration of the experiment. Treatments: 0, 1, 2, 4, 8, 16, 32 and 64 µg/ml of MIT. Mean error is negative because estimations of final hatch underrate the real mean cumulative number of larvae hatched (between 400 and 800).

the expected error should be minimized by using as many cysts and eggs as possible to obtain enough larvae. Cysts originating from field plots tend to have lower hatching percentages than cysts reared in the laboratory. Sometimes cysts originating from the field are parasitized; sometimes they have been subjected to a nematicide treatment in a previous year. Therefore, it is recommended to precede each experiment by testing a batch of 1000 untreated larvae for hatchability. By adjusting, by way of a small pilot test, the concentration of the hatching agent so that the largest possible fraction of the pipetted larvae actually hatches, a larger number of larvae can be counted. If, for some reason, hatchability of the control is low the number of eggs to be used in the test tubes has to be increased over all treatments. Adjustment of the number of hatched larvae to be counted per test tube to 700-1000 in the final experiment is advisable. The easiest way to minimize error even more is by increasing the number of eggs counted when estimating the population density of the stock solution.

The most critical point in using egg suspensions is the process of keeping eggs and larvae suspended in the stock solution without causing segregation (e.g. by stirring); in other words to obtain a stock solution with Poisson distributed eggs and larvae. The method of grinding cysts and making egg suspensions is now in use in most Dutch Re-

search Institutes, not only for hatching tests but also to obtain precise initial population densities for population dynamics research (e.g. Schomaker *et al*, 1995, Beniers *et al*, 1995) and to estimate the partial resistance of potato varieties (Schomaker *et al*, 1994). Recently, medical apparatus using air bubbles has successfully been applied to keep eggs and larvae in continuous random movement (Molendijk, personal communication).

Experimental error

The variation between replications of nematicidal treatments proved to be larger than between replications of the hatching process. For field experiments this is not a great surprise as the applied dosages vary vertically in the tilth because of the injection depth and of redistribution of the fumigant and horizontally by fluctuations of driving speed of the injector and differences in soil structure as well as the accessibility of cysts in clods of soil. However, the large variation in laboratory experiments between identical concentrations of 1,3-dichloropropene and MIT was a surprise. As these replications came from the same stock solution of the fumigant this error originates from the transfer of a sample from the fumigant solution to the vial in which the cysts were exposed. This problem can only be addressed by a more sophisticated methodology of exposing nematodes to pesticides or by a larger number of replicates to reduce the standard error. When hatching tests are performed in the manner described in this paper, no replications of the hatching process are required. Instead, an adequate number of replications of treatments can be incorporated in the test. In the second field experiment (SPIJ 2) the number of replicates per treatment was quadrupled and replicates of hatch drastically reduced. The *cv* of the mean mortality by the fumigant application could be reduced from 14.% to 8% (reduction by 43%) as expected.

Hatching curve

Fenwick (1951b) already stated the futility of allowing a hatching test to continue for any pre-determined time period as these tests can last from 10 days up to more than three month depending on age, origin and treatment imposed on the cysts investigated. He stated that individual counts of emerged larvae after several time periods should be made to obtain cumulative data for fitting the hatching curve to a validated model, thereby avoiding errors as shown in Figure 4.3. Only then, accurate estimations of final hatch can be obtained even when fungal and bacterial growth interferes with the later stages of the hatching process.

Modelling the hatching process proved valuable to describe the various ways nematicides act upon nematodes with more subtlety. Schomaker and Been (1998*a*) showed that, although low dosages of nematicides increased the final cumulative number of hatched larvae (λ) compared to the control, the start of the hatching process, after immerging the eggs into the hatching agent, was delayed as expected after application of a nematicide treatment. Thus, the hypothesis that increased final numbers at low dosages were artifacts caused by variability could be refuted.

The introduction of the delay factor improved the accuracy of the fit. The duration of the delay period increased with the increase of the dosage 1,3-dichloropropene the nematodes were exposed to (Schomaker & Been, 1998*a*). These findings contributed to the development of sophisticated models describing the effect of nematicides.
Thorough investigation of the hatching curves allowed to infer that the hatching process of fumigated cysts originating from field experiments on marine clay soils (Been & Schomaker, 1998) could be described by a compound curve constructed from more than one log-logistic curve. The phenomenon can only be explained by the presence of two or more distinct groups of cysts in the same sample. Exposure to different dosages of nematicide, as a result of one group being incorporated in clumps of clay and another not, as described by Been & Schomaker (1998), is not the cause as the controls displayed exactly the same behaviour. Fields B (SPIJ1) and field V (SPIJ2) displayed normal hatching curves like all laboratory experiments. Both fields were never fumigated prior to the field experiment in contrast to the four fields which did display compound hatching curves and had been subjected to one up to three earlier soil fumigations. If soil fumigation is considered the cause of the phenomenon the conclusion is warranted that the inhibition of hatch in time as shown in Figure 4. 2. persists over several years.

According to Fenwick (1951b), application of the probit transformation to the hatching process supplies a method to reduce the duration of these stratified tests by estimating the 'point of inflection' of the sigmoidal hatching curve. His method produced errors between 5-10%, saving about 70% of the required time to perform the hatching test. However, the critical part of this method was the eyeball estimation of the point of inflection. This parameter can be estimated using a maximum likelihood algorithm (GENSTAT; Payne, 1993). Fitting the log-logistic curve including the delay parameter has to be carried out with caution. By adding the delay factor γ, 4 parameters have to be estimated simultaneously. Starting with a least square fit of the logistic curve the estimates of λ , α and β can be obtained. No start parameter for the delay factor can be supplied and optimization can fail to converge. Sometimes a seemingly good fit (high R^2) produces nonsense estimates of the parameters, especially of the final cumulative hatch (λ). Even when a start parameter is estimated (by intelligently looking at your data), optimization can fail and sometimes several reruns are necessary to obtain a good fit. When a start parameter for the delay has to be provided, or when one is obtained from a successful fit, it does not necessarily coincide with the time interval after which the first larvae actually were counted. These first larvae may not have hatched but have been freed from the eggs when crushing the cysts to prepare the stock solution. Problems concerning the maximization process involved with the maximum likelihood estimation will increase when incomplete data sets are fitted,
The present research confirms Fenwick's findings, with the added restriction that application, in the case of nematicide experiments, is limited to laboratory experiments only. Field experiments have to be hatched over the full period of the hatching process.

Final remarks

The major disadvantage of hatching tests is not their variability but the length of time required to finish these tests and the amount of time to count the hatched larvae. The counting can easily be carried out using image analysis (Been *et al*, 1996). Recent improvements in hardware and software permits real time analysis of several hundred specimens per day. The time length required to finish a hatching test can be substantially reduced when experiments are carried out with laboratory reared cysts under controlled conditions.

The methodology described in this paper for hatching experiments can be used to push the minimum error under a certain threshold and even to stabilise variability over all treatments according to the expected mortality. To obtain the same *cv* at all dosages the number of cysts to be collected (assumption t3) and the number of eggs exposed in the hatching test has to be increased with increasing dosages of a nematicide. Appendix A provides a short list of predictable errors in hatching tests, the way to calculate them and the method to cope with them.

Appendix 4.1.

Sources of variation

STEP 1: Batches of cysts are exposed to different concentrations of a nematicide or are collected from the field after a nematicide trial. The basic assumption in these tests can be that cysts are the experimental units and that the cyst content (eggs and larvae) will either be completely dead or completely alive. After collection of a certain number of cysts, the numbers of dead and live cysts in that batch (which will be used in a hatching test) will suffer an error according to a binomial distribution.

Example:

$i := 1, 2 .. 100$ counter

$p_i := i \cdot 0.01$ 100 fractions of cysts surviving the treatment

$cysts := 1000$ number of cysts collected per batch for each treatment

$reptreat := 5$ number of replications of this treatment

expected number of live cysts: (m) $m_i := cysts \cdot (p_i)$

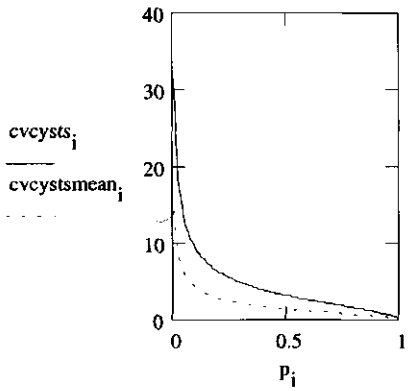
variance: (σ) $\sigma_i := cysts \cdot [p_i \cdot (1 - p_i)]$

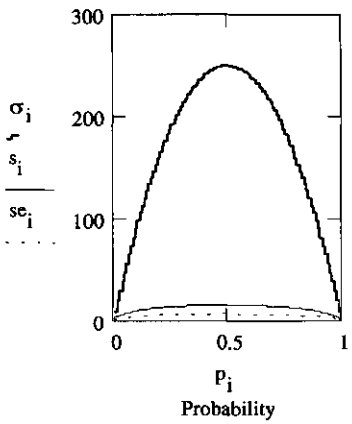
stdeviation: (s) $s_i := \sqrt{\sigma_i}$

standard error: (se) $se_i := \dfrac{s_i}{\sqrt{reptreat}}$

coefficient of variation: (cv) $cvcysts_i := \dfrac{s_i}{m_i} \cdot 100$

cv of the mean: (cvmean) $cvcystsmean_i := \dfrac{\frac{s_i}{\sqrt{reptreat}}}{m_i} \cdot 100$





If cysts are not the experimental units but eggs (so a certain amount of eggs are killed in each cyst) STEP 1 will be obsolete as then only the number of larvae used in the test will be of importance.
In field tests the first assumption definitely applies, however in vitro tests the second assumption seems to be more valid. As the first assumption causes the addition of a binomial error to the overall variation it is safer to include this error regardless of the test situation.

STEP 2: Every batch of cysts is crushed and a suspension of eggs and larvae is made. A certain volume (e.g. 0.5 ml) of this stock solution is used to estimate the density of eggs and larvae in that suspension. A minimum of 300 to 400 eggs and larvae is counted. If less are present another volume of 0.5 ml has to be counted fully. The error made when sampling the stock solution is in accordance with a Poisson distribution. Although afterwards maybe 1000 or more eggs and larvae will be pipetted into a hatching device, the error of the actual number pipetted will remain the same as the estimation error (expressed as coefficient of variation) as only the volume is adjusted to obtain the required number.

Example:

$count := 300$ number of eggs counted

$vc_{count} := \frac{\sqrt{(count)}}{count} \cdot 100$ coefficient of variation

$vc_{count} = 5.774$ result

The fraction of the larvae in the hatching vial that will respond to the hatching agent is by no means 100%. In hatching tests a percentage of 70 to 80% is feasible. So the number of larvae that actually will hatch depends on the hatchable fraction and another error is introduced. Just like with nematicide treated cysts, there will be the theoretical possibility that whole cyst contents are hatchable/non hatchable or that larvae are hatchable/non hatchable. There is, however, ample evidence that the first assumption is not valid (e.g. Fenwick, 1950a, b).

STEP 3: Using the first assumption an error similar to STEP 1 has to be included since the batch of cysts collected will consist of hatchable and non hatchable cysts. However, only the fraction of live cysts will be submitted to this error. Therefore, if 1000 cysts are used and mortality of cysts = 0.5 then n in STEP 3 will be 500.
When using the second assumption STEP 3 can be disregarded and only STEP 4 applies.

STEP 4: After pipetting, the fraction of larvae actually hatchable will add to the overall variability. Again this error will be in accordance with a binomial distribution:

Example:

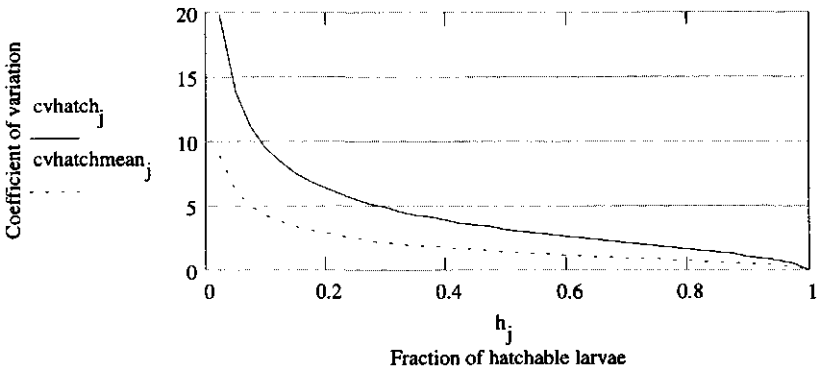
$j := 1, 2 .. 40$ counter

$h_j := j \cdot 0.025$ 40 fractions of untreated larvae (control) hatchable

$eggs := 1000$ number of eggs pipetted into the hatching vial

$hatchs := 5$ number of replications of hatching test per treatment

expected number of hatchable larvae:	(m2)	$m2_j := eggs \cdot h_j$
variance:	(σ2)	$\sigma2_j := eggs \cdot \left[h_j \cdot \left(1 - h_j \right) \right]$
stdeviation:	(s2)	$s2_j := \sqrt{\sigma2_j}$
cv of hatchability:	(cvhatch)	$cvhatch_j := \frac{s2_j}{m2_j} \cdot 100$
cv of the mean:	(cvhatchmean)	$cvhatchmean_j := \frac{\frac{s2_j}{\left(\sqrt{hatchs} \right)}}{m2_j} \cdot 100$



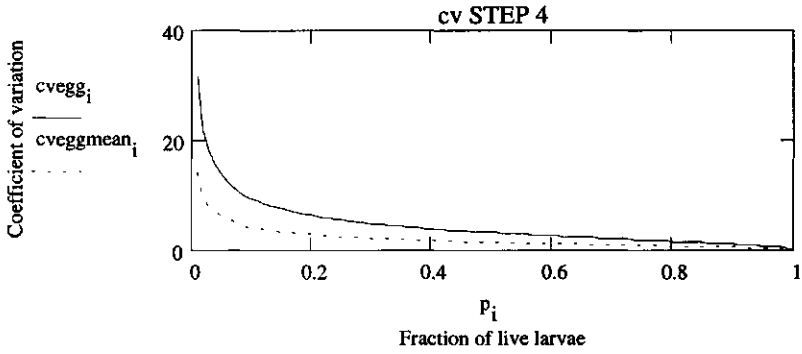
STEP 5: When cysts have been exposed to a nematicide, the percentage of larvae that will actually hatch depends in part on the expected fraction of the population that survived the treatment. STEP 1 already dealt with the unit cyst. After pipettation, however, the number of surviving larvae in the hatching device will also suffer an error according to a binomial distribution. This error has to be added whether or not STEP 1 applies.
NOTE: when eggs are treated STEP 4 only applies to the surviving larvae (increasing the error as n decreases).

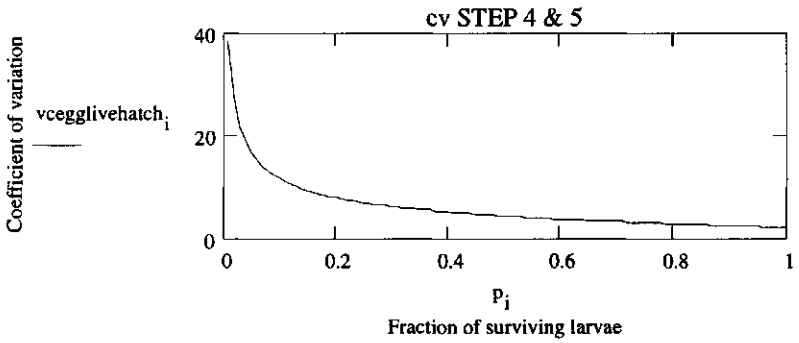
Example:

expected number of live eggs:	(m3)	$m3_i := eggs \cdot p_i$
variance:	(σ3)	$\sigma3_i := eggs \cdot \left[p_i \cdot \left(1 - p_i \right) \right]$
stdeviation:	(s3)	$s3_i := \sqrt{\sigma3_i}$
coefficient of variation of live eggs	(cveggs)	$cvegg_i := \frac{s3_i}{m3_i} \cdot 100$
cv of the mean:	(cveggmean)	$cveggmean_i := \frac{\frac{s3_i}{\left(\sqrt{hatchs} \right)}}{m3_i} \cdot 100$

step 4 and 5 combined:

$$vceggliveh atch_i := \frac{\sqrt{p_i \cdot h_{27} \cdot (1 - p_i \cdot h_{27}) \cdot eggs}}{p_i \cdot h_{27} \cdot eggs} \cdot 100$$





Components of variability can be added in two ways for untreated larvae and four ways for treated larvae, as following:

Untreated larvae:

u1: assumption: % cysts hatchable
Add: STEP 2 + STEP 3 + STEP 4

u2: assumption: % eggs hatchable
Add: STEP 2 + STEP 4

Treated larvae:

t1 assumption: % cysts hatchable; cysts dead/alive
Add: STEP 1 + STEP 2 + STEP 3 + STEP 4 + STEP 5

t2: assumption: % cysts hatchable; eggs dead/alive
Add: STEP 2 + STEP 3 + STEP 4 + STEP 5

t3: assumption: % eggs hatchable; cysts dead/alive
Add: STEP 1 + STEP 2 + STEP 4 + STEP 5

t4: assumption: % eggs hatchable; eggs dead/alive
Add: STEP 2 + STEP 4 + STEP 5

In view of the evidence that treated cysts from field experiments are either dead or alive and that hatchability is more likely to correspondent with larvae than with cysts, usually assumption u2 will apply to untreated larvae and assumption t3 for treated larvae.

Combining all sources of variation

Untreated larvae:

First let us calculate the coefficient of variation of the hatching process for the untreated batches, using the assumptions valid for u2:

cysts collected: $cysts := 1000$

eggs counted when estimating density of stock solution: $count = 300$

hatchability: $h_{27} = 0.675$

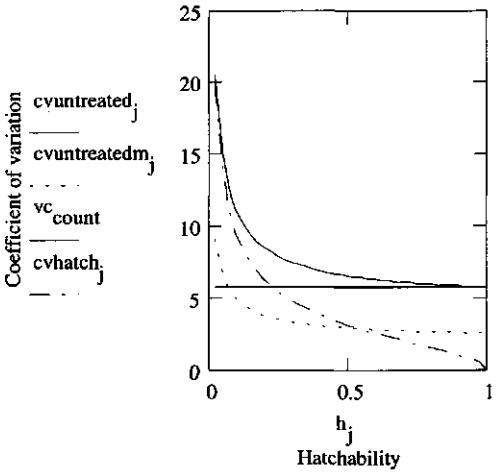
eggs pipetted into hatching device: $eggs = 1 \cdot 10^3$

coefficient of variation of one hatching vial:

$$cvuntreated_j := \sqrt{\left(cvhatch_j\right)^2 + \left(vc_{count}\right)^2}$$

coefficient of variation of all hatching vials:

$$cvuntreatedm_j := \frac{\sqrt{\left(cvhatch_j\right)^2 + \left(vc_{count}\right)^2}}{\left(\sqrt{hatchs}\right)}$$



Note: Although the coefficient of variation decreases as the fraction of hatchable larvae increases, the major error term is caused by the density estimation of the stock solution. A great deal of intrinsic variability can be reduced by a better estimation of this density, that is by counting more larvae.

Treated larvae:

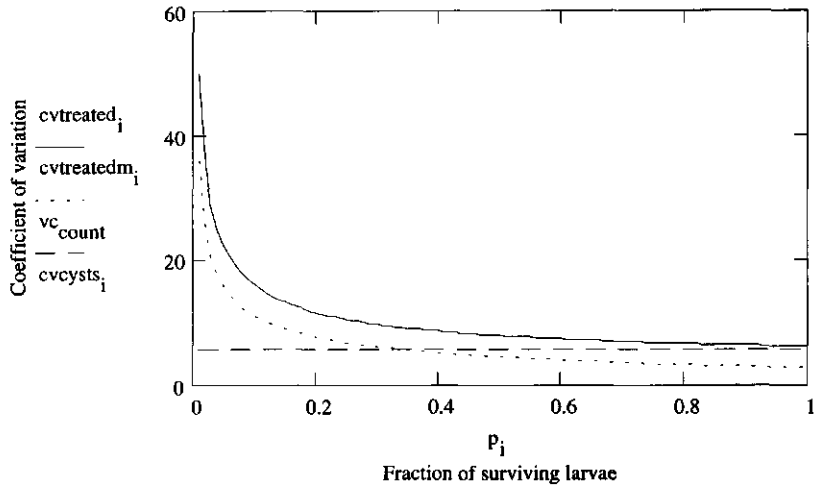
For the treated larvae the same steps apply as for t3.

coefficient of variation of one treated vial:

$$cvtreated_i := \sqrt{\left(vc_{count}\right)^2 + \left(cvcysts_i\right)^2 + \left(vcegglivehatch_i\right)^2}$$

coefficient of variation of all treated vials:

$$cvtreatedm_i := \sqrt{\left(cvcysts_i\right)^2 + \left[\frac{\sqrt{\left(vc_{count}\right)^2 + \left(vcegglivehatch_i\right)^2}}{\sqrt{hatchs}}\right]^2}$$

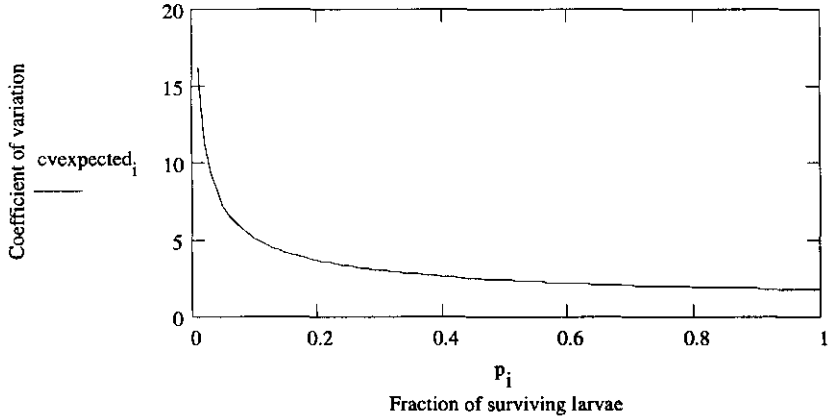


Finally:

A hatching test will be designed using several replications per treatment and, in this case, several replications of the hatching process per treatment. Moreover, survival is expressed as relative hatch (treated larvae are compared to untreated larvae). Therefore the final expected minimal coefficient of variation will be:

cysts collected:	$cysts = 1 \cdot 10^3$	eggs pipetted:	$eggs = 1 \cdot 10^3$
hatchability:	$h_{27} = 0.675$	number of replications:	$hatchs = 5$
eggs counted:	$count = 300$	number of treatments:	$reptreat = 5$

$$cvexpected_i := \sqrt{\left(\frac{cvuntreatedm_{27}}{\sqrt{reptreat}}\right)^2 + \left(\frac{cvtreatedm_i}{\sqrt{reptreat}}\right)^2}$$



Chapter 5

Compound models, describing the relationship between dosage (Z)- or (E)-isomer of 1,3-dichloropropene and hatching behaviour of *Globodera rostochiensis* (Wollenweber)

C.H. Schomaker & T.H. Been

5.1. Summary - Batches of increasing numbers of *Globodera rostochiensis* cysts were exposed to a range of concentrations of the (E)- and (Z)-isomers of 1,3-dichloropropene. The cysts were of identical origin. Temperature during treatment was 10°C, humidity 100% and time of exposure 8 days. The integrals of concentration time products (*CT*) created were 0, 3, 7, 14, 31, 60, 125, 242, and 437 µg/ml•day for the (E)-isomer and 0, 3, 16, 59, 240, and 419 µg/ml•day for the (Z)-isomer. Survival was estimated with hatching tests 1.5, 3, and 7 months after treatment. The relationship between dosage of (E)-isomer and numbers of hatchable nematodes followed a log-logistic equation at all hatching dates. Hatchability, and therefore lethal dosages, increased as hatching tests were more delayed. Seven months after treatment, practically all treated nematodes had recovered and hatchability of treated and untreated nematodes was the same. A log-logistic relationship was also found for dosage (Z)-isomer and numbers of hatchable nematodes 1.5 month after treatment. When hatching tests of nematodes treated with the (Z)-isomer were delayed till 3 and 7 months after treatment, the results were better explained by a compound model, assuming two independent log-logistic effects, one stimulating hatch at low dosages and one reducing hatch at all dosages. Only the (Z)-isomer of 1,3-dichloropropene was effective as a nematicide.

5.2. Introduction

Until recently, nematicides were widely used to control potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). In The Netherlands, where short crop rotations are common (starch potatoes are mostly grown every other year), such nematicide applications represented more than 60% of the total amount of pesticides used in agriculture. One of these nematicides is 1,3-dichloropropene, an approximately 1:1 mixture of its cis (Z) and trans (E)-isomers. These isomers differ in physico-chemical properties, such as vapour pressure, water solubility, distribution over water and gas phases, adsorption to soil, decomposition rate (Leistra, 1972), and probably also in toxicity to nematodes (Youngson & Goring, 1970). The effectiveness of soil fumigation depends not only on these properties, but also on application techniques, soil type and condition, and weather conditions. Leistra (1972) developed a theoretical model to calculate integrals of concentration time products (*CT*) at different depths in the soil after measuring concentrations at various times after application. The model incorporates both the physico-chemical properties of a fumigant and environmental factors such as temperature and humidity. If the relationship between *CT* and mortality of potato cyst nematodes were available, Leistra's model could be used to quickly evaluate new techniques of nematicide application, soil treatment before application, etc. Without such a model, patterns of *CT* estimates in different soil layers are

Table 5.1. - Definitions

Time	t	Period of exposure to the test compounds (in days).
	t	Period of exposure to the hatching agent (in days).
	τ	Time interval between the end of the treatment and the start of the hatching test (in months).
Concentration	c_0	The initial concentration of a test compound per unit of volume in the water phase (in µg/ml), at the beginning (time $t = 0$ days) of nematode exposure.
	c_t	Concentration at time t.
Dosage	CT	The amount of test compound placed in the environment of the nematodes for a period of exposure t. It is the integral of concentration time products, expressed in µg/ml•day.
Dose	-	The amount of test compound received by an individual nematode, when it is exposed to a certain dosage.
	k	Rate of decrease of the concentration c during time t of exposure.
Hatching curve	F(t)	Numbers of hatched nematodes as a function of time t.
	λ	Cumulative final hatch.
	α	Value of ^{10}log t at which the cumulative hatch of the nematodes is $0.5 \bullet \lambda_{CT}$.
	β	Slope parameter.
	γ	Time t needed for the hatching agent to reach the nematode and for the nematode to weaken the egg shell, to rupture it and to pass the 22.4-µm sieve.
	$\lambda_{CT}/\lambda_0 \bullet 100\%$	Relative hatch. Percentage cumulative final hatch at a dosage $CT>0$ of the cumulative final hatch at dosage $CT=0$.
Models 1 and 2	D	Total number of nematodes per cup exposed to the hatching agent.
	$H(CT)$	Hatchability (potential response to hatch inducing stimuli), independent of $S(CT)$, as a function of CT.
	$H_1(CT)$	equals λ_0, the cumulative final hatch at $CT = 0$, at all dosages CT in *model 1*.
	$H_2(CT)$	The sum of λ_0 and a log-logistic function of dosage CT in *model 2*.
	$S_1(CT)$, $S_2(CT)$	Survival of all D nematodes as a function of dosage CT for model 1 and model 2 respectively.
	$F(CT)$	Cumulative final hatch λ as a function of dosage CT.
	$F_1(CT)$	equals $H_1(CT) \bullet S_1(CT)/D$.
	$F_2(CT)$	equals $H_2(CT) \bullet S_2(CT)/D$.
	a_H	Value of $^{10}\log(CT)$ for which $H_2(CT) = 0.5 \bullet (D-\lambda_0)$.
	b_H	Slope parameter in $H_2(CT)$.
	a	Value of $^{10}\log(CT-d)$ for which $S(CT) = 0.5 \bullet D$ and $F_1(CT) = 0.5 \bullet \lambda_0$.
	LD_1 50	Value of dosage $(CT-d)$ for which $F_1(CT) = 0.5 \bullet \lambda_0$ or $S(CT) = 0.5 \bullet D$. It equals $d+10^a$.
	LD_1 80	Value of dosage $(CT-d)$ for which $F_1(CT) = 0.2 \bullet \lambda_0$ or $S(CT) = 0.2 \bullet D$.
	LD_2 50	Value of dosage CT for which $S(CT) = 0.5 \bullet D$ in model 2. It equals 10^a
	LD_2 80	Value of dosage CT for which $S(CT) = 0.2 \bullet D$ in model 2.
	b	Slope parameter in $S_1(CT)$ and $F_1(CT)$.
	d	Maximum dosage for which $S_1(CT) = D$ and/or $F_1(CT) = \lambda_0$.

compared without understanding their nematicidal effects.

To investigate the dosage/response relationships of the two isomers of 1,3-dichloropropene and potato cyst nematodes, a bio-assay was designed exposing batches of increasing numbers of cysts to logarithmically increasing dosages (CT) of the (Z)- and the (E)-isomers.

5.3. Materials and methods

Experimental design

The experiment was designed so that the conditions during nematode exposure closely approximated those of soil during fumigation: a moist, gas-filled porous system. It is generally assumed that in such a habitat the concentration in the water-phase is the best measure for nematicidal activity of a compound (Leistra, 1972).

The tests were done with cysts of *G. rostochiensis*, pathotype Ro 1, reared in the glasshouse on potato cv. Irene, and kept in dry storage at 4 °C for about 1 year. The nematodes had never been in contact with the test compounds before, so that differences in tolerance between the nematodes were highly improbable. Before treatment with the pesticides, cysts were exposed to water vapour at 10 °C during 48 h, to equilibrate them to the experimental conditions and to create a water-film around the cyst walls.

The chosen nematicide concentrations in the water phase were 0, 0.5, 1, 2, 4, 8, 16, 32, and 64 µg/ml for the (E)-isomer and 0, 0.5, 2, 8, 32, and 64 µg/ml for the (Z)-isomer. More dosages were included for the (E)-isomer because of reports, (*e.g.* McKenry & Thomason, 1974), that this isomer might be less toxic than the (Z)-isomer The purity of both test compounds was larger than 99%. Numbers of cysts (at least 500 cysts) per batch were chosen so that at any dosage the contents of at least 100 cysts (either the content of whole cysts or distributed over all cysts) would be expected to survive exposure (Been & Schomaker, in preparation). Expected percentages of mortality were derived from the literature (Kaai & Windrich, 1971; Seinhorst & Den Ouden, 1973).

The batches of cysts were enclosed in glass tubes (5 cm long and 2.5 cm wide) each placed in a 100-ml DURAN glass jar, with an air tight cap, containing 20 ml of a solution with the required concentration (µg/ml) of the test compound in double distilled water (Table 5.1.). The cysts were not in direct contact with the solution. They were fumigated during a period t of 8 days at a temperature of 10 °C in a saturated atmosphere. There were five replicates per dosage, but the zero concentration had ten replicates.

After 8 days, the cysts were transferred to 5-µm sieves, rinsed thoroughly with water and exposed for seven days to air at room temperature to remove traces of pesticide. Then the cysts from each treatment were divided into three sub-batches that were stored at 4°C. After a time interval τ of 1.5, 3 and 7 months, the sub-batches of cysts were soaked in tap water during 7 days and crushed to obtain egg suspensions (Seinhorst & Den Ouden, 1966). Cyst walls were removed from the suspensions by means of a 250-µm sieve and, assuming (from a preliminary estimation) an average cyst content of approximately 200 eggs with nematode juveniles, water was added so that the suspensions contained roughly 1000 eggs per ml. At this or lower densities, the total egg volume is negligible compared to the water volume and a Poisson distribution of egg numbers in small samples drawn from this suspension can be expected if the suspension is heavily agitated in a non-systematic manner. Moreover,

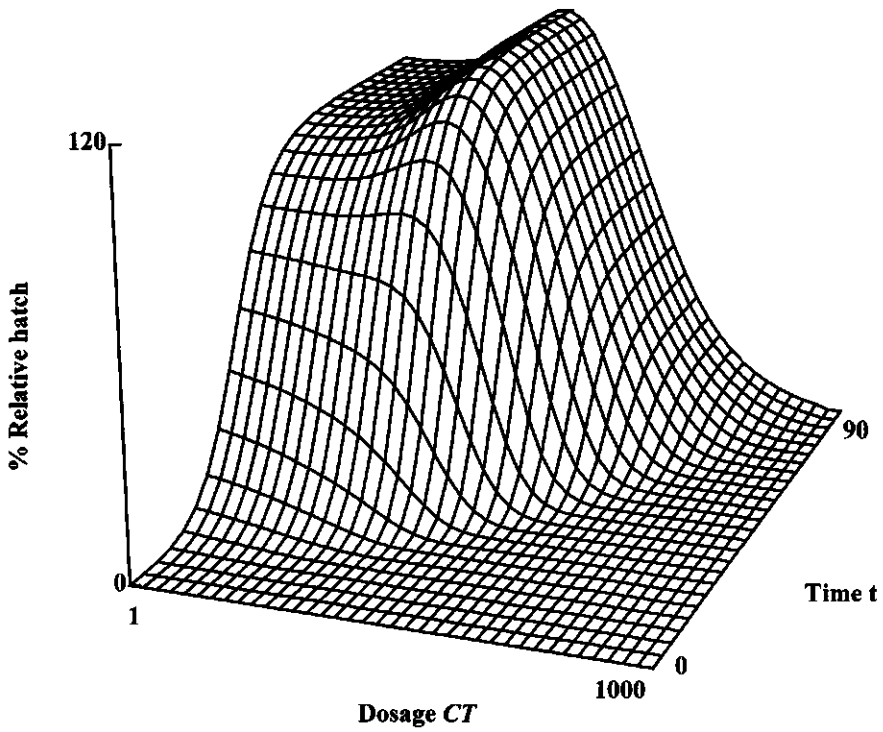


Figure 5.1. - A 3-dimensional presentation of dosage/response relations for the (Z)-isomer, consisting of % relative hatch on the Z-Axis as a function of dosage *CT* (in μg/ml•day) on the X-axis and of exposure time t (in days) to the hatching agent on the Y-axis. The number of nematodes hatched at dosage $CT = 0$, λ_0, was considered to be 100%; in this presentation τ, the time interval between the end of treatment and the start of the hatching test, is 7 months; the scales on the X and Y-axes are logarithmic.

at this density the desired numbers of eggs can be obtained by a single pipet transfer. A 0.5-ml sample from each suspension was counted and the volume needed to obtain 1000 nematodes was pipetted onto 22.4-μm sieves. The sieves were placed in glass cups with 1 ml of concentrated, purified hatching agent (Janzen & van der Tuin, 1956). There were five replicates per sub-batch to estimate the parameters of the hatching curves. Once a week the glass cups with the solution of hatching agent and hatched nematodes were replaced with other ones containing a fresh solution and the hatched nematodes were counted. In case of fast hatching, determined by counting hatched nematodes from separately prepared tubes, hatched nematodes were also counted at intervals shorter than a week. Nematodes were allowed to hatch as long as

necessary to reach their maximum hatch, which numbers were estimated from preliminary hatching curves for nematodes at each dosage. As nematicides delay hatch proportionally to their dosage (Seinhorst & Den Ouden, 1973) hatch was recorded for 50 days at all dosages (E)-isomer and dosages (Z)-isomer smaller than 59 µg/ml•day; for 50 till 70 days for nematodes at 59 µg/ml•day; while nematodes at the two highest dosages of the (Z)-isomer hatched for 80 till 100 days. A full description of the method and the hatching devices is given by Been & Schomaker (in preparation).

Calculation of the dosages

To check the initial concentrations c_i and to calculate the dosages *CT*, eighteen jars without cysts were filled with 20 ml solution of each isomer at three concentrations (0.5, 8, and 64 µg/ml), six jars per concentration, and their contents analysed (Smelt *et al.*, 1989) after a few hours and after 8 and 14 days. Assuming that nematicide concentrations decrease exponentially, the rates of decrease k at the three different concentrations could be estimated (Leistra, 1972) and dosages *CT* were calculated as follows:

$$c_t = c_0 \bullet \exp(-k.t) \tag{1}$$

$$CT = \int_0^t c_t dt = (c_0/k) \bullet (1 - \exp(-k \bullet t)) \tag{2}$$

Model construction

Effect was expressed as hatching behaviour of the nematodes and represented as a four-dimensional model, consisting of hatch or relative hatch, time t of exposure to the hatching agent and dosage *CT* of test compound, obtained after three time intervals τ between the end of the treatment and the start of the hatching test (Figure 5.1.).

To describe the effect of the isomers on the final cumulative hatch λ, at a dosage *CT*, two models were applied. Both models assume that a response of an individual nematode to a certain stimulus can be described by a log-normal (Finney, 1978) or a log-logistic (Ashton, 1972) distribution function. Fenwick (1950), Seinhorst & Den Ouden (1973) demonstrated that the log-normal model also gives a good description of the hatching response of cyst nematodes as a function of time of exposure to the hatching stimulus. Been & Schomaker (in preparation) did the same for the log-logistic model. For convenience, log-logistic functions were chosen for both purposes. By comparing the general patterns in the observed responses to the doses nematicides with the pattern of a singular response, the log-logistic distribution, we also made assumptions about the number of stimuli that acted upon the nematodes and the mutual dependency of the nematode responses to these stimuli. Model 1 assumes one stimulus (the hatching agent) affecting hatchability and one (the test compound), independent of the first one, affecting survival. Model 2 assumes two stimuli (the hatching agent and the test compound) affecting hatchability and one affecting

survival. Subsequently, two or three responses were discriminated: $S(CT)$ reducing survival beyond a certain dosage $CT = d$, and $H_1(CT)$ the hatching response in model 1 or $H_2(CT)$ the sum of two hatching responses (in model 2), this sum increasing with dosage CT. The two or three responses combined result in $F_1(CT)$ for model 1 and $F_2(CT)$ for model 2. Nematodes were assumed to be in either of four different states: i) surviving and hatchable, ii) surviving and not hatchable, iii) not surviving but hatchable in case of survival or iv) not surviving and not hatchable. Only the first category, described by $F(CT) = H(CT) \cdot S(CT)/D$, can empirically be assessed. The other categories can only be distinguished by extrapolation. Category ii) is then represented by $\{D-H(CT)\} \cdot S(CT)/D$; category iii) by $\{D-S(CT)\} \cdot \{D-H(CT)\}/D$; and category iv) by $\{D-S(CT)\} \cdot H(CT)/D$.

Nematode hatch as a function of time t is described by:

$$F(t) = \lambda/[1+exp\{\beta \cdot (log(t-\gamma)-log\alpha)\}] \quad \text{if } t > \gamma \quad (3)$$

$$F(t) = 0 \quad \text{if } t \leq \gamma \quad (4)$$

Survival, as a function of dosage CT is described by:

$$S(CT) = D/[1+exp\{b \cdot (^{10}log(CT-d)-a)\}] \quad \text{at } CT > d \quad (5)$$

$$S(CT) = D \quad \text{at } CT \leq d \quad (6)$$

The parameter d equals zero in model 2.

Hatch, as a function of CT, is represented by the product of hatchability and survival:

$$F(CT) = S(CT) \cdot H(CT)/D \quad (7)$$

In model 1

$$H_1(CT) = \lambda_0 \quad \text{at all dosages } CT \quad (8)$$

$$F_1(CT) = S_1(CT) \cdot H_1(CT)/D \quad (9)$$

In model 2

$$H_2(CT) = \lambda_0 + (D-\lambda_0)/[1+exp\{-b_H \cdot (^{10}logCT)-a_H\}] \quad \text{at } CT > 0 \quad (10)$$

$$H_2(CT) = \lambda_0 \quad \text{at } CT = 0 \quad (11)$$

$$F_2(CT) = S_2(CT) \cdot H_2(CT)/D \quad (12)$$

The construction of $F(CT)$ as a result of $H(CT)$ and $S(CT)$ is illustrated in Figure 5.2A. and 5.2B. for model 1 and model 2 respectively. Definitions of variables and parameters are given in Table 5.1.

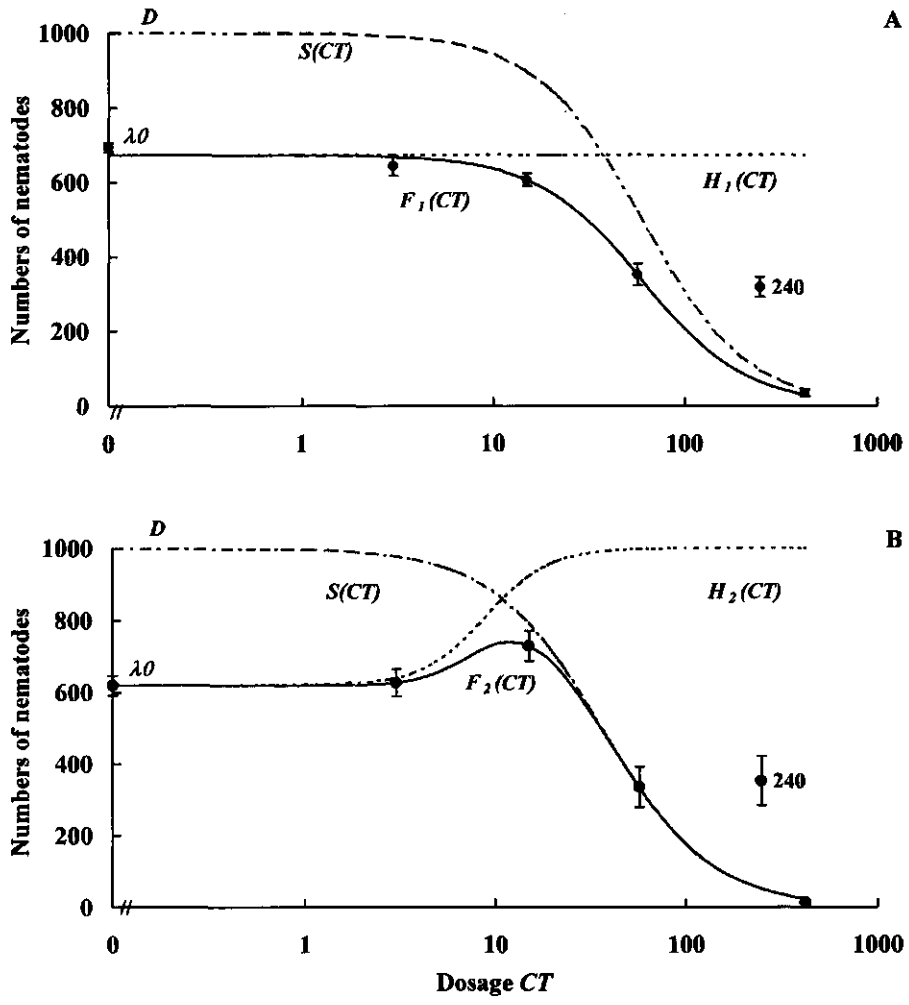


Figure 5.2. - A: Dosage/response relations (model 1) for the (Z)-isomer at τ = 1.5 months, expressed in numbers of hatched nematodes. The expected number of hatched nematodes $F_1(CT)$ is explained as a product of hatchable nematodes $H_1(CT)$ and surviving nematodes $S(CT)$. $H_1(CT)$ is constant at all dosages CT; $S(CT)$ is a function of dosage CT; there were 1000 (D) nematodes per cup involved in the hatching test; the symbols (●) with bars indicate means of 5 x 5 replications (except for CT = 0 with 10 x 5 replications) with standard errors. B: Dosage/response relations (model 2) for the (Z)-isomer at τ = 3 month, expressed in numbers of nematodes. The number of hatched nematodes $F_2(CT)$ is explained as a product of hatchable nematodes $H_2(CT)$ and surviving nematodes $S(CT)$, both dosage (CT) dependent; there were 1000 (D) nematodes per cup involved in the hatching test; the symbols (●) with bars indicate the averages of 5 x 5 observations (except for CT = 0 with 10 x 5 replications) with standard errors.

claimed to be feasible, which would justify the method economically. Since then, soil fumigation is applied to almost every soil type, including marine clay soils, to control potato cyst nematodes. Two nematicidal fumigants are used; metam-sodium (standard dosage: 300 l/ha) and 1,3-dichloropropene (standard dosage: 150 l/ha). Because metam-sodium has the lowest vapour pressure of the two nematicides, it is commonly used on sandy soils which have a looser and drier structure than marine clay soils. The latter soils have a higher moisture content, which makes 1,3-dichloropropene, with a higher vapour pressure, the most frequently used compound there.

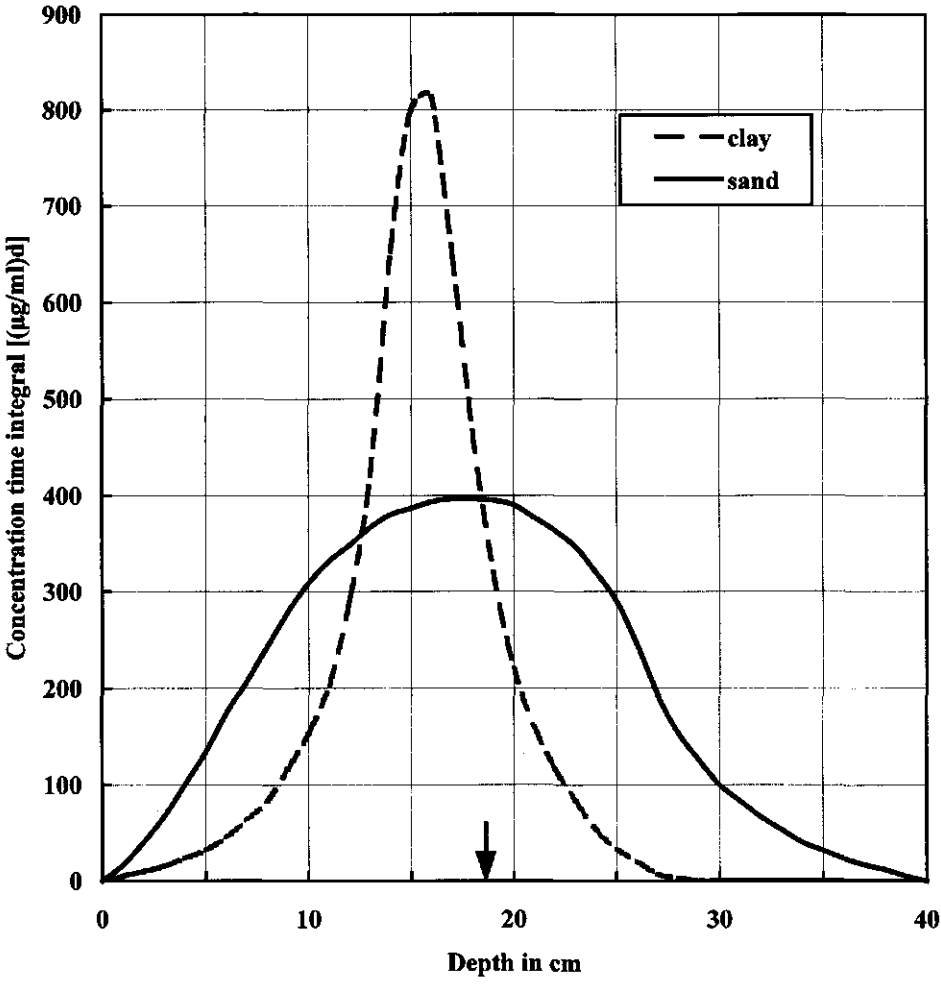


Figure 6.1. - Characteristic integrals of concentration time products ([µg/ml]d) of the (Z)-isomer of 1,3-dichloropropene versus depth obtained after fumigation of a sandy soil (solid line) and of a marine clay soil (broken line). Arrow indicates depth of injection layer in sandy soil (*e.g.*, Leistra, 1971; 1972; Rops & Smelt, 1985; Smelt *et al.*, 1989).

The efficiency of nematicides with high vapour pressure depends on the vapour concentration to which a nematode is exposed and the time of exposure of the nematode. The concentration of a fumigant is not the same at every depth in the soil and, moreover, is not stable over time (Leistra, 1972). In order to obtain high mortality the fumigant must not disperse through the soil too quickly or too slowly. Thus, the soil must not be too dry or too moist and it must have a good texture. Evaporation to the atmosphere must be limited by sealing the soil surface as well as possible. Leistra (1972) developed a model to calculate the integrals of the different concentration time (*CT*) products which will occur at any depth, based on concentration measurements in the field at different times and fumigant decay rates in the soil determined in the laboratory.

Favourable soil physical conditions are frequently found in sandy soils. Figure 6.1. (solid line) illustrates a typical integral of *CT* products of the (Z)-isomer of 1,3-dichloropropene over a sandy soil profile (after Leistra, 1971, 1972). *CT* products were highest in the injection layer and lowest in the upper and lower layers. In a laboratory test, Schomaker & Been (1998) determined that an exposure to 139 µg/ml.day of the (Z)-isomer of 1,3-dichloropropene resulted in a mortality of about 80% of the juveniles in eggs. The (E)-isomer had hardly any effect (LD50 = 169,824 µg/ml.day). If the curve of Figure 6.1. applies, a mortality of 80 % or more can be expected in a sandy soil between depths of 5 and 28 cm.

The cloddy structure of heavy marine clay soils makes optimum conditions for soil fumigation rare. Rops & Smelt (1985) estimated that *CT* products for various fields on marine clay soils after treatment with the standard dosage of 150 l/ha 1,3-dichloropropene were lower than those obtained for sandy and loamy soils. In the upper and lower soil layers, *CT* products were low and probably too small to kill sufficient, if any, nematodes (Figure 6.1., broken line). Only between 9 and 21 cm concentrations exceeded 138 µg/ml.day to provide an expected mortality of 80 % or more of the nematodes.

To verify these projections, information about nematode mortality after fumigation is needed from field trials. Therefore, field and laboratory trials were conducted to assess mortality of potato cyst nematodes in heavy marine clay soils, and - in cooperation with the Institute for Pesticide Research (SC-DLO) - to calculate *CT* products of 1,3-dichloropropene in these soils.

Because expectations about the effectiveness of the nematicide treatment were low, the possibility of an additional top soil treatment before autumn ploughing to increase mortality in the top soil was explored, thus increasing overall mortality throughout the tilth. Seinhorst (1973*b*) and Seinhorst & Van Hoof (1976) reported high mortality rates of *Rotylenchus uniformus* and *Paratrichodorus pachydermus* on sandy soils after a combined treatment of 1,3-dichloropropene/dichloropropane (DD mixture) and dazomet (3-5-dimethyltetrahydro-2H-1-3-5-thiadiazine-2-thione). Kaai & Windrich (1971) had similar results on a heavy soil (40% < 2µ) where the dazomet treatment killed an additional 99.5% of the fraction of *Ditylenchus dipsaci* which survived the

DD mixture treatment. Therefore, dazomet and metam-sodium were used for the top soil treatment; both formulations produce methyl isothiocyanate as the active compound.

6.3. Materials and methods

Field selection

In the spring of 1985, several fields known to be infested with potato cyst nematodes, were sampled using a grid pattern. The sampled area, ≈1/3 ha (300 × 12 m), was divided into app. 150 rectangles of 8 × 3 m, with the longer axis in the direction of cultivation. The central square metre of each rectangle (0.75 × 1.33 m in the direction of cultivation) was sampled. Approximately 1000 g of soil was extracted by collecting 40 cores of 25 g with a 25 cm long × 1 cm diameter auger according to a stratified plan. This procedure permitted the accurate location of the infestation focus and an adjustment of the soil sample size to collect the required number of cysts needed for the experiment in order to minimize sampling error. In September of 1985 three of these fields containing the highest potato cyst nematode population densities were chosen for the study (Table 6.1.).

Sampling and treatments

Selected plots (1.33 × 0.75 m) were sampled to determine hatchability before the soil fumigation. The bulk sample size was adjusted so that at least 200 cysts were collected per plot. The potato cyst nematode infestation in field 2 was so small in size that only five plots could be selected for sampling; on all other fields ten plots were sampled. After sampling the plots were shallowly rotavated to smooth the soil surface and to obtain enough loose soil to seal the surface after fumigation. Additional soil treatments only would cause a deterioration of soil structure. 150 l/ha 1,3-dichloropropene (Teleone II™ or Shell 95™) was applied using a Combiject shear injector (Rumptstad) with subsoil sprayers and two power-driven rollers in sequence to compact and seal the soil surface. Concentrations of both isomers of 1,3-dichloropropene were measured at 5 cm increments to a depth of 40 cm. Measurements were made three times after application, with four replications per field. For a detailed report describing climatic conditions, methods used and the concentrations measured in each field see Smelt *et al.* (1989*a*). To avoid disrupting the integrity of the tilth and allowing evaporation of the fumigant, these soil samples were not taken in the plots used to sample for potato cyst nematodes, but within 10 m distance from them.

Table 6.1. - Characteristics of the three test fields selected in 1985.

	Field 1	Field 2	Field 3
Mean population density (eggs/g soil)	79.97	65.15	109.08
Previously fumigated	1989	no	1979, 1982
Clay ($< 2\mu$)	30%	31%	36%
Silt ($2\mu <> 50\mu$)	59%	54%	45%
Organic matter	2.5%	2.8%	4.2%
$CaCO_3$	10%	10%	8%
pH KCl	7.4	7.3	7.3

Table 6.2. - Treatments and application data for three test fields selected in 1985.

Treatments	Field 1	Field 2	Field 3
Replicates DD-treatment	10	5	10
Replicates metam-sodium-treatment	5	5	5
Replicates dazomet-treatment	5	-	5
Replicates in time, not treated	-	-	5
Fumigation			
Sampling date (before DD)	2-9-85	2-9-85	4-9-85
Date of fumigation	11-9-85	25-9-85	13-9-85
pF at injection time	2.0	1.9	2.0
injection depth in metres	0.17	0.18	0.15-0.18
Driving speed in km/hour	3.5	3.4	2.5-2.7
Nozzle type	KSS02	KSS01	KSS01
1,3-dichloropropene in l/ha	150	150	156
Sampling date (effect DD)	10-10-85	23-10-85	11-10-85
Top soil treatment			
Date of treatment	30-10-85	25-10-85	6-11-85
Soil temperature at 5 and 15 cm depth	5-7	6.5-9.6	5-6
Time lapse application-ploughing (min.)	15	15	120
Ploughing depth (cm)	22-24	22	22-23
Sampling date (effect top soil treatment)	4-4-86	4-4-86	4-4-86

When the fumigant was no longer detectable, plots were resampled to collect cysts to determine the effect of the nematicide application. Five plots per field were sampled using a cylindrical auger (5 cm diameter × 80 cm length) with 5 cm markings. Separate samples were taken per 5 cm, to a depth of 30 cm. The remaining five plots in fields 1 and 3 were sampled using the standard 25 cm auger. Fields were again precoditioned using a rotavator (5 cm depth) one day before the top soil treatment in October or November to improve the distribution of the nematicide through the top soil. In fields 1 and 3, one half of the fumigated plots (partly randomly and partly contiguously chosen) received 150 l/ha metam-sodium (AA Monam GC™) sprayed to the soil surface, the other halves were intended to receive 100 kg/ha dazomet powder (Basamid™, equivalent to 10 g per m^2), also applied to the soil surface. Dosages were chosen so that equivalent amounts of the active compound, methyl isothyocianate, were applied. However, the quantity of dazomet applied was almost twice the required 100 kg/ha (180 kg/ha) due to malfunctioning of the distributor. Field 2 only received the metam-sodium treatment. Both chemicals were shallowly (8 cm) incorporated into the soil after which the treated soil was ploughed (22-24 cm, Table 6.2.). In April 1986, 6 months after treatment, soil samples were taken to measure the extra effect of the top soil treatment. Each plot was sampled with a 25 cm long auger.

The amount of soil taken from each plot/layer after fumigation and top soil treatment was adjusted so that at least 1000 cysts were collected. It was assumed (Hietbrink, pers. com., who investigated eggs of individual cysts), that all eggs within a cyst, exposed to a dosage *CT*, will either be completely killed or completely unaffected by the nematicide. The binomial response leads to a binomial distribution for dead and live cysts in a batch of cysts originating from a treated plot. To aim for the greatest possible differentiation between treatments, the coefficient of variation was reduced by collecting at least 1000 cysts per plot (Been & Schomaker, submitted).

Although it was not likely that population densities or viability would decrease during the winter months, five plots of field 3, which were not treated with chemicals, were sampled parallel with the treated plots to monitor such changes.

In several fields, including the three trial fields, the vertical distribution of cysts was determined once in 1986 prior to the soil fumigation. Per layer of 5 cm soil and to a depth of 80 cm several cores were collected to obtain at least 1 kg of soil per layer.

Hatching tests

Cysts were extracted using a Pollähne Karoussel (Pollähne, Hannover, Germany), and stored at 4 C° for 6 month after the nematicide treatment. Schomaker & Been (1988) found an inhibition of hatch by the (E)-isomer of 1,3-dichloropropene, lasting for more than three months, but less than six, whereas the (Z)-isomer appeared to be nematicidal. May, 1986, survival was estimated by means of an improved hatching test (Been & Schomaker, submitted). Cysts from each plot or soil layer were soaked in tap water and crushed in suspension using a plunger (Seinhorst & Den Ouden, 1966). Eggs and larvae (on average 97 and 3%, respectively) were separated from cyst walls using a

250 µm sieve. Assuming an egg content of 200 per cyst, a stock suspension of approximately 1000 eggs and larvae per ml was prepared of which 0.5 ml was counted to estimate the actual nematode density. A volume containing 2000 eggs and larvae was pipetted into glass tubes sealed at the bottom by gauze (22.4 µm, Monadur), which withholds eggs but not active larvae. The tubes were placed into glass cups containing 1 ml of the natural hatching agent G of potato plants (Janzen & Van der Tuin, 1956). Groups of twenty glass cups were stored in a plastic carrier, placed in a plastic box to reduce evaporation and incubated at 20 C° in the dark. Tubes were placed in new cups containing fresh hatching agent at least weekly, while cups containing hatched larvae were stored at 4 °C until counting. At low nematicide concentrations, when hatching is fast, nematodes were counted at shorter intervals (Been & Schomaker, submitted). A method to automatically count hatched larvae is described by Been *et al.*, 1996a.
Mortality was defined as the complement of the % survival. Survival was estimated by the relative hatch defined as the (number of hatched treated larvae/number of hatched untreated larvae) × 100.

Viability test
Hatched larvae, from soil samples collected from around the injection layer (layers 3 and 4) and larvae originating from non-fumigated plots were collected after counting and used in a glasshouse test of their reproductive capacity. They were inoculated at a density of 0.6 larvae/g soil in 2-kg pots, with five replications, containing an artificial soil mixture of silversand, claypowder and ground earthenware with Steiner's nutrient solution (Been & Schomaker, 1986). After inoculation, a piece of tuber of the susceptible cultivar Irene was planted and pots were tended in a glasshouse until plants had matured and died. A minimum of 500 g soil, but more if needed to count at least 200 cysts, was elutriated in a Seinhorst elutriator and cleaned using the '*mini* Fenwick can' (Seinhorts, 1974; 1988). Cysts were counted, ground and eggs counted as described above. Estimates of the maximum multiplication rate were calculated as well as the number of eggs/cyst and cysts/kg soil.

6. 4. Results

Sampling date
Soil samples collected from untreated plots of field 3, sampled simultaneously with the treated plots at all sampling dates, displayed no differences over time in the number of cysts/kg, eggs/cyst and percentage hatch (60%) of potato cyst nematodes. Therefore, no bias is to be expected in the data of the treated plots caused by different sampling dates during autumn and winter.

Table 6.3. - Vertical distribution of cysts per 1000 g in the three test fields (1-3) and in several other fields (4-6) in the same region. Fields are ranged from left to right according to decreasing population densities in the surface soil layer to demonstrate the link between these densities and infestation levels in the lower soil layers.

Layer	Depth (cm)	Cysts/ 1000 g of soil					
		Field 3	Field 2	Field 5	Field 6	Field 1	Field 4
1	0-5	1230	1003	622	198	191	20
2	5-10	1398	1147	1493	197	249	30
3	10-15	1612	1219	1205	140	112	16
4	15-20	1417	951	1115	121	76	13
5	20-25	1141	625	815	112	75	0
6	25-30	1048	132	405	146	77	15
7	30-35	478	44	55	66	26	2
8	35-40	305	17	69	10	4	1
9	40-45	142	32	10	1	2	0
10	45-50	65	50	4	3	4	0
11	50-55	20	51	7	0	2	0
12	55-60	46	77	5	3	2	0
13	60-65	49	29	7	1	0	0
14	65-70	37	19	5	0	0	0
15	70-75	58	45	7	1	2	n.s.
16	75-80	53	65	n.s.	1	3	n.s.
1-5	$\sum$ 0-25	6797	4946	5250	768	703	78
1-16	$\sum$ 0-80	9098	5506	5823	999	824	96

n.s. = no sample available

Vertical distribution

The vertical distribution of cysts/1000g in the three test fields (1-3) and in three other fields (4-6) in the same region is presented in Table 6.3. Cysts could be recovered from depths of 40 cm in most fields while those with heavy infestations contained cysts as deep as 80 cm. In general, the higher the population densities in the upper soil layers the higher the infestations of the deeper soil layers.

Fumigation

Fumigation killed 48, 72 and 48% of the potato cyst nematodes in the upper 30 cm of the tilth (Figure 6.2.). Weighed total mortality was calculated by correcting mortality of each layer for the actual population density of that layer (Figure 6.3.). Mortality was

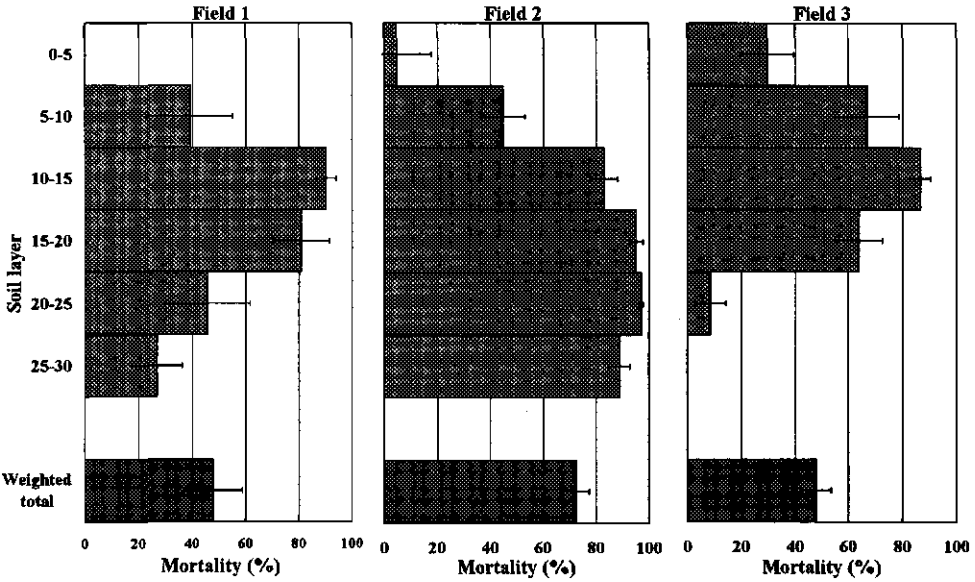


Figure 6.2. - Mortality (%) per soil layer and weighted total mortality for the six soil layers (5 cm) of the tilth (30 cm) for three fields. Error bars: standard deviation of the mean (n = 5). 'Weighed total mortality' is defined as the average mortality over all layers corrected for the actual population density of each layer (Figure 6.3.).

poor in the upper and lower soil layers where variation of mortality between fields was highest. The pattern of field 3 differs from that of the other two fields. A relatively high mortality was found in the first and second soil layer and almost no mortality in layers five and six. This field was unique in the heaviness of the soil combined with a compacted soil structure, making it difficult to keep the fumigant injection mechanism (4 subsoil sprayers; one at each shear) at the appropriate depth. The injection layer varied between 15 and 18 cm depth and caused a vertically upward shift of mortality rates (Figure 6.2.). The top layers of fields 1 and 2 show the low mortality characteristic of these clay soils. Field 2 differs from the other two in the high mortality in the two deepest layers. In this field the injection was comparatively deep (18 cm) and many large, deep cracks were present.

In fields 1 and 3 (fields with two different top soil treatments), another five plots were sampled using the 25 cm auger. To combine these data with those from Figure 6.2. the weighted total mortality of layers 1 to 5 was used. Mortality in fields 1 and 3 changed slightly to 56% (was 49%) and 53% (was 55%), respectively.

Fields 1 and 3, which were fumigated for the second and third time in their history,

respectively, suffered from a rapid decline of the concentration of 1,3-dichloropropene after several days. The main cause for this phenomenon was an accelerated breakdown of the active ingredient caused by adapted microorganisms (Smelt *et al.*, 1989*b*). Field 2 was fumigated for the first time and microbial degradation was only of minor importance here, although Smelt *et al.*, 1989*b* reported fast transformation even in fields never fumigated before.

The horizontal variation of the concentration of Z 1,3-dichloropropene per layer was large. Smelt *et al.* (1989*a*) reported coefficients of variation of 14 to 98% indicating a non-uniform spread of the fumigant through the soil. The same order of variation applied to the mortality rates which differed considerably between the five replications per layer (Figure 6.2.). The results show that mortality varied greatly due to variation of injection depth and driving speed, as well as by local differences in soil structure (clumps and cracks).

Estimated *CT* products of the (Z)-isomer and mortalities per soil layer were compared to a model describing their relation. As there were few data at low *CT* products where stimulation is to be expected according, the data of each field were fitted using a model without a stimulatory effect (Schomaker & Been, 1998). In Figure 6.4.A this model is fitted to the data of field 1 and compared to the extended model with stimulatory effect after 7 months (dotted line) according to Schomaker & Been (1998). The data from

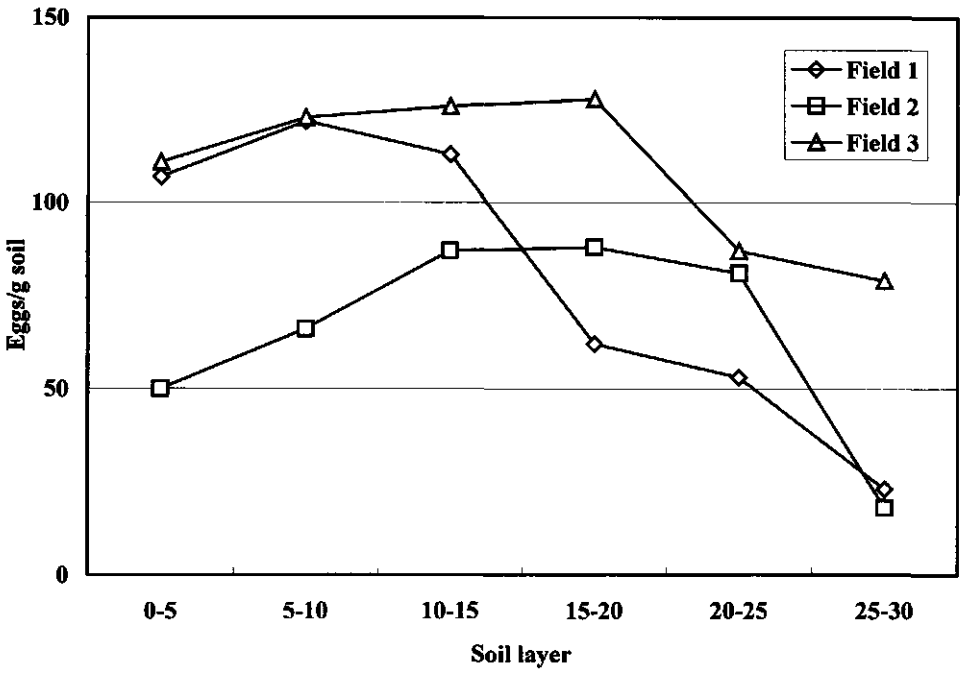


Figure 6.3. - Population densities (eggs/g soil) per soil layer of 5 cm for each of the three test fields of Figure 6.2.

fields 1 and 2 fit reasonably well and produce the same LD50 values (Table 6.4). Field 3, however, displays a poor fit to the model. A closer inspection of the data revealed a systematic difference between layers 1, 2, and 3 and layers 4, 5, and 6. In fact, completely different fits can be produced for the two groups. Figure 6.4.B shows high mortalities in the upper layers and low mortalities in the lower layers at the same dosages. By combining the data of field 1 and 2 and that of the lower layers of field 3, Figure 6.5. is produced providing a 'general' dosage/response relationship for marine clay soils. A LD50 value of 136 could be calculated for this field test, twice as much as that observed by Schomaker & Been (1988) in a laboratory test.

Top soil treatment

Autumn ploughing of the treated top soil to a depth of 20 cm was expected to contribute to the mortality of the first 25 cm of the tilth. Therefore, soil sampling following the top soil treatment was restricted to that depth. The result was compared with the corresponding weighted total mortality of the 1,3-dichloropropene treatment. Metam-sodium increased mortality significantly ($P \leq 0.005$) in two out of three fields (Table 6.5.). Final mortality rates of 89 and 95% were obtained. Field 3 was autumn ploughed perpendicular to the direction of application and a considerable time lapse between metam-sodium application and autumn ploughing occurred (Table 6.2), resulting in high evaporation of the active ingredient and low 'Extra' mortality. Although the amount of methyl isothiocyanate in the dazomet treatment was nearly double that of metam sodium, the treatment efficiency did not increase.

Viability test

The multiplication rate of the hatched larvae from cysts extracted from the vicinity of the injection layer, where dosages *CT* of 1,3-dichloropropene were highest, was 25 % lower ($P \leq 0.05$) than of those collected from untreated soil (Figure 6.6.). The difference is the result of a reduced number of larvae maturing into cysts ($P \leq 0.05$) and not of a reduced number of eggs and larvae per produced cyst ($P \leq 0.20$).

Table 6.4. - Some characteristics of the model fitted in Figs. 4 and 5. Model 1 in Schomaker & Been (1998); LD50: dosage at which hatch is reduced by 50%, c: no effect level, *b*: slope parameter, R^2: % variance explained.

Data	LD50 (µg/ml.day)	c (µg/ml.day)	*b*	R^2
Field 1	135	0	3.6	89.7
Field 2	141	0	6.9	87.2
Field 3	69	0	2	60.0
Field 3: upper layers	28	0	2.3	94,3
Field 3: lower layers	240	20	3.5	76.2
Combined	136	10	4	90.9

Table 6.5. - The effect of top soil application of metam-sodium and dazomet on the mortality of potato cyst nematodes in the first 25 cm of the tilth in three selected clay soils.

Treatment	Field 1			Field 2			Field 3		
	'Before'	'After'	'Extra'	'Before'	'After'	'Extra'	'Before'	'After'	Extra
Metam-sodium	49	89	78	72	95	82	55	64	22
Dazomet	46	80	62	-	-	-	36	53	27

'Before': Weighted total mortality in % after DD treatment. Averages of fields 1 and 3 are the result of combining ten replications per field into two groups and therefore they are slightly different from the data in Figure 6.2.
'After': Average mortality in % after additional top soil treatment.
'Extra': Percentage of the surviving nematodes killed by the additional top soil treatment. [1-{(100-'After')/(100-'Before')}]× 100.

6.5. Discussion

Fumigation
As expected, soil fumigation was insufficient to obtain the mortality of 80 to 90% of the potato cyst nematode population in the tilth of marine clay soils which was considered necessary to compensate for the average maximum multiplication rate of potato cyst nematodes (20 for *Globodera pallida* and 25 for *G. rostochiensis*) in a 1:3 or 1:4 crop rotation with an average annual population decline of 35% when a non-host crop is grown. The poor result is mostly due to low mortality in the upper and lower layers of the tilth. The highest mortality (0 - 30 cm) attained was 72% (field 2) where no accelerated degradation occurred (Smelt *et al*, 1989*b*), otherwise it was barely 50%. A replication of this study in cooperation with the Research Station for Arable Farming and Field Production (PAGV) on three other fields in 1988 yielded mortalities of 40, 50, and 60% for the first 25 cm of the tilth (Molendijk, pers. com.).
Although Seinhorst (1973*a*) summarized several dosage/response curves from various sources, no *CT* products were measured in the soil. Therefore, only the experiment by Schomaker & Been (1998) is available for comparison. They showed that the (Z)- and (E)-isomers of 1,3-dichloropropene differed in efficacy. Only the former compound proved to be active, while the latter temporarily inhibited hatching of larvae. Therefore, only data of the (Z)-isomer of 1,3-dichloropropene were used to fit a dosage/response relationship. In general, the model by Schomaker & Been (1998), without stimulatory effect, describes the results well, except for field 3 were high

mortalities were found in the upper layers and low ones in the lower layers at the same dosages. No explanation can be given for the former which are even higher than found in the laboratory test; the latter could be explained by the combination of a high clay content and the compacted soil structure of this field making it more difficult for the fumigant to penetrate. The average dosage required for the LD50 is twice as high as observed in the laboratory.

Apparently, in these heavy soils with their cloddy structure part of the cysts escape fumigation by being incorporated in clumps of clay impenetrable to the fumigant while in adjacent parts relatively high concentrations of the active compound can be measured. Therefore, measurements of *CT* products obtained in laboratory and even in field experiments cannot be easily translated into mortality levels.

Top soil treatment

The combination of a standard 1,3-dichloropropene fumigation, a top soil treatment with metam-sodium and autumn ploughing seems to offer a method to reduce nematode populations in the first 25 cm of the tilth by more than 80 % with a high degree of reliability (Table 6.5.) when carried out properly. In the three trial fields, adaptation to metam-sodium was absent (Smelt *et al.*, 1990). The net effect of the combination of the two nematicides tended to equal that of two subsequent, successful, standard applications of 1,3-dichloropropene on an unadapted field and three consecutive, less successful, treatments on an adapted field. Application of metam-sodium in combination with autumn ploughing - on marine clay soils commonly performed to improve the soil structure by the winter's freezing and breaking up of large clumps - allows to take advantage of the low dispersion rate of this nematicide caused by its lower vapour pressure. When used as a top soil treatment the characteristics of metam sodium are beneficial. The soil layers with the lowest mortality of potato cyst nematodes together with the active compound are transported to a depth of approximately 20 cm Low dispersion rate and slow breakdown at the prevailing low temperatures in October/November (8.5-14.7 and 4.3-10.3 C respectively, average min/max values over 5 years), almost inhibits movement of the compound while its presence is prolonged.

The application of dazomet as a top soil treatment proved to be far less effective than reported by other researchers. Kaai & Windrich (1971) injected 12.5, 25, 50, and 100 ml/m^2 of DD mixture at 15 cm depth of a clay soil and killed 23, 46, 58, and 73% of *D. dipsaci* in the top 20 cm of the soil. Application of 30 g dazomet per m^2 increased mortality to 99% in all cases. Seinhorst (1973*b*) reported an additional 80 and 96% mortality when using 5 g and 10 g dazomet per m^2 respectively for *R. uniformis*, surviving treatment with DD mixture, on sandy soils. Seinhorst & Van Hoof (1976) reported similar effects for *R. uniformis* and trichodorids (mainly *P. pachydermus*) with the same dosages of dazomet in sandy soil.

Seinhorst & Den Ouden (1973) reported the need of a 1.7 times as high a dosage of

DD necessary to kill the same proportion of eggs of *Heterodera rostochiensis* as active *R. uniformis*. In the present research, the dosage of dazomet (18 g/m^2 dazomet) could be expected to produce similar results. However, in the former experiments dazomet was dispersed on the soil surface and was converted to the active compound by rain. In this experiment the dazomet, transferred to a depth of 20 cm by autumn ploughing, was activated by soil moisture.

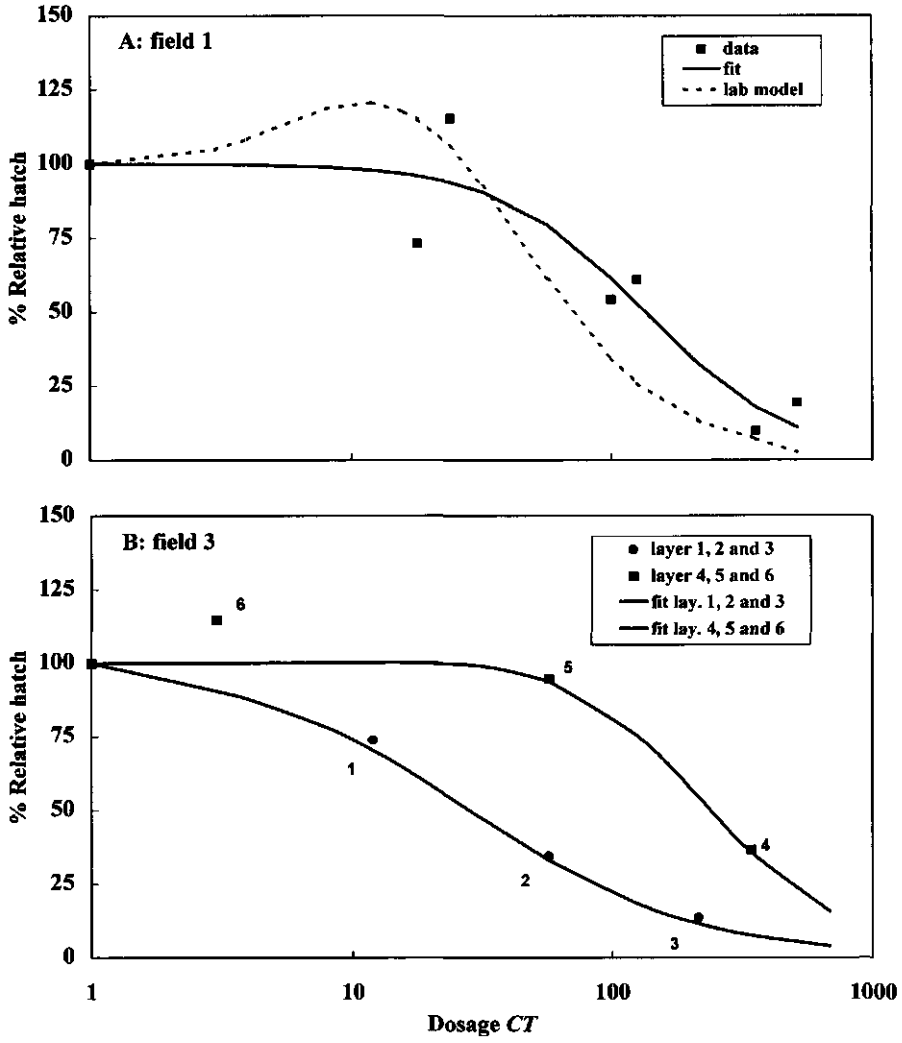


Figure 6.4. - Dosage/response relation for relative hatch of *G. pallida* cysts treated with 1,3-dichloropropene in field 1 (A), and for layers 1, 2 and 3 and layers 4, 5 and 6 of field 3 (B). Squares represent average relative hatch per layer. Solid line: model according to Schomaker & Been (1998) without stimulatory effect. Dotted line: laboratory dosage/response relationship for the (Z)-isomer, 7 months after application (Schomaker & Been, 1988).

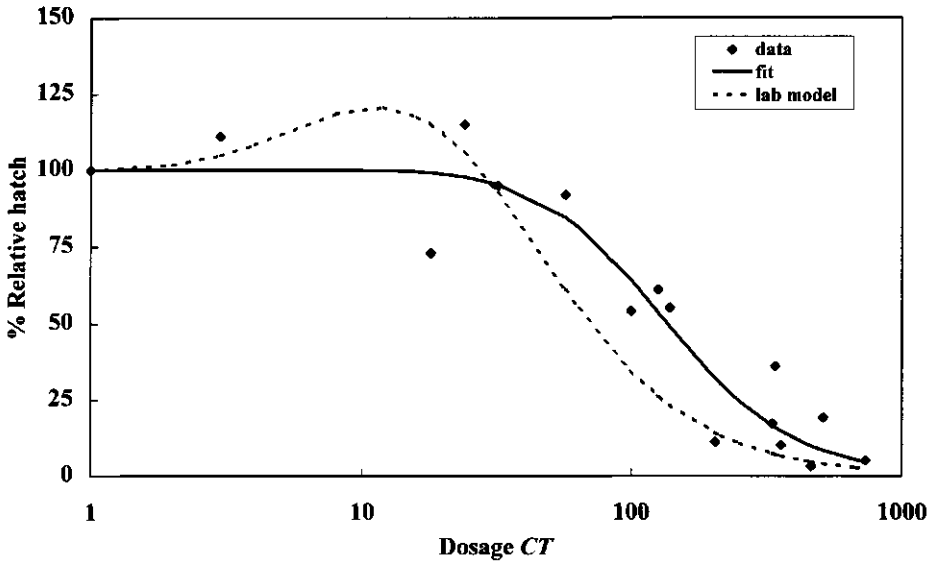


Figure 6.5. -Dosage/response relation for the survival of G. pallida of the combined data sets of field 1, 2, and lower layers of field 3.

Viability test

From the results presented in Figure 6.6. we conclude that the viability of the surviving larvae is reduced but not their capability to produce offspring. Initial population densities in this pot experiment were so small (0.6 eggs/g soil) that intra specific competition of the nematodes can be assumed to be negligible (Seinhorst, 1993) and multiplication rates for treated and untreated larvae the same. The decrease of the final population density of the treated and surviving larvae from layer 3 and 4 can be translated in a 25% reduction of the initial population density (0.45 instead of 0.6 eggs/g soil). The reduced population density increases the mortality caused by the soil fumigation, but will not be of great significance when translated to the upper 30 cm of the tilth. Estimated mortality increases from 48, 72, and 48% to 49, 74 and 50% for fields 1, 2 and 3, respectively.

Considerations with soil fumigation

Several considerations suggest that soil fumigation as a standard method to control potato cyst nematodes in marine clay soils is unpractical:

1 Favourable climatological conditions and physical soil properties do not occur often or for long in regions with marine clay soils (Smelt *et al*, 1989*a*).

2 These soils are prone to microbial adaption as even untreated soils had the possibility of instant adaption to the fumigant used (Smelt *et al.*, 1989*b*). The

minimum of 50% mortality in the field trials discussed here was obtained in spite of rapid breakdown. As breakdown of 1,3-dichloropropene is negatively linked to the concentration of the fumigant, mortality around the injection layer will always be high; the effect mainly decreases results in those layers where concentrations are lower anyhow. Farmers, therefore, should be aware that application of a fumigant as a preventive measure decreases the effect of a nematicide application when fields are truly infested.

3 Even when fumigation is successful, only nematodes to 25 - 30 cm depth will be affected. In these fumigated fields and others in the same area, cysts could be recovered from depths as deep as 80 cm (Table 6.3.). In field 3, 14% of the total number of cysts were found beneath 30 cm depth and, assuming the same cyst content as in the upper layers, mortality due to treatment is reduced to 42% over the whole 80 cm. Since roots of potatoes reach depths between 80 and 100 cm (Vos & Groenwold, 1986), even successful chemical treatment will not prevent continual nematode increase, especially in the lower soil layers.

4 On marine clay soils it is almost impossible to maintain the proper injection depth of 18 cm driving at speeds over 3,5 km/h which is normal for commercial contractors, who try to drive as fast as possible to save time. Moreover, contractors frequently utilize nozzles which are too large, even for the high speed aimed at. As a result the fumigant was not properly dispersed in the injection layer but deposited as a fluid in bands. Therefore, results of commercial fumigation will likely be inferior to those reported here.

5 Proper cleaning of fumigation equipment is extremely difficult and time consuming. Even when a high pressure hose was used, some parts of the machinery could not be cleaned. In commercial situations no such efforts will be made. Soil fumigation equipment will therefore be one of the major contributors to the dispersal of potato cyst nematodes between fields as adhering soil is constantly being refreshed in the course of the work (Hofmeester, 1991).

6 Five fields infested with potato cyst nematode were sampled to locate the site of infestation and to define its size and intensity. Four natural infestations (field 3 was a more or less uniform infestation caused by the spread of sandy soil over its surface) proved to be rather small (about 500 m^2) compared to the size of the area which is statutorily sampled (1/3 ha) and far smaller than the area which will be fumigated when a standard soil fumigation is carried out (1 ha). Probably, 90% of the active ingredient would be wasted on uninfected soil.

7 Schomaker & Been (1993, submitted) defined a simple exponential model describing the expected cyst numbers within an infestation, applying to foci in all growing areas in the Netherlands and irrespective of control measures previously taken. In combination with a relationship between population densities before planting and yield reduction of tubers at harvest (Seinhorst, 1982) yield losses can be calculated for any focus. Been *et al.* (1996*b*) calculated maximum yield reductions of less than 1% per ha for even the largest single focus found

when no fumigation would be applied. These equalled less than 100 Guilders (about 58 US$) when seed potatoes are grown, far less than the cost of a soil fumigation (approximately 1300 Guilders). Even if fumigation was 100% successful it would be unprofitable.

We conclude that typical soil fumigation has no practical value on marine clay soils. Even when a combined treatment of 1,3 dichloropropene and metam-sodium is successfully conducted only two out of seven considerations mentioned above are met. Only in cases of severe infestations causing yield reductions exceeding the costs of a combined treatment with 1,3-dichloropropene and metam-sodium or when a seed potato farmer wants to reduce a detected infestation as soon as possible before a new statutory soil sampling is carried out, could this combined soil treatment have any value.

To apply nematicides rationally, a sampling method is required which can detect small infestations with high reliability. Such a monitoring system could make preventive soil fumigation obsolete. Furthermore, if potato cyst nematode infestations can be located when damage to the potato crop is still low, the effect of growing a resistant potato variety (80 % reduction of the population throughout the rooted area) will greatly surpass the effect of a nematicide treatment. Improved sampling combined with an

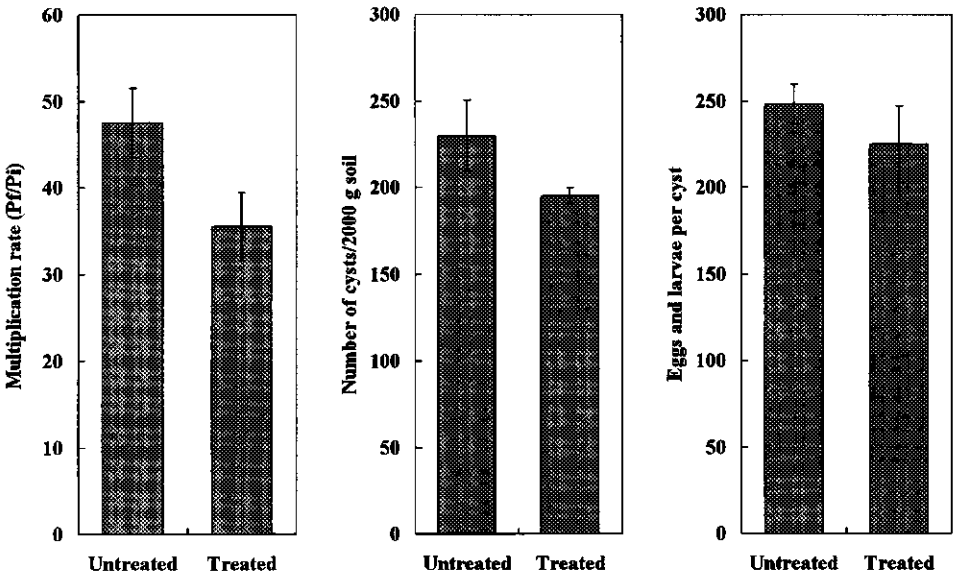


Figure 6.6. - Multiplication rates, expressed as the ratio between final and initial population densities (eggs/g soil), number of newly formed cysts per 2000 g soil and average cyst content (eggs and larvae) after growing the susceptible cv. Irene in a pot experiment on hatched larvae originating from 1,3-dichloropropene treated and untreated soil.

ELISA test to determine the species of the potato cyst nematode (Schots, 1988) will permit selection of the proper resistant cultivars. If a nematicide treatment is inevitable, the area of application can be minimized and the above described combination of 1,3-dichloropropene and metam-sodium could be used. Research on sampling strategies has been rewarding (Schomaker & Been, 1993; Been & Schomaker, 1996). Research on application techniques for the topsoil treatment, however, was less successful and eventually was discontinued as the need for soil fumigation in these areas declined.

Chapter 7

A model for infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*)

C.H. Schomaker & T.H. Been

7.1. Summary - In 1990 a research program was initiated to develop new sampling methods for the detection of patchy infestations of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) with known accuracy in all potato cropping areas of The Netherlands. Patchy infestations in cropping areas of the provinces of Zeeland, Friesland, Groningen and Drente were sampled to validate a model based on data from cropping areas in Flevoland and to determine whether one detection method could meet the requirements of all cropping areas in The Netherlands. Eighty two fields were presampled to locate patchy infestations using a coarse sampling grid (8 · 3 m). Parts of thirty seven fields, containing one or more foci, were sampled intensively by extracting at least 1.5 kg of soil per square metre (1.33 · 0.75 m). Forty foci were analysed for spatial distribution characteristics of cysts using Generalized Linear Models (GLM's) and classical Multiple Linear Regression Analysis, differing in assumptions about the distribution of the input variable (number of cysts per kg of soil). The results showed that the data from all investigated cropping areas fit well to an exponential model with two parameters, the length and width gradient parameters. Significant differences in these parameter values between cropping areas could not be demonstrated. As both parameters follow a normal distribution, the probability of any combination of these parameters can be described by a bivariate normal distribution. Gradient parameters were correlated but significant correlations between these parameters and certain variables, such as the nematode species involved (*G. pallida* or *G. rostochiensis*), the time interval between sampling and the last potato crop, soil type, cropping frequency and cyst density in the focus centre could not be demonstrated. It can be concluded that one detection method for small infestation foci suffices for all investigated cropping areas. Its expected accuracy is independent of soil type, potato cyst nematode species, cropping frequency or time interval between sampling and last potato crop.

7.2. Introduction

Since 1968 the use of nematicides (mostly 1,3-dichloropropene and methyl isothiocyanate) was promoted in The Netherlands as a major solution to all problems caused by plant parasitic nematodes, especially potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). Soil fumigation on light, sandy soils under optimum conditions, presumably, could reduce populations of plant parasitic nematodes by an average of 80%, thus enabling narrow rotations (once in three years or less) while maintaining high yields of economically important crops, especially potatoes (1). At the time, the input of nematicides was thought to be a temporary one, as the availability of other control measures such as biological control and potato cultivars resistant to newly discovered pathotypes of *Globodera rostochiensis* 2 and 3 would make the use

of nematicides obsolete. However, the expectations of effective biological control agents were not fulfilled. Potato cultivars resistant to the new pathotypes were introduced, but new virulent pathotypes of potato cyst nematodes emerged (pathotypes 2 and 3 of *G. pallida*) and the use of nematicides increased to $12{,}535 \bullet 10^3$ kg in 1986, 60% of the total pesticide use in The Netherlands (Anonymous, 1991).

Since 1968, nematicide applications after each potato crop has become common practice in the starch potato area of the provinces Groningen and Drente as they were strongly recommended by the provincial research stations and extension services. In the late seventies nematicides were also applied, mostly as a precaution, in the province of Flevoland where seed and ware potatoes were grown on a much heavier soil type. Farmers had two reasons for application of fumigants. First, at the time, potato cyst nematodes were quarantine organisms and government legislation did not allow farmers to grow potatoes on infested fields. Second, it was almost impossible for farmers to find out whether their fields were infested or not, as neither accuracy nor precision, as defined by Campbell and Madden, 1990, of the current sampling methods was known. Therefore, farmers tended to apply nematicides as a precaution. Only in 1985 was research directed towards the efficiency of nematicides on marine clay soils where most of the Dutch seed and ware potatoes are grown. Research into the effectiveness of these nematicides revealed an accelerated breakdown of the active component by microorganisms (Smelt *et al* 1989a, b), not only in fields that had been fumigated in previous years but also in fields treated for the first time. Mortality was about 50% in fields containing adapted microorganisms and did not exceed 70% in fields without adaptation (Been & Schomaker, 1987). These figures applied to the first 30 cm of the tilth, but cysts in these and other fields were often found to be evenly distributed down to 40 cm depth and to occur, in decreasing numbers, in soil layers down to 60 cm depth. Therefore, the percentage mortality achieved throughout the root zone, down to 1.2 metre on these soils (Vos and Groenwold, 1986), was insufficient to compensate for the density-dependent multiplication rate of potato cyst nematodes on potatoes. Moreover, all infestations, which were mapped during these nematicide trials, proved to be rather small in size compared to the area commonly treated with a soil disinfectant. Almost 90% of the nematicide was wasted on uninfested soil; the rest did not reach all infested soil strata (Been & Schomaker, 1998). Therefore, the benefit/cost ratio of soil fumigation was poor.

To reduce the use of nematicides a sampling method was needed which detects a certain standard infestation with a predefined probability and minimizes the area of a soil fumigation if one is needed, thus providing the means to make intelligent decisions on the nature and extent of control measures. This requirement implies that a more effective sampling method should be based on a general model describing size and shape of an infestation. Using data available from nematicide trials and a few intensively sampled fields in Flevoland, Schomaker & Been (1992) developed a prototype detection method for infestations in that area. An evaluation of De Groene Vlieg Ltd and the Ministry of Housing, Physical Planning and the Environment (VROM) of the

Table 7.1. - Definitions of terms used.

Infestation focus	-	Patchy infestation originating from a point infection with small numbers of cysts, transmitted by seed potatoes of agricultural machinery. These numbers are increased by multiplication on hosts and spread by farmer's practices.
Length direction	-	The direction of cultivation.
Width direction	-	The direction perpendicular to the direction of cultivation.
Cyst density	-	Cyst counts from samples of 1.5-2.5 kg of soil, converted into numbers of cysts per unit dry weight of soil. For mathematical analysis mean sample dry weight was used, for tabulating, graphs and mapping 1 kg was used.
Position in focus	-	Area in the focus of length 1.33 m by width 0.75 m ($\approx$ 1m^2), with (assumed) average cyst density *E[p(x,y)]*.
Focus centre	-	Position in the focus with the highest cyst density.
l	-	Gradient parameter, the ratio of the cyst density at a position with length coordinate x and the density at a position with length coordinate x-1.
w	-	Gradient parameter, the ratio of cyst density at a position with width coordinate y and the density at a position with width coordinate y-1.
x	-	Length coordinate of a given position in the focus: the distance in meters between the focus centre and that position in length direction.
y	-	Width coordinate of a given position on the focus: the distance in meters between the focus centre and that position in width direction.
p(0,0)	-	Cyst density in a sample from the focus centre. This position has the predefined length and width coordinates x = 0 and y = 0.
p(x,y)	-	Cyst density in a sample from a position in the focus with length coordinate x and width coordinate y.
E[p(x,y)]	-	Expected value of p(x,y). Parameter of the negative binomial distribution.
st	-	Subscript relating a parameter or variable to the steep side of the focus.
sh	-	Subscript relating a parameter or variable to the shallow side of the focus.
k	-	Coefficient of aggregation. Parameter of the negative binomial distribution.
k'	-	Estimate for *k*. $k' \approx k$ if variation due to errors in laboratory procedures and random disturbances of *E[p(x,y)]* from the gradient described by eq. (i) or eq. (ii) is negligible.

effect of the prototype sampling method in Flevoland proved that a decrease of 86% or more of the volume of nematicide use could be realised in the tested area (Schomaker & Been, 1992; NOVEM, 1995). At the same time the Dutch government proclaimed a drastic, mandatory, reduction of nematicides in the near future (Anonymous, 1991). As preliminary results with the new sampling method indicated the potential to reduce the use of nematicides substantially, the prototype was used throughout the country.

To establish whether the developed model for infestation foci applies to cropping areas with different soil types, cropping histories and cropping frequencies and to find out whether the same parameter values are valid, patchy infestations in other parts of The Netherlands had to be mapped and analysed. In 1990, a research program was initiated to sample patchy infestations in Zeeland, Friesland, Groningen and Drente to answer these questions. Sampling data of Schomaker & Been (1992) from Flevoland, originating from nematicide trials, were added to the data set. The statistical analysis of the data and the conclusions are presented in this paper.

7.3. Material and methods

Experimental procedure

Infested fields were selected in the cropping areas of Groningen, Drente, Friesland, Zeeland, and Flevoland using sampling results provided by the Dutch Plant Protection Service (PD) originating from their statutory soil sampling survey of potato fields. The survey gives the number of cysts found in soil samples of 200 ml originating from 1/3 ha (Oostenbrink, 1950) and the dimensions of the field sampled. Sampling units were always strips covering the full length of a field in the direction of cultivation with a width varying between 8 and 18 metres to obtain the approximate area needed.

First sampling: Location of the foci

The first sampling involved 82 fields. For this purpose infested strips of these fields were divided into rectangular plots of 8 · 3 m (length · width). These plot dimensions were chosen because changes in nematode densities per unit of distance are smaller in the length than in the width direction as potato cyst nematodes are mostly dispersed by farmers' practices (harvesting, tillage and planting) and therefore primarily in the direction of cultivation. From the central square metre (0.75 m wide and 1.33 m long) of these 8 by 3 m plots, a sample of approximately 1 kg soil, consisting of 40 cores of 25 g, was collected with a 25 cm long, 1 cm diameter auger. In The Netherlands it is common practice to grow potatoes in rows with a distance between rows of 0.75 m and an average distance between plants in a row of 0.66 m. Immediately after harvest, without further disturbance of the tilth, the majority of cysts are in the ridges (Seinhorst and Den Ouden, 1980). Therefore, the dimensions of the central square metre were chosen so that the relative amount of soil retrieved from ridges and furrows was approximately the same. Soil samples were transferred to special paper bags (type Harmonika, manufacturer Burgers BV, Apeldoorn, Netherlands) which allow slow evaporation of water from moist soil and do not rupture during this process. Samples in the bags were dried at temperatures not exceeding 20 °C to keep the nematodes alive. They were elutriated at a station of the Dutch General Inspection Service for Agricultural Seeds and Seed Potatoes (NAK) in the region where the samples were taken. For clay soils a carousel (Pollähne, Hannover, Germany) was used, for sandy and peaty soils the Schuiling centrifuge was used (Folkerts, Pancreas, Netherlands). Elutriation residues of the sandy and peaty soils were cleaned with acetone, using the improved Seinhorst device (Seinhorst, 1974, 1988). Finally, cysts were removed from the remaining organic debris and counted. An example of a result from this first sampling is given in Figure 7.1. Not all 82 sampled fields produced results as in Figure 7.1. Eleven fields were excluded from further investigation for the following reasons:

1. No infestation was found. The positive result by the statutory soil sampling was probably due to cross contamination of samples in the laboratory.

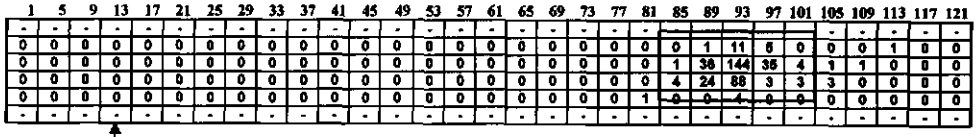


Figure 7.1. - Cyst densities (cysts/kg) in the central square metre of each of 124 plots of 8 by 3 m (length x width) covering approximately 0.33 ha (248 by 12 m) of field F27B resulting from the first sampling to locate the focus.

2. Infestations were not caused by the regular point infections, but by filling up (by farmers) of ditches or dips in fields with infested soil removed from potatoes during grading.

Another thirty-four fields were excluded because the foci were too small to yield data sets suitable for statistical analysis or because of an excess of fields with suitable foci in some cropping areas.

Second sampling: Mapping of the focus

At least five fields per cropping area were selected for the second sampling. Depending on the size of the infestation detected by the first sampling, a decision was made whether to sample every square metre of the focus or to use a wider grid (*e.g.*, every second square metre in the length and every square metre in the width direction).

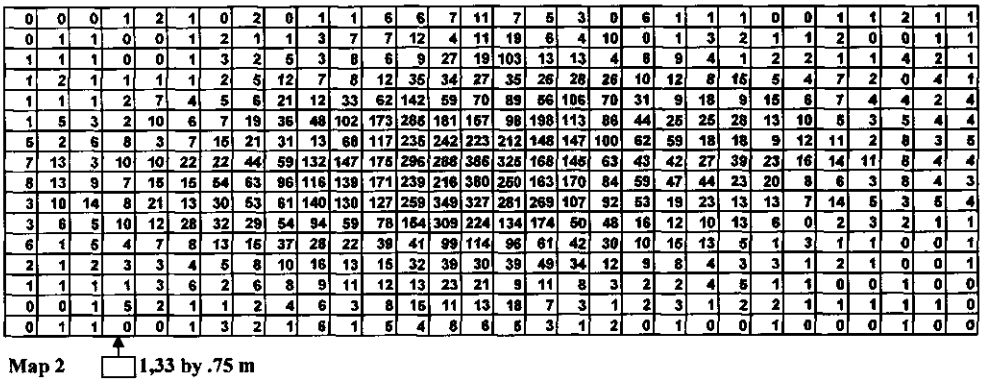


Figure 7.2. - Cyst densities (cysts/kg) in every square metre within the rectangle outlined in Figure 7.1. (field F27B) resulting from the second sampling.

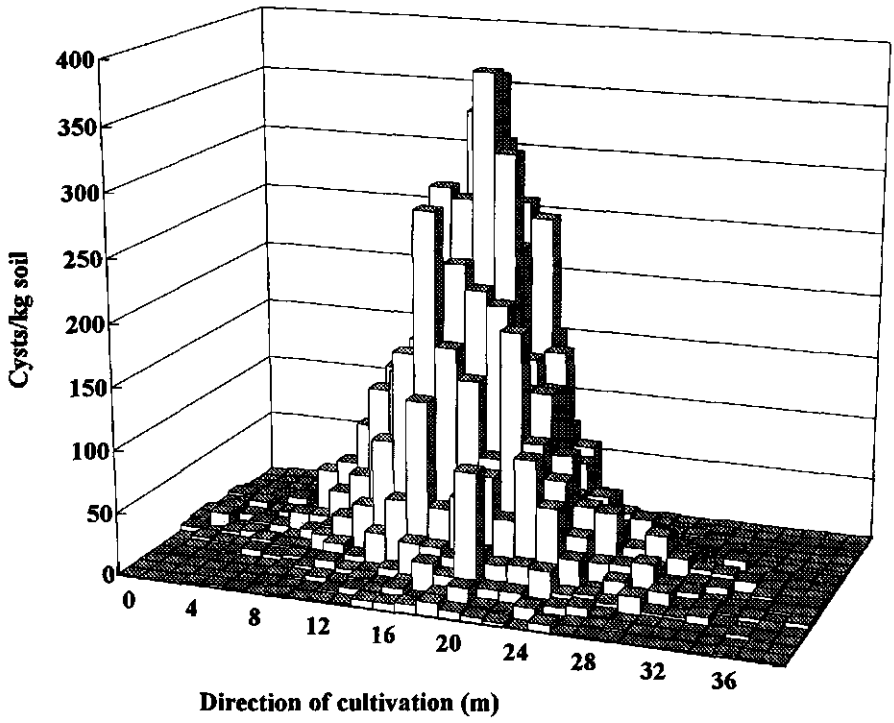


Figure 7.3. - Three-dimensional representation of the cyst densities (cysts/kg) shown in Figure 7.2. (field 27B). Note the approximately exponential increase of cyst densities towards the focus center.

Samples of 1.5 to 2.5 kg of soil were collected from each square metre (length x width = 1.33 x 0.75 m) by taking 80 cores with the above described auger in a stratified plan. Samples were divided into two or three subsamples and elutriated separately because of capacity limitations of the equipment. After extraction, all cysts were counted, subsamples were reunited and foci were mapped. Figure 7.2. and Figure 7.3. give two- and three-dimensional representations of a typical focus.

Mathematical analysis

An overview of sampled fields, grids used and other data of relevance in the first and second sampling is given in Table 7.2.

Adjustments in data sets:

Some data had to be excluded from the statistical analysis for the following reasons:

- Some fields contained several foci. Fig 4 shows examples of infestations ranging

from a single focus to a conglomerate of several foci. If possible, the secondary foci in the conglomerates were analysed separately. When foci in a conglomerate overlapped, the data points involved (often a plateau of identical cyst densities) were excluded.

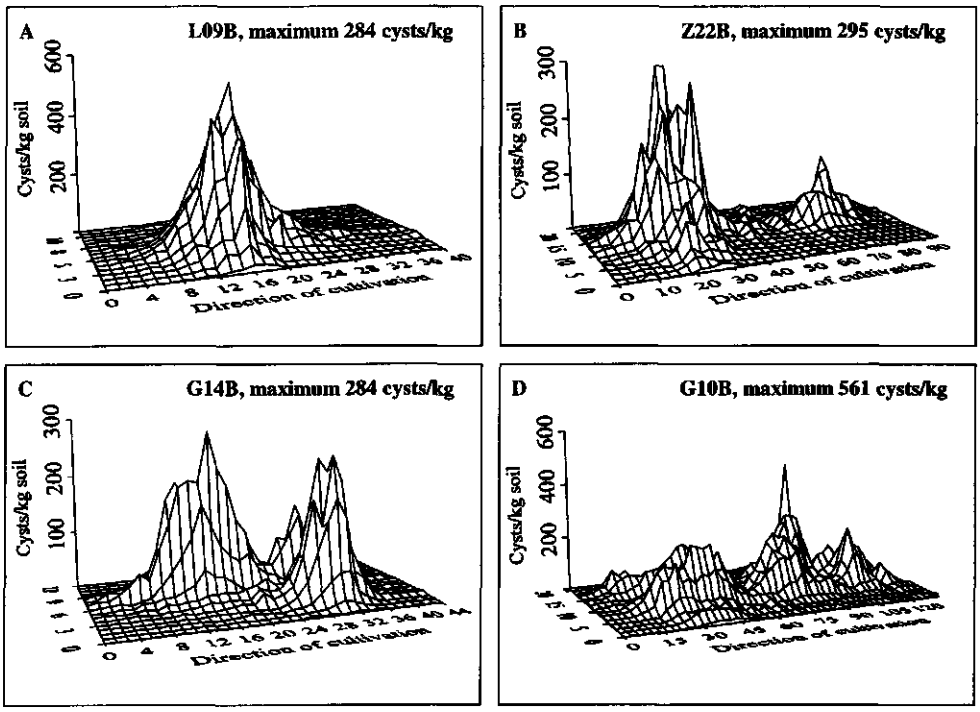


Figure 7.4 - Several infestation foci ranging from a single infestation focus (A) to an emerging secondary focus (B) and two confluent foci (C) to a conglomerate of foci (D). Densities (cysts/kg soil) are maximum densities of the primary focus in each sampled field from the second sampling.

Table 7.2. - Fields sampled to map infestation foci and some corollary data. a: "Grid" indicates the distance between the sample units (*e.g.*, a 1.33 · 0.75 grid implies that every square metre of the infestation focus is sampled). The sampled area of focus FL02B was a cross of samples running over the estimated centre of the infestation. b: The sample unit is 1 square metre (1.33 by 0.75 m). Eighty cores of approximately 20 g were collected in a stratified way, resulting in 1.5 kg bulk samples.

Name of field	Province	Cropping frequency	Year sampled	Grid[a] (m•m)	Number of rows	Number of cols	Number of samples[b]	Size of sample (kg)	Last potato crop	Max. pop. density (kg^{-1})	Used for analysis
FL01B	Flevoland	1 : 4	1987	1.33 • 0.75	4	37	148	2.5	1984	296	Yes
FL02B	Flevoland	1 : 4	1989	1.33 • 0.75	21	27	47	2.5	1988	481	No
FL04B	Flevoland	1 : 4	1987	1.33 • 0.75	16	37	592	2.0	1984	85	Yes
FL05B	Flevoland	1 : 4	1987	4.00 • 3.00	4	8	20	1.5-10.0	1986	1423	No
FL09B	Flevoland	1 : 3	1987	1.33 • 0.75	16	31	496	2.5	1985	527	Yes
FL09C	Flevoland	1 : 3	1988	4.00 • 2.25	8	13	104	2.5	1985	330	Yes
FL10B	Flevoland	1 : 4	1988	2.66 • 2.25	9	16	128	2.5	1988	184	No
F01B	Friesland	1 : 3	1991	1.33 • 0.75	9	24	216	1.5	1988	21	No
F11B	Friesland	1 : 2.5	1991	2.66 • 1.50	14	27	378	1.5	1989	468	Yes
F26B	Friesland	1 : 3.5	1991	2.66 • 1.50	16	49	784	1.5	1989	1217	Yes
F27B	Friesland	1 : 2	1991	1.33 • 0.75	16	30	480	1.5	1989	386	Yes
F28B	Friesland	1 : 3	1991	2.66 • 1.50	20	34	680	1.5	1989	808	Yes
G10B	Groningen	1 : 4	1990	2.66 • 1.50	15	50	750	1.5	1989	561	Yes
G14B	Groningen	1 : 4	1990	2.66 • 1.50	17	34	578	1.5	1989	284	Yes
G15B	Groningen	1 : 3	1990	2.66 • 1.50	11	15	165	1.5	1989	34	No
G18B	Groningen	1 : 3.5	1990	2.66 • 1.50	15	19	285	1.5	1989	235	Yes
G19B	Groningen	1 : 3	1990	2.66 • 1.50	13	13	169	1.5	1989	150	Yes
Z01B	Zeeland	1 : 4	1992	2.66 • 0.75	24	18	429	1.5	1990	113	Yes
Z02B	Zeeland	1 : 4	1992	1.33 • 0.75	21	18	378	1.5	1990	2	No
Z04B	Zeeland	1 : 4	1992	1.33 • 0.75	20	30	600	1.5	1990	189	Yes
Z09B	Zeeland	1 : 2	1991	2.66 • 1.50	15	23	345	1.5	1989	55	No
Z10B	Zeeland	1 : 4	1991	2.66 • 1.50	20	21	420	1.5	1989	565	Yes
Z17B	Zeeland	1 : 4	1991	1.50 • 2.66	14	20	280	1.5	1989	1209	Yes
Z21B	Zeeland	1 : 6	1991	2.66 • 0.75	24	14	336	1.5	1989	237	Yes
Z22B	Zeeland	1 : 4	1991	2.66 • 1.50	16	37	592	1.5	1989	295	Yes
Z29B	Zeeland	1 : 4	1991	2.66 • 1.50	14	18	252	1.5	1989	55	No
Z34B	Zeeland	1 : 8	1991	2.66 • 1.50	14	12	168	1.5	1989	53	No
D02B	Drenthe	1 : 2.25	1991	2.66 • 1.50	14	28	392	1.5	1989	532	Yes
D02C	Drenthe	1 : 2.25	1993	1.33 • 0.75	16	18	288	1.5	1989	307	Yes
D03B	Drenthe	1 : 5	1993	1.33 • 0.75	12	24	288	1.5	1987	164	No
D04B	Drenthe	1 : 3	1993	2.66 • 0.75	18	22	324	1.5	1992	12	No
D06B	Drenthe	1 : 3	1991	1.33 • 0.75	20	18	360	1.5	1991	571	Yes
D07B	Drenthe	1 : 2.67	1991	2.66 • 1.50	14	21	294	1.5	1991	1164	Yes
D11B	Drenthe	1 : 3	1991	2.66 • 1.50	14	19	266	1.5	1991	845	Yes
D12B	Drenthe	1 : 3.33	1991	1.33 • 0.75	14	27	342	1.5	1991	576	Yes
D13B	Drenthe	1 : 3	1993	1.33 • 0.75	20	24	480	1.5	1992	627	Yes
D17B	Drenthe	1 : 3	1993	2.66 • 0.75	20	21	420	1.5	1992	810	Yes

- Extremely small or high densities of one data point in a focus, mostly caused by elutriation errors, which were reported by the controllers.

Some fields had to be excluded from data analysis for the following reasons:

- During the second sampling the sampling team did not succeed fully in relocating the infestation on the fields Z02B, Z29B and D04B. The foci in these fields were only partly mapped or even completely missed.
- In most samples from the fields D03B, F01B, G15B, L02B, L05B, Z09B and Z34B only small numbers of potato cyst nematodes were found or none at all.
- Field FL10B was rejected because the farmer changed the direction of cultivation recently. As a result the length and width gradient parameters were similar.

The remaining 26 fields contained 46 foci of which 6 had to be rejected because of too few data points. Data sets from 40 foci (Table 7.3.) were suitable for analysis.

The models

All foci analysed by Schomaker and Been (1992) appeared to be more or less elliptical and cyst densities decreased exponentially away from the focus centre, but more slowly in the length than in the width direction. Consequently, the relation between log cyst numbers and distance from the centre was linear. The model for the general shape of a focus, described by Schomaker and Been (1992), is a symmetrical one for which the expected cyst density E in all directions is:

$$E[p(x,y)] = p(0,0) \cdot l^{|x|} \cdot w^{|y|} \qquad \text{(i)}$$

with terms as previously defined (Table 7.1.)

However, if foci are mirrored in a plane through the focus centre in the length or width direction, it appears that they are often not symmetrical. In most foci one side is steeper than the other in both length and width directions. This is thought to be caused by one-directional harvest and cultivation practices of farmers. Because of this phenomenon, an extension of eq. 1 with four parameters, w_{st}, w_{sh}, l_{st} and l_{sh}, was also fitted:

$$E[P(x,y)] = p(0,0) \cdot l_{sh}^{|x(sh)|} \cdot w_{sh}^{|y(sh)|} \qquad \text{for } x > 0 \text{ and } y > 0$$

$$E[P(x,y)] = p(0,0) \cdot l_{st}^{|x(st)|} \cdot w_{st}^{|y(st)|} \qquad \text{for } x < 0 \text{ and } y < 0$$

$$E[P(x,y)] = p(0,0) \cdot l_{sh}^{|x(sh)|} \cdot w_{st}^{|y(st)|} \qquad \text{for } x > 0 \text{ and } y < 0$$

$$E[P(x,y)] = p(0,0) \cdot l_{st}^{|x(st)|} \cdot w_{sh}^{|y(sh)|} \qquad \text{for } x < 0 \text{ and } y > 0 \qquad \text{(ii)}$$

Table 7.3. - Tabulated results of the Multiple Regression Analysis and GLM analyses of all sampled foci for the comprehensive (eq. ii) and the simple model (eq. i). '-' in cells indicate that parameter estimation was not possible. Gradient parameters *l* (length) and *w* (width) with subscript st and sh relate to the steep and shallow side of the focus, respectively. R^2 and D present goodness of fit of the Multiple Regression Analysis and GLM analyses, respectively.

Name of field	Data points per focus	Months between last pot. Crop and 2nd sampling	Multiple regression								Generalized Linear Models							
			Comprehensive model					Simple model			Comprehensive model					Simple model		
			l_{st}	l_{sh}	w_{st}	w_{sh}	R^2	l	w	R^2	l_{st}	l_{sh}	w_{st}	w_{sh}	D	l	w	D
FL01B	222	33	0.9	0.86	0.60	-	0.92	0.9	0.6	0.9	0.8	0.86	0.57	-	1	0.85	0.57	1.02
FL04B		33	0.67	*0.83	0.6	0.6	0.87	0.75	0.58	0.81	0.65	*0.83	0.6	0.6	1	0.75	0.58	1.36
FL04B/2	66	33	0.74	*0.86	0.51	0.66	0.76	0.78	0.61	0.70	0.72	*0.85	0.50	0.68	1.00	0.8	0.62	1.14
FL09B	285	21	0.69	*0.82	0.46	*0.58	0.92	0.80	0.54	0.86	0.68	*0.81	0.48	*0.57	1.00	0.8	0.54	1.66
FL09C	42	34	0.78	0.82	0.44	0.65	0.83	0.81	0.57	0.82	0.78	0.83	0.44	*0.66	0.96	0.8	0.6	1.20
F11B	160	21	0.83	*0.87	0.59	*0.64	0.90	0.86	0.62	0.88	0.83	*0.87	0.59	*0.64	1.01	0.85	0.6	1.16
F26B	259	22	0.89	*0.91	0.48	*0.68	0.90	0.90	0.64	0.87	0.89	*0.91	0.48	*0.68	0.94	0.9	0.6	1.21
F26B/2	118	22	0.67	*0.86	0.72	0.77	0.85	0.78	0.73	0.79	0.68	*0.86	0.73	0.79	1.06	0.78	0.7	1.42
F26B/3	46	22	0.79	*0.86	0.51	*0.69	0.93	0.82	0.60	0.89	0.77	*0.86	0.52	*0.67	1.00	0.81	0.6	2
F27B	258	21	0.79	0.79	0.49	0.50	0.92	0.79	0.49	0.91	0.74	0.77	0.36	0.49	1.02	0.76	0.5	1
F28B	197	21	0.86	*0.89	0.66	*0.75	0.88	0.88	0.71	0.86	0.85	*0.90	0.69	*0.72	1.00	0.87	0.7	1.1
F28B/2	80	21	0.84	*0.89	0.49	*0.81	0.89	0.86	0.79	0.73	0.83	*0.88	0.45	*0.81	0.99	0.86	0.77	2.5
G10B/4	148	15	0.88	0.91	0.68	0.75	0.78	0.90	0.72	0.78	0.93	0.94	0.69	*0.78	1.03	0.92	0.7	1.1
G10B/5	65	15	0.79	0.84	0.49	*0.79	0.72	0.8	0.70	0.64	0.73	*0.94	0.47	*0.78	1.06	0.84	0.69	1.4
G10B/7	113	15	0.90	0.91	0.66	*0.70	0.85	0.90	0.68	0.84	0.90	0.91	0.66	*0.70	0.97	0.90	0.7	1
G10B/11	51	15	0.75	0.83	0.62	0.68	0.80	0.83	0.65	0.79	0.75	0.84	0.63	0.67	1.00	0.84	0.65	1
G14B	139	27	0.88	0.89	0.60	*0.67	0.87	0.89	0.64	0.86	0.9	0.90	0.60	*0.68	1.04	0.89	0.6	1.18
G14B/2	59	27	0.84	*0.93	0.65	0.78	0.86	0.86	0.67	0.85	0.83	0.92	0.64	*0.75	1.02	0.85	0.66	1.1
G14B/4	71	27	0.79	0.81	0.46	*0.78	0.90	0.80	0.60	0.87	0.79	0.81	0.45	*0.77	0.97	0.80	0.61	1.2
G18B	47	15	0.76	0.77	0.51	0.54	0.83	0.77	0.53	0.83	0.76	0.77	0.50	0.55	0.95	0.76	0.54	0.94
G19B	50	15	0.73	0.75	0.69	0.72	0.81	0.74	0.71	0.81	0.72	0.74	0.69	0.72	1.01	0.73	0.70	1
Z01B	79	27	0.76	0.81	0.57	0.63	0.79	0.79	0.61	0.8	0.76	0.81	0.56	0.63	0.98	0.79	0.60	1
Z04B	135	27	0.77	*0.82	-	0.46	0.87	0.78	0.46	0.86	0.77	*0.78	-	0.48	0.97	0.78	0.48	1
Z10B	153	28	0.80	*0.88	0.70	0.71	0.81	0.84	0.71	0.78	0.79	*0.88	0.70	0.71	1.01	0.84	0.71	1.2
Z17B	186	28	0.87	-	0.67	*0.81	0.93	0.87	0.75	0.87	0.88	-	0.68	*0.81	1.05	0.87	0.75	1.9
Z21B	183	8	0.82	*0.92	0.74	0.76	0.71	0.88	0.74	0.69	0.82	*0.93	0.73	0.78	1.01	0.88	0.74	1.1
Z22B	156	28	0.84	0.85	0.64	*0.77	0.89	0.84	0.73	0.85	0.83	*0.85	0.64	*0.76	0.99	0.84	0.73	1.2
Z22B/1	57	28	0.85	0.86	0.58	0.62	0.82	0.86	0.61	0.82	0.85	0.86	0.57	0.61	1.00	0.85	0.60	1
D02B	77	27	0.77	*0.85	0.60	0.67	0.82	0.82	0.62	0.80	0.76	*0.84	0.59	0.65	0.95	0.80	0.61	1.07
D02B/1	60	27	0.80	0.83	0.55	0.59	0.89	0.81	0.59	0.88	0.79	0.82	0.52	0.57	0.99	0.80	0.57	1
D02C	131	45	0.80	*0.86	0.56	*0.67	0.88	0.81	0.59	0.87	0.80	*0.86	0.57	*0.67	1.00	0.81	0.60	1.1
D06B	169	3	0.75	0.78	0.50	*0.65	0.87	0.76	0.62	0.86	0.75	0.78	0.50	*0.65	0.95	0.76	0.61	1.03
D07B	140	3	0.81	*0.88	0.65	*0.85	0.92	0.85	0.71	0.86	0.82	*0.88	0.65	*0.85	0.95	0.84	0.72	1.54
D07B/2	79	3	0.86	*0.94	0.61	*0.75	0.92	0.90	0.67	0.89	0.87	*0.95	0.62	*0.76	0.97	0.90	0.68	1.18
D11B	73	4	0.80	*0.84	0.52	0.65	0.87	0.82	0.63	0.86	0.84	-	0.54	*0.64	0.94	0.84	0.64	1.12
D11B/2	58	4	-	-	-	-	-	-	-	-	-	0.77	0.59	*0.73	0.92	0.77	0.61	
D12B	200	21	0.83	*0.88	0.41	*0.76	0.91	0.86	0.59	0.80	0.84	*0.88	0.33	*0.82	1.01	0.86	0.58	2.48
D13B	116	10	0.74	*0.84	0.55	*0.75	0.90	0.82	0.73	0.89	0.78	*0.84	-	0.74	1.00	0.8	0.7	1.25
D13B/3	57	10	0.82	0.83	0.61	*0.81	0.85	0.82	0.65	0.84	0.83	0.83	0.62	0.82	1.01	0.8	0.7	1.05
D17B	163	15	0.85	*0.90	0.65	0.65	0.86	0.88	0.65	0.84	0.85	*0.90	0.65	0.65	1.05	0.9	0.7	1.2

The gradients in a focus with the smallest values of *l* or *w* were defined as 'steep' gradients; those with the largest values as 'shallow' gradients. The subscripts *st* and *sh*

relate parameters and variables to the steep side and the shallow side of the focus, respectively. Both models (eq. i) and (eq. ii) were fitted to each focus. By means of an F-test it was decided if model (ii) indeed explained the sampling results better than model (i).

Cyst counts, estimated by P(x,y), in samples from a small area (position) of about one square metre in a focus with coordinates x and y, are assumed to follow a negative binomial distribution (20). This distribution and its fitting to biological data has been extensively described by many workers, among others by Bliss & Fisher (1953).

$$Pr[p(x,y)=\alpha] = \binom{\alpha+k-1}{k-1}\left(\frac{E[p(x,y)]}{E[p(x,y)]+k}\right)^{\alpha}\left(\frac{k}{E[p(x,y)]+k}\right)^{k} \qquad \text{(iii)}$$

where Pr indicates the probability of finding a certain number (α) of cysts and α is a an integer ≥ 0. Both model (i) and (ii) enable calculation of the expected cyst density *E[p(x,y)]* at any location in the focus, if the cyst density of the focus centre p(0,0) is given. Model (iii) calculates the probability of any cyst count in a sample from a given position in the focus with coordinates x and y if *k* and *E[p(x,y)]* are known.

Estimation of parameters and statistical inference

Only four data sets from Flevoland, analysed by Schomaker & Been (1992) represented completely mapped infestations; the remainder consisted of only two or three length or width transects through foci or results from the first sampling (locating of the focus). In the latter case multiple regression analysis is impossible. Therefore, they applied simple linear regression analysis to the log transformed cyst density. From a statistical point of view, however, simple linear regression is not an appropriate tool to evaluate a three-dimensional model. As the combined data set (Table 7.3.) now consisted of a large number of completely mapped infestations, all sampling data by Schomaker & Been (1992) unsuitable to multiple regression could be omitted.

Manipulating data, for instance toggling outlier points in or out of the data set or removing a secondary focus and performing a rerun of the analysis was very cumbersome with GENSTAT (version 4.2) on a mainframe Vax computer. Therefore, a computer program, FOCUS V1.0 (Brouwer *et al*, 1993), was written to handle, display (in two and three dimensions) and analyse the chosen data sets. FOCUS also performs single and multiple regression analyses.

Multiple Linear Regression

FOCUS applies a log transformation to the cyst density to satisfy the classical conditions for linear regression: constancy of variance, approximate normality of errors and additivity of systematic effects (Mc Cullagh & Nelder, 1992, Chatterjee & Price). Equation (i) then becomes:

$$E(\log[p(x,y)]) = p(0,0) + |x| \cdot \log(l) + |y| \cdot \log(w) \quad \text{(iv)}$$

A potential problem with this method of analysis may be that the log transformation of cyst density may not be the most appropriate one, and the requirements for linear regression might not be entirely met. Seinhorst (1988) distinguishes three causes of plot to plot variation of cysts density: (i) exponential density gradients, (ii) (random) disturbances of these gradients and (iii) sampling error due to a negative binomial distribution. The distribution of deviations from mean log cyst density, caused by (ii) and (iii), approximate a normal distribution well enough, except that at log cyst density of 1.3-2 the upper tail of the normal frequency distribution contained fewer observations than the lower tail and at log cyst densities of 2-3 there were too many observations in both tails (Seinhorst, 1988). From this normal distribution an estimate (k') of k can be derived (Seinhorst, 1988), which is smaller than or equal to k. The value of k' equals k only if the second cause of variation, random disturbances of gradients, is small. Another condition for k' to approximate k is that the variation connected with laboratory procedures must be a negligible compound of the total amount of variance.
Another problem to be solved before analysis is the fact that the exact position of the centre of the focus is not known *a priori*. It cannot simply be derived from the two-dimensional map of the focus. Not every square metre of all foci was sampled and the grid quadrat with the highest cysts density may not represent the true maximum density in the focus. Even when every square metre of the focus had be sampled it would be unlikely that any quadrat of the sampling grid over the focus fully coincided with the position of the focus centre. Thus, a series of regression analyses were performed at variable positions of the putative focus centre to estimate the location of the true focus centre by an iterative procedure. The position of the focus centre was estimated as the position with the best fitting result (*i.e.* the smallest sum of squares).
The iteration in FOCUS proceeded as follows: (i) The area with the expected centre was identified; (ii) This area is subdivided; usually in 5 by 5 squares, resulting in 25 possible centre positions; (iii) For each position a multiple regression analysis is done; (iv) The position with the best fit is again subdivided into 25 areas. Steps (iii) and (iv) are repeated as often as necessary until the best fit was obtained. The number of iterations was determined by examining the distance between centre positions in the last two iterations. If this distance was smaller than a predefined value (down to 1 mm is possible), the iteration was ended. The minimum number of iterations depended upon the size of the chosen area and the dimensions of the grid quadrats.

Generalized Linear Models (GLM's)
Mc Cullagh & Nelder (1992) point out that in most cases no single scale satisfies all the conditions for classical regression. In our data set the log scale satisfied the condition of additivity, but possibly did not meet the requirements of normality and constancy of variance. With GLM's the conditions of normality and constancy of variance are no longer required, so that any distribution from the exponential family

can be used as an error distribution function, whereas the function that satisfies the condition of additivity need not be the same as the distribution for error (Mc Cullagh & Nelder, 1992). Because of the constraints mentioned above of the classical multiple regression analysis, the data set was also analysed by means of GLM's. In the GLM analysis the position of the focus centre as estimated by multiple regression analysis was used. The negative binomial distribution was chosen as the error distribution; the log transformation was used as a link function to linearize the models. Goodness of fit of the models was assessed by the mean deviance, which should be close to unity. The deviance (D) is defined as:

$$D = 2l(\alpha;p(x,y)) - 2l(\alpha;E[p(x,y)]) =$$

$$2\Sigma[p(x,y)\log\frac{p(x,y)}{p(x,y)+k'} + k'\log\frac{k'}{p(x,y)+k'} - p(x,y)\log\frac{E[p(x,y)]}{E[p(x,y)]+k'} - k'\log\frac{k'}{E[p(x,y)]+k'}]$$

$$2\Sigma[p(x,y)\log\frac{p(x,y)}{p(x,y)+k'} - p(x,y)\log\frac{E[p(x,y)]}{E[p(x,y)]+k'} + k\log\frac{E[p(x,y)]+k'}{p(x,y)+k'}] \qquad \text{(v)}$$

The mean deviance is then represented by eq. (v) divided by the number of degrees of freedom (*i.e.*, the number of data points minus the number of parameters estimated).
If k is fixed, the negative binomial distribution can be considered as a member of the exponential family. Earlier observations (Seinhorst, 1982, 1988; Schomaker & Been, 1992) support the assumption that the error distribution of cysts in samples from small plots in foci is well described by the negative binomial distribution with variable $E[p(x,y)]$ between plots of one square metre but a common k for all plots of the focus (and k dependent on sample size and not, with a certain minimum, on the number of cores). So it was safe to assume that k is a constant.
As maximum likelihood estimation was not possible, k' was estimated iteratively. An estimate for k that produced a value of the mean deviance closest to unity was considered the best (Mc Cullagh & Nelder, 1992). The value of k' obtained for the comprehensive model (eq. ii) was regarded as the best estimator and, therefore, also used as input parameter for the simple model (eq. i). The GLM-analyses were done with GENSTAT, release 5.2. (Payne, 1993), the first release which included the negative binomial distribution as an error distribution for this method of multiple linear regression.

Conversion of cyst counts
Because of inevitable variation in the sample size (weight) when 80 cores are collected per square metre, each data set had to be recalculated to a standard unit of soil. Conversion of actual counts (cysts/actual weight of soil) into considerably smaller units of soil would decrease the estimated variance and increase the estimated k, whereas conver-

sion into larger units of soil would have the opposite effect and the more so, as the coefficient of variation (standard error/mean) is smaller. Of course, in both cases the coefficient of variation itself remains unchanged. Thus, for all analyses, both with multiple regression and GLM's, cyst counts were converted to number of cysts per mean sample weight per data set (as close as possible to the actual counts) to avoid distortion of k and its estimates.

7.4. Results

Goodness of fit

Table 7.3. presents the results of both statistical methods for each field for the two models, the simple two-parameter model (eq. i) and the comprehensive four-parameter model (eq. ii).

Important characteristics in this table are:

- The number of data points in a focus
- The time interval (in months) between the second sampling and the last potato harvest
- The estimates of w_{sh}, w_{st}, l_{sh} and l_{st} by either method of analysis
- Significance of differences, indicated by *, between the steep and the shallow gradient parameters l_{st}, w_{st}, l_{sh} and w_{sh}, calculated by either method of analysis
- Goodness of fit: calculated by R^2 in Multiple Regression Analysis and by the mean deviance, D, in the analysis with GLM's.

Both models fitted well to the data sets from all foci (Table 7.3.). For the simple model (eq. i) the average values of R^2 and mean D were 0.83 and 1.25 and for the extended model (eq. ii) 0.86 and 1, respectively. These differences in goodness of fit were small, but significant ($P < 0.05$) with either method of analysis. Twenty five out of 34 foci were better described by the extended model (eq. ii) than by the simple model (eq. i) which means that 74% of the foci were asymmetric.

Parameter estimation

Per focus

There was little difference in parameter estimates of the gradient parameters between the multiple regression analysis and the GLM analysis. An asterisk indicates that a gradient parameter on the shallow side of a focus differs significantly from its equivalent on the steep side. Such a difference occurred in 55 (58)% of the foci in the parameter l, in 51 (60)% of the foci in the parameter w and in 31 (35)% of the foci in both gradient parameters l and w. In 25 (21)% of the foci only the gradient parameters l_{sh} and l_{st} and in 19 (21)% only the gradients parameter w_{sh} and w_{st} differed significantly while the other gradient parameters did not. In 25(23)% of the foci l nor w gradient parameters differed significantly. The percentages mentioned first come from the

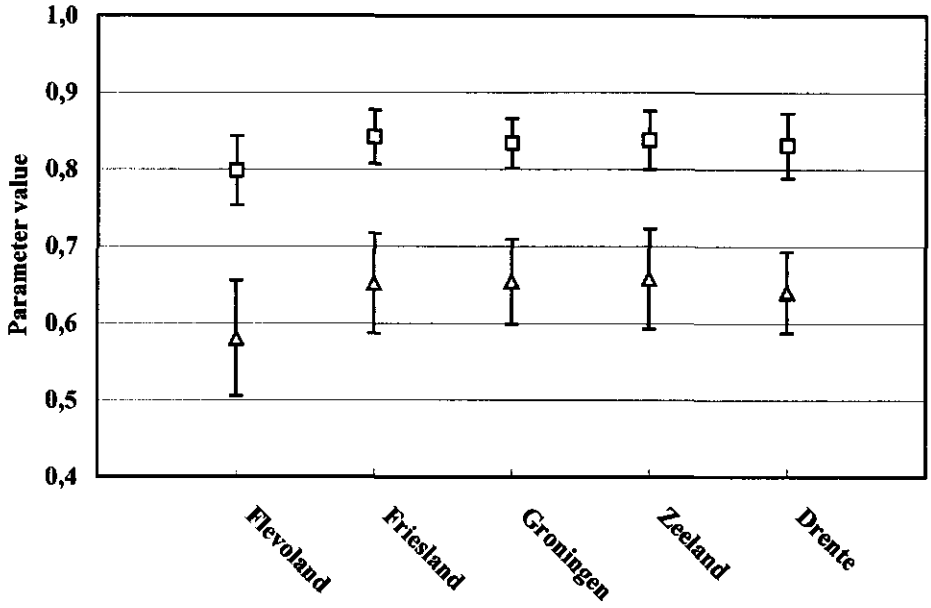


Figure 7.5. - Averages of the gradient parameters l (□) and w (△) for foci in five cropping areas in The Netherlands with their standard deviations (bar represents $\pm \sigma$). Soils of the first four cropping areas are mainly (heavy) marine clays, those of the last sand and peat.

multiple regression analyses, those between brackets from analyses by GLM's. These results indicate that most differences between the shallow and steep gradient parameters reflect true aberrations from the symmetrical model of eq (i).

Per cropping area

For the simple model (eq. i) the average parameter estimates were calculated for every cropping area: Drente, Flevoland, Friesland, Groningen and Zeeland. In Figure 7.5./Table 7.4. the averages of the gradient parameters l and w with their standard deviations are summarized for the five cropping areas. One way analysis of variance was used to investigate whether there were differences in gradient parameters between cropping areas. As probabilities of significant differences between cropping areas were 0.32 (for w) and 0.48 (for l), the answer to this question was negative. So, the parameter estimations of all foci can be averaged and considered to apply to all cropping areas, which implies that one detection or sampling method for infestation foci will suffice in all cropping areas of The Netherlands. In Table 7.5. the mean values of the parameters l and w of eq. (i) are given with their 95% confidence intervals. Mean values are virtually the same for both multiple regression analysis and GLM's.

Table 7.4. - Summary statistics for GLM results for each cropping area.

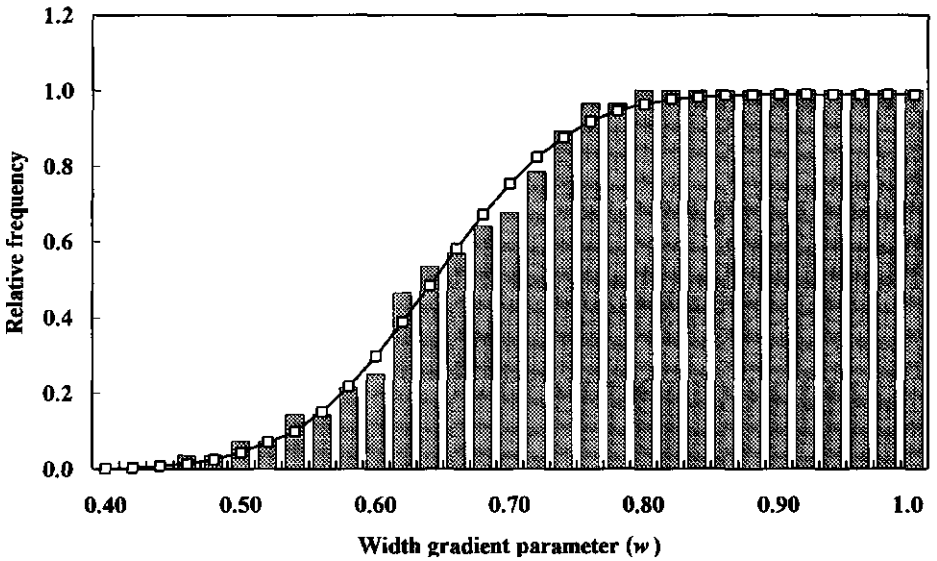
Province	Average gradient param. *l*	Standard deviation of *l*	Average gradient param. *w*	Standard deviation of *w*	Confidence limits $l_{st\ 95\%}$	$l_{sh\ 5\%}$	$w_{st\ 95\%}$	$w_{sh\ 5\%}$
Drente	0.83	0.042	0.64	0.053	0.90	0.76	0.73	0.55
Flevoland	0.80	0.045	0.58	0.075	0.87	0.73	0.70	0.46
Friesland	0.84	0.035	0.65	0.065	0.90	0.78	0.76	0.54
Groningen	0.83	0.032	0.65	0.055	0.88	0.78	0.74	0.56
Zeeland	0.84	0.038	0.66	0.065	0.90	0.78	0.77	0.55
All data	0.83	0.048	0.64	0.075	0.91	0.75	0.76	0.52

Table 7.5. - Summary statistics for two methods of analysis for the simple model (eq. i) pooled over all data.

	Multiple linear regression		Generalized linear models (GLM)	
	Gradient parameter *w*	Gradient parameter *l*	Gradient parameter *w*	Gradient parameter *l*
Number of values	39	39	40	40
Mean	0.642	0.832	0.640	0.831
Minimum value	0.459	0.739	0.447	0.731
Maximum value	0.791	0.904	0.773	0.926
Standard deviation	0.073	0.044	0.075	0.048
Upper 5% conf. limit	0.76	0.73	0.76	0.91
Lower 5% conf. limit	0.52	0.76	0.52	0.75
R^2/Deviance (averaged)		0.83		1.26

Probability distribution of the gradient parameters l and w

The probabilities of the length and width gradient parameters in any focus appeared to be well described by the normal distribution $N(\mu,\sigma^2)$. For *l*: $N(0.83, 0.0023)$ and for *w*: $N(0.64, 0.0056)$. Figure 7.6. shows the cumulative normal distributions as drawn lines and the cumulative relative frequencies of *l* and *w* plotted as grey bars. The probability of any combination of parameters *l* and *w* in a given focus can be described by a bivariate normal distribution with parameters $l = 0.83$, $w = 0.64$, $\sigma_l^2 = 0.0023$, $\sigma_w^2 =$



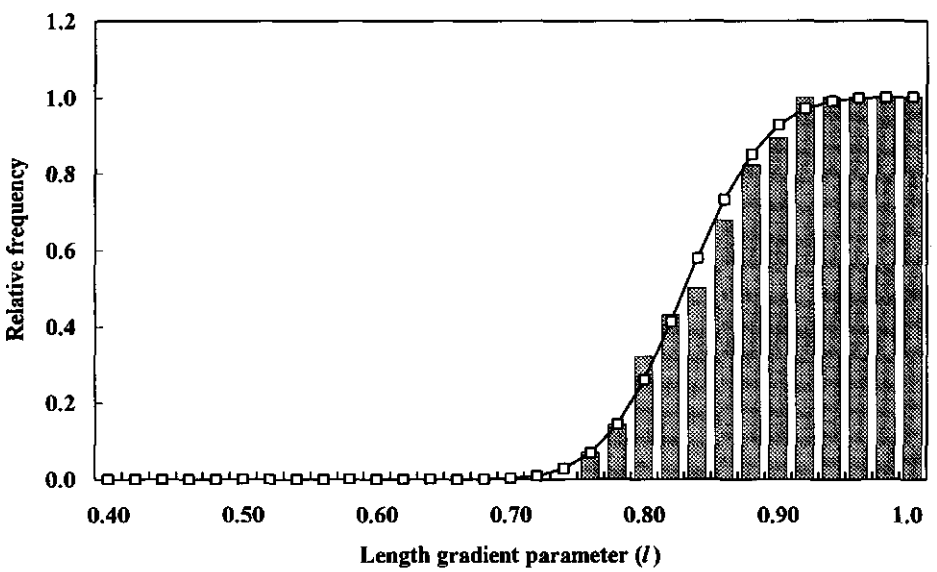


Figure 7.6. - Check for normality of the two gradient parameters *l* and *w*, describing an infestation focus. Bars: relative frequencies of the estimates of *l* and *w*; line: relative frequencies of *l* and *w* according to a normal distribution.

0.0056 and correlation coefficient $\varphi = 0.5$. Figure 7.7. gives a two-dimensional display of the actual combinations of *w* and *l* for all 40 foci studied and the 90% and 95% contours of the bivariate normal distribution (Morrison, 1990).

Correlation of parameter values and field characteristics
Several variables related to the sampled fields were listed and correlated pair-wise, such as cropping frequency, time interval (months) between last potato crop and the second sampling, cyst density of the focus centre, nematode species, and cropping frequency. Apart from the correlation already mentioned between l and w, no other correlations were found.

7.5. Discussion

Focus
Both the multiple regression analysis and the GLM provided good fits of the available

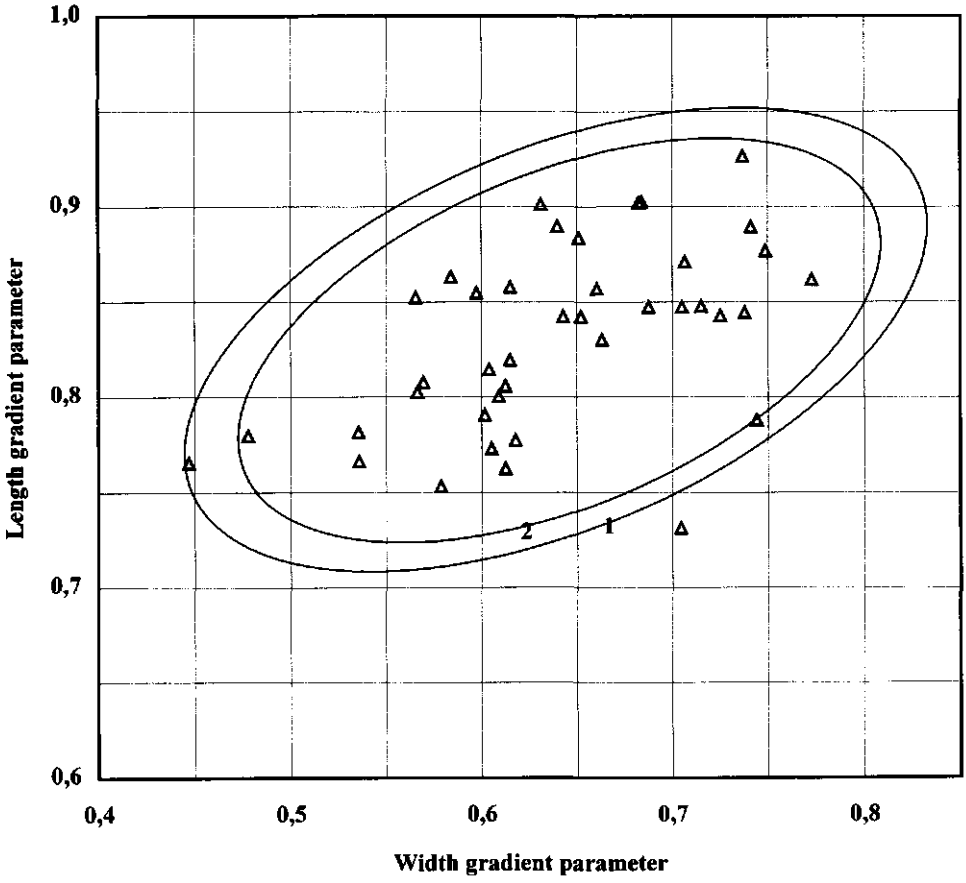


Figure 7.7. - Two-dimensional display of the bivariate distribution of the gradient parameters l and w. Triangles: actual estimates of l and w per focus; curves 1 and 2: 95 and 90% probability contours.

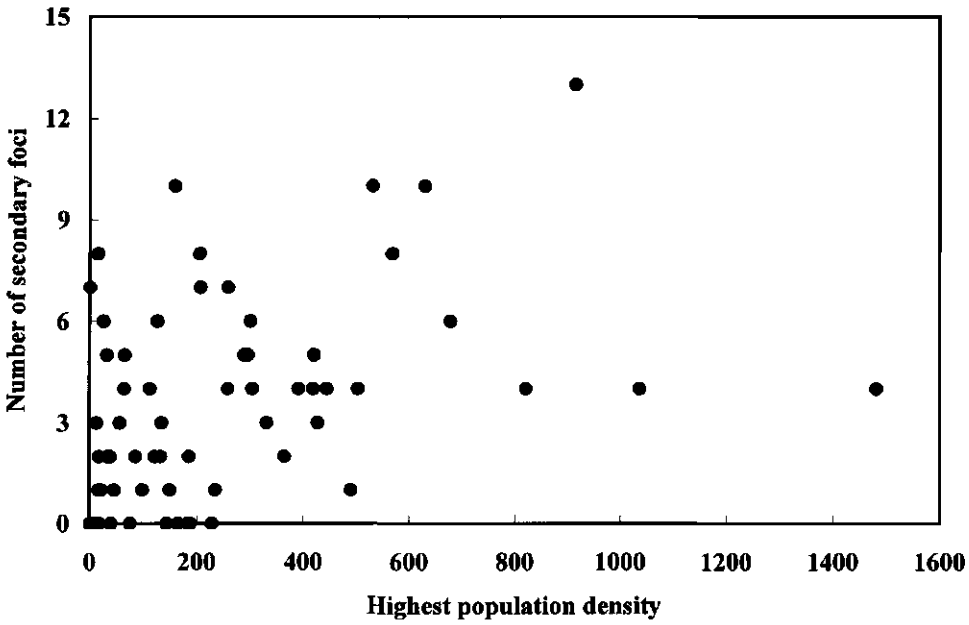


Figure 7.8. - The number of secondary infestations in a field (average area 0.33 ha) plotted against the highest cyst density of the primary (= largest) infestation focus in that field. The data are obtained from the first sampling.

data sets to both models, eq. (i) and (ii), with almost identical results (Table 7.3.). The requirements for multiple regression analysis, normality of residuals and constancy of variance were sufficiently met. For the latter requirement, constancy of variance, this is probably due to the large cyst counts, estimated by p(x,y), relative to the value of *k*' (Anscombe, 1948).

Both models described by eq. (i) and (ii) provided a good description of the shape and size of an infection focus, the comprehensive model explaining the observations best. From a statistical point of view it seems correct to choose the comprehensive model represented by eq. (ii). For scientific purposes, such as the disentangling of sources of variance in cyst counts or studying the processes contributing to the shape of a focus, eq. (ii) indeed is the best model. For practical purposes however, such as the development of detection methods, simplicity is required as long as there are no large deviations from reality. The R^2 values of the multiple regression analysis of the simple model (eq. i) are only slightly reduced compared to those of the comprehensive model (eq. ii) and the mean deviance D from the analyses with GLM's fit never moves dramatically from unity. Both the fitting results and the robustness of the simple two-parameter model (eq. i) make it pre-eminently suitable for extension purposes.

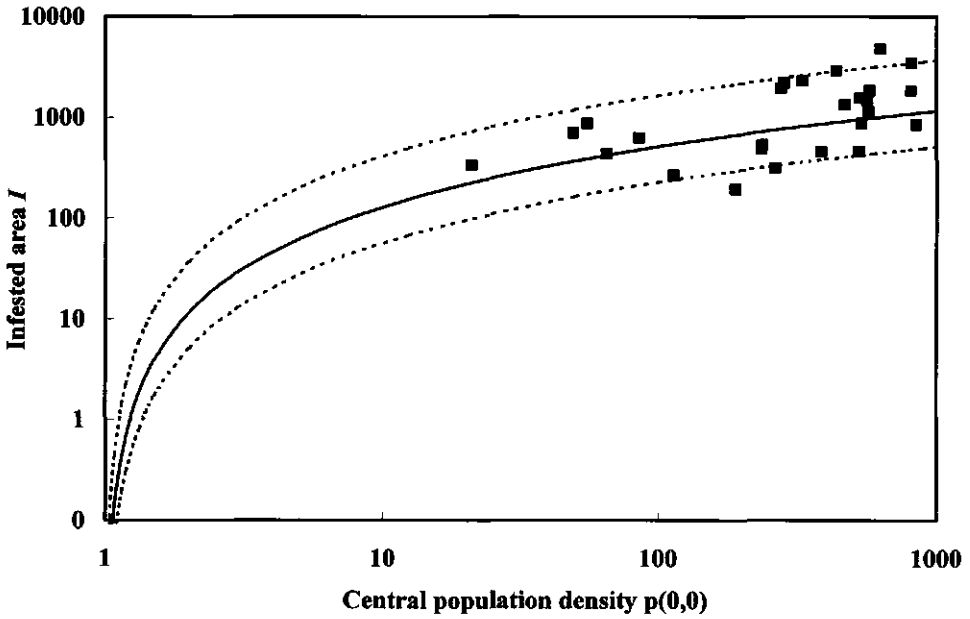


Figure 7.9. - Infested area *I* (m^2) of fields plotted against the cyst density of the primary foci in these fields. The data are obtained from the first sampling. Solid line and dotted lines represent the infested area for one focus, calculated using the mean and the 95% confidence limits, respectively, of both the length and width gradient parameters according to eq. (vi).

Cropping areas

The fact that no differences in parameter values between cropping areas were found implies that any sampling method developed on the basis of the pooled data set will be useful for all cropping areas in The Netherlands. Been & Schomaker, (submitted) describe the use of the simple model (eq. i) in the development of new sampling methods for detection. As the two gradient parameters *l* and *w* both follow a normal distributions, the probability for any combination of these parameters can be described by a bivariate normal distribution. This frequency distribution can be used in several ways. For instance the overall performance of a detection method can be assessed by calculating the average detection probability of a predefined focus for any possible combination of parameter values (Been & Schomaker, unpublished). Conversely, a sampling method can be adjusted in order to detect a certain volume of this distribution with predefined probability or to interpret sampling results obtained by these methods. The latter feature has already been incorporated in a prototype IPM system for growers of seed and consumption potatoes (Been *et al*, 1996).

Correlations

Notwithstanding the 40 foci available for analysis, no correlation could be found between focal size and the two gradient parameters of the model or the time interval between sampling and the last potato harvest. This implies that any detection method utilized to find infestation foci of potato cyst nematodes is not subject to these criteria. For instance, the detection probability will not suffer from the time interval passed between harvest and the date of carrying out the sampling procedure. Of course this interval will be restricted by the natural decline of the population density if no potatoes are grown and a safe margin would be within three to four years at most.

Secondary foci and infested area

With the negative binomial distribution describing the small scale distribution of cysts (per square metre) and the models according to eq. (i) and (ii) describing the medium scale distribution (foci) an attempt was made to estimate the large scale distribution within fields (number of foci per area) with data of the first sampling, which primary purpose was locating the focus. Secondary foci were found in 67 out of 71 (94%) pre-sampled fields. No simple relation could be found between the number of secondary foci and the highest cyst density found (*i.e.*, estimate for the cyst density in the centre of the focus) in the primary focus and therefore no predictions could be made about the number of secondary foci if the size of the primary focus was known (Figure 8.8.). Irrespective of the highest cyst density in the primary focus, an average of three and a maximum of 13 secondary foci per field were found. However, it was possible to establish a relationship between the infested area of a field and the highest cyst density of the primary focus. To evaluate its pattern and magnitude, it was compared with the relation between infested area (I) of a particular focus and cyst density in the centre of that focus, derived from the focus model:

$$I=2\cdot\log(p(0,0))^2/\log(l)\cdot\log(w) \qquad \text{(vi)}$$

It then appeared that, although the mathematical patterns of both relations were quite simular, the infested area of both primary and secondary foci estimated from the cyst density in the centre of the primary focus was greatly underestimated with the data set of the first sampling (Fig 9). It should have been larger than the infested area I of the primary focus as, on average, three secondary foci were present in each field.

For lack of a model of the distribution of foci in a field, which obviously cannot be derived from the first sampling, probably because of its incompleteness and the coarseness of the sampling grid, the total infested area can be estimated with equation (vi) assuming that

1) any primary infestation found is accompanied by three secondary foci;
2) these secondary foci have an average cyst density in the centre of 50 cysts/kg;

3) the overlap of the foci is negligible.

For the time being it can serve as a basis for advice to farmers about the control measures to be taken once an infestation is found. If for instance, a primary focus is detected with a cyst density of 200 cysts/kg in the centre (estimated from highest cyst count and the number of 5 metre width infested strips (Been & Schomaker, 1992)) the average infested area is estimated as 675 m^2 for the primary infestation plus 3 x 368 m^2 for the secondary infestation, amounting together to 1,779 m^2, which is more than 50% of a, for Dutch concepts regular, 0.33 ha field* (*defined as an 0.33 ha area with average length of 220 m and an average width of 15 m). It is useless then to spend time and money in a more precise location of the focus to take local control measures. It would be more appropriate to plant the whole field* with a potato cultivar with a degree of resistance sufficient for the growing frequency of potatoes and the virulence of the nematode population (Been *et al.*, 1995).

Chapter 8

Sampling methods for fields with patchy infestations of the potato cyst nematode (*Globodera* spp.): A simulation model to develop and evaluate sampling methods

T. H. Been & C. H. Schomaker

8.1. Summary - A computer program called SAMPLE was developed to evaluate existing and create new sampling methods for the detection of patchy infestations or 'foci' of the potato cyst nematode (*Globodera* spp.). By combining a model for the medium scale distribution of cysts, which provides the expected population densities at each position within the focus, and a model for the small scale distribution within square metres (negative binomial distribution) SAMPLE allows to simulate sampling procedures. The importance of the parameters of the two distribution models - the length and width gradient parameters for the medium scale distribution and the aggregation factor k of the negative binomial distribution for the small scale distribution - was investigated by sensitivity analyses. The aggregation factor k proved to be less important when calculating the average detection probability of a focus than the length and width gradient parameters. Several existing versions of the statutory sampling method used in The Netherlands were tested for their performance on a standard infestation focus with a central population density of 50 cysts/kg soil. The standard focus is small enough to use resistant potato varieties as a control measure without noticeable yield reductions in a 1:3 potato crop rotation. As the statutory soil sampling methods did not perform with the desired average detection probability, set at 90%, the program was used to develop several new sampling methods for focus detection and to investigate their performance. SAMPLE is a tool to develop sampling methods on demand for every possible combination of characteristics required for use by seed and ware potato growers (recommendations for optimum control measures leading to maximum returns, Integrated Pest Management) and by governments (legislation, quarantine and export protection).

8.2. Introduction

In the 1980's, 60% of the total volume of pesticides used in The Netherlands consisted of soil disinfectants for the control of potato cyst nematodes (*Globodera* spp.). Approximately half of this volume was used for soil fumigation in seed and ware potato areas, located mostly on heavy marine clay soils. Been & Schomaker (1987, submitted) conducted field trials with 1,3-dichloropropene on these soils to investigate the efficiency of soil fumigation and reported average mortalities of 60% in the upper 25 to 30 cm of the tilth. However, potato roots and cysts were found down to a depth of 80 cm. The area of the infestations was small compared to the area commonly treated with a fumigant. As a result, more than 90% of the active compound was wasted on uninfected soils. Large numbers of fields in these areas were treated with fumigants without the demonstrated presence of potato cyst nematodes. The fields were treated as a result of government regulation or as a prevention of an eventual detection of potato cyst nematodes by statutory soil sampling.

To reduce this high, ineffective and in most cases unnecessary input of soil fumigants, sampling methods are needed to detect infestation foci having low population densities with a high detection probability in order to help farmers in making decisions on the nature of the control measures required for optimal economic returns. Knowledge of both the medium scale (shape and size of foci) and the small scale distribution (within square metres) of potato cyst nematodes can be combined in a simulation model to evaluate existing sampling methods and to develop new ones if necessary. As the intended computations are too comprehensive to do by hand, a computer program, SAMPLE, was developed using the BASIC Compiler V.7 to automate development and testing of sampling methods for detection of patchy infestations.

8.3. Material and Methods

Spatial distribution models

The spatial distribution of potato cyst nematodes in the field is not uniform. Fields are free of potato cyst nematodes until an initial infestation occurs. Localized populations will increase every year in which potatoes are grown and cluster at the location where plants have grown, leading to a hot spot or focus (Zadoks & van den Bosch, 1994). Redistribution of nematodes in the soil depends on farming practices, mainly the use of machinery, which cause the growth of the primary focus and the appearance of secondary foci elsewhere in the field. A large scale, medium scale and small scale distribution can be distinguished. The large scale distribution refers to a whole field and its number of foci. The medium scale distribution pattern refers to the size and shape of a focus as a result of farming practices. The small scale distribution of potato cyst nematodes is the result of multiplication on the roots of evenly spaced potato plants and local effects of redistribution; the result can be described by a density probability function.

Schomaker & Been (submitted) developed a simple exponential model describing the general shape of a focus based on the analysis of 39 infestations, mapped in square metre units, in various cropping areas of The Netherlands. The general shape of a focus is described by:

$$E[P(x,y)] = p(0,0) \cdot l^{|x|} \cdot w^{|y|} \qquad (1)$$

For explanation of symbols see Table 8.1.

With this model the expected population density at any location in a focus can be calculated given a certain central population density $p(0,0)$ and the values of the length gradient parameter (l) and the width gradient parameter (w). These parameter values proved to be the same in all cropping areas (Schomaker & Been, 1998) so that any sampling method for detection will have the same performance throughout The Netherlands.

Table 8.1. - Definition of terms used

Sampling grid	
grid point	Location where a core sample will be taken.
grid quadrat	Area between four grid points placed in a rectangle; also area represented by one grid point in a rectangular grid.
grid quadrat length	Distance between two grid points in the direction of cultivation.
grid quadrat width	Distance between two grid points perpendicular to the direction of cultivation.
grid pattern	Pattern of grid points of a rectangular grid, defined by a combination of grid quadrat length and width.
grid quadrat area	Area 'covered' by one core sample in a grid point = grid quadrat length . grid quadrat width.
grid position	Position of grid with respect to centre of focus.
Soil samples	
core sample	Single sample taken with a sampling auger at a certain grid point.
bulk sample	Soil sample taken per standard area (m^2, ha) consisting of several core samples and processed as one sample.
field sample	Soil sample taken per standard area (ha) consisting of several bulk samples which are separately processed.
core sample size	Weight of soil collected by one core sample.
bulk sample size	Total weight of all core samples to be processed as one bulk sample.
field sample size	Total weight of all core samples to be processed as several bulk samples.
Detection probability	
Pr	Probability of detecting 1 or more cysts by collecting one core sample.
Pr'	Probability of detecting 1 or more cysts by collecting all core samples taken within a focus.
Pr'_{aver}	Average detection probability; average of all detection probabilities per focus for all possible grid positions.
$Pr'_{overall}$	Overall detection probability; average of all average detection probabilities for all combinations of the length and width gradient parameters according to the bivariate normal distribution.
Equations	
Length direction	The direction of cultivation.
Width direction	The direction perpendicular to the direction of cultivation.
$p(x,y)$	Cyst count at a position in the focus with length coordinate x and width coordinate y.
$E[p(x,y)]$	Expected population density at a certain position in the focus with length coordinate x and width coordinate y [cysts/kg soil]. Also parameter of the Negative Binomial Distribution.
$p(0,0)$	Population density in the centre of the focus [cysts/kg]. This position has the predefined length and width coordinates x = 0 and y = 0.
l	Length gradient parameter, the ratio of the cyst density at a position with length coordinate x and that density at a position with length coordinate x-1.
w	Width gradient parameter, the ratio of cyst density at a position with width coordinate y and that density at a position with width coordinate y-1.
x	Length coordinate of a given position in the focus: the distance in metres between the focus centre and that position in length direction.
y	Width coordinate of a given position in the focus: the distance in metres between the focus centre and that position in width direction.
k	Coefficient of aggregation. Parameter of the Negative Binomial Distribution.
k'	Common k according to Bliss & Owen (1953).
k''	Coefficient of aggregation for the core sample size; proportional to the size of the sample used to estimate k'

Cyst counts, estimated by $p(x,y)$, in samples from a small area of about one square metre in a focus with coordinates x and y, are assumed to follow a Negative Binomial Distribution (Seinhorst 1988). This distribution and its fitting to biological data is extensively described by many workers, among others by Bliss & Fisher (1953). Considering available data from the literature (summarized by Seinhorst, 1988) and k values calculated from more than 40 foci and from numerous small scale distribution patterns, Schomaker & Been (1992, in prep) an operational value resembling a 'common k' (Bliss & Owen, 1953), denoted as k', of 70 could be established for bulk samples of 1.5 kg (80 core samples of approximately 20 g) originating from one square metre. The aggregation factor used in the calculations, k'', is proportional to the (core) sample size taken from that square metre (Seinhorst, 1988).

The probability of detection within a square metre can be defined as the probability of extracting one or more cysts from this area, or 1 minus the probability of finding no cysts at all. For this purpose the negative binomial distribution function:

$$Pr[p(x,y)=\alpha] = \binom{\alpha+k-1}{k-1} \cdot \left(\frac{E[p(x,y)]}{E[p(x,y)]+k}\right)^{\alpha} \cdot \left(\frac{k}{E[p(x,y)]+k}\right)^{k} \qquad (2)$$

where Pr indicates the probability of finding a ceratin number (α) of cysts and α is a integer ≥ 0 can be simplified to the following equation when $\alpha = 0$:

$$Pr[p(x,y)=\alpha] = \left(\frac{k}{E[p(x,y)]+k}\right)^{k} \qquad (3)$$

The combination of equations (1) and (3) allows to calculate the probability of detection when applying a certain sampling method defined by grid pattern and core sample size on the one hand and focus size defined by the central population density and the values of the length and width gradient parameter on the other hand.

Simulation model

Table 8.1. defines the terms used in this paper while Table 8.2. lists the input parameters required for the simulation program.

The finite focus

The exponential model according to equation (1) is infinite so that population densities will decrease asymptotically towards zero in all directions. In real life, a focus is limited in size and therefore focus dimensions have to be established for use in the calculations. Two different methods are possible, the probability model and the density model. The first method sets a limit to the focus when the probability of detecting no

Table 8.2. - Program input

central population density $P(0,0)$	from equation (1) in cysts/kg soil
length gradient parameter l	from equation (1)
width gradient parameter w	from equation (1)
grid quadrat length	dimension of the sampling grid in the direction of cultivation e.g. 5 m
grid quadrat width	dimension of the sampling grid in the direction perpendicular to the direction of cultivation e.g. 5 m
core sample size	weight of soil collected by one core sample (g)
step size grid	distance in cm used to shift the sampling grid in l and/or w direction (default 0.1 m)
aggregation factor	k' for 1.5 kg soil
threshold value	value which is used to limit focus dimension in length and width direction; Pr[p(x,y)=0] > 0.99 (probability model) , 1 cyst/kg soil (density model).

cysts at all in the volume of soil collected by a core sample will exceed a set value according to equation (3), e.g. $Pr[p(x,y) = 0] \geq 0.99$. The second method limits the area of the focus to that area where the population density is above a set value e.g. 1 cyst/kg soil. Both methods have been implemented in SAMPLE.

The probability model is elegant but it has some severe drawbacks. When the core sample size increases in the course of an iteration process to attain a predefined average detection probability, the probability of detecting no cysts will decrease at any location within and just outside the focus as defined by the set value. Thus the probability model increases the size of the focus. When this increase occurs at a critical point in the iteration process, close to the desired average detection probability, optimisation will fail. Core sample size increment and decrement will cause the average detection probability to jump around the required probability, outside the tolerance value set for the difference between the required and the calculated average detection probability. In addition, the size of a focus can grow disproportionately by increasing the core sample size. Considering these drawbacks the probability model was discarded in favour of the density model.

Valid sampling grid points

The first step of any calculation will be to match the size of the sampling grid - the number of grid points which will actually be considered in the calculation of probabilities - with the size of the focus under investigation. Starting at the centre of the focus and stepping along both axes (length and width), using as increment values the grid quadrat length and width, the numbers of relevant grid points (those within the finite focus) in both directions, g_x and g_y, will be calculated. In this way the size of a rectangle ($2g_x + 1 \cdot 2g_y + 1 = n$ grid points) covering the whole focus is calculated. The program only computes probabilities for the calculated area of the focus and therefore is not hampered by the size and shape (such as blocks, strips or squares) of the spatial unit from which the core samples are collected to constitute the bulk sample. In view

of the shape of the focus, most grid points at the corners of the calculated rectangle will be below the threshold value of the density model (Figure 8.1.). The calculated population density at each grid point will be tested against the threshold value and grid points below the threshold will be disregarded in further calculations.
In every grid point where a core sample is taken the expected population density is calculated using equation (1). The probability of finding zero cysts in a soil sample from a focus as a whole is defined as the product of the outcomes of equation (3) for all core samples taken within the focus. The complement of this product is the detection probability of a focus, Pr^f. If n subsamples are taken:

$$Pr^f[p\geq 1] = 1 - \prod_{i=1}^{n} Pr[p(x,y)_i = 0] \qquad (4)$$

The position of the focal centre
As the exact position of the focus within the field is unknown, the sampling grid can overlay a focus in many different ways and a separate detection probability can be related to all of them. Therefore, SAMPLE systematically shifts the grid in both directions using 'step size grid' (Table 8.2.) and calculates the detection probability of the focus for each new position of the grid. As the above calculated rectangle of valid grid points has its central grid point in the centre of the focus the sampling grid will be shifted by half a grid quadrat length and width to the left and upwards respectively before actual simulations start, see Figure 8.1. where a 5 x 5 m sampling grid is superimposed over a focus with a central population density of 50 cysts/kg of soil (standard focus), with arrows indicating the directions in which the grid will be shifted. When the sampling grid has been shifted in both directions using 'step size' for distances equivalent to grid quadrat length and width, all possible grid positions will have been evaluated. As the model chosen for the shape of the focus is symmetrical over both axes (Schomaker & Been, submitted), only one quadrant needs to be evaluated, thus saving nearly 75% of calculation time.

Finally, a frequency distribution of detection probabilities per focus is obtained over all grid positions. If the grid was shifted N times, the average detection probability is

$$Pr^f_{aver}[p\geq 1] = \frac{\sum_{i=1}^{N} Pr^f_i[p\geq 1]}{N} \qquad (5)$$

Table 8.3. shows a sampling routine in which, for simplicity, code for restricting calaculations to one quadrant and valid grid points has been omitted. Gridnumber counts the number of grid positions evaluated (maximum = [grid quadrat length/step

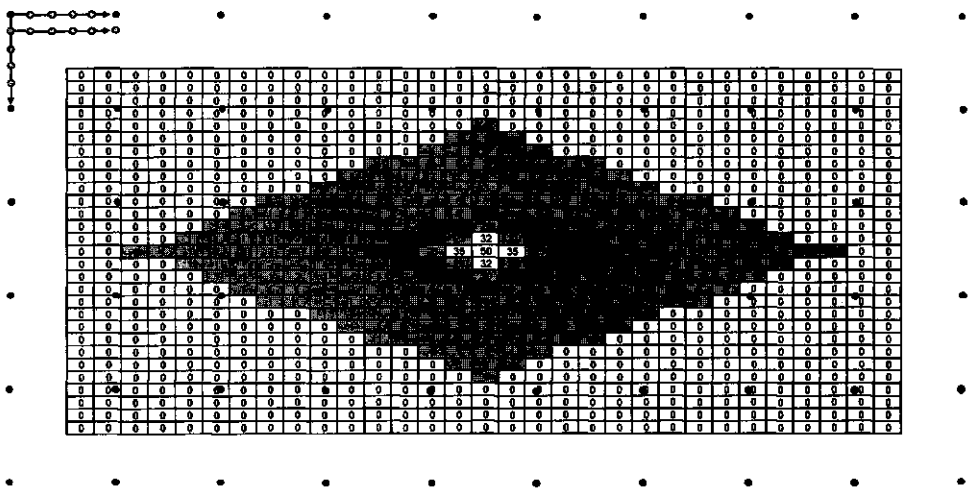


Figure 8.1. - Map of a focus with a central population density of 50 cysts/kg soil and length and width gradient parameters according to the 10% lower percentile of the bivariate normal distribution (standard focus). Population densities (cysts/kg) per square metre (1.33 x 0.75 m) calculated according to equation (1). A 5 by 5 m grid pattern is superimposed. Arrows indicate the shift of the grid in both directions to calculate the average detection probability and its variance. The shift distance (called step size) of the grid can be set to any value from one cm upwards (default 10 cm).

Table 8.3. - Simplified sampling routine in BASIC

```
SUB monster
    gridnumber = 0:
    CONS = (centralpopdensity/kg)/1000*coresamplesize
    FOR i = 0 TO gridstepnumberinwidth - 1
        FOR j = 0 TO gridstepnumberinlength - 1
        probability = 1!: coresamplenumber = 0
            FOR k = -coresamplenumberinwidth TO coresamplenumberinwidth
                FOR l = -coresamplenumberinlength TO coresamplenumberinplength
                    M! = CONS * (lengthgradient parameter ^ ABS(j*step size + (l * gridquadrat length))) *
                    (widthgradient parameter ^ ABS(i*step size + (k * gridquadratwidth)))
                    Zpsub = (k / (M! + k)) ^ k
                    ZPTOT = ZPTOT * Zpsub: coresamplenumber = coresamplenumber + 1
                NEXT l
            NEXT k
            gridnumber = gridnumber + 1: probability(gridnumber) = 1! - ZPTOT
            gridaverage = gridaverage + probability(gridnumber)
        NEXT j
    NEXT i
    gridaverage = gridaverage / gridnumber
END SUB
```

size] • [grid quadrat width/step size]). First the expected number of cysts per core in the centre of the focus (CONS) will be calculated using the central population density and the core sample size. Two FOR loops will take care of every grid position possible. The detection probability will be set to one and coresamplenumber to zero. Two FOR loops will go through all possible grid points according to the current grid position, calculate the expected number of cysts according to equation (1) and the probability of $Pr[p_{(x,y)}] = 0$ according to equation (3). At the end of the inner loops the detection probability for each grid position is calculated. At the end of the subroutine the average detection probability of the focus (Pr_{aver}) is calculated.

8.4. Results

Sensitivity analysis

With the algorithm described above, sensitivity analyses were carried out for various parameters of equations (1) and (3) using the statutory soil sampling methods used in The Netherlands (Table 8.4.).

These sensitivity analyses (Figures 8.2.A and B) teach that the length and width gradient parameters both strongly influence the average detection probability. This influence is the stronger the greater the bulk sample, since the increase of the bulk sample from 200 to 600 ml increases the average detection probability by a factor varying from 2 to 4. Schomaker & Been (1992) decided to set the length and the width gradient parameters (data originating only from one cropping area) to the 10% lower percentile of their distributions when running simulations: for the length gradient parameter this value was 0.76, for the width gradient parameter 0.60. When these values are used for the calculation of average detection probabilities of e.g. 70%, 90% of the foci will have these gradient parameters or larger ones and therewith an average detection probability equal to or larger than 70%.

Sensitivity analysis of the effect of variation in k (Figure 8.2.C) on the average detection probability revealed that the latter hardly changed when k (defined for samples of 1.5 kg soil per m^2) increased from 40 to 100. The impact of k on the detection probability is low because the aggregation factor belonging to a core sample depends on its size, k" being proportional to the size of the sample used to estimate the 'common k'. As generally core sample sizes for statutory soil sampling are only several grams or decigrams (Table 8.4., Southey, 1973), k" for the core sample size will almost always be below 1. This implies considerable aggregation which hardly changes when the k value for 1.5 kg bulk samples is increased. It is concluded that, for the present purpose, some variation of k can be accepted so that a 'common k', k', can be used as described by Seinhorst (1988).

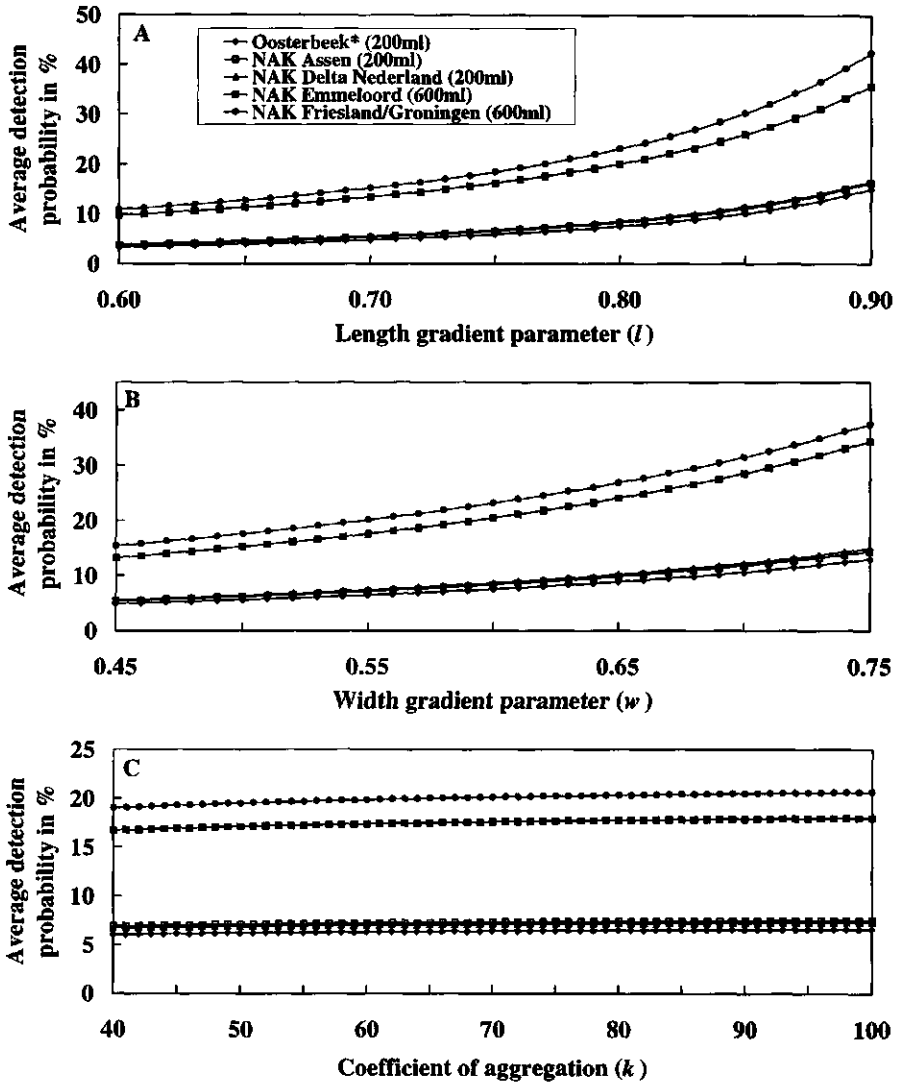


Figure 8.2. - A: Sensitivity analysis of the effect of the length gradient parameter in the exponential model (medium scale distribution, equation(1)) on the average detection probability of a standard focus. B: Sensitivity analysis of the effect of the width gradient parameter in the exponential model (medium scale distribution, equation(1)) on the average detection probability of a standard focus. C: Sensitivity analysis of the effect of the coefficient of aggregation k (negative binomial distribution, equation (3)) on the average detection probability of a standard focus. Focus: Central population density 50 cysts/kg, $l = 0.77$, $w = 0.55$ (Schomaker & Been, 1998), $k = 70$. Sampling method: Grid patterns, core sample sizes and bulk sample sizes according to Table 8.4.

* Laboratory for Soil and Crop testing, Oosterbeek.

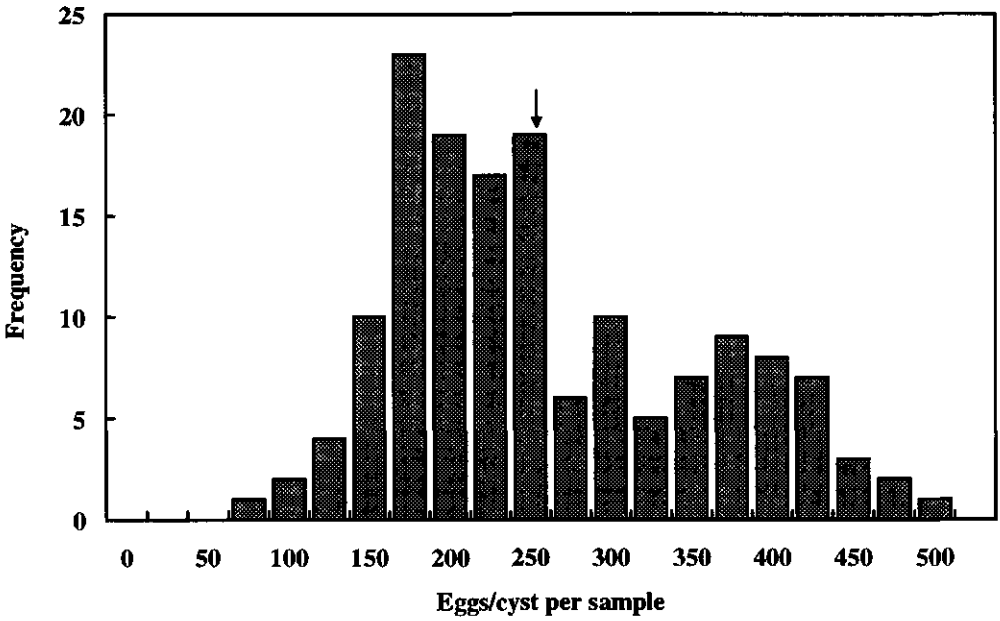


Figure 8.3. - Frequency distribution of eggs/cyst per batch of cysts from square metre samples with population densities between 30 and 70 cysts/kg soil originating from 9 foci.

Defining a standard focus

Before sampling methods for detection can be evaluated or new methods developed a standard focus must be chosen which one wants to detect with a set probability. Three requirements can be postulated too help defining the standard focus. Firstly, the level of infestation at detection should be so low that farmers can take control measures as soon as possible, but at least one crop rotation period before the infestation becomes visible in the next potato crop. Secondly, the population densities in the centre of the focus should not exceed the population threshold for damage at the time of planting a new potato crop. Then, resistant potato cultivars, which differ from susceptible ones only in their quality as a host, will suffer hardly any growth reduction with as a direct result no visibility of the infestation in the crop. Thirdly, detection of a focus should occur early, so that the probability of secondary foci and point infestations caused by infested clods of soil adhering to and transported by machinery (Hofmeester, 1990) would be low.

To establish the average number of eggs/cyst immediately after the susceptible potato cultivar Bintje is grown, the contents of batches of cysts, originating from square metre samples from 9 foci were determined. Assuming that a number of 50 cysts/kg soil

should approximately contain the number of eggs to approach the damage threshold (T = 2 eggs/g soil at planting - Seinhorst, 1982b), population densities between 30 to 70 cysts/kg soil were analysed. The resulting frequency distribution of eggs/cyst is slightly skewed (Figure 8.3.). An average of 248 eggs/cyst was calculated, and a value of 250 eggs/cyst was used for further calculations. As the actual sampling is done immediately after the potato harvest, two to three years of non-host crops follow in which the population density decreases. This decline, investigated among others by Den Ouden (1960), is averaged at 50%/year in the first year after a potato crop and 35%/year in subsequent years (Been, Schomaker & Seinhorst, 1995).

With the information presented above, a focus with a central population density of 50 cysts/kg soil was calculated as being a suitable standard focus. Figure 8.1. shows a two-dimensional image of the standard focus which, apart from its central population density, is defined by length and width gradient parameters according to the 10% lower percentile of their frequency distribution (Schomaker & Been, 1993). The population density at the centre of the standard focus is 50 cysts/kg soil or about 12.5 eggs/g soil. Population densities would be expected to decrease to 4.1 and 2.6 eggs/g soil in 1:3 and 1:4 crop rotations, respectively. These densities are only slightly above the tolerance limit (T) of 2 eggs/g soil (Seinhorst, 1982). No yield reductions will occur and the infestation will not be visible in the crop. For the accuracy of detection of the standard focus a 90% probability was arbitrarily chosen as being a minimum requirement for performance of a sampling method, keeping costs to collect and process the required field sample size within reasonable limits.

Table 8.4. - Characteristics of several Dutch versions of the statutory soil sampling method. The method of the Laboratory for Soil and Crop Testing (Oosterbeek) is in accordance with the regulations issued by the Dutch Plant Protection Service.

Sampling agency	Core numbers (n)	Core size (ml)	Grid quadrat length (cm)	Grid quadrat width (cm)	Bulk sample size (ml/0.33 ha)
Oosterbeek	60	3.33	750	750	200
NAK Delta Nederland	60	3.33	400	1200	200
NAK Assen	60	3.33	500	1000	200
NAK Emmeloord	120	5.00	250	1100	600
NAK Friesland/Groningen	120	5.00	500	500	600

Statutory sampling methods

Several Dutch sampling methods, defined by grid pattern and core sample size (Table 8.4.), were evaluated for their efficiency to detect the standard focus. In the 1990's five methods were used in The Netherlands. The method used by the Laboratory for Soil and Crop Testing (Oosterbeek) is the statutory sampling method according to the regulations issued by the Dutch Plant Protection Service. The Dutch General Inspection Service for Agricultural Seeds and Seed Potatoes (NAK) Delta Nederland and NAK Assen only changed the grid pattern to reduce the walking distance for the soil sampler and thus the costs. NAK Emmeloord and NAK Friesland-/Groningen doubted the efficiency of collecting only 200 ml per 0.33 ha and tripled the bulk sample size. Both agencies covered prime seed potato areas in the Netherlands and wanted to contribute to keeping these areas free from potato cyst nematode. Most other statutory sampling methods within the European Community are derived from the Dutch methods although there is a tendency to sample larger areas. For the Dutch methods the average detection probability of the standard focus was calculated (column three of Table 8.5.). The 200 ml sampling methods detect the standard focus only with an average detection probability of about 6%, while the 600 ml methods yield average detection probabilities of about 17%.

Table 8.5. - Performance of several Dutch versions of the statutory soil sampling method. Average detection probability according to the 10% lower percentile of the length and width gradient parameters (0.77 and 0.55) according to Schomaker & Been (submitted). Overall detection probability according to the bivariate normal distribution of the length and width gradient parameters.

Sampling agency	bulk sample size (ml/0.33 ha)	Average detection probability in %	90% average detection probability at a central population density: (cysts/kg)	Required sample size for 90% average detection probability of standard focus		Overall detection probability of standard focus in %
				core sample size (g)	field sample size (kg/0.33ha)	
Oosterbeek	200	5.8	9200	172	10.0	12.2
NAK	200	6.6	8300	468	32.2	13.8
NAK	200	6.4	7850	242	16.0	13.4
NAK	600	15.9	1650	176	21.2	30.8
NAK	600	18.4	1000	61	8.0	34.6

In the fourth column of Table 8.5. the required number of cysts/kg in the centre of the focus is given at which detection will occur with an average detection probability of 90% using the respective methods. The 200 ml methods require extremely high central population densities to detect foci with this probability, much higher than population densities will be under field conditions. 2500 to 3500 cysts/kg were the highest densities ever found, while Schomaker & Been (1998) in a survey of almost 100 infested fields did not detect central population densities higher than 1500 cysts/kg. Two sample sizes were calculated needed to detect the standard focus with 90% average detection probability, the core sample size and the associated field sample size per 0.33 ha. A surprising result is that two methods, NAK Emmeloord and NAK Friesland/Groningen, which collect the same bulk sample size (600 g soil) and have almost the same average detection probability for the standard focus (about 17%), require core sample sizes and bulk sample sizes differing a factor 3 to detect the standard focus at the set probability. The explanation for this difference lies in the grid patterns. The NAK Friesland/Groningen grid pattern of 5 by 5 m is more favourable than the 2.5 by 11 m grid pattern of NAK Emmeloord, the latter being incongruent with the shape of the focus.

Schomaker & Been (1998), having analysed foci scattered over many cropping areas in The Netherlands, found that both the length and width gradient parameters are normally distributed. As a result the frequency of occurrence of any combination of both gradient parameters in the field can be described by a bivariate normal distribution. Using this distribution, an overall detection probability for the standard focus can be calculated. The overall detection probability, defined as the sum of the products of the average detection probability of that focus for each possible combination of the length and width gradient parameters and the probability of occurrence of that combination, is listed in the last column of Table 8.5. If there are n_l possible values for the length gradient parameter and m_w possible values for the width gradient parameter and each combination of the length and width gradient parameters has the frequency of occurrence $freq_{i,j}$

$$Pr^f_{overall} = \frac{\sum_{i=1}^{n_l} \sum_{j=1}^{m_w} (Pr^f_{aver_{i,j}} \cdot freq_{i,j})}{(n \cdot m)} \qquad (6)$$

The overall detection probability has a value between zero and one, $0 \leq Pr^f_{overall\,i,j} \leq 1$. Figure 8.4. gives an example of the products of the average detection probability at all possible combinations of the length and width gradient parameters and the probability of occurrence of those combinations according to the bivariate normal distribution ($Pr^f_{aver\,i,j} \bullet freq_{i,j}$) for the NAK Groningen/Friesland method. From Table 8.5. it is evident that, although the overall detection probabilities are higher than the average detection probabilities based on the 10% lower percentile, none of the statutory soil sampling methods qualifies for the required 90% detection level.

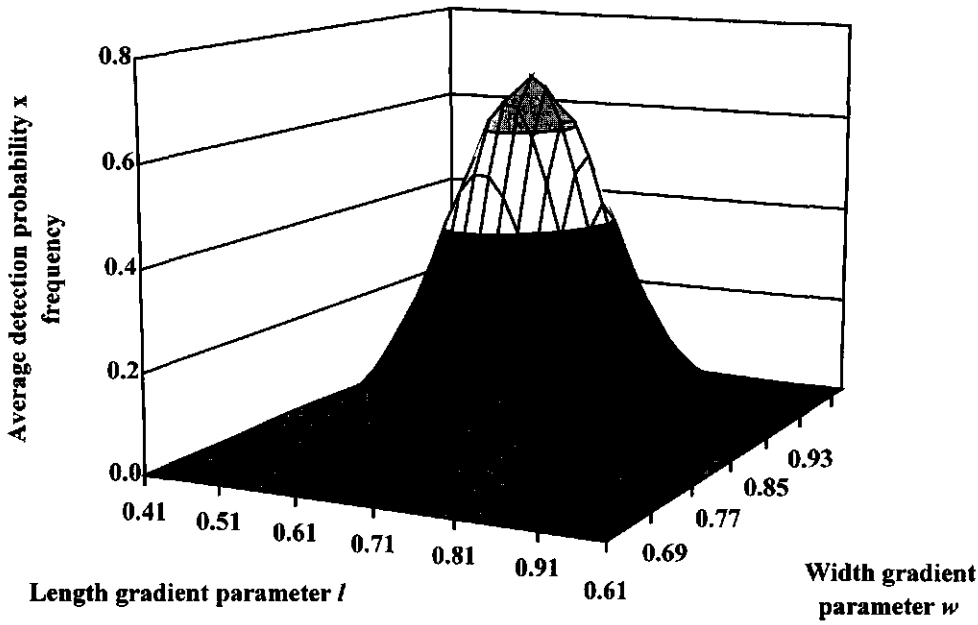


Figure 8.4. - Average detection probability of a standard focus at all possible combinations of the length and width gradient parameters times the frequency of occurrence of those combinations according to the bivariate normal distribution using the sampling method of NAK Friesland/Groningen, which results in an overall detection probability of 34.6% (Table 8.5.).

Efficiency of sampling methods

Since the dimensions of the grid pattern obviously affect the field sample size necessary to obtain a certain average detection probability, SAMPLE was used to establish the optimum grid pattern. The sample size required for a 90% detection probability was calculated for a variety of grid patterns from a 2 by 2 m to a 11 by 11 m grid pattern and for several combinations of the length and width gradient parameters. Figure 8.5. shows that the wider the grid pattern the larger the soil sample needed to detect the standard focus with 90% average detection probability, and the relation is by no means linear. Further, an increase in grid quadrant length is less sensitive than the same increase in grid quadrant width. The effect becomes less noticeable when higher values for the length and width gradient parameter are used (foci covering a larger area). Thus, the field sample size required to detect the standard focus with an average detection probability of 90% varies from 6.7 kg for a 2 by 2 m grid pattern to 33 kg for a 12 by 12 m grid pattern when the lower 10% percentile of the bivariate distribution of the length and width gradient parameters is used ($l = 0.77$, $w = 0.55$; Figure 8.5.A), from 3.9 to 5.9 kg when the average values are used ($l = 0.83$, $w = 0.64$; Figure 8.5.B)

and from 1.6 to 1.7 kg when the upper 10% percentile (l = 0.91, w = 0.76; Figure 8.5.C) is used.

In Figure 8.6. another consequence of differences in grid patterns is demonstrated by comparing the frequency distributions (= number of occurrences) of detection probabilities of the 2 by 2 m and the 11 by 11 m grid patterns for the same three combinations of the length- and width gradient parameters as in Figure 8.5. Although sample sizes were adjusted so that for both grid patterns the standard focus is detected with an average detection probability of 90%, the variance of the detection probabilities for various grid positions is much smaller for the 2 by 2 m grid pattern than for the 11 by 11 m grid pattern. For the individual farmer, who draws only once from this frequency distribution, a sampling method with a closely-spaced grid pattern is more reliable. The frequency distribution of the 11 by 11 m grid pattern in Figure 8.6.A. (for the lower 10% percentile) displays a large number of zero probability detections. In these cases grid positions occur without any grid point touching the focus. As a result, the core sample size has to be drastically increased for those grid positions that do touch the focus in order to obtain the desired average of 90% detection probability. The field sample size has to be increased by 13 kg when stepping from the 11 by 10 grid pattern to the 11 by 11 grid pattern. An 11 by 12 m grid pattern has 25% of the grid positions with zero probability of detection, and an average detection probability of 90% is impossible.

According to these analyses, grid patterns of 2 by 2 m would be the best of those calculated and smaller ones probably even better. However, reducing the grid quadrat in size to obtain denser grid patterns only yields low profit in reducing the field sample size and it cannot significantly improve the frequency distribution of detection probabilities, whereas the number of core samples required increases prohibitively. A range of grid patterns is acceptable when considering only the field sample size but a compromise has to be found for two conflicting aims: minimizing the sample size and the variance of the detection probability on the one hand and minimizing the time needed to collect and process the samples on the other hand. The prototype sampling method by Schomaker & Been (1992) and Been & Schomaker (1996) tried to achieve this compromise by utilising a 5 by 5 m grid pattern.

8.5. Discussion

Optimization of sampling methods

The results of Table 8.5. indicate that the statutory soil sampling methods used in The Netherlands do not fulfil the requirements for a sampling method which can detect a small infestation by potato cyst nematodes with a high probability and which can be employed as a monitoring tool in an IPM system. New soil sampling methods for detection had to be developed. A start was made in 1989 when a prototype version, called AMI-50, based on a limited data set originating from the province Flevoland, was introduced in The Netherlands (Schomaker & Been , 1992).

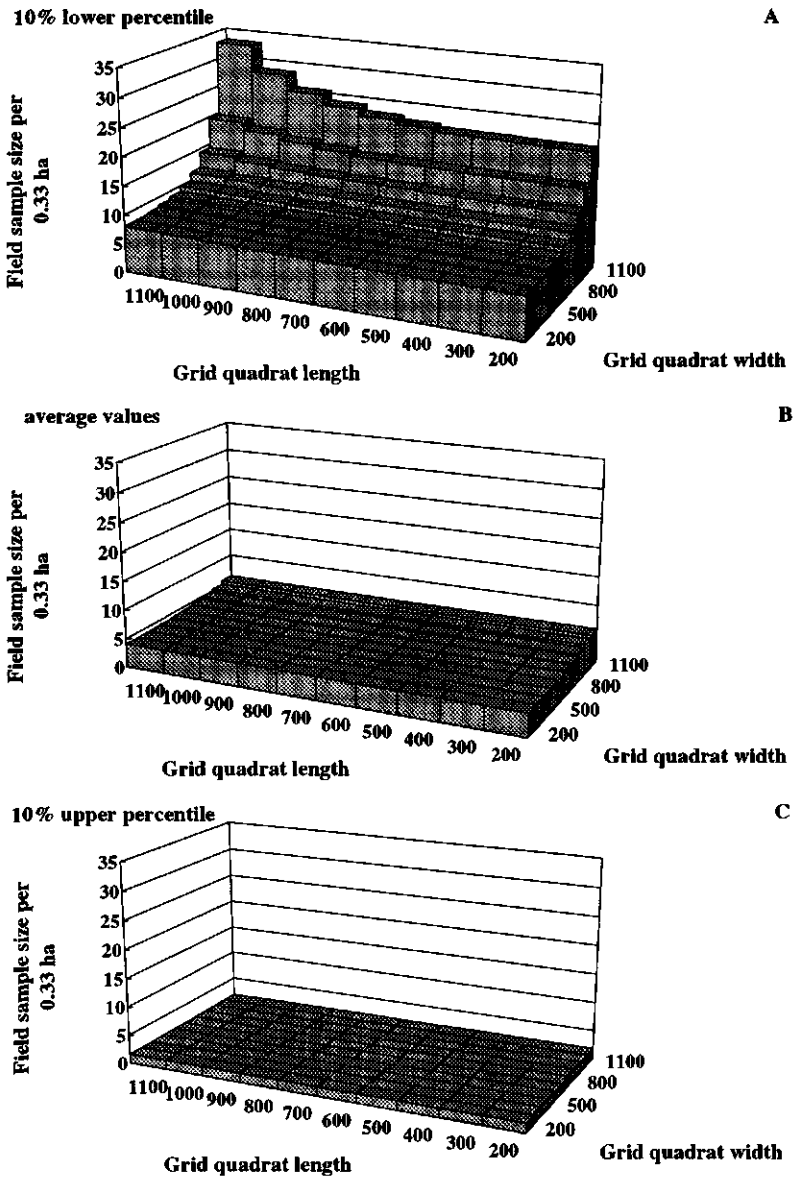


Figure 8.5. - Sensitivity analyses for grid patterns. The field sample size per 0.33 ha required to detect the standard focus with an average detection probability of 90% was calculated for grid patterns varying from 2 x 2 m to 11 x 11 m, using the 10% lower (A), the mean (B) and the 10% upper percentile (C) of the bivariate normal distribution of the length and width gradient parameters.

Table 8.6. - Features of several versions of a new sampling method for the detection of potato cyst nematodes. The prototype method is in use in Dutch agriculture since 1989 (Schomaker & Been, 1993). Adapted method 1 is an adjustment of the prototype method due to research carried out in all potato cropping areas of The Netherlands (Schomaker & Been, submitted). Adjusted method 2 is the result of applying the bivariate normal distribution (set to 90% overall detection probability) instead of the 10% lower percentile of the length and width gradient parameters. Adjusted method 3 takes into account the requirement of a basic registration unit (6 metres) compatible with farm operations.

Sampling method	Length gradient parameter	Width gradient parameter	Grid quadrat length (m)	Grid quadrat width (m)	Core sample size (g)	Field sample size (kg/ ha)	Bulk sample size (kg per 1/7 ha)	Overall detection probability (%)
Prototype method	0.76	0.60	5	5	53	21	3	96.4
Adapted method 1	0.77	0.55	5	5	61	24.1	3.4	97.5
Adapted method 2	bivariate distribution		5	5	34	13.5	1.9	90.0
Adapted method 3	bivariate distribution		5	6	42	13.9	2	90.0

In Table 8.6. the characteristics of this prototype method are displayed. According to Schomaker & Been (1998), who analysed a more comprehensive data set, the values of the 10% lower percentiles of the length and width gradient parameters, which were used to develop the prototype method, had to be adapted. The core sample size had to be increased from 53 to 61 g and the field sample size from 21.0 to 24.1 kg to ensure the desired average detection probability of 90% (adapted method 1). Using the bivariate distribution of the two parameters, a method could be developed with an overall detection probability of 90%. Instead of optimizing the detection method to the 10% lower percentile of both the length and width gradient parameters, it was optimized to detect an average of 90% of all possible combinations of these parameters (adapted method 2). A substantial reduction in core and field sample sizes is the result.

Because of the large amount of soil that had to be collected, bulk samples of areas smaller than the standard 0.33 ha were collected and processed. As secondary infestations and point infections occurred upwards and downwards in the direction of cultivation, the almost square area from which to collect a bulk sample was replaced by a elongated rectangular area or 'strip'. On average, strips are 300 m long covering the complete field in the direction of cultivation and as wide as the grid quadrat width. The prototype sampling method utilized a 5 by 5 m grid pattern. By combining all core

samples over areas 5 m wide and 300 m long into one bulk sample, 7 bulk samples per ha are collected. This new grid pattern was inconvenient as most agricultural machinery has a working width which goes by multiples of three metre. As a result, when growing a resistant cultivar as a control measure, the area in the width of the field will generally not cover the same width as the sampled and infested area. Deviant dimensions pose problems with the registration systems currently under development which need a standard width to store information. Consequently, the final sampling method for detection of foci (adapted method 3) was based on a 5 long by 6 m wide grid pattern.

The soil sampling methods of Table 8.4. are applied for different purposes in The Netherlands. They are used as statutory sampling methods by the government to protect the export of seed potatoes or are part of regulations on quarantine and control of potato cyst nematodes. Farmers want sampling methods for optimum control measures which lead to maximum returns, preferably before detection occurs by statutory soil sampling methods. In the Multi-Year Crop Protection Plan of the Dutch government (Anonymous, 1991) monitoring by soil sampling is advocated as a method to restrict the input of nematicides by avoiding preventive soil fumigation and applying need-based nematicide treatments only. SAMPLE can be used to develop optimum sampling methods for such purposes.

Seed potato growers

The prototype sampling method was developed specifically for seed potato growers. Although government regulation concerning potato cyst nematodes has drastically changed in The Netherlands - primarily to reduce the input of nematicides - it is still prohibited to grow seed potatoes on infested soils. Farmers, confronted with such a situation, are allowed to grow resistant potato varieties but only for human consumption or industrial processing. The financial loss in a prime seed potato area, such as the province Flevoland, amounts to 2.600 - 3800 guilders (about 1400 - 2000 US$) net per ha (Anonymous, 1995), far more than the costs of the new sampling method (about 200 guilders or 100 US$ per ha). Since the proposed sampling methods detect foci long before the statutory soil sampling method will do so, farmers will be able to take control measures to prevent that detection. As precise information on the width of the focus is supplied, the size of the focus can be estimated and customer-designed recommendations for control can be provided (Been *et al*, 1996).

Ware potato growers

When ware potatoes are grown, almost no regulatory restrictions are applied. No statutory soil sampling is carried out. Instead, the Plant Protection Service performs aerial surveys of potato fields and registers the locations where bare patches or bad growth is visible. Soil samples are taken on the spot and when the presence of potato cyst nematodes is demonstrated resistant varieties have to be grown until subsequent soil sampling proves the field to be free from cysts. The use of resistant varieties

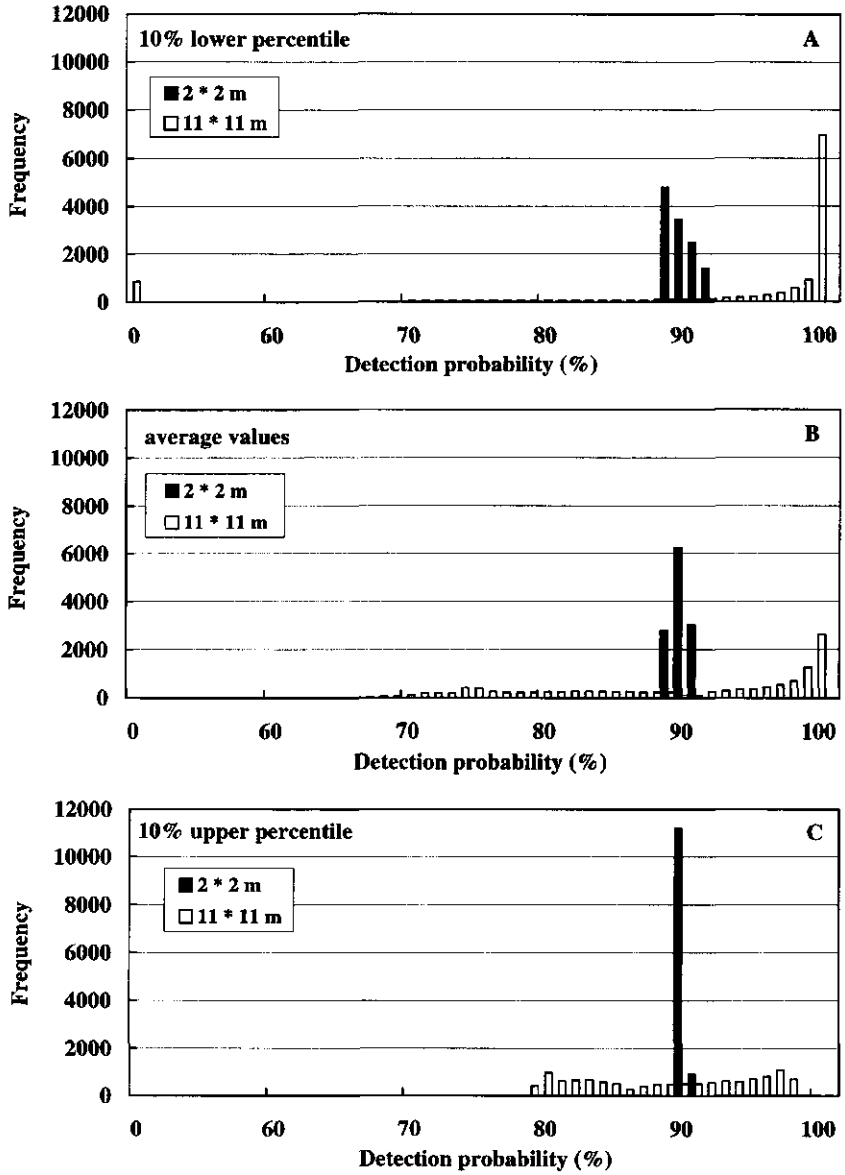


Figure 8.6. - A comparison of the frequency distributions of detection probabilities for 2 x 2 m (□) and 11 x 11 m (■) grid patterns (length x width) at the 10% lower (A), the mean (B) and the 10% upper percentile (C) of the bivariate normal distribution of the length and width gradient parameters. The two grid patterns produce the same average detection probability (90%) for the standard focus (using different core sample sizes) but they differ in their variance. Note: A grid quadrat of 11 by 11 m and 'step size grid' of 10 cm for the sampling grid (Table 8.2.) yields 110 * 110 = 12100 simulated sampling results; the frequencies of the 2 by 2 m grid were converted to the same number for comparison.

amounts to a net loss of 600 - 1800 guilders (about 300 - 950 US$) per ha (Anonymous, 1995). For a typical bare patch to be spotted from the air, an area of at least 25 square metres of reduced growth is necessary. Any reasonably attentive farmer will spot such an infestation himself in the preceding potato crop when growth reduction is still small. When rotations of 1:4 or wider are practised, visible damage in the next potato crop will become harder to notice as a result of redistribution and population decrease. However, the infestation will spread; secondary infestations will occur and slowly the complete field will be contaminated. Schomaker & Been (submitted) demonstrated the presence of secondary foci in 94% of the fields sampled. The average area contaminated by the primary focus increases from 370 to 1150 m^2 when $P(0,0)$ increases from 50 to 1000 cysts/kg soil. Most of the infested area contains low population densities of the potato cyst nematode. When a field becomes more or less uniformly infested, densities will build up. At this stage yield reductions can be suffered without being seen as comparison with uninfected areas in the same field is not possible. Growing seed potatoes or export crops (e.g. flower bulbs), which are only allowed to grow on nematode-free soil, will become impossible. Sampling, therefore, is still required although not as intensive as for seed potato growers.

An option is to link the size of the infestation focus to be detected to the cropping frequency. The less frequently potatoes are grown the bigger the focus may develop before detection by the sampling method is required as a longer period of non-host years will follow. For every extra non-host crop season the acceptable central population density of the focus to be detected may increase by the average reduction of population densities during that extra year (35%). A maximum focus size can be established above which yield reduction by or spread of potato cyst nematodes is considered too high. The latter criterium is given more emphasis as yield reductions of even large foci (1500 cysts/kg soil in the centre) are of no consequence when yield is calculated per ha (Been et al, 1996) but an increasing area will be contaminated. A arbitrary limit was set at 225 cysts/kg soil in the centre when approximately 700 m^2 is contaminated by the primary focus alone and approximately 900 m^2 by secondary foci (Schomaker & Been, submitted). Table 8.7. shows the features of such sampling methods for ware potato growers. As a result, ware potato farmers can make use of less intensive sampling methods which will be accordingly cheaper.

Table 8.7. - Sampling methods suitable to farmers growing ware potatoes at different cropping frequencies. All methods use a 5 by 6 metre (length x width) grid pattern and have an overall detection probability of 90%.

Cropping frequency	Central population density of focus	Core sample size (g)	Field sample size (kg/ha)
1 : 3	100	21	7.2
1 : 4	150	15	5.0
1 : 5 and higher	225	10	3.3

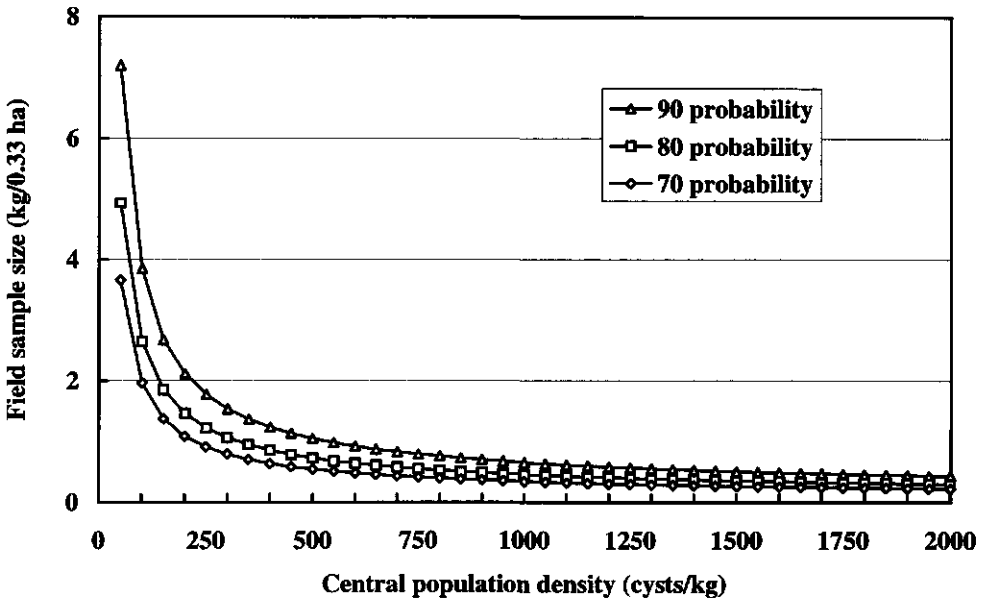


Figure 8.7. - Field sample sizes required to detect foci of increasing central population densities at average detection probabilities of 70, 80 and 90%. 5 by 5 m grid pattern, $l = 0.77$, $w = 0.55$ and $k = 70$.

Statutory soil sampling

The statuary soil sampling methods were not developed for the purpose of IPM. They are used, in combination with other control measures, e.g. sampling adhering soil, to ensure the position of The Netherlands on the export market of seed potatoes. Implicitly, these methods are not so efficient that too many farmers will be caught and not so inefficient that too many export lots will be contaminated. From a commercial point of view the statutory sampling methods seem to be adequate but a scientific foundation of these methods is lacking. Their effectiveness greatly depends on the willingness of the importing country to invest in checking import lots. Many countries within the European Union do not respond any more to *G. rostochiensis* contamination of import lots. As is generally known, only a slight increase of the intensity of sampling or of the fraction of lots sampled will cause the (increase of) detection of *G. pallida* abroad.

The simulation model described in this paper provides the possibility to develop sampling methods for detection on demand for any set of conditions. For example to provide for statutory soil sampling methods with predefined characteristics e.g. to facilitate that only a certain percentage of export lots will be contaminated or that foci above a certain threshold level will be detected. In Figure 8.7. the field sample size,

required for a range of possible infestation foci, a grid pattern of 5 by 5 metre, and three average detection probabilities for the 10% lower percentile of the bivariate normal distribution of the length and width gradient parameters, is displayed to show one possibility.

The use of statutory soil sampling methods for detection and population density estimation poses a problem since they were not developed and certainly not validated for those purposes. Farmers react with confusion, when fields have been declared nematode-free as a result of statutory soil sampling, and research scientists nonetheless urge them to apply a more intensive sampling method. In 1989, the Dutch Plant Protection Service propagated the new sampling method by stating that control measures should be based on the new method, since the statutory soil sampling method does fulfil EPPO regulations but has no added value for the farmer (Toussaint, 1989).

Environment

The statutory soil sampling methods using a 200 ml bulk sample have such poor performances that only extremely large foci will be detected (Table 8.5.). Most detections will occur when infestations were already visible in the foregoing potato crop. At the moment of detection these infestations will be so serious that farmers are likely to take chemical control measures. The new sampling method enables farmers to detect infestations when densities are low enough to grow (partially) resistant potato cultivars without yield reduction. As potato cyst nematodes can occur down to depths of up to 80 cm (Been & Schomaker, submitted), as do potato roots (Vos & Groenwold, 1986), the effect of resistant cultivars greatly surpasses the effect of soil fumigation since the latter only reduces population densities by an average of 60% and only in the upper 25 to 30 cm of the tilled zone (Been & Schomaker, 1987, submitted). It is possible to control potato cyst nematode infestation without severe crop losses by growing (partially) resistant potato cultivars in the usual rotations (1:3 and 1:4).

Since 1989 the application area of the prototype sampling method as discussed in this paper increased rapidly up to 13,000 ha per annum and in 1994 soil fumigation was reduced by more than 80% in those areas where seed and ware potatoes are grown. As a result the total reduction of the volume of nematicides throughout The Netherlands (including the starch potato cropping areas where soil fumigation was recently restricted to once in four years instead of once in two years) was 77% in 1995, far more than the 45% which was required for the year 1995 by the Multi-Year Crop Protection Plan (Anonymous, 1991).

Integrated Pest Management

As the statutory soil sampling method with a 7.5 by 7.5 m grid pattern and a core sample size of 3.3 g (bulk sample size 200 g/0.33 ha) is still used because of EPPO regulations, it is compared with the prototype sampling method (Schomaker & Been,1992) and the new method (adapted 3) for two aspects: 1) the size of the focus detected with a 90% overall detection probability (Figure 8.8.) and 2) their overall

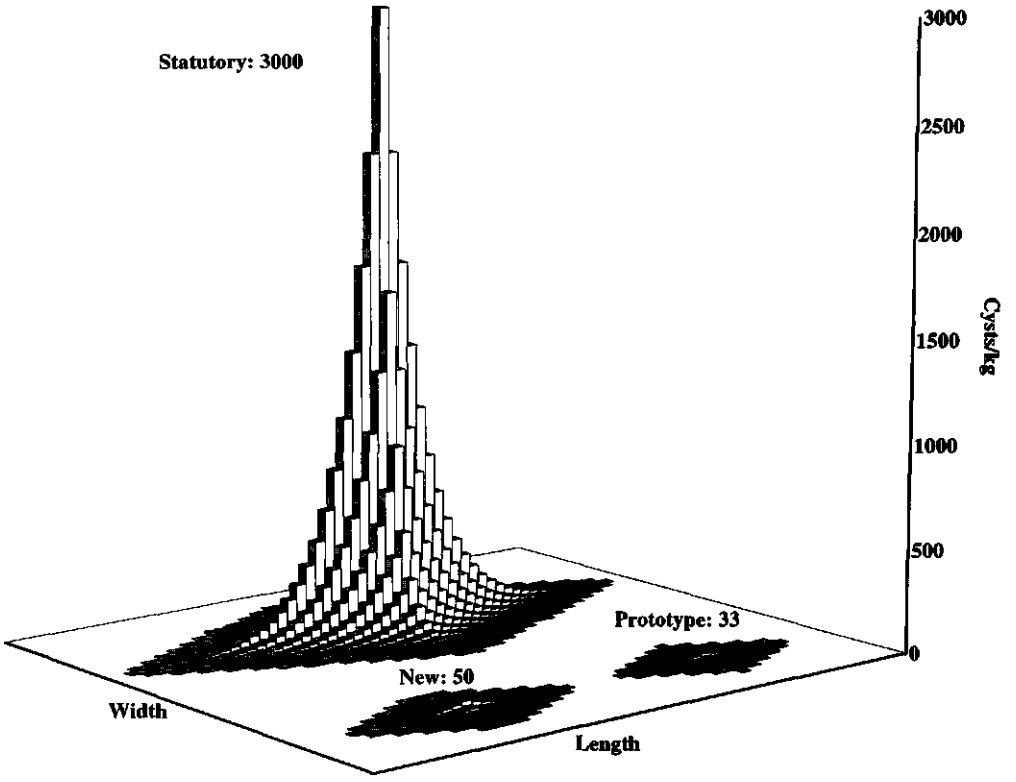


Figure 8.8. - Comparison of foci detected with a 90% average detection probability by the statutory, the prototype and the new (adapted 3) sampling methods (3000, 33 and 50 cysts/kg in the centre respectively) at the lower 10% percentile of the bivariate normal distribution of the length and width gradient parameters.

detection probabilities for foci of different central population densities (Figure 8.9.).

Given an overall detection probability of 90%, the new sampling method detects foci with central densities sixty times smaller than does the statutory soil sampling method. The new method detects foci with a central density of 200 cysts/kg soil and higher with high (approximating 100%) probability. Therefore, if a focus is present in a field, but not detected with the new sampling method, its size can only be very small. The probability of detection by statutory soil sampling is low (e.g conditional overall detection probability 0.012 for the standard focus) whereas the probability of a yield reduction larger than 1% in the next potato crop is nil.

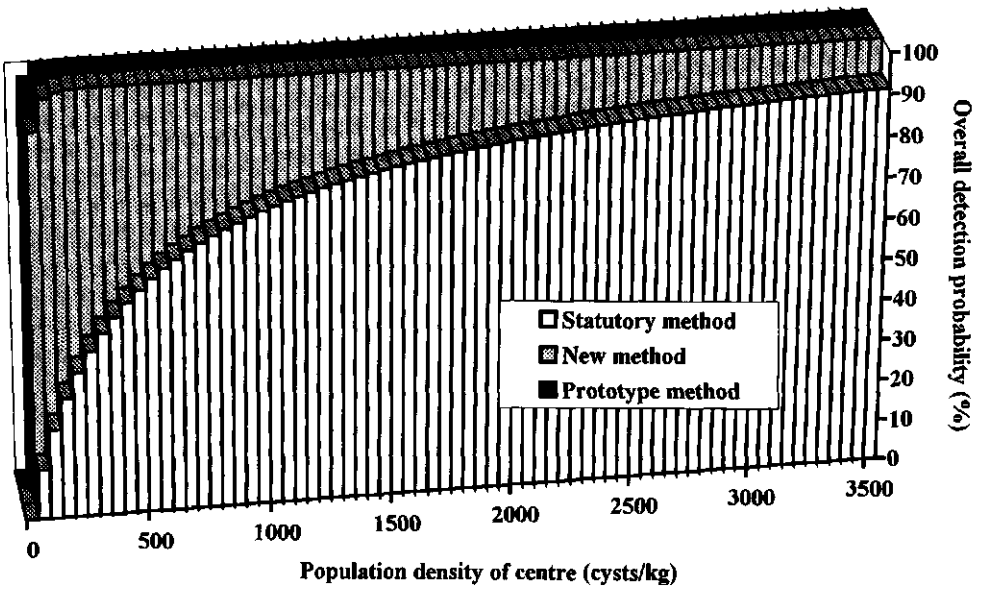


Figure 8.9. - Overall detection probabilities with the statutory, the prototype and the new (adapted 3) sampling methods of foci with central population densities ranging from zero to 3500 cysts/kg.

Using the new sampling methods seed potato growers can take control measures before the infestation reaches a magnitude that causes it to be detected by any of the statutory soil sampling methods or becomes noticeable in seed potato lots. Sampling results can be used to calculate the size of the infestation detected and to generate advice for optimum control (Been *et al*, 1996).

It is impossible to check the spread of potato cyst nematodes, but good detection methods allow the farmer to take immediate action to reduce infestations by *Globodera rostochiensis* with resistant cultivars and to contain infestations by *G. pallida* by growing (partially) resistant cultivars (Been *et al*, 1995). Without soil sampling methods no proper action can be taken.

Chapter 9

A growth model for plants attacked by nematodes

C.H. Schomaker, T.H. Been, & J.W. Seinhorst[†]

9.1. Summary - The relation between small and medium initial population densities and the relative total plant weight was derived as cross sections at right angles to the time axis of a growth model with three dimensions: time after planting t, relative total plant weight Y and relative growth rate r_p/r_0. The relative growth rate is the (constant) ratio between the growth rate r_p of plants of a certain weight at a nematode density P and the growth rate r_0 of (younger) plants of the same weight without nematodes. Therefore, the ratio between the time after planting that plants need to reach a certain weight in the absence of nematodes and at nematode density P, $t_0/t_p = r_p/r_o$ (2).
The relative growth rate $r_p/r_o = k + (1\text{-}k)0.95^{P/T\text{-}1}$ for $P > T$ and = 1 for $P \leq T$ (3). Formally, k is the minimum relative growth rate as $P \rightarrow \infty$. As a result the arbitrary equation $y = m + (1\text{-}m)0.95^{P/T\text{-}1}$ for $P > T$ and = 1 for $P \leq T$ (6) also applies to the relation between small and medium initial population densities and relative total plant weight. T is the tolerance limit, below which growth and yield are not reduced by nematodes; m is the relative minimum yield.
The relations between small and medium initial population densities of potato cyst nematodes and relative tuber weight of potatoes can be derived from the growth model in an analogous way. However, there is one complication: tuber initiation does not start at the same haulm weight in plants with and without nematodes, but at the smaller haulm weight the larger the nematode density. As a consequence, tuber weights of plants with a certain total weight at nematode density P are not equal to those of plants with the same total weight without nematodes, but $r_p\Delta t$ units of weight larger, Δt being the difference between the actual time of tuber initiation and the time total plant weight becomes the same as that of plants without nematodes at the initiation of tuber formation.
Relative total and tuber weights of plants with 'early senescence' and at large nematode densities are smaller than estimated by the model and equation (2). This indicates that at large initial population densities growth reducing mechanism(s) become active that were not operating at smaller densities.

9.2. Introduction

"I shall take the simpleminded view that a theory is just a model of the universe or a restricted part of it, and a set of rules that relate quantities in the model to observations that we make. It exists only in our minds and does not have any other reality (whatever that may mean). A theory is good theory if it satisfies two requirements: It must accurately describe a large class of observations on the basis of a model that contains only few arbitrary elements, and it must make definite predictions about the results of future observations. For example, Aristotle's theory that everything was

made out of four elements () was simple enough to qualify, but did not make any definite predictions. On the other hand, Newton's theory of gravity was based on an even simpler model (). Yet it predicts the motions of the sun, the moon and the planets to a high degree of accuracy." (Stephen Hawking, 1988. A brief History of Time).

In many biological and nematological studies one is not very particular about these requirements. Several nematologists or biologists have formulated mathematical relations between pre-plant nematode densities and crop yield that were indeed based on observations - although mostly limited ones - but that lacked a theoretical base and predictive value. Examples are the linear and log-linear functions (Lownsberry & Peters, 1956; Hesling, 1957; Seinhorst, 1960; Hoestra & Oostenbrink, 1962; Brown, 1969), the quadratic curve (Peters, 1961); the inverse linear function (Elston *et al.*, 1991) and an exponential function (van Haren *et al.*, 1993). These equations do not represent a vision of the mechanisms involved in nematode-plant interactions. Calculations of limits of variables or parameters in some of them produce bizarre results.
Others (e.g. Evans & Haydock, 1990; Trudgill, 1992) tried to build theories on the effect of nematode attack on plants without using a model. This approach makes it difficult to study a (biological) problem methodically or to detect flaws in one's chain of reasoning. Moreover, there is no efficient transfer of these theories to fellow-nematologists for further development or criticism.
An approach which at least partly satisfies one of Hawking's requirements is the 'comprehensive simulation model'. This type of model tries to describe an often-limited research area with detailed information in a large number of sub-models and parameters. They were first applied in nematology by Ward *et al.*, 1985 in a preliminary model on the population dynamics of potato cyst nematodes and were later extended by Schans (1993), who tried to describe the population dynamics of potato cyst nematodes and the associated damage to potatoes with information about some plant physiological processes and their changes with environmental conditions. Only because of the arbitrary nature of the sub-models and the multitude of parameters, which, although treated as constants, are more often variable than not, these models did not contribute much to theorem building and have a limited predictive value. In the model of Schans this was the more so as description of plant physiology was confined to short-term measurements of photosynthesis, respiration and transpiration on one or a few leaves, which did not explain differences in yield, at a limited range of nematode densities which, moreover, were not always constant during plant growth. Besides, as external conditions during crop growth are indispensable data in these models, they are unsuitable as a base for recommendations about control measures for nematode pests, which, unlike those for fungi or insects, must be taken, at the latest, at the time of sowing or planting of the crop to be protected (nematostatics), but generally much earlier (nematicides, crop rotation, resistant cultivars). Therefore, these simulation models would not qualify as good models.

In nematology Seinhorst's models, especially those for potato cyst nematodes (Seinhorst 1986a, 1986b, 1993) come closest to the classical approach recommended by Hawking. The models are based on a theory which describes the principal mechanisms of nematode/nematode and nematode/plant interactions, they don't conflict with a large number of observations in different countries on different nematode species over a large period of time. The integrated, simplified, stochastic versions of the models (Been *et al.*, 1994) deduced from more complicated ones that suit mainly scientific purposes, contain only a few parameters with known distribution functions and allow predictions to be made within sufficiently narrow limits, albeit with some constraints. The predictive value of the models, which makes them suitable for an advisory system for the control of potato cyst nematodes on farmer's fields, will be demonstrated by Been *et al.* (1994), elsewhere in this book; the theorem of one of Seinhorst's models: the effect of nematodes on growth and yield of plants, is explained in this article.

9.3. Causes of yield reduction by nematodes

Crop returns are reduced by nematode attack as a result of reduction of crop weight per unit area (which is mostly equivalent to average weight of marketable product per plant) and reduction of the value of the product per unit weight. For example, carrots attacked by rootknot nematodes (*Meloidogyne*) may be worthless because of branching and deformation of the tap root, although they have the same weight per unit area as carrots without nematodes. Onions of normal weight but infested with few stem nematodes (*Ditylenchus dipsaci*) at harvest will, nevertheless, be lost in storage. Attack of crop plants by potato cyst nematodes (*Globodera rostochiensis*, *G. pallida*) does not only reduce potato tuber weight, but may also reduce tuber size. However, potato cyst nematodes attacking potatoes and almost all root infesting nematodes attacking crop plants of which the above ground parts are harvested, hardly ever affect the value per unit weight of harvested product, unless crop weight is reduced so much by large initial population densities that growing it was uneconomical from the start. Therefore, prediction of crop reduction by these nematodes can, in general, be based on models of the relation between nematode density at sowing or planting and average weight of single plants at harvest. In the following the term 'yield' will be avoided. The yield in the agronomic sense must be derived from individual plant weights, taking the restrictions mentioned above into account.

To make a model of the relation between population density before planting (P) and the proportion (y) of uninfested plants (onions, flower bulbs) or relative plant weight (y) after a certain period of growth of the plants (the ratio between the yield of plants at density P and the yield of plants without nematodes, grown under the same conditions) a theory was developed about the mechanisms involved. The theory was tested by comparing it with the patterns of observed relations between nematode density and

plant growth and plant weight at a certain time after sowing or planting. This also allows the estimation of values of system parameters under various experimental conditions.

Seinhorst's (1965) model for the relation between the population densities of stem nematodes (*Ditylenchus dipsaci*) at sowing and the proportion of infested onion plants is identical to that of Nicholson (1933) for the infection of caterpillar pupae by parasite wasps. The chain of reasoning is as follows: Only infested and non-infested onions are distinguished. The degree of infestation of single plants is irrelevant as only nematode-free onions are marketable. Only three assumptions (which in a slightly different formulation also apply to the root infesting species) were needed to formulate the model:

1) The average nematode is the same at all densities. This means that population density does not affect the average size or activity of the nematodes.
2) Nematodes do not affect each other's behaviour. They do not attract or repel each other directly or indirectly.
3) Nematodes are distributed randomly over the plants in a certain area.

If now a density of one nematode per unit area or weight of soil attacks a proportion d of the onion plants in a certain area (and leaves a proportion $1\text{-}d$ uninfested), then a density of two nematodes per unit area or weight of soil will attack, on the average, a proportion d of already damaged plants (which has no additional effect on yield reduction), plus a proportion d of the still uninfested proportion $(1\text{-}d)$, leaving a proportion $1\text{-}d\text{-}d(1\text{-}d) = (1\text{-}d)^2$ of the plants uninfested. Generalized: a nematode density P leaves a proportion

$$y = (1 - d)^P = z^P \qquad (1)$$

of the onions uninfested. In Figure 9.1. values of z^P are plotted, not against P, but against log P. This has not only the advantage that the shape of the curves is the same for all z ($d(\log P)/dy$ is only determined by y), but also that, if P is estimated by counting nematodes in a soil sample, the variance of log P depends less on true P than the variances of P. The values of z is determined by conditions that affect the efficiency of the nematodes in finding and penetrating plants. In patchy infestations of stem nematodes these conditions for attack appeared to be more favourable in the centre of the patch than towards the borders, resulting in an increase of z with increase of the distance from this centre. This results in persistency of the patchiness. The model also applies when nematodes spread from randomly distributed infested plants to neighbouring ones, which results in overlapping circular patches of infested plants.

Patchy infestations of nematodes do not always develop this way. Infestation foci with potato cyst nematodes, for instance, occur by introduction of cysts in fields: small numbers of cysts are mostly transmitted by seed potatoes and larger numbers of cysts by agricultural machinery. After multiplication on potatoes they are spread by tillage and harvesting machines. As a result the patches have the same shape in all potato

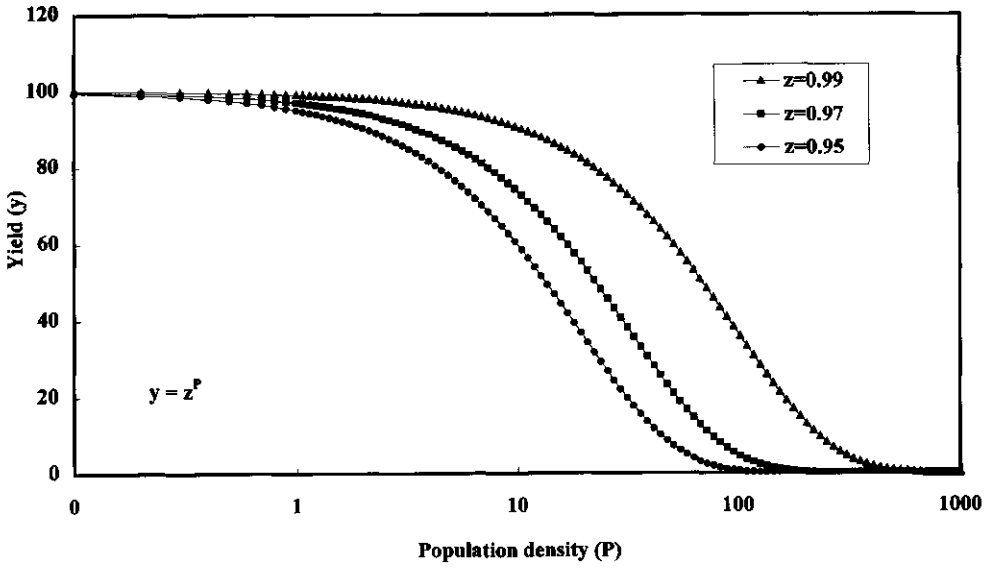


Figure 9.1. - The relation $y = z^P$ (eq. (1)) between the initial population density (P) of stem nematodes and the proportion y of the onions that is not attacked.

growing areas in the Netherlands (Seinhorst, 1982*b*, 1988; Schomaker & Been, 1992). If good host plants are grown in short rotations, patchy infestations can become larger until the whole field is infested more or less uniformly. Examples of such infestations are found in south-east Groningen and east Drenthe, where starch potatoes are grown every other year.

9.4. Reduction of plant growth

Tylenchid root infesting nematodes (for instance cyst nematodes, root knot nematodes and *Pratylenchus* species) generally reduce crop yield in a less direct way than stem nematodes. The rate of growth and development of attacked plants is reduced, resulting in smaller weights than of plants without nematodes at given times after sowing or planting or, in exceptional cases, in reaching the same final weight as of plants without nematodes later. In general such a delay of the ripening of the crop is prevented by the external conditions at the end of the growing season of the plants.
Seinhorst (1979, 1986*b*) and Seinhorst *et al.* (submitted) based a growth model on two simple concepts: one of the nature of the plant; an element that increases in weight in the course of time, and one of the nature of plant parasitic nematode: elements that

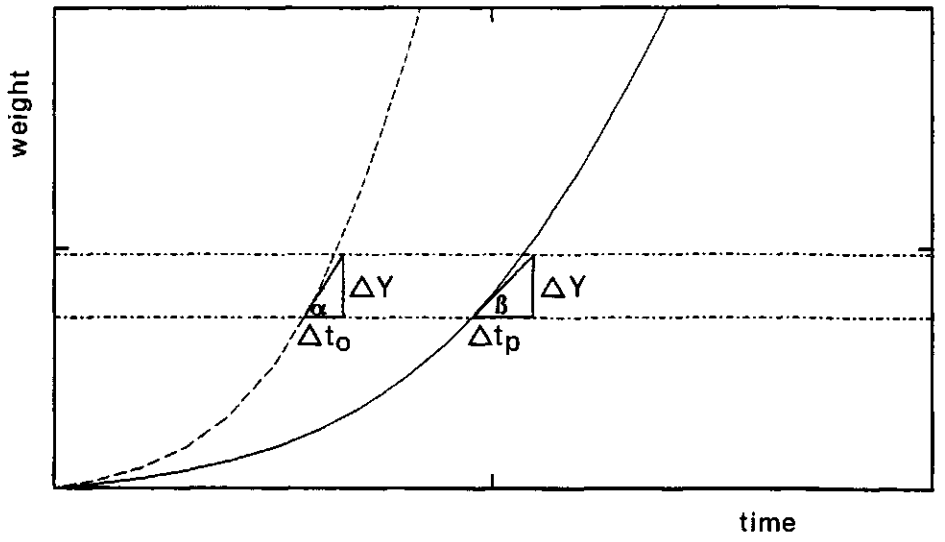


Figure 9.2. - Growth curves of plants without nematodes and at nematode density P. Y = total plant weight; t = time after sowing or planting; t_0 and t_p are the times plants without and with nematodes need to reach the same total weight Y; r_0 (= tanα = $\Delta Y/\Delta t_0$) and r_p (= tanβ = $\Delta Y/\Delta t_p$) are the growth rates of plants without nematodes and at nematode density P respectively.

reduce the rate of increase of this weight and, in principle, more so the larger the population density. Further the following principles are applied in addition to those mentioned above for the model on the relation between stem nematode density and proportion of onions attacked:

4) Root infesting nematodes are distributed randomly in the soil.
5) Nematodes enter the roots of plants randomly in space and time. Therefore the ave-rage number of nematodes entering per quantity of root and time is constant. This number is proportional to the nematode density P (number of nematodes per unit weight or volume of soil).
6) If the growth rate of plants at a given time t after sowing or planting is the increase in total weight (Y) per unit time (dY/dt), represented by r_0 for plants without nematodes and r_P for plants at nematode density P, then the ratio r_P/r_0 for plants of the same total weight (and, therefore, of different age) without nematodes and at nematode density P, is constant throughout the growing period. Further, according to Figure 9.2., r_0 = tanα = $\Delta Y/\Delta t_0$ and r_P = tanβ = $\Delta Y/\Delta t_p$. Therefore,

$$r_P/r_0 = t_0/t_P \qquad (2)$$

The relation between population density of the nematodes and its total effect on the growth rate of the plants is also considered to be according to Nicholson's competition model (eq. (1), Figure 9.1.). Eq. (1) is a continuous function for $0 \leq P \leq \infty$. However, all sufficiently accurate observations on the relation between the population density P of various nematode species, including potato cyst nematodes, on the weight of various plant species demonstrate the existence of a minimum density T, below which the nematodes do not reduce plant weight. It may be concluded that at densities $< T$ they also do not reduce the growth rate of plants. This is corroborated by the results of the few experiments in which growth rates were actually determined. Moreover, at large nematode densities plant weight approached zero in only few of these experiments and growth rates never did. Therefore, eq. (1) is adapted by replacing P by P-T and introducing the minimum relative growth rate $r_P/r_0 = k$ for $P \rightarrow \infty$. The second equation constituting the model then becomes:

$$\begin{aligned} & r_P/r_0 = \mathrm{k} + (1\text{-k})z^{P-T} && \text{for } P > T \\ \text{and} \quad & r_P/r_0 = 1 && \text{for } P \leq T \end{aligned} \tag{3}$$

in which z is a constant smaller than 1. The value of k is independent of nematode density and time after sowing or planting, but may vary between experiments. Growth curves of plants for different nematode densities can be derived from a growth curve of plants without nematodes with the help of the equations (2) and (3). These curves may vary in shape, as long as they are continuous and the growth rate decreases monotonously from shortly after sowing or planting. Figure 9.3. gives an impression of the three dimensional model with axes for total plant weight Y, relative nematode density P/T and time after planting t for a given value of k.

9.5. A simple model for the relation between nematode density and relative plant weight.

The primary results of experiments are almost always weights of plants attacked by known nematode densities at a given time after sowing or planting. To investigate whether these relations are in accordance with the model, they must be compared with cross sections orthogonal to the time axis of the model, through growth curves of plants for ranges of densities P/T and different values of k. These cross sections were in close accordance with the now arbitrary equation:

$$y = m + (1\text{-}m)z^{P-T} \qquad \text{for } P > T \tag{4}$$

in which m is the minimum relative plant weight, z is a constant < 1 with the same or a slightly smaller value than in eq. (3) and T is the tolerance limit with the same value as in eq. (3).

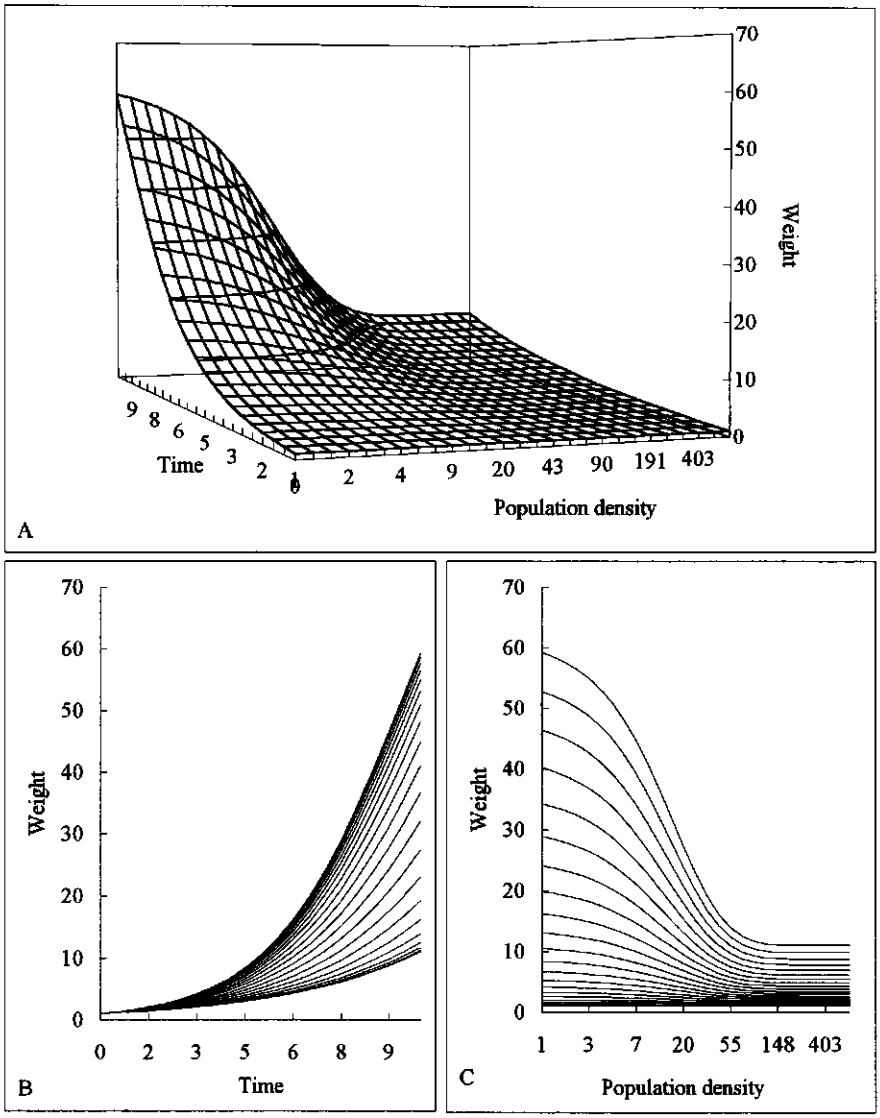


Figure 9.3. - Surface plots of the three-dimensional model which represents the relation between total weight (Y) and relative nematode density P/T as cross sections at right angles with the time axis (t) of growth functions of plants at different nematode densities, $Y(r_p,t)$, and that without nematodes, $Y(r_0,t)$:

A. at 230° rotation, which illustrates the relation between (Y,P) and (Y,t).

B. at 0° rotation, which shows the relation between Y and t at different nematode densities.

C. at 270° rotation, which exposes the relation between Y and population density P.

All rotations are clockwise. The growth rates of plants of the same weight without nematodes (r_0) and at nematode densities P (r_p) are related by eq. (1) and (2)

Seinhorst (1986*b*) gives the relations in pot experiments between *P/T* of different tylenchid nematode species, including *Heterodera avenae* (Seinhorst, 1981), *G. rostochiensis* and *G. pallida* (Greco *et al.*, 1982) and the relative dry plant weight (y) several months after sowing or planting. Values of $y' = (y\text{-}m)/(1\text{-}m) = z^{P\text{-}T}$ (5) in thirteen pot experiments are plotted against *P/T* (Figure 9.4.). The relation between average y' and *P/T* is in close accor-dance with $y' = z^{P\text{-}T}$ which suggests that the deviations of individual observations in the different experiments from values according to this relation were due to experimental error. Average relative plant and tuber weights of different resistant and susceptible potato cultivars at different times after planting were in close agreement with those according to eq. (5) with $T = 1.8$ eggs/g soil. As eqs. (4) and (5) are identical, we may conclude that the results of Figure 9.4. and the other experiments are well described by the model, which apparently applies to tylenchid nematodes that feed and multiply in as different ways as cyst nematodes, *Pratylenchus penetrans* and *Tylenchorhynchus dubius*. It also indicates that the general relation between nematode density of tylenchids and relative plant weight of attacked plants some time after sowing or planting is independent of the shape of the growth curve of plants without nematodes and of external conditions. Although values of z and T differed between nematode species and plant species and m also between experiments with the same combination of nematode and plant species, z^T differed too little from 0.95 between experiments to distinguish the variation from that caused by experimental error. Therefore, for fitting curves according to eq. (3) to experimental data, z^T is generally assumed to be 0.95, which transforms eq. (4) to:

$$y = m + (1\text{-}m)0.95^{P/T\cdot 1} \tag{6}$$

The value of T depends on nematode species and pathotype and on the plant species, but seems in general not to be affected by external conditions. For potatoes with potato cyst nematodes, planted in spring, with T was about 1.8 eggs/g soil. An exception is the sensitivity to day length of the value of T for potato cyst nematodes on potatoes (Been & Schomaker, 1986; Greco & Moreno, 1992). In contrast, m for a given nematode species varies between cultivars or plant species within experiments and for the same cultivar between experiments under the influence of so far unknown external conditions. It is not established whether there are consistent differences between cultivars or even plant species. Therefore, T is the main measure of the degree of tolerance of a plant species or cultivar to a certain nematode species or pathotype, whereas the importance of m remains to be investigated. The shortcoming of most discussions on differences in tolerance between cultivars or crop plants is, that they do not refer to these two parameters.

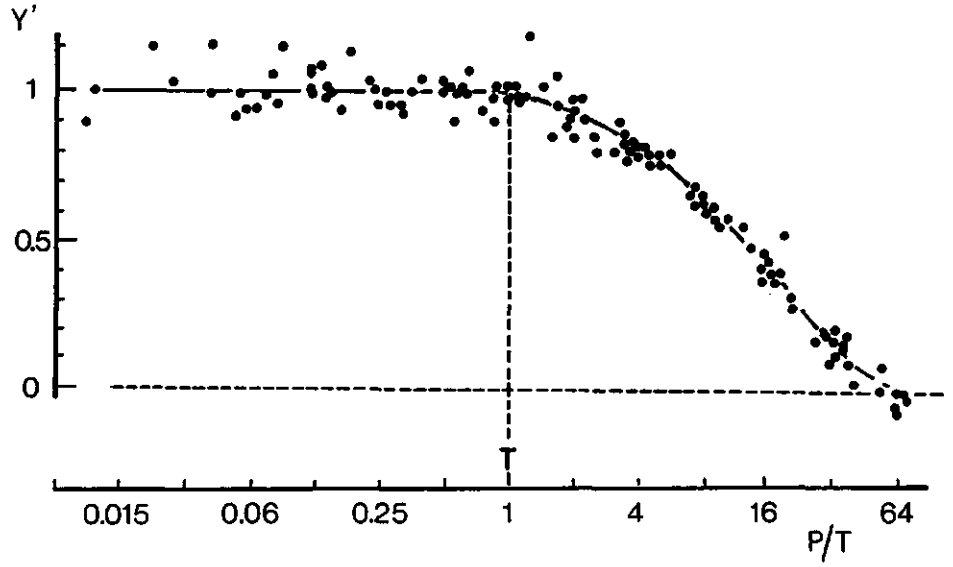


Figure 9.4. - The relation between nematode density P/T at sowing or planting and the relative weight y' of plants after a certain growing time in thirteen experiments with several tylenchid nematode species and several plant species.
$y' = (y-m_x)/(1-m_x) = 0.95^{P/T-1}$, in which $y = m_x + (1-m)0.95^{P/Tx-1}$ for the x-th combination of nematode species and plant species and all values T_x are coinciding.

9.6. Implications of the model

The similarity between very different nematode species in their reaction upon nematode attack is not restricted to its effect on the size of the plant. Nematode densities up to at least $16T$, but generally more than $32T$, affect neither water consumption per unit weight of plant and per unit duration of time, nor dry matter content (Seinhorst, 1981). There are indications that this also applies to shoot to root weight ratio's (Seinhorst, 1979; Been & Schomaker, 1986). The only difference between plants without nematodes and those at small and medium nematode densities of the same weight stated so far is that the latter may be slightly taller.
The model implies comparison of growth rates, as affected by nematode attack, of plants of the same weight and not of the same age, because the latter also differ in developmental stage. The importance of this consequence of the model is evident from the investigation on the effect of *H. avenae* on the shoot to root ratio of young oat plants (Seinhorst, 1979) and on the relation between weight of potato plants with and without *G. pallida* and nitrate and potassium content (Been & Schomaker, 1986).
Seinhorst (1986*b*) rated the mechanisms by which nematodes should reduce growth rates of plants (extraction of nutrients, mechanical damage to root tissue resulting in a

hampered uptake of water and minerals, obstruction of plant vessels causing wilting, extraction of food), suggested in the literature, as myths and fairy tales. A more probable mechanism is the production of a growth reducing substance only during the penetration of the nematodes in the roots but not any more when they have settled. Because of the constant number of nematodes penetrating per unit quantity of root and per unit duration of time, the growth reducing stimulus will then remain constant per unit weight of plant. It remains to be investigated how the reduction of the efficiency per nematode with the increase of population density comes about. Further major problems to be investigated are the way growth reduction is prevented up to a certain nematode density and what determines the ratio k, keeping it independent of T.
Strictly taken, the model only applies to nematode species with a single generation per growing season, as of potato cyst nematodes, oat cyst nematodes and *Meloidogyne naasi*. However, Figure 9.3. demonstrates that it also applies at least to nematodes with small rates of reproduction (e.g. ten to twenty fold in a growing season). Seinhorst (1995) shows that the reason why may not be simple.

9.7. The effect of potato cyst nematode attack on tuber growth

After the start of tuber initiation the rate of weight increase of the haulms decreases strongly to change finally to a decrease. In the model increase of haulm weight is assumed to stop shortly after the initiation of tuber growth, after which increase of total weight is due entirely to that of tuber weight.
If the effect of nematode attack on potato plants would only be retardation of development, tuber initiation of plants without and with nematodes would start at the same haulm (= total) weight of plants, irrespective of the retardation of the development. If t_{s0} is the time tuber initiation of plants without nematodes starts, then tuber initiation at nematode density P would start at a time

$$t_{sP} = r_0\, t_{s0} / r_P \tag{7}$$

However, the ratio between tuber weights of plants of different age but of the same total weight at nematode density P and without nematodes (W_P/W_0) increases most probably from a density T, and there are indications that the initiation of tuber growth is delayed less than according to eq. (7) (Seinhorst *et al.*, submitted) and therefore starts at a time

$$t'_{sP} = (r_0\, t_{s0} / r_P) - \Delta t \tag{8}$$

Δt increases with r_P/r_0 in such a way that t'_{sP} approaches a maximum of t_{s0} + about 2 weeks, possibly because the external conditions inducing the initiation of tuber growth become stronger than the delaying effect of nematode attack (relation between r_P/r_0

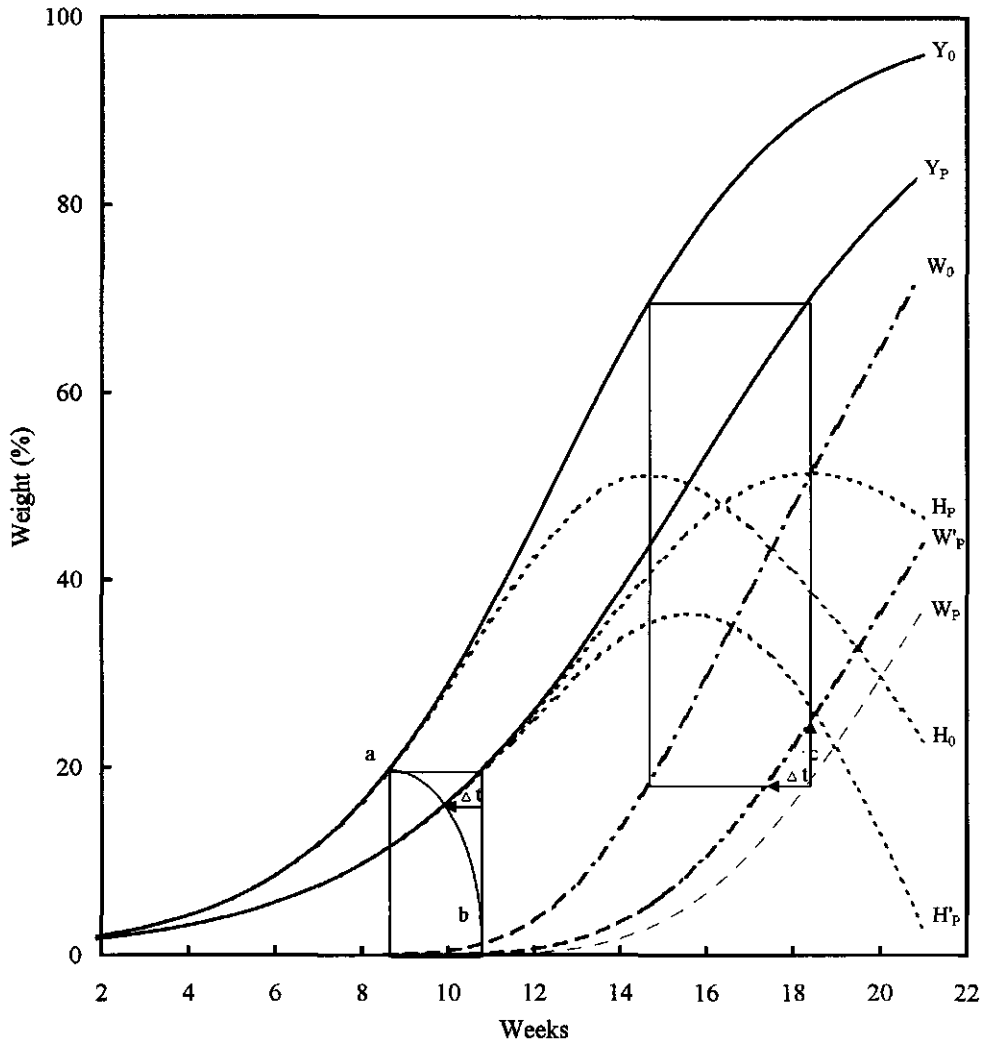


Figure 9.5. - Growth curves for total, haulm and tuber weight of potatoes with and without nematodes. The growth rates for plants of the same total weight or the same tuber weight (measured some time after tuber initiation) with and without nematodes are related by eq. (2): $r_p/r_0 = t_0/t_p$. The curves for tuber weights of plants of the same total weight with and without nematodes are related by $W'_p = W_0 + r_p\Delta t$. In the figure, the curve ab represents the relation between haulm weight and the time tuber growth starts; c = $r_p\Delta t$; Y_0 and Y_p represent total weight of plants without nematodes and at density P respectively; W_0 and W_p are tuber weights of plants without nematodes and at nematode density P if tuber initiation had started at the same haulm weight as in plants without nematodes; H_0 and H_p are haulm weights of plants without nematodes and at nematode density P, if tuber initiation had started at the same haulm weight as in plants without nematodes. H_p represents the actual haulm weight at nematode density P.

and t'_{sP} according to line *ab* in Figure 9.5.). As a result tuber weights of plants at nematode density P (W_P') are r_p Δt units larger than tuber weights of plants without nematodes of the same total weight.
The relation at given times t between nematode density and relative tuber weights in a model, constructed as described above, are again according to eq. (6) up to $P/T = 50$ eggs/g soil and with $k>0.4$ ($m>0.05$) from the time tuber to total dry weight ratio's larger than 0.5 are reached and with the same value of T but a smaller one of m than for total plant weight. Within these ranges of tuber to total dry weight ratio's and values of P/T and k actual relations between nematode density and relative tuber weights were according to those in the model, if not affected by growth reduction by other mechanisms than that described by the model.

9.8. More than one mechanism of growth reduction

According to Seinhorst (1981) nematodes cause two kinds of growth reduction: the 'first mechanism of growth reduction' operating at all population densities, and a 'second mechanism of growth reduction' additional to that of the 'first mechanism', with a noticeable effect only at medium to large nematode densities. The model only applies to growth reduction caused by the 'first mechanism' that retards growth of plants and, occasionally, also increases the length of haulms compared to those of plants of the same weight without nematodes. As long as only the first mechanism is active, water consumption during short periods is proportional to plant weight and, therefore, relative water consumption at different nematode densities and times after sowing or planting is a measure of relative plant weight. Actual plant weights are these relative weights times the actual weights of plants of the same age without nematodes, determined at the same time (Seinhorst 1981).

The 'second mechanism' reduces water consumption per unit plant weight and the (active) uptake or excretion of K^+ and Na^+ and increases the (passive) uptake of Ca^{2+} and dry matter content of plants (Seinhorst 1981; Been & Schomaker, 1986). There probably is a negative correlation between age of the plant and nematode density at which the effect of the 'second mechanism' becomes noticeable. For potato cyst nematodes this density rarely is as small as 16T, but more commonly, also for other nematode species on other plant species >32T. Contrary to the 'first mechanism' it tends to advance the initiation of tuber growth.
A 'third mechanism of growth reduction' is 'early senescence' of potato plants attacked by *G. pallida*: a sudden ending of the increase of haulm length and weight. The time after planting at which it occurs may be negatively correlated with nematode density. The earliest occurrence was nine weeks after planting in the early cultivar Ehud and the smallest nematode density 25T in cultivar Darwina. Not all cultivars are equally sensitive (Seinhorst *et al.*, submitted). The cause of 'early senescence' is unknown.

9.9. The estimation of T and m

There are strong indications that the value of T for potatoes planted in spring is not affected by differences in external conditions and can, therefore, be determined in pot experiments in both greenhouse and (much more labourious) field experiments. The only requirement of greenhouse tests is that large enough pots are used to guarantee about the same root density in the soil as in the field and to prevent the plants from becoming pot bound, which affects the relation between nematode density and plant weight and obscures the true value of T (Seinhorst & Kozłowska, 1976). According to the model and the results of pot experiments, T for total weight, a short time after planting, suffices as an estimate of T for final tuber weight, which allows the use of smaller pots. Experiments should be done with ranges of nematode (egg) densities with ratios not larger than 1 to 2 between successive densities and a sufficient number of densities $<T$ and pots to provide an accurate estimate of plant weight at $P<T$. The largest density in the range should be about $30T$. The accuracy of the estimates is mainly a matter of uniformity of plant material and growth conditions (light, water content) and carefully filling and handling of the pots.

At best m is characteristic of a combination of nematode and plant species with a very large variability between experiments. Therefore, a small number of tests in greenhouses, as will suffice to obtain a reliable estimate of T, will not produce such an estimate for a combination of a potato cultivar and a potato cyst nematode pathotype. However, it may be possible to derive differences, if they exist, between host varieties from the results of greenhouse experiments. In addition, of at least some host varieties per nematode pathotype, a sufficient number of values of m must be estimated in field experiments to establish a distribution function.

As growing potatoes at large potato cyst nematode densities is uneconomical, the relation between nematode density and growth and yield reduction by the 'second mechanism' can be ignored in a model for advisory purposes.

Estimation of T and m in field experiments is much more labourious than estimation in pot experiments. The same range of densities is needed and nematode density must be the only variable. Ranges of nematode densities in patches of potato cyst nematode infestation (but not necessarily of other species) come closest to this requirement, if free of other causes of variation of tuber yield with intractable spatial distributions. Ranges of nematode densities cannot be created by applying different dosages of nematicide or other biocide, as has often been done, as this has unpredictable effects on crop yield, other than by killing nematodes. The ranges of nematode densities, that are of interest for the estimation of the parameters in question, must be determined in sufficiently large samples from each field plot to guarantee a coefficient of variation of egg counts not larger than 15% (density differences 1:2 just distinguishable). According to Seinhorst (1988) soil samples of 4 kg per plot are then needed to estimate population densities of 1 egg per g of soil, given a coefficient of variation of the number of eggs per cyst of 16% and a negative binomial distribution of egg densities in

samples from small plots with a coefficient of aggregation of 50 for a kg soil. As the coefficient of variation per unit weight of soil is negatively correlated with nematode density, required sample size also is. For instance, to estimate densities of 0.5 or 0.25 eggs/g soil with the same accuracy, eggs from soil samples of 10 and 20 kg must be counted respectively. Another requirement is that plots must on the one hand be small (e.g. 1 m^2) to reduce the effect of medium scale density variation, whereas on the other hand a large enough area per small density interval must be available to guarantee a small variability of tuber weight per unit area. Again, there must be a sufficient number of plots at densities smaller than T to estimate the maximum yield accurately.
Therefore, to avoid unnecessary handling of large soil samples, T and m should, as much as possible, be estimated from greenhouse experiments. Field trials are most efficiently used to confirm of falsify these estimates or ratios of estimates for different combinations of pathotypes and cultivars under more natural external conditions.

9.10. Final considerations

As a result of the striking conformity between the effects of all root infesting nematodes investigated upon the growth of attacked plants, whatever the host status of the plants, the ways the nematodes attack, and the reactions of the affected tissues, the growth model discussed above applies to small and medium population densities of all tylenchids. It also results in the same formal relation during the year of sowing or planting between nematode density at sowing or planting and relative total plant weight and, in potatoes, also relative tuber weight, from some time after planting, as long as later generations of species with large rates of reproduction (e.g. *Meloidogyne* species) do not cause additional growth reduction. This is corroborted by a large number of pot experiments and the few field experiments, that were suitable for the purpose. It allows a characterization of the sensitivity to growth and yield reduction (the tolerance) of a crop plant (annuals and perennials during the first year after sowing or planting) by small and medium population densities at sowing or planting of a particular nematode species by the values of two parameters, which is preferable to the (customary) single characterization without reference to nematode density. The most important of the two parameters, the tolerance limit T, most probably is insensitive to variation in external conditions that normally occur during the period the crop is grown. That of potatoes to potato cyst nematode attack depends on change of day length during the plant growth. The values of these parameters, especially that of the tolerance limit, are key factors in the calculation of combinations of cost of reduction by control measures of a given nematode population density, expected at the time of sowing or planting of a crop to be protected, and the cost of crop loss, caused by the surviving nematodes, resulting in maximum net returns. These calculations are again formally the same for all tylenchid nematodes, annual crops and control measures.
On the other hand the model gives strong indications of the nature of the mechanisms

resulting in the growth reduction by nematode attack and is, therefore, a base for investigations on its biochemistry, the counteraction of this reduction, resulting in the existence of tolerance limits, and the adding up of the effects of very large numbers of nematodes to a maximum reduction of the growth rate of the attacked plant by an amount which, in general, is smaller than the actual growth rate of plants without nematodes. So far, the physiological mechanism(s) resulting in growth reduction by nematode attack have hardly been investigated seriously. Guided by this growth model, this type of research, which may open new ways for the management of these nematodes, could make a better progress.

Chapter 10

An advisory system for the management of potato cyst nematodes (*Globodera* spp.)

T.H Been, C.H. Schomaker & J.W Seinhorst†

10.1. Summary - An advisory system is presented for the management of potato cyst nematodes (*Globodera pallida*). It emphasizes the use of partially resistant potato cultivars, which provide the possibility of keeping population densities of potato cyst nematodes at a low level in short fixed rotations. Using stochastic models based on the population dynamics of potato cyst nematodes and the relation between pre-plant nematode densities and relative yield it is possible to calculate the probabilities of population development and the reductions in yield caused by these population densities. A simulation model is developed which integrates both models, using the frequency distributions of some of the most variable parameters relevant to a particular combination of potato cultivar and nematode population. Also, the natural decline in population density when non-hosts are grown is incorporated in the model. The model makes it possible to calculate the probability of a certain yield reduction, given a certain potato cultivar, nematode population and rotation. Therefore, it becomes feasible for a farmer to evaluate risks and the costs of different control measures in fixed rotations. The application of this model in the starch potato growing areas could lead to significant improvements in financial returns and a major reduction of the use of nematicides.

10.2. Introduction

Potatoes are among the most profitable agricultural crops in arable farming in the Netherlands. Therefore, they are grown as frequently as possible, especially in those areas, where farmers have almost no choice of other profitable crops. This frequency is limited by build up of potato cyst nematodes which, without control, leads to considerable crop losses. Possible control measures other than crop rotation (growing susceptible potato cultivars in rotation with five to seven years of non-hosts) are growing highly- or partially-resistant potato cultivars and chemical control. Chemical control not only has a poor cost-to-benefit ratio, its use is also increasingly restricted by legislation. A 50% reduction by 1995 and a 80% reduction by the year 2000 in the use of the so-called fumigants are main objectives in the Multi-Year Crop Protection Plan of the Dutch government. Cultivars that are highly resistant to the pathotypes Pa 2 and Pa 3 of *Globodera pallida* are rare (industrial processing) or not available (human consumption). As crop loss is strongly associated with nematode density in the field at the time of planting, control should aim at preventing nematode densities from becoming too large. Once the population density in the field is reduced to an acceptable level a proper combination of relative susceptibility of a potato cultivar and crop rotation can keep it small. It is in the interest of potato growers and the environment to integrate the use of partially-resistant potato cultivars and other control measures in farming practices in such a way that a maximum return is obtained by minimizing the sum of cost of control and cost of yield reduction. This requires an advisory system based on

prediction of population development and yield reduction in given rotations which emphasizes the use of partially resistant potato cultivars. As treatments with nematicides are expensive (about 1200 Dutch guilders/ha) and their effectiveness in reducing population densities is overrated, net returns will be considerably larger than with chemical control, when partially resistant potato cultivars are grown in the proper short rotation and, therefore, with only minor crop losses due to the resulting small nematode densities. As a result the greater part of the reduction of the use of chemicals required in the Multi-Year Crop Protection Plan can be achieved without adverse economical consequences, if advices according to the advisory system are followed.

An advisory system has been developed based on a stochastic simulation model which can include a number of sub-models ranging from those of spatial distribution of population densities (resulting in new soil sampling methods), to economics for calculation of maximum returns. This paper is restricted to a discussion of the possibilities of using partially-resistant potato cultivars in certain crop rotations to minimize yield reductions and the cost of control. Four sub-models in the integrated simulation model will be discussed:

1 The simplified version of population dynamics of potato cyst nematodes applying at small to medium nematode densities at planting;
2 The concept of relative susceptibility. ('susceptibility' = 'host status');
3 The relation between pre-plant nematode densities and relative yield (yield as fraction of the yield when potatoes are grown without nematodes);
4 Population decrease as a result of growing non-host crops.

10.3. Population dynamics

The relation between population density at planting or sowing (*Pi*) and the population density of the new generation at harvest (*Pf*) for nematode species with only one generation per season (potato-, oat-, white clover cyst nematodes, *Meloidogyne naasi*) is described by Seinhorst 1967, 1970, 1986*a* and 1993 (Figure 10.1). Seinhorst's (1993) most extended equation for this relation contains ten parameters of which the most important ones are the maximum rate of reproduction (occurring at very small initial population densities) and a theoretical maximum density, the number of eggs that would have been produced per unit weight of soil at very large initial nematode densities if the size of the plant would not have been reduced by the nematodes. Because of the reduction of the size of the plants by nematodes the actual maximum nematode density after a potato crop in the field occurs at medium initial densities and also includes non hatched eggs of the parent generation (Seinhorst, 1967, 1984, 1986*a*).
Seinhorst's (1993) equation for this relation, if extended to incorporate the part of the parent population surviving in soil that was not exploited by roots (Seinhorts, 1986*a*), contains eight parameters; too many to be useful for the prediction of population den-

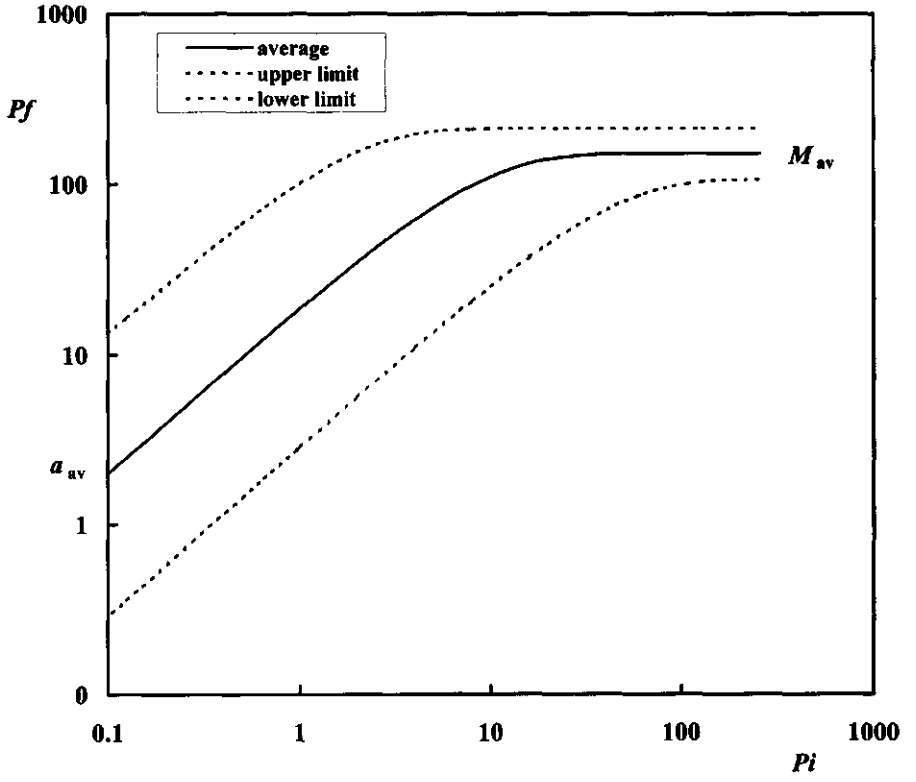


Figure 10.1. - The relation between initial and final egg densities according to eq. (l). Dotted lines = 95% confidence interval of the log-normal distribution of a and M ($0{,}145a < a_{av} < 6{,}9a$ and $0{,}71M < Mg_{av} < 1{,}41M$). The scale of Pf (eggs/g soil) applies to *G. pallida* ($a_{av} = 20$ and $M_{av} = 150$ eggs/g soil on susceptible cultivars).

sities and their frequency distributions after growing a cultivar with a certain degree of resistance. However, it can be used as a basis for the formulation of the constraints on the simpler equation:

$$Pf = M \bullet (1 - e^{-aPi/M}) \qquad (1)$$

in which:

Pi initial egg densities (before planting) in eggs/g soil;
Pf final egg densities (after harvest) in eggs/g soil;
a the maximum rate of reproduction;
M a theoretical maximum final population density.

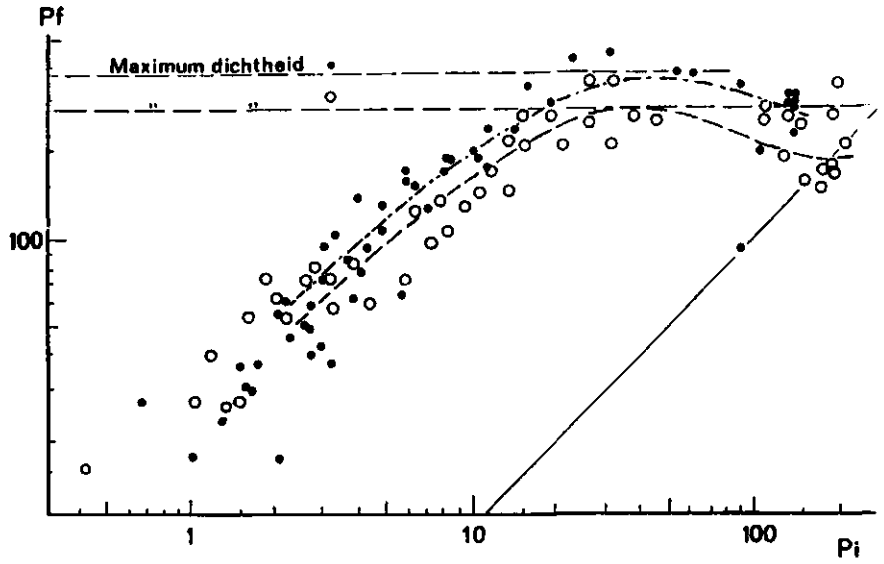


Figure 10. 2. - The relation between *Pi* and *Pf* of pathotype Ro 1 of *G. rostochiensis* on cultivar Bintje on two parts of a farmer's field (Seinhorst, 1986a). *Pi* and *Pf* in eggs/g soil. The solid line indicates $Pf/Pi = 1$.

This equation fits to the relation between initial and final nematode densities up to the *Pi* value where the maximum *Pf* value is reached according to the extended equation. Taking this maximum as an estimate of *M* did not result in appreciable differences between *Pf* values according to eq. (1) and observed ones in field experiments (e.g. Seinhorst, 1986*a*), where a considerable plot-to-plot variation existed.

As the values of *a* and *M* are not only determined by the potato cultivar but also by external conditions, the final population density at one initial population density can vary strongly between fields and years. Both parameters not only differ between years and fields; variation can occur within a single field (Figure 10.2). Therefore, it is impossible to predict the development of population densities in individual fields using only average values of *a* and *M*, let alone from *a* only, as is common practice in contemporary advisory systems and legislation. A better approach is to establish the frequency distributions of *a* and *M* and to calculate the probability of all possible combinations of *a* and *M* and of the resulting densities *Pf* and their probabilities. According to observations on seventeen farmers' fields on several soil types in several years *a* for *G. rostochiensis* varied between 3 and 157 with a geometric mean of 25 and *M* varied between 200 and 400 eggs/g soil with a mean of 300 eggs/g soil (Seinhorst, 1986*c*). In

further calculations, the frequency distribution of both parameters is assumed to be log-normal. There are no indications that these distributions are different for partially-resistant cultivars. Therefore, the calculations for these cultivars are based on smaller average values (depending on their degree of resistance) with the same variability as for fully-susceptible cultivars.

There are not enough observations from field experiments to estimate mean values and their variances of a and M for *G. pallida*. A value of 0.8 for the ratio between a for *Globodera rostochiensis* pathotype Ro 1 and for *G. pallida* pathotype Pa 3 and a value of 0.5 for that between M for these pathotypes could be deduced from values on the susceptible cultivar Irene grown in pot experiments (den Ouden, 1974*a*; Seinhorst and Oostrom, 1984; Seinhorst, 1986*b*). Therefore, it is assumed that a_{av} and M_{av} for *G. pallida* on susceptible cultivars in the field are 20 and 150 eggs/g soil respectively. These assumptions must be verified against observations from field experiments when these are available.

10.4. Measures of partial resistance and their relation

When partially-resistant potato cultivars are grown fewer females will mature than on susceptible cultivars; also the number of eggs/cyst may be smaller. Therefore, nematodes multiply less strongly on these cultivars than on susceptible ones and sustain a smaller maximum population density. However, both a and M are too variable to be suitable as a measure for partial-resistance. Therefore, the concept 'relative susceptibility', rs, was introduced, based on the population dynamics of the potato cyst nematode. The relative susceptibility is the ratio between the maximum multiplication rate a of the nematode population on the tested cultivar and on a susceptible reference cultivar or the equivalent ratio of the maximum population density M on these cultivars. These present two measures of partial resistance or relative susceptibility, provided that the tested cultivar and the susceptible reference are grown under the same conditions in the same experiment. Relative susceptibility is independent of external conditions which influence both a and M and contribute to their large variability. Figure 10.3. shows the relation between *Pi* and *Pf* of pathotype Pa 3 of *G. pallida* on the partially-resistant cultivar Darwina and on the susceptible cultivar Irene according to equation (1).

Jones *et al.* (1981) and Phillips (1984) assumed that the ratios $(a_{partially\ resistant}/a_{susceptible}) \cdot 100\%$ and $(M_{partially\ resistant}/M_{susceptible}) \cdot 100\%$ are numerically equal. According to Seinhorst (1984), Seinhorst and Oostrom (1984) and Seinhorst *et al.* (1995) this also applied to 9 out of 11 cultivars tested with pathotype Pa 3. However, rs_M was smaller than rs_a with cv. Activa and breeding line Karna 77/281 (Seinhorst *et al.*, 1995) and cv. Ehud (Seinhorst, 1984). Seinhorst and Oostrom's 1984 data also indicate that, despite considerable differences in rates of reproduction at small initial egg densities of the same pathotype on the same cultivar in different experiments, the variation of the relative susceptibility was largely if not entirely due to experimental error, resulting

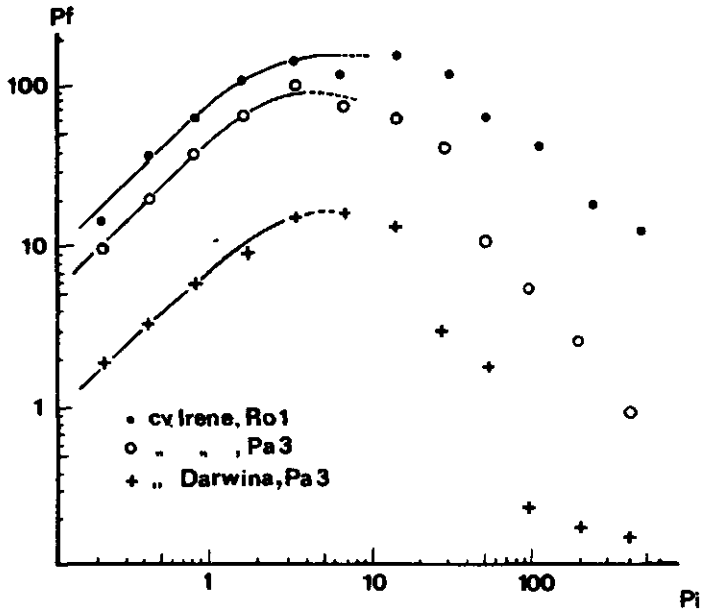


Figure 10.3. - The relation between initial and final population density *Pi* and *Pf* of *Globodera pallida*, pathotype Pa 3 on cv.'s Irene (o) and Darwina (+), (after Seinhorst and Oostrom, 1984). *Pi* and *Pf* in eggs/g soil. Lines according to equation (1).

from limited numbers of pots and insufficient number of cysts and eggs counted per replication.

The great similarity between rankings of cultivars according to degrees of susceptibility in pot and field experiments observed by Forrest and Holliday (1979), Phillips and Trudgill (1983, 1985), Phillips *et al.* (1987) supports the conclusion that relative susceptibilities do not depend on external conditions. The methods to determine the relative susceptibility of a potato cultivar with a high degree of accuracy are described in detail by Seinhorst *et al.*, 1993.

Table 10.1. presents the relative susceptibilities of sixteen potato cultivars to different pathotypes of *G. pallida*, measured in 10 l pots at the IPO-DLO. The pathotypes include one of the most virulent populations found so far: Pa 3 (1) (the 'Rookmaker' population). A wide and continuous range of relative susceptibilities is apparently available in the cultivars tested.

Table 10.1. - Relative susceptibilities (a/a_s) of sixteen potato cultivars for pathotype Pa 1, Pa 2 and Pa 3 of *Globodera pallida* expressed in percentages of the susceptibility of the susceptible cultivar Irene for these pathotypes. Pa 3 (1), also known as the 'Rookmaker' population, is highly virulent, Pa 3 (2) is a population with average virulence.

Cultivar	Pa 1	Pa 2	Pa 3 (1)	Pa 3 (2)
Irene 100	100	100	100	
Amalfi	-	3	-	39
Amera	76	63	-	98
Producent	15	3	40	34
Multa	-	12	43	40
Pansta	-	6	32	36
Promesse	-	4.5	35	21
Proton	-	1	31	18
Darwina	4.7	0.3	12	5
Santé	-	1	18	8
Atrela	-	0.7	20	9
Karna 77/270	-	-	28	-
Karna 77/281	-	-	12	-
Activa	-	-	25	-
Elles	-	-	17	-
Seresta	-	-	2	-

- = relative susceptibility not measured.
Pa 1 does not occur in the Netherlands

10.5. Relation between nematode density and yield

Another essential part of the integrated simulation model is the relation between population density of eggs at the time of planting and tuber yield at harvest expressed as a proportion of the yield in the absence of nematodes. As large yield losses must be avoided, large *Pi* values must always be reduced by extra control measures to acceptable *Pi* values before a crop is grown. Therefore, the relation at small to medium nematode densities (Seinhorst, 1986*b*) suffices:

$$y = m + (1 - m) \bullet 0.95^{(P-T)/T} \quad \text{for} \quad P > T$$
$$\text{and} \quad y = 1 \quad \text{for} \quad P \leq T \qquad (2)$$

in which:

y the yield at egg density P at planting as a proportion of that at $P \leq T$;

m the minimum relative yield (therefore a constant <1);

T the tolerance limit, the density P up to which no yield reduction is caused.

T for susceptible cultivars varied little between experiments about an average of 2 eggs/g soil in field experiments with pathotype Ro 1 (Seinhorst, 1982*a*, 1986*c*), micro plots with pathotypes Ro 1 and Pa 3 (Greco *et al.*, 1982) and pot experiments with pathotypes Ro 1, Ro 3 and Pa 3 (Seinhorst, 1982*c*). Values of m varied more; 0.2 - 0.6 according to Seinhorst, 1982*a*. But using an average of $m = 0.4$ in predictions does not result in unacceptable deviations between actual and calculated losses at densities ≤ 15 eggs/g soil. Over- or under-estimations do not exceed 5% (Figure 10.4.). Predicting relative yield losses at larger nematode densities has no practical value as yield reductions at these densities are too high to be tolerated. Farmers will not grow a potato crop at such nematode densities without first taking control measures. In the Netherlands this implies the application of a nematicide to reduce *Pi* ánd also, as fumigation is mostly not sufficient to attain acceptable nematode densities, the use of a systemic nematicide just before planting. The latter provide a temporal protection by delaying nematode invasion, thereby increasing the minimum yield m.
Little is known about the tolerance limit of partially-resistant cultivars except that T for pathotype Pa 3 on cv. Darwina is, also, 2 eggs per g soil and that results of field tests do not contradict such a value of T for other cultivars. The similarity of ratios M_r/M_s to a_r/a_s for the cultivars including cv Darwina, partially-resistant to pathotype Pa 3 (Seinhorst *et al.*, 1995), suggest that T for pathotype Pa 3 on these cultivars is not a multiple of the 2 eggs/g soil for cv. Darwina. An accurate assessment of the tolerance limit is desirable for two reasons. It is required for the calculation of losses to be expected at given egg population densities at the time of planting and, as the maximum population density tends to become proportional to the size of the plant when *Pf* approaches M, this maximum will be correlated positively with the tolerance limit. However, small differences in tolerance (e. g. a ratio of tolerance limits of 1 to 2) would not have a detectable effect on M.

10.6. Population decline in the absence of a host

The nematode density at planting of potatoes is the density left by the previous potato crop multiplied by the survival rate over the period during which no potatoes were grown. This rate can be considered to be independent of nematode population density. Reduction of the population in the absence of a host crop is largely due to spontaneous hatching during a short period in spring of an apparently fixed proportion of the eggs. According to Huijsman (1961) the survival rates over a period of six years was 65% per year and this rate was (largely) independent of the age of the population as was the viability of the surviving population (den Ouden, 1963). Cole and Howard (1962) found a survival of 80 % per year during three years, and den Ouden (1970) reported an average of 79% over 14 fields in 1969 but only 51% over six fields in 1973 (den

Ouden, 1974*b*). However, the magnitude of field-to-field and year-to-year variation in the survival rate is unknown as, generally, sampling error was either ignored or was so large that differences could not be distinguished. However, there are indications that the survival rate during the first year after a potato crop is lower (den Ouden, 1960, Cole and Howard, 1962, Andersson, 1987, 1989) than in subsequent years. Therefore, in the calculations of the simulation model, a lower survival rate is used the first year after the potato crop than during later years (about 50% changing to 65%). The survival rate is used to calculate a factor for crop rotation, *c*, having a value of 0.5 for a 1:2 rotation; $0.5 \bullet 0.65 = 0.32$ for a 1:3 rotation; $0.5 \bullet 0.65^2 = 0.21$ for a 1:4 rotation and $0.5 \bullet 0.65^3$ for 1:5 rotation. But more information on survival rates in the absence of potatoes is required to adjust the model if necessary.

10.7. Simulation model

Basics

To make computations the frequency distributions of *a* and *M* were assumed to be log-normal with means 1.30103 and 2.17609 and standard deviations 0.419 and 0.07525 respectively for *Globodera pallida* pathotype Pa 3 on susceptible cultivars. Both frequency distributions were divided into 24 classes with a width of 0.25 times the standard deviation (s). Hence, a range of classes between (log *a* or *M*) + 3*s* and (log *a* or

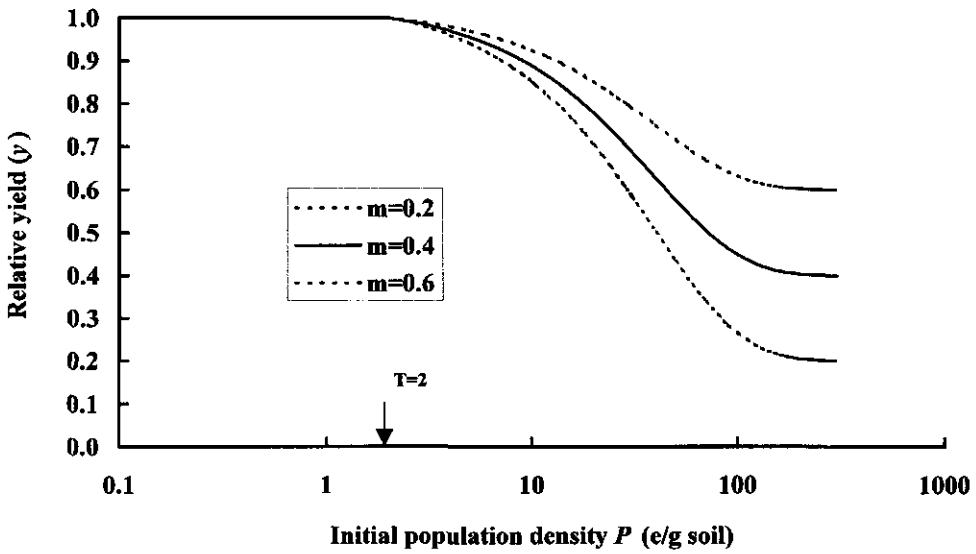


Figure 10.4. - The relation between the initial population density (*P*) and the relative yield *y* according to the equation: $y = m+(1-m)\bullet 0.95^{(P-T)/T}$ with $T = 2$ eggs/g soil and $m = 0.4$ (the mean) and $m = 0.2$ and 0.6 (the boundaries of the 95% confidence interval).

M) - 3*s* were used, comprising 99.7 of the frequency distribution. Each class of *a* and *M* is presented by the antilog of its class mid-mark and divided by a_{av} and M_{av} respectively and a relative frequency. Values of log *a* and *M* beyond this range are ignored. All relative frequencies are multiplied by 0.997^{-1} to obtain a total of 1 for the cumulative probability of the range considered.

Pf values following a given *Pi* for a potato cultivar with a given *rs*, are calculated according to eq. (1) with every class mid-mark of the distribution of *a* times $rs \bullet a_{av}$ combined with all class mid-marks of the distribution of *M* times $rs \bullet M_{av}$. The relative frequency of each *Pf* is the product of those of the *a* and *M* used. Calculating *Pf* values for the next crop rotation as indicated above with each of these 24^2 values as *Pi* would require an unnecessarily excessive amount of work. Therefore, the *Pf* values, also, are divided into 24 classes and new mid-marks are calculated. The relative frequency of each of these class mid-marks is the sum of relative frequencies of *Pf* values belonging to that class. Now, there are 24 *Pi* values to start with, resulting in 24^3 = 13824 *Pf* values after the second potato crop. This procedure is repeated until the required number of potato crops has been simulated.

As this paper is limited to the use of partial resistance to keep nematode densities small, actual population densities as determined by soil sampling are not used as input. Instead, the almost stable frequency distribution of population densities established after five years of growing partially-resistant potatoes of the same relative susceptibility is used. These are practically independent of population densities in the first year of cropping. A more extensive description of this part of the simulation model will be presented in another paper.

Crop losses in rotations with potato cultivars of given relative susceptibility.

For the assessment of relative crop losses (1 - *y*) the relation between nematode density and relative tuber yield according to eq. (2) is used to calculate *y* for the mid-marks of classes of *Pf* values. For the tolerance limit *T* a value of 2 eggs/g soil is used and for the minimum yield *m* = 0.4. Estimates of percentages of fields with nematode densities and crop losses exceeding certain limits occurring in a given year or percentages of years in which these occur in a given field when a potato cultivar with a certain *rs* is grown in a certain rotation are given in Figure 10.5. The probability of suffering a crop loss larger or equal than a certain value (on the x-axis) can be read off from the y-axis. From these data the average yield reduction can be calculated. When crop rotation (and the same applies to other control measures) is used to reduce the population density between two potato cultivars with a factor *c* then the *Pi* at the next potato crop is *cPf* of the population density after the last potato crop. The development of population densities then depends on ca_{gem} and cM_{gem}. This implies that the frequency distributions of expected crop losses in Figure 10.5., which are obtained when cropping potatoes with a certain *rs* continuously, are the same when a potato cultivar with *rs/c* is cropped in the corresponding rotation cycle. For a better overview concerning a range of relative susceptibilities Table 10.2. shows the different average yield reductions, the probabilities

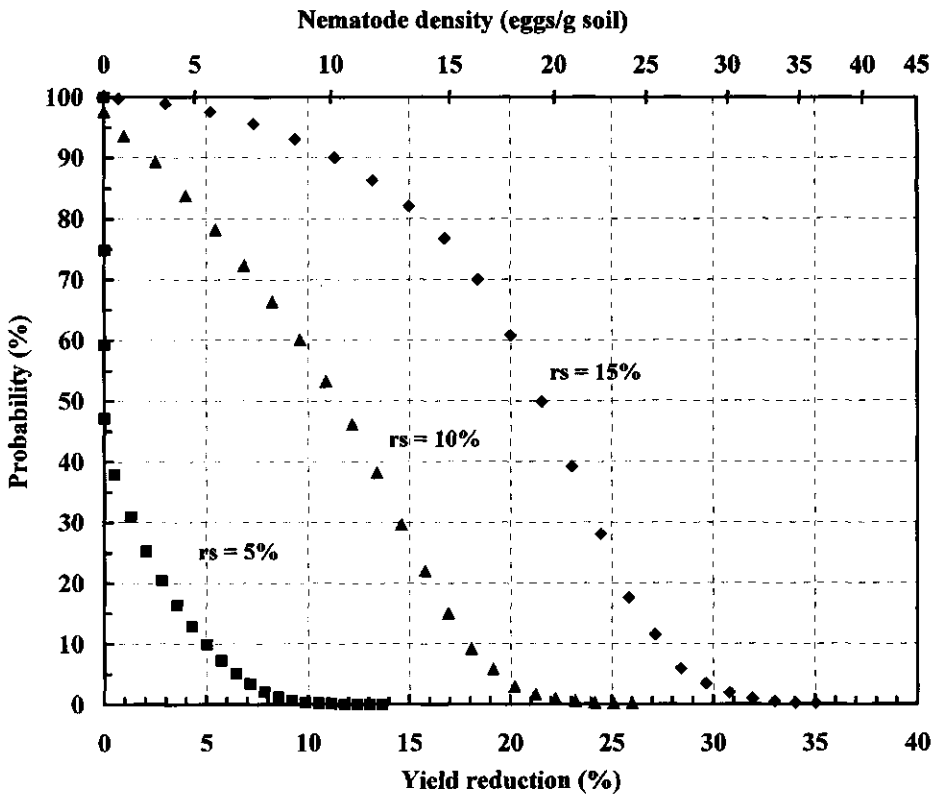


Figure 10.5. - Probabilities of nematode densities (top x-axis) and resulting relative yield reduction ((1-*y*)•100%) (bottom x-axis) after five croppings of a partially-resistant potato cultivar with *rs* of 5/*c* (■); 10/*c* (▲); and 15/*c* (♦) respectively at an initial density of 5 eggs/g soil in the first year (*c* = 0.5 at 1:2 cropping frequency; 0.32 at 1:3 frequency; 0.21 at 1:4 frequency and 0.14 at 1:5 frequency). The relative yield (*y*) according to equation (2) and Figure 10.4. with $T = 2$ eggs/g soil and $m = 0.4$. $M_{av} = 150$ and $a_{av} = 20$ eggs/g soil.

of more than 0%, 5%, 10%, 12.5% and 15% yield reduction and the maximum yield reduction in 95% of the fields in 5 different crop rotations and with different values of *rs*. From these figures it can be concluded that cultivars with a partial resistance of $\leq$ 8/*c* % always control potato cyst nematodes sufficiently without the need for additional control measures. However, the level of *rs* that is still useful depends on the costs of control and the extra yield as a result of applying that control. It also depends on the financial situation of the potato grower. If he has a healthy financial reserve he can choose relative susceptibility on the basis of average yield reductions predicted. However, if no financial reserves are available and crop losses may not exceed a certain

amount, risks of certain yield reductions have to be considered and the choice of relative susceptibilities becomes more limited. The percentages in Figure 10.5. and Table 10.2. can also be interpreted as probabilities that such densities and losses will occur in any field and year.

By applying a fixed amount of control on all fields, after cropping a susceptible cultivar, calculated to prevent unacceptable yield losses even in the most heavily infested fields, too much control will be applied on a large number of fields. If the amount of control is adapted to obtain maximum average returns, yield losses in a certain proportion of the fields will be too high, while in the other fields still too much control is applied. Therefore, it would be justifiable to adjust the amount of control, after growing susceptible potatoes, to actual population densities, estimated by taking soil samples (Seinhorst, 1982*c*; Schomaker and Been, 1992). Sampling methods are then needed which provide estimates of densities of eggs and larvae with only a small error.

Table 10.2. - Relation between relative susceptibility, cropping frequency, average yield reduction and probabilities of a certain yield reductions after five croppings of a partial resistant potato cultivars at a *Pi* of 5 eggs/g soil in the first year.

Relative susceptibilities (%) at cropping frequencies of					Average yield reduction (%)	Prob (%) of yield reduction larger than					Percentage yield reduction in 95% of the fields smaller than
1:1	1:2	1:3	1:4	1:5		0%	5%	10%	12.5	15%	
1	2	3	5	7	0,0	0,0	0,0	0,0	0,0	0,0	0,0
2	4	6	9	14	0,0	0,1	0,0	0,0	0,0	0,0	0,0
3	6	9	14	20	0,1	3,7	0,0	0,0	0,0	0,0	0,0
4	8	12	18	27	0,4	18,0	1,2	0,0	0,0	0,0	2,6
5	10	15	23	34	1,2	37,8	7,4	0,0	0,0	0,0	5,8
6	12	18	27	41	2,5	60,0	20,0	1,8	0,0	0,0	8,6
7	14	21	33	47	4,2	70,9	35,4	8,9	2,1	0,0	11,1
8	16	24	36	54	6,1	86,4	51,1	21,9	9,2	2,3	13,7
9	18	27	41	61	8,1	90,5	63,8	37,0	21,4	7,9	16,1
10	20	30	45	67	10,1	93,3	74,0	51,0	35,4	19,5	18,2
11	22	33	50	74	12,2	95,7	81,4	62,7	49,7	32,9	20,2
12	24	36	54	81	14,1	96,8	86,6	72,3	61,6	47,0	22,2
13	26	39	59	88	16,1	100	91,0	79,3	70,8	59,5	24,2
14	28	42	63	94	17,9	100	93,8	84,4	77,8	69,3	25,7
15	30	45	68	100	19,7	100	95,6	88,4	83,5	76,6	27,5

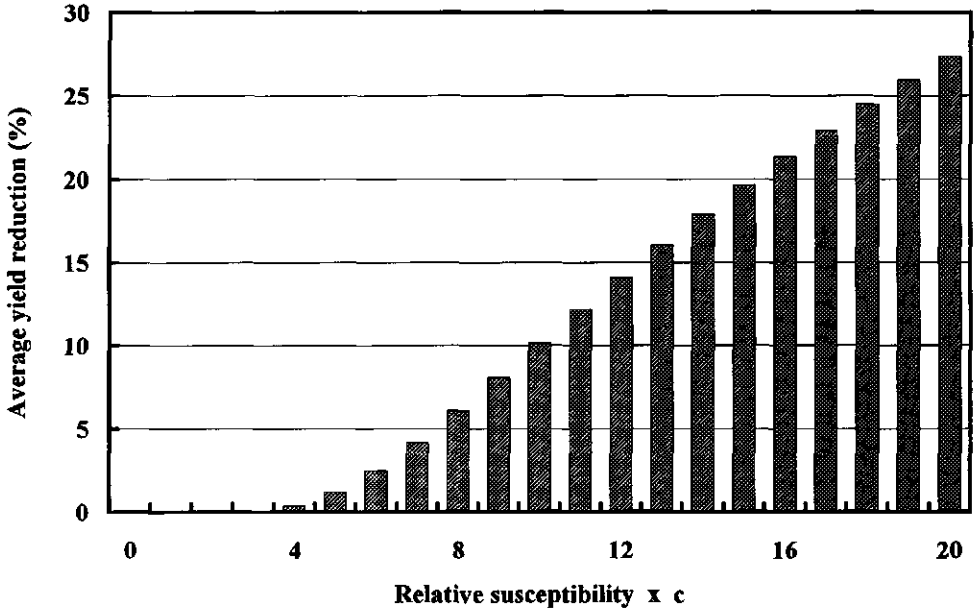


Figure 10.6. - Sensitivity analysis of the effect of variation of estimated $rs \bullet c$ on the average yield reduction expressed as percentage of the expected yield when no nematodes are present after 5 croppings of a partially-resistant cultivar with the same ca_{av} and cM_{av} and $Pi = 5$ eggs/g soil in the first year.

However, the need for soil sampling becomes obsolete when potato cultivars with high partial resistance are grown as then the small maximum population density M on these cultivars limits population increase sufficiently to densities where the cost of additional control exceeds the increase of net return obtained, whereas the required cropping frequency for potatoes is determined by other factors than crop loss caused by potato cyst nematodes. Soil sampling could then be restricted to once during a couple of rotation cycles to check for the presence of a more virulent nematode pathotype.

Sensitivity analysis

The values of ca_{av} and cM_{av} which are supposed to apply to a certain partially-resistant cultivar grown in a certain rotation in a field with a given nematode pathotype depend on several factors: The susceptibility (rs) of the cultivar, the values of a_{av} and M_{av} of the pathotype involved on the susceptible reference cultivar (of which a_{av} is the hardest to estimate in field trials), and the survival rate c of the nematode pathotype during the years without potatoes are all subject to experimental error. For practical application the indirectly derived values of a_{av} and M_{av} for pathotype Pa 3 on susceptible cultivars should be replaced, as soon as possible, by accurate averages and estimated frequency distributions derived from sufficient numbers of directly observed values of a and M from several fields and years.

Table 10.3. - Relative susceptibilities of different potato cultivars on two populations of pathotype Pa 3, average yield reduction and probabilities of a yield reduction larger than 10% at two values of M_{av}/a_{av}. *Pi* = 5 eggs/g soil; - = < 0.5%. The relative susceptibilities are averages of all measurements up to now and can differ from previous publications.

Pa3 (1) population	%	Average yield reduction (%)								Prob. (%) of yield reduction > 10%							
		$a = 20$				$a = 25$				$a = 20$				$a = 25$			
	rs	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4
Irene	100	58	50	41	30	58	50	42	31	100	100	100	99	100	100	100	100
Astarte	44	48	30	19	10	48	31	21	12	100	99	87	48	100	100	94	63
Producent	40	45	27	17	8	46	29	19	10	100	98	81	35	100	99	90	50
Proton	31	39	21	11	4	40	22	13	6	100	90	55	8	100	95	70	15
Karnico	30	38	20	10	4	39	21	12	5	100	88	51	6	100	94	66	11
Santé	25	33	15	7	2	34	17	9	3	100	76	27	1	100	86	40	2
Ellen	22	30	12	5	1	31	14	7	2	98	63	13	-	100	75	22	-
Atrela	20	27	10	4	-	29	12	5	1	97	51	6	-	99	65	11	-
Karna 77/281	16	21	6	2	-	23	8	3	-	92	22	1	-	96	34	1	-
Darwina	15	20	5	1	-	21	7	2	-	88	15	-	-	94	24	-	-
Seresta	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pa3 (2) population	%	Average yield reduction (%)								Prob. (%) of yield reduction > 10%							
		$a = 20$				$a = 25$				$a = 20$				$a = 25$			
	rs	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4	1:1	1:2	1:3	1:4
Irene	100	58	50	41	30	58	50	42	31	100	100	100	99	100	100	100	100
Astarte	40	45	27	17	8	46	29	19	10	100	97	81	35	100	99	90	50
Proton	18	25	8	3	-	26	10	4	1	96	37	2	-	98	51	4	-
Atrela	9	8	1	-	-	10	1	-	-	37	-	-	-	51	-	-	-
Santé	8	6	-	-	-	8	1	-	-	22	-	-	-	34	-	-	-
Darwina	5	1	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Seresta	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Average reduction in yield (%) and the probabilities of reductions in yield > 10% were calculated using the best estimate of a_{av} of a susceptible control (20) and using the value of 25 (presuming an underestimation of the best estimate by 20%). The values for several cultivars with different *rs* are presented in Table 10.3. The underestimation of a_{av} resulted in only slightly larger calculated average crop losses than those using the best estimate of a_{av}. However, the underestimation of a_{av} resulted in a considerable underestimation of the probabilities of crop losses $\geq$ 10 % in single fields. Presuming an overestimation of the best estimate of a_{av} by 20% (a_{av} then is 16) resulted in equivalent changes; calculated average crop losses were only slightly smaller, but crop losses $\geq$ 10% have considerable smaller probabilities than originally estimated.

Figure 10.6. shows the sensitivity of the relative yield to the value taken for the relative susceptibility of a cultivar. It can be deduced that an over- or under-estimation of *rs* has a far greater effect on the average yield reduction than the same error in the estimation of a_{av}. Up to an *rs*/*c* of 4% the average yield reduction is negligible. Between 5% < *rs* • *c* < 15% the relation becomes linear and average yield reduction increases by 2% per unit *rs* • *c*. Therefore, an error of 5 percentage points in the estimation of *rs* causes a deviation of 10% • *c* in the predicted average yield reduction. It demonstrates the necessity of minimizing testing errors by conducting potato cultivar testing for relative susceptibility with the utmost care.

Which relative susceptibilities for which rotation schemes

The yield of potato cultivars, with the same characteristics grown in fixed rotations in fields infested with potato cyst nematodes, is negatively correlated with their relative susceptibility but there will be no difference in cost of nematode control. Therefore, the least susceptible of otherwise similar cultivars is always the best choice either because losses caused by the nematodes are smaller or because a shorter and more profitable rotation cycle can be practised. To determine the upper limit of susceptibility that is still acceptable in a given rotation not only the average loss but also the probability of more than a certain percentage loss of a single crop is important, as, once a large nematode density has built up, the probability of larger than average population densities will be increased during the following years. Table 10.2. provides general information.

Table 10.3. presents a list of cultivars with relative susceptibilities for two different pathotypes of Pa 3 ranging from 2 to 44% with average yield losses and probabilities of more than 10% yield loss.

A farmer can decide to use this information in different ways. Assume he wants to grow potatoes in a fixed rotation, for instance once in two years. He chooses which risk of yield reduction he is prepared to accept, taking into account the amount of money he could save by applying no other control measure (for instance the use of a nematicide), and chooses a cultivar with the required or better relative susceptibility that promises the largest net return because of other cultural characteristics. Another possibility is to determine in which crop rotation his favourite potato cultivar can be grown with the largest net return with or without additional control.

An example: Let's investigate whether a cultivar with a relative susceptibility of 18% yields a better net return in a 1:2 crop rotation than in a 1:3 crop rotation over a period of six years. In both cases no nematicides will be used. It is assumed that the net return of potatoes is 47% and that of a certain, extra, non-host crop is 27% of the gross return of potatoes (Kwantitatieve informatie 1990-1991, IKC-agv & PAV). According to Table 10.2. the potato crop suffers an average yield reduction of 8.1% in a 1:2 rotation and of 2.5% in a 1:3 rotation. The only difference between the two rotations is that one potato crop in the 1:2 rotation is exchanged for the extra non-host crop. Therefore, the

other three non-host crops can be disregarded in the calculation. Then the average net return in the remaining three years will be 3 • (47% - 8.1%)/3 = 38.9% using a 1:2 rotation and (2 • (47% - 2.5%) + 27%)/3 = 38.7% using a 1:3 rotation. So, there is still a slight advantage in growing potatoes once in two years with a cultivar with 18% relative susceptibility. However, at larger susceptibilities the balance will tip in favour of a 1:3 crop rotation.

10.8. Conclusion

By combining eqs. (1) and (2) and using the frequency distributions of the relevant parameters, probabilities of different relative yield reductions can be calculated. As input for eq. (1) sampling results can be used provided that these estimates of population density give a good approximation of the real density within the sampled area. When highly partially-resistant potato cultivars are grown in the proper rotation, sampling data become obsolete, as at the maximum population density M no such increase of yield can be obtained, that can balance the cost of sampling and control. The calculated frequency distribution of a and M for *G. pallida* and the rate of decrease of population density in the absence of potatoes are now verified in field experiments by the IPO-DLO on 20 farmers' fields during several years.

As the tolerance limit T is 2 eggs/g soil for most combinations of potato variety and nematode pathotype, the two important variables for equation (2) are $P = Pf$ of the previous potato crop (the output from eq. (1)) times c (crop rotation factor) and m. When using a value of 0.4 for m in the Netherlands, reliable predictions of yield loss can be made at economically interesting population densities. With the results of these calculations the farmer can evaluate the risks associated with the cropping of potato cultivars with a known relative susceptibility in a certain cropping frequency and choose combinations with the greatest probability of a maximum financial return based on net returns from potatoes and alternative crops in his fields.

Sensitivity analysis demonstrates that the accurate estimation of relative susceptibility of a cultivar is more important than an equally precise estimation of a and M (eqn. (1)). Emphasis should be put on stabilizing experimental error when screening potatoes for partial resistance. Presently, the CPRO-DLO is testing more than 40 cultivars for their relative susceptibility against a number of populations of *G. pallida*, pathotype Pa 3, ranging in virulence from moderate to high. Field tests with some of these cultivars are being performed by the Applied Research for Arable Farming and Field Production of Vegetables (PAV).

The use of an advisory system as described above requires a mental reorientation by farmers, who still are inclined to aim at attaining maximum yields and, as a consequence, tend to opt for maximum security as actual yield losses are considered to be unpredictable. Nematicide treatments are, therefore, seen as a necessary insurance. However, in the Netherlands the use of nematicides is, at present limited to once in four years by statutory measures. Moreover, the frequent application of nematicides causes

adaptation of the microflora in soils resulting in accelerated breakdown of the fumigant (Smelt *et al*, 1989*a*, 1989*c*). Therefore, chemical control cannot be used any further as an 'insurance' against losses by nematode attack in the areas producing potatoes for industrial processing, where a 1:2 cropping frequency is prevalent.

Farmers should strive to optimize returns instead of yields, not only to decrease the use of nematicides, but also to make more profit. An advisory system would provide the necessary information to apply a more profitable method of control, but whether it will be used depends on acceptance of the advice by the potato growers. The primary impulse to use the information generated by this advisory system will be the need to prevent yield reductions in those cropping years when potatoes are grown but nematicide application is not allowed.

Chapter 11

The Seinhorst Research Program

C.H. Schomaker & T.H. Been

11.1. Summary - We postulate a 'Seinhorst Research Program', derived from Seinhorst's empirical philosophy. All theories of the Seinhorst research program are developed by searching for recurring regularities (patterns) in a collection of observations, named 'the empirical base'. To prevent "*ghost theories from sloppy data*" all assumptions underlying the empirical base are carefully described in theories with respect to methodology and technology, including statistics. The patterns to be recognized are summarized by mathematical equations, which must be connected with biological processes to bridge the gap between 'normal' language and mathematical language for the description of biological theories. Often, the patterns result from more than one biological process. If so, the basic patterns are disentangled from one another using a method of pattern analysis. The procedure is best carried out when only a limited number of more or less congruent patterns are involved. Therefore, attention must be given to the choice of the hierarchic level and the complexity of the investigated system. Investigations proceed from simple experimental systems to complex natural systems at a hierarchic level that is neither so high that manifesting processes are very dissimilar nor so low that one runs the risk of describing processes irrelevant for the purpose of the investigation. In the 'Seinhorst Research Program' this purpose is finding methods for improvement of financial returns of host crops attacked by plant-parasitic nematodes through calculating risks of nematode population development and subsequent yield reduction. Pattern analysis yields theories about causes of phenomena observed at the investigated hierarchic level and about properties of processes at the nearest lower hierarchic level. Predictions at the next higher hierarchic level are made by synthesizing several patterns in (stochastic) simulation models. Synthesis is also applied to compound patterns of processes in simple experimental systems, with the objective to explain complicated patterns in complex systems.

11.2. Introduction

In this paper we try to describe Seinhorst's empirical philosophy in some detail. He has never put his ideas about this subject into writing, probably because he considered them to be part of a classic philosophy developed and sufficiently described by others. To some extent this may be true but, first, not all interpretations of the classical empirical philosophy are equally satisfactory (Koyré, 1997) and, second, comment of fellow-nematologists on his work suggest that the nature of this philosophy and the way Seinhorst interpreted it in every-day nematological practice might not be quite clear to everybody.

Seinhorst's personal interests (natural sciences, philosophy, modelling) as well as his ideas on what should be the true purpose of science played an important role in his

work. These ideas began to take shape in his 'underground period' during the last year of the Second World War, when he was 26 years old and had ample opportunity for reflection and studies in philosophy, natural sciences, theology, and linguistics.
In his personal diary of this period, he formulated his opinions on the ultimate purpose of the (natural) sciences: *"Not extended factual knowledge, which can be such a nuisance in ambitious schoolmasters, but the deeper understanding, the possibility of a view over an unknown landscape must be the purpose of all work. Therefore careful examination of the work of the great scientists is also an instigating part of the study"* (Personal diary Seinhorst, March 10, 1945; translated from Dutch by the authors). On the role of philosophy in natural sciences he wrote: *"I am more interested in natural sciences than in philosophy. But without philosophy understanding is impossible. To me natural sciences and all that consists of separate observations, including art, is a passion. Philosophy is a duty, a necessity and an ambition"* (Personal diary Seinhorst, March 12th, 1945).
These considerations, combined with a set of special conditions at the beginning of his career, were to become the backbone of his work.

11.3. Conditions

Apart from his personal interests, the research themes and the empirical philosophy which form part and parcel of the 'Seinhorst Research Program' are also logical consequences of a number of circumstances and conditions at the time of the foundation of this program in the early fifties. Many of these conditions are still valid today. They were described by Seinhorst (1996):

1. PURPOSE OF RESEARCH - MISSION OF THE IPO
   At the time of its foundation in 1949 the mission of the Research Institute for Plant Protection (IPO-DLO), commonly known as IPO, was formulated as "*Finding methods, by means of scientific research on pests and diseases in crops, to improve the economic returns of these crops*". Seinhorst, responsible for the nematological research at IPO, interpreted the IPO mission for his discipline as follows: providing information to weight the costs of control against its benefits and find the optimal balance in individual cases.

2. THE TENDER AGE OF NEMATOLOGY AS A QUANTITATIVE NATURAL SCIENCE
   When Seinhorst began his research at IPO almost all scientific tools for accomplishing the IPO mission were lacking. This was even the case for stem, potato cyst and beet cyst nematodes, that is, for species that were generally considered as harmful ones. There were no quantitative methods for measuring yield reduction by nematode populations in crops. Although the existence of a negative correlation between nematode density at the time of planting of a crop and its expected yield was generally accepted, no mathematical function was available to describe this correlation accurately. Yield reductions by nematodes rotation or soil

Glossary

Accuracy	The closeness of a sample estimate to its true value.
Analysis	*"..consists in making experiments and observations and in drawing general conclusions from them by induction, and admitting of no objections against conclusions, but such as are taken from experiments and other certain truths. For hypothesis are not to be regarded in experimental philosophy."* Analysis enables us to *"proceed from effects to their causes"* (Newton in Querie 31 of the Opticks, as interpreted by Cohen, 1995).
Anomaly	A manifest phenomenon in a system that is not explained by the theory with respect to that system.
Deduction	Inference - only by logical rules - of hypotheses or new theories from fundamental theories.
Deterministic model	Model in which parameters are considered to be true constants.
Empirical base	Collection of observations that are free from theories, except methodological theories.
Empirical cycle	Reconstruction of working methods in observations and theory building in science.
Empirical philosophy	Philosophy with respect to scientific working methods, especially those with respect to observations and theory building.
Falsification	Elimination of theories or parts of theories that are contradicted by recurring patterns in the empirical base.
Hierarchic levels	Order in the organisation of a system from low (molecule) to high (ecosystem).
Hypothesis	General statement about causes attributed to phenomena, insufficiently supported by a model.
Induction	Way of reasoning that derives general causes of phenomena from recurring regularities in an empirical base.
Model	Empirical base, pattern, theory and a set of rules to connect them mutually.
Parameter	Biologically relevant quantity in an equation that determines its outcome over a certain range of values of the independent variable.
Pattern	Regularity, recurring in the empirical base about natural phenomena manifest in a certain system, from which causes are induced by mathematical analysis and predictions about future phenomena in that same system are deduced. Mathematical analogue of a theory.
Philosophy	In this paper philosophy is used as described by Wittgenstein (1918) in his Tractatus 4.112. *"The purpose of philosophy is the logic clarification of thoughts. Philosophy is not a science but an occupation.* *A philosophical work consists basically of elucidations.* *The results of philosophy do not consist of 'philosophical propositions' but of clarification of propositions. Philosophy should clarify and demarcate thoughts that otherwise would be troubled and vague."* (This choice of definition does not imply that in our opinion there is no justification for philosophy as a science. S & B)
Precision	The repeatability or variability of a sample estimate.
Process	Spatial and/or temporal changes in phenomena.
Reductionism	The concept that phenomena at any hierarchic level can be explained by studying the phenomena at the lowest level.
Research program	Lakatos (1978): A complex of logical coherent theories named after its founder and used to explain natural phenomena. The theories consist of methodological and fundamental theories and new theories. Falsification of theories in case of anomalies is done only if it brings scientific progress. Apart from the theories, the program also includes: • An empirical philosophy • Methodology (methods and instruments) • Directions for further development of the program. New theories must be logical consistent with the fundamental theories that represent the core of the research program.

Glossary continued

Stochastic model	Model in which parameters are considered to vary under influence of changing known and unknown environmental factors
Synthesis	or 'composition' "*..consists in assuming the causes discovered and established as principles, and by them explaining the phenomena proceeding from them, and proving the explanations.*" (Newton in query 31 of the Optics, as interpreted by Cohen 1995).
System	Specific surroundings in which observations are made. These surroundings can vary from simple (experimental systems) to complex (natural systems)
Theory	General pronouncements (in 'normal' language) about causes of phenomena in a certain system, sufficiently supported by a model, from which future phenomena in that same system are predicted. Natural analogue of a pattern

fumigation and by growing resistant varieties, but the true effects of these control measures and the causes to these effects were largely unknown. The lack of knowledge of quantitative relations between nematodes and plants was closely connected to an almost complete lack of reliable methods for quantifying numbers of nematodes in soil samples and in plant parts. Although cysts could be extracted from the soil with Fenwick's (1940) can, no reliable methods were available to estimate numbers of eggs within cysts. Free-living nematodes were separated from soil by Baermann's (1917) funnel which is only suitable for small soil samples, or by Cobb's (1918) sieving and decanting method, both methods of unknown efficiency and accuracy (Seinhorst, 1988).

3. PREJUDICES AND FANTASIES

The lack of quantitative knowledge on plant/nematode relations gave ample room for phantasies about causes of yield reduction by nematodes and about nematode control. Even today the situation has not much improved because of the reluctance of most nematologists to involve in quantitative research.

Reductionism

In The Netherlands, this situation was mainly due to the fact that quantitative nematology was not included in the education of students. Internationally, there is a shift of nematological research to low hierarchic levels (*e.g.*, molecular level) and a tendency to interpret low level results as causes of phenomena at a higher level. The same tendency has been observed in other natural sciences. In physics, it was called "reductionism" by Lagendijk (1989). Reductionism assumes that all biological processes follow the same laws and that all phenomena can be explained by studying only the building stones at a low hierarchic level. True reductionists consider that the translation of genetic or molecular information to processes in space and time, resulting in an adult organism, is superfluous and they think that theories on nematode/plant interactions and organisation patterns that exist within and between the intermediate hierarchic levels from low (molecular) to high

(farmer's field) are irrelevant. However, new concepts become manifest at higher hierarchic levels. These concepts must be consistent with those at the lower hierarchic levels but they cannot be deduced from them (Kooijman, 1987; Lagendijk, 1989). "*More is different*" (Anderson *et al.*, 1988). Just as the question of whether or not quarks are locked-in is irrelevant to a brain surgeon (Lagendijk, 1989), the point of protein-based similarity dendrograms for pathogens is irrelevant to breeders and farmers.

Yield reduction

Seinhorst (1986*b*) rated most causes attributed to growth reduction by nematodes in the literature as "*myths and fairytales*". He mentioned obstruction of plant vessels causing wilting (Oostenbrink, 1950), withdrawal of nutrients, mechanical damage to root tissue resulting in a hampered uptake of water and minerals, and decreased shoot-root ratio causing insufficient mineral uptake (Trudgill *et al.*, 1975*a,b,c*; Evans *et al.*, 1975; Trudgill, 1980; Trudgill & Cotes, 1983).

Control measures

Believe and ideals on the ultimate solution for nematode problems range from frequent applications of both fumigant and non-fumigant nematicides (Mulder, 1979; Mulder *et al.*, 1979) - which were wrongly supposed to have favourable cost/benefit ratios -, to the use of late maturing potato cultivars (Trudgill *et al.*, 1990; Haverkort *et al.*, 1992) - which were wrongly presumed to be more tolerant than early maturing cultivars - and to a balanced bio-diverse (agro)-ecosystem - which was supposed to suppress harmful organisms (Sikora, 1992). The true nature - structural or functional - of such an equilibrium, or homeostasis, in which many biologists tend to believe, is still controversial. As 'normal' ecosystems are characterized by large structural fluctuations there seems to be more reason to believe in a functional (with respect to food chains) homeostasis (Odum & Biever, 1984) than in a structural one (with respect to numbers of species) (Rosenzweig & McArthur, 1963). However, the hypothesis of homeostasis is basically disputable because reliable quantitative methods, based on identification and distribution patterns of all relevant organisms, to describe 'bio-diversity' or 'equilibrated' ecosystems are conspicuous by their absence.

11.4. Research themes

Because of these conditions, which to some extent still persist (especially for *Meloidogyne*, *Pratylenchus* and *Thrichodorus* species), the 'Seinhorst Research Program' on plant parasitic nematodes consists of the following four themes (Seinhorst, 1996).

1. METHODOLOGY

Methods for the estimation of numbers of the various nematode species in plant and soil samples and other experimental methods, with known accuracy, both for research and extension purposes, including:

- Methods for extraction of nematodes from plant and soil samples with known efficiency.
- Identification methods, including fixation techniques and microscopy.
- Nematode distribution patterns at different scales, varying from centimetres to several metres in farmers' fields.
- Methods to identify and quantify sources of variance.

2. YIELD REDUCTION

General relation between nematode densities at the time of planting and the relative yield (yield of plants with nematodes as a proportion of the yield in absence of nematodes, all other conditions being identical).

3. POPULATION DYNAMICS

General relation between nematode densities in soil or plant samples at successive observation dates during the vegetation period of the host plants (for instance at planting and at any time afterwards, such as after ripening of the plants). This relation must take into account the degree of plant growth reduction by the nematodes.

4. CONTROL MEASURES

Relations between control measures (nematicides, biological control, resistant cultivars, crop rotation) and nematode population dynamics of nematodes and crop growth.

A proper integration of control measures and farmers' practices requires integration of all relations in an operational model, which implies that they must be available as mathematical equations. The model must predict, within adequate and specified limits, the consequences of control measures against nematodes in an individual field. These control measures must be taken, at the latest, at the time of planting of the host crop to be protected, but generally much earlier. Such a requirement makes inclusion in the model of most external conditions during crop growth useless. Therefore, deterministic models with their high dependance on external conditions can at best predict average effects in large areas. They are unfit to predict nematological phenomena and their consequences on the individual farmer's field. As a consequence, Seinhorst chose to develop stochastic equations with few parameters, the distribution functions of the latter to be estimated by detailed research.

11.5. Empirical philosophy

To exclude as far as possible preconceived ideas and biassed conclusions on causes to nematological phenomena as much as possible, both from the literature and from sloppy empirical methods, and to make all nematological equations and theories consistent, including those influencing the empirical base, such as methodology, technology, chemistry, mathematics and statistics, Seinhorst applied the Newtonian empirical philosophy, excellently described by Cohen & Westfall (1995), in a consistent manner. According to this philosophy, complex natural (here nematological) situations are reduced to mathematical simplicity by studying the properties of a mathematical analogue. The methods of analysis and synthesis are applied in the order described by Newton, the former always preceding the latter, to be sure that relevant principles are assumed. "*Analysis proceeds from effects to their causes and from particular causes to more general ones, guided by mathematical properties of recurring regularities (patterns). Synthesis consists in explaining phenomena from their discovered causes, which are then regarded as principles, thus confirming the explanations*" (Newton interpreted by Cohen (1995). The philosophy further includes correspondence rules to link the results of mathematical analysis to nematological theories in 'normal' language. These rules are:

I. The mathematical analogue should describe biological processes.
II. The variables and parameters in the mathematical analogue should have clear nematological interpretations.

We shall use the empirical cycle as a model to clarify each and every step during investigation in the 'Seinhorst research program' and to reveal all external theories used. In some respects the present empirical cycle differs from those described by others, for instance by Zadoks (1978) or by Campbell & Madden (1990). The reasons why will be explained.

To avoid confusion, some terms used in this paper, such as hypothesis, theory, model, accuracy, precision etc. that do not have an unequivocal meaning for all scientists, are explained in a Glossary.

11.6. Empirical cycle

The empirical cycle (Figure 11.1.) is divided into four empirical sub-cycles, covering the following subjects:

1. Methodological models.
2. Fundamental models.
3. Compound models.
4. Models of causes.

At the beginning of a new investigation, when hardly any quantitative information is available, the sequence indicated above is followed, the development of

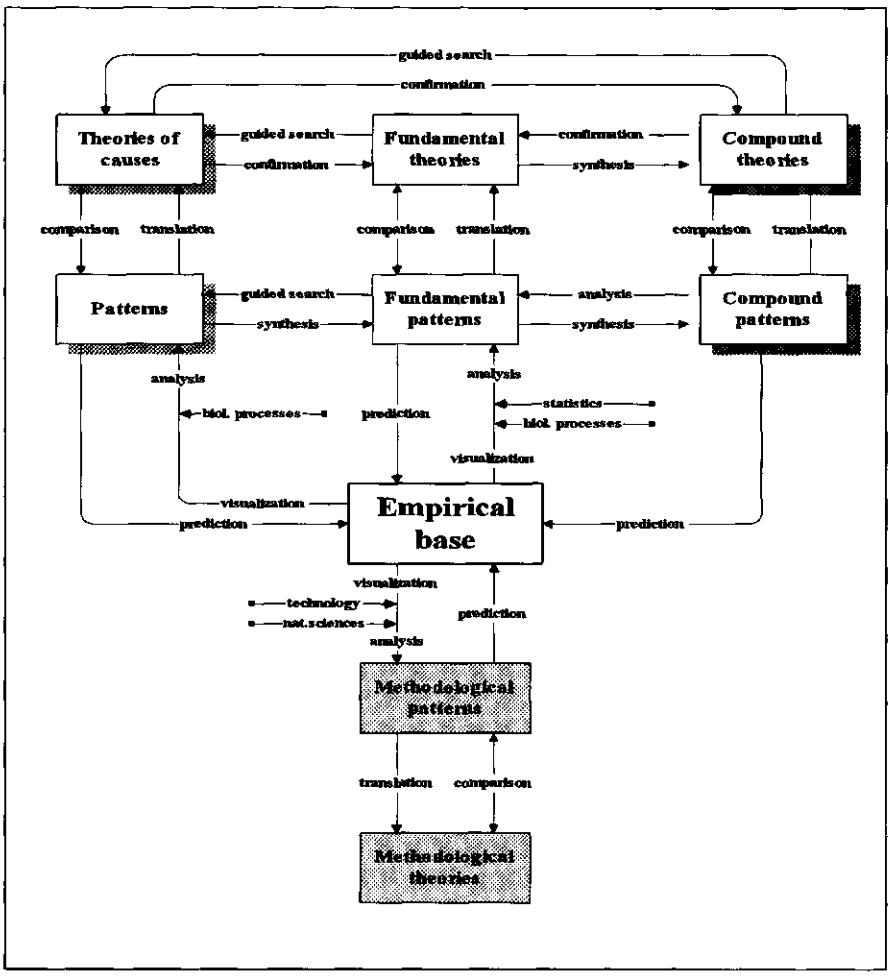


Figure 11.1. - A model of the empirical cycle of the 'Seinhorst research program', divided in four empirical sub-cycles. Patterns and theories are placed in different box types for each sub-cycle, as indicated below.

Methodological models

Fundamental nematological models

Compound nematological models

Models of causes

Empirical base — All sub-cycles originate from and return to the empirical base.

■→ Imported external information (statistics, biological processes etc.)

→ Processes leading from one (intermediate) result to another (analysis, synthesis etc.)

methodological theories with respect to nematological observations preceding that of fundamental nematological theories and theories deduced from these fundamental theories. Later on, the theories in the cycles are considered as jig-saw puzzles. Whenever a piece becomes available, often because of research questions on agricultural problems, sometimes by coincidence, it is fitted in. When a pattern appears in the puzzle, it can be used in three ways, first to improve calculations on risks of unwanted phenomena in farmers' fields, second to discover the causes (at a lower hierarchic level) to these phenomena, guided by the newly discovered properties of these causes, and third to develop new ways of control (prevention or counteraction) based on these causes, for instance by manipulating plant properties in biotechnology programs.

New nematological theories can only be derived from fundamental nematological theories and their mathematical analogues. In all sub-cycles, the following steps are taken from observation to theory and back.

1. EMPIRICAL BASE (OBSERVATIONS)

Assumptions

All empirical knowledge goes ultimately back to an empirical base, with records of details of experiments and observations. As this base serves as an impartial arbiter in accepting or rejecting theories, it should be independent of these theories. The requirement of independence also applies to the observation language. Thus, replacement of one theory by another would be of no consequence for the observation terminology or the truth of the basic conclusions (Koningsveld, 1976). It may be feasible to make observations not loaded with nematological theories, but it is impossible to perform theory-free observations when theories from other disciplines are needed to do any observations at all. Examples are physical or statistical theories needed if microscopy or counting problems play a role in the observations and in the basic conclusions drawn from them. Generally speaking, all methods and measuring instruments used to obtain the facts on which theories are to be based contain their own theories and assumptions that will influence the conclusions from observations if these theories should be changed (Popper, 1968). To handle these biases as carefully as possible, all necessary 'external' theories are formulated in a separate methodological empirical sub-cycle (Figure 11.1.) and are carefully checked for their consistency with fundamental nematological theories during the progress of the research process. Observations from the literature, either on methodology or nematology, are handled in the same way. Nematological observations are added to the empirical base only when the theories underlying the methods are recognized as fundamental in their own disciplines and are consistent with the fundamental nematological theories. If not, the observations cannot be used in the research program discussed here.

The requirement of 'theory free' observations does not mean that nematological theories cannot be used to decide which observations are relevant and which are not (Koningsveld, 1976). For instance, when nematode/plant relations are studied, one

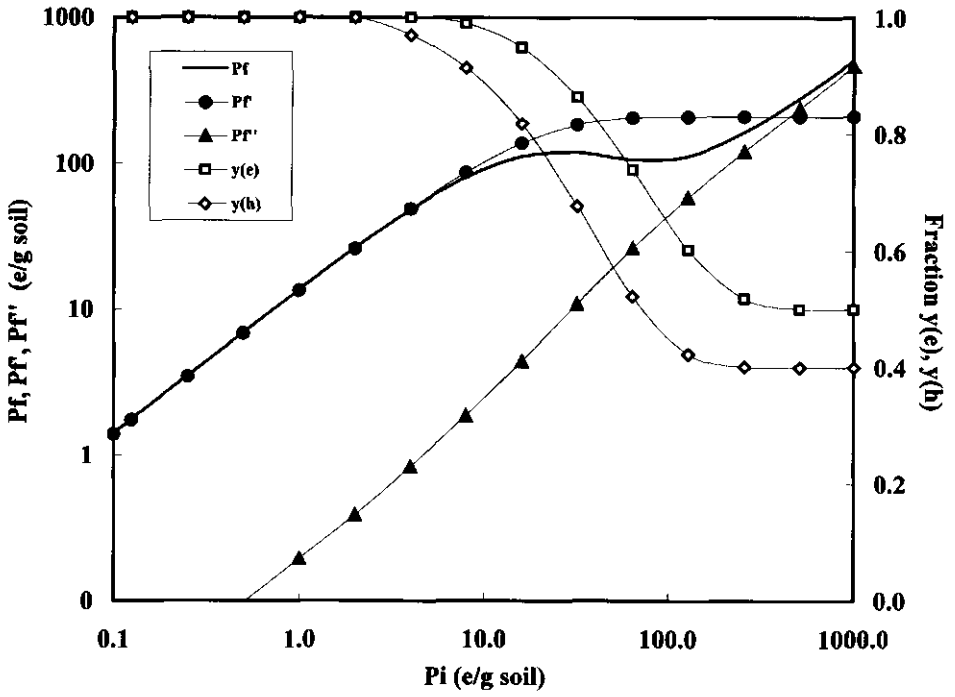


Figure 11.2. - The compound model Pf, represented by the heavy line, for population dynamics of tylenchid nematodes with one generation per growing season. It consists of four non-linear equations. Three of them, ●Pf', ◊y_h and □y_e , are multiplicative and one, ▲Pf'', is additive. ◊y_h and □y_e represent fractions; ●Pf' and ▲Pf'' eggs per gram of soil (e/g).

Biological processes

In the 'Seinhorst Research Program' patterns are condensed into one or more mathematical equations describing biological processes. The equation parameters must have clear biological meanings so that theory building (transfer of mathematical properties to nematological theories formulated in normal language and vice versa) is possible. The number of parameters in these equations is as small as possible but sufficient to explain the effects of the full range of the independent variable under investigation. *"As a rule, no more causes of effects are admitted than is sufficient for their explanation and the same causes are assigned, as far as possible, to the same effects"* (Newton interpreted by Cohen, 1995).

To recognize and describe patterns in the empirical base under investigation, one must be familiar with the mathematical patterns belonging to the biological processes discovered up to now and with all underlying assumptions. Pattern analysis opens the possibility to trace biological processes that have not yet been described.

Example 4. Growth reduction

The mathematical analogue of Seinhorst's theory on growth of plants affected by small and medium nematode densities (Seinhorst, 1986*b*; Schomaker *et al.*, 1995) shows that:

$r_p/r_0 = t_0/t_p$ for $Y_0 = Y_p$ (2)

$= k + (1-k).z^{P-T}$ for $P > T$ (3)

$= 1$ for $P \leq T$ and

$= k$ for $P \rightarrow \infty$

$z^T = 0.95$ (4)

As a consequence

$y = m + (1-m).0.95^{P/T-1}$ for $P > T$ (5)

$= 1$ for $P \leq T$ and

$= m$ for $P \rightarrow \infty$

The parameters *m, k, T,* r_p, r_p and *z* have a clear biological meaning.

Y_0	g	weight of whole plants or parts of plants without nematodes.
Yp	g	weight of whole plants or parts of plants at nematode density P
y	-	relative plant weight Y_p/Y_0
t_0; t_p	day	time needed for plants respectively without and with nematodes to reach the same weight Y.
r_p/r_0	-	relative growth rate
m	-	minimum relative plant weight.
k	-	minimum relative growth rate.
T	e/g	largest nematode density not affecting the relative growth rate and relative plant weight
r_p	g/day	growth rate of plants at nematode density P
r_0	g/day	growth rate of plants without nematodes
z	-	the degree to which plants can prevent growth and weight reduction by nematodes.

Equation (3) describes the relevant nematological process at play when nematodes compete for effect on plant growth.

Statistics

In the 'Seinhorst Research Program' statistical theories are developed with the same caution as nematological theories, but nematological theories always come first, before statistical theories. Statistical theories are hardly ever used during pattern analysis as they usually demand drastic additional assumptions that later-on, when more data are available, are often found to be conflicting with the observations. Moreover, information from the usual regression analysis, such as the amount of variance explained or the confidence intervals of parameters, are irrelevant for the explanation of biological patterns.

The aims for pattern analysis in the 'Seinhorst Research Program' do not comply with the common practise of many biologists and nematologists who only try to find

Example 7. Dose-response relations.

Compound response curves of nematodes to doses of nematicides (Schomaker & Been, 1998) are shown in Figure 5.2.B. They indicate two stimulating and one reducing effect at different ranges of doses of a nematicide, the reducing effect and at least one of the stimulating effects being independent, meaning that they act on different nematode receptors.

In Example 4, the mathematical analogue of Seinhorst's theory on growth reduction of plants attacked by small and medium densities of nematodes is summarized. The theory is more fully described by Seinhorst (1986*b*) and Schomaker *et al.* (1995). It can be transcribed in 'normal' language because the correspondence rules required in the 'Seinhorst Research Program' are obeyed.

3. COMPOUND PATTERNS

Often, a pattern cannot be explained by a single process but only by a combination of two or more processes. In such a case, analysis of the pattern makes it possible to split it into separate patterns, each belonging to separate biological processes. Then, patterns are regrouped by synthesis and compared with patterns from new observations. The choice of the hierarchical level at which observations are made is crucial for the success of this procedure of alternate analysis and synthesis. It should not be too high, because manifest but overlapping processes would become too numerous and too different from each other to be seperated. Nor should it be too low, as then one is at risk to describe biological processes that are irrelevant for the purpose of the investigation. The analysis and synthesis of compound comprehensive models on population dynamics, hatching processes and dose-response relations into separate processes is illustrated in the Examples 5, 6 and 7. Full descriptions of these models are given by Seinhorst (1993), Been & Schomaker (unpub.) and Schomaker & Been (1998).

In the methodological empirical sub-cycle, alternate analysis and synthesis are also applied to compound variance from different sources to make more efficient experimental schemes or practical tests for resistence and tolerance (getting more information from less work) or to choose tests with an optimal cost/uncertainty ratio for extension purposes. In the latter case the financial consequences of the uncertainty in predictions by a test, due to a certain amount of variance, are weighted against the costs of the test to find the optimum.

Examples of the use of compound pattern analysis, to identify and quantify sources of variance, using parametric statistics (not to be confused with ANOVA or Multivariate Analysis) are presented in a posthumous paper by Seinhorst *et al* (unpub.) on tests for partial resistance to potato cultivars for potato cyst nematodes and in Chapter 4 of this thesis, on effects of pesticides on hatching behaviour of potato cyst nematodes.

Example 8. A stochastic simulation model.

A stochastic dynamic simulation model for potato cyst nematodes was compounded from theories about growth reduction and population dynamics in partially resistant and non-host crops to serve as the basis for an advisory system, enabling farmers to choose agricultural scenarios with maximum financial returns (Been *et al.*, 1995). The extended equation on population dynamics contains ten parameters; too many to be useful in an advisory system. For those values of P_i where potatoes can be grown with acceptable yield reductions ($P_i/T < 100$) the relation between P_i and P_f can be simplified to:

$$P_f = \varphi \cdot M' (1 - e^{-\alpha}) \qquad (11)$$

in which

$\alpha = a \cdot P_f / M'$

φ - degree of susceptibility of the potato cultivar for the nematode population

a - the maximum multiplication rate

M' - the hybrid maximum population density ($y_h \cdot y_e \cdot M$, see Example 5) which does not differ much from M under the given constraints ($P_f/T < 100$)

M - the maximum egg density per gram soil

The population dynamics under non-hosts is given by

$$Pf' = P_i \cdot (b_1) \cdot (b_2)^{n-1} \qquad (12)$$

b_1 - fraction of unhatched nematodes during the first year of a non-host crop.

b_2 - fraction of unhatched nematodes during the second and next years of a non-host crop

n - number of years with a non-host crop

The fraction b_1 in the first year of a non-host is smaller than the fraction b_2 in the following years. Schomaker & Been (unpubl.) found on twenty experimental fields an average fraction of 30-40% unhatched nematodes in the first year after a potato crop and one of 65% in the second year.

The value of the maximum multiplication rate, a, varies strongly from year to year and from field to field, while the variation of M is more restricted as M is closely connected with the size of the food source (estimated by dry haulm weight). The large variation of the maximum multiplication rate, a, makes the population dynamics and subsequent yield losses in susceptible potato crops too unpredictable to recommend a fixed rotation, with or without chemical control. Control measures must then be based on nematode numbers in samples using methods with known accuracy and precision. However, the probabilities of densities P_f and their subsequent yield reductions in years following partially resistant potato crops can be predicted from equations (5), (11) and (12) and the probabilities of all possible combination of a and M, based on their distribution functions.

Another possible use of compound patterns (and theories) are simulation models that synthesize several fundamental (simplified) patterns at a given hierarchic level into a comprehensive model generating predictions and new theories about relevant nematological scenarios at the same and at higher hierarchic levels. An instance of a stochastic simulation model for advisory purposes is described in Example 8.

Example 10. Population dynamics.

Equation (6) $P'_f = M(1 - e^{-\alpha})$

The occurrence of discrete, random events in space and/or time, such as the random encounters of nematodes and plant roots, are described by the Poisson distribution. The first term in this distribution function, the likelihood for a plant root to escape nematode attack (zero encounters), is given by e^{α}. The probability of one or more encounters is given by $1- e^{\alpha}$. In its strictest interpretation the presence of the factor $1-e^{\alpha}$ suggests that plant roots can be imagined as a cylindrical surfaces divided into equal compartments, that are per cross section randomly penetrated by juvenile nematodes, the cross sections moving up along the cylinder as time goes on. The juveniles can settle in only one compartment at the same time. If they could partially settle in more than one compartment, this would result in overlapping territories and a decrease in eggs per settled nematode, which is in contradiction with the observed patterns. Only one juvenile per compartment can survive. The size of the compartments depends on the place of the root in the root system and the growing conditions of the plant, but not on the density of the surviving juveniles. Juveniles trying to settle in an engaged compartment will remain unsuccessful and eventually die from starvation. In other words, juveniles that successfully enter a root possess a territory that is inaccessible for others. This mechanism prevents females from decreasing in size because of competition for food and, at high densities or in case of coincidental clustering, remain too small to become adults and reproduce.

Equation (7) $y_e = m_e + (1-m_e)\cdot 0.9^{\gamma}$ for $P_i > T_e$

At higher densities P_i the numbers of eggs per cyst are decreasing, but not because of competition for space between the growing females. The appropriateness and the form of Equation (7) suggest that the quality of the territory degenerates because of random overlapping area's of damaged root tissue, from which these territories are to be developed. (see also Example 8 'growth reduction'). The factor 0.9^{γ} implies that the territories can still reach their maximum quality by using undamaged root cells if they are less than 10% damaged.

Equation (8) $y_h = m_h + (1-m_h)\cdot 0.95^{\gamma}$ for $P_i > T_h$

This equation describes the relative size of the food source, estimated by relative haulm dry weight. Its theory is described under Example 8 'growth reduction'.

Equation (9) $P''_f = b_2\cdot(1-s\cdot y_h)\cdot P_i$

In case of cyst nematodes, the theory must be extended by a term for the number of nematodes per unit soil that did not hatch spontaneously or under influence of the root system. This number of nematodes is determined by:

- The proportion, s, of soil with roots at nematode densities smaller than T_h. The proportion of nematodes stimulated to hatch in the presence of plant roots is proportional to the relative size of the root system.
- The relative size of the root system at nematode density P_i, described by y_h (Equation 8). The nematode density per unit root weight does not change because of this reduction.
- The fraction b of unhatched nematodes.

The correspondence rules in the 'Seinhorst Research Program' make it possible to transfer the conclusions from the mathematical analogue to the corresponding 'natural' analogue in 'normal' language. Here, 'normal' means that the language is understandable and useful for those who are not involved in the 'Seinhorst Research Program'. Therefore, jargon and technical terms are avoided as much as possible. If technical terms are inevitable, they are explained in 'normal' language.

In Examples 9 and 10, Seinhorst's theories on growth reduction of annual crops attacked by nematodes and on population dynamics of nematodes with one generation per year (for instance *Globodera rostochiensis, G. pallida, Heterodera avenae, H. schachtii, Meloidogyne naasi*) are summarized. For more details, the reader is referred to Seinhorst (1986*a*, *b*, 1993, 1998) and Schomaker *et al.* (1995).

Comparison

The correspondence rules in the 'Seinhorst Research Program' make it possible to compare new and current theories. Patterns from simple experimental systems are compared with patterns from more complex systems, and the theories on causes of effects are compared in both systems.

Falsification - These comparisons usually give rise to extensions and/or modifications of the original theory. Not every aberrant pattern leads to changes in the theory. Four conditions must be satisfied before a theory is replaced by another:

1. The aberrant pattern must come from observations based on sound methodological theories described in the methodological empirical cycle.
2. The aberrant pattern must be a recurring one.
3. The aberrant pattern must reveal new processes or clarify ones already known.
4. The new theory connected with the aberrant pattern must be consistent with the fundamental theories in the 'Seinhorst Research Program'.

All conditions for falsification, except perhaps the second condition, agree well with those described by Lakatos (1978). An 'old' theory is only replaced by a 'new' one if it brings scientific progress. The 'new' theory must be able to explain and predict all phenomena that were satisfactorily explained and predicted by the 'old' theory and it must also explain and predict new phenomena.

Confirmation - Comparisons between new and old patterns from observations and the theories connected to these patterns are repeated whenever additions to the empirical base give cause. If the same process is involved, combining a large number of observations enables an improved separation between 'signal' and 'noise' and results in a more complete and clear emergence of the patterns, thus confirming the theories about the causes to the manifesting phenomena. Example 11 illustrates this for the pattern of equation (1): $y' = 0.95^{\alpha}$.

The fundamental theories are also confirmed if new theories from compound fundamental patterns or 'theories of causes' derived from the properties indicated by the 'fundamental theories' in turn successfully predict observations in the empirical base.

Working thus from high hierarchic levels to lower ones, applied, fundamental physiological and molecular biologic research can meet and formulate together

Example 11. Growth reduction.

The existence of a tolerance limit T and its constancy for a given nematode/plant combination under almost all conditions (except short days conditions combined with high light intensity) is often met with doubts or even disbelieve. However, Seinhorst did not start his modelling of yield reductions with the concept of a tolerance limit (the fewer parameters the better) but was forced to add this parameter because it gave a better description of the patterns found. The analysis of 29 nematode/plant combinations in Seinhorst's (1998) paper is bound to end all doubts. The combination of such a large number of observations reduced variance and clarified the pattern to an extent that the existence and constancy of T (z^T =0.95 for all nematode/plant combinations) becomes abundantly clear.

coherent, consistent theories. This approach has the advantage that every new theory at a low hierarchic level that is relevant to the explanatory level can be directly implemented.

Deduction

When theory building has progressed so far that the theory on the explanatory system agrees satisfactorily with the patterns derived from the observations of simple and more complex systems, unobserved effects of nematodes in host plants can be deduced from the general causes in the theory, which now are considered as principles. For instance general relationships of un-investigated or newly discovered nematode species, such as *Meloidogyne chitwoodi* or *M. fallax* can be predicted from relationships known from fifteen experiments with other *Meloidogyne* species and their hosts (Example 12. Growth reduction).

11.7. Future research

In the very near future, the authors, and some of their Dutch colleagues will be involved in a research program (not in the sense meant by Lakatos, 1978), financed by the Dutch government, on population dynamics and distribution patterns of *Pratylenchus penetrans, Meloidogyne chitwoodi* and *Trichodorus* spp. and growth reduction caused by these species in some relevant host plants. An attempt will be made to describe distribution patterns of viruses transmitted by trichodorid nematodes. This approach could later be used with other plant viruses. The part of this program under our responsibility will be done in much the same way as is described in this paper and summarized in Figure 11.1. Methodological theories will be developed before nematological theories, all theories will be derived from mathematical properties of patterns describing biological processes. Analysis will always come first, then alternated with synthesis. We will also apply Seinhorst's empirical philosophy to integrate our knowledge and that of colleagues from the various research stations in The Netherlands into a limited number of consistent theories and hypotheses. Later in the program our attention will turn to the subjects discussed below.

Example 12. Growth reduction.

Seinhorst (1998) proved that the relation between P/T and y', based on his theory about growth reduction by nematodes, closely agrees with $y' = z^{P-T}$ (eq. 1); with $z^T = 0.95$ for all investigated 29 combinations of nematodes and plants. From his theory, growth reduction can be deduced for host plants and tylenchid nematode species other than those investigated, for instance, growth reduction of host plants of the newly discovered nematode species *Meloidogyne chitwoodi* and *M. fallax*.

1. CONNECTION WITH OTHER RESEARCH PROGRAMS

In 'Deterministic Dynamic Simulation Models', applied to potato cyst nematodes in potatoes by several authors (Ward *et al.*, 1985; Schans, 1993; Van Oijen *et al.* 1995*a*, *b* and to *Tylenchorhynchus dubius* in *Lolium perenne* by Den Toom (1989, 1990), the working method is the opposite of Seinhorst's. Synthesis goes before analysis, which undermines and questions the relevance, truth and sufficiency of the assumed principles (biological processes, equations describing them and external conditions influencing them) to explain the nematological phenomena and their consequences in farmers' fields. The situation is exacerbated as these models represent not just one mathematical equation but numerous equations with dozens of parameters. Both the equations and the parameters can be adjusted flexibly to the observations. Consequently, the deterministic simulation models can predict almost any phenomenon, albeit only *after the fact*. Therefore, and because the mathematical equations are often purely descriptive (see also Example 13), the 'Deterministic Dynamic Simulation Models' in their present quality do not contribute much to theory building.

The assumptions leading to the Dynamic Energy Budget (DEB) model, which tries to "*capture the diversity of the energetics of the different species into one model with different parameter values and to build theories for the parameter values*" (Kooijman, 1993), interpreted for potato cyst nematodes by Van Haren *et al.* (1993), proved not to be sufficient and true causes to population dynamics of these nematodes at the explanatory level. The causes are insufficient as they ignore the 'all or nothing' principle in population dynamics of potato cyst nematodes. The causes are non-true causes because they attribute differences in relative susceptibility only to differences in egg numbers produced per female and ascribe decrease in plant weight to withdrawal of food by the nematodes (Seinhorst, 1986*a*, *b*, 1993).

The 'Deterministic Dynamic Simulation Models' as interpreted and applied by various authors (Ward *et al.*, 1985; Den Toom, 1989, 1990; Schans, 1993; Van Oijen *et al.*, 1995*a*, *b*) and the 'Dynamic Energy Budget Model' as interpreted and applied by Van Haren *et al.* (1993) are trying to deduce effects from assumed causes, but these causes are derived from a jumble of loose facts reported in the literature, many of them questionable with respect to their relevance and validity.

Van Oijen *et al.* (1995*a*) carry off the palm as they managed to incorporate only wrong assumptions on plant-nematode relations into the LINTUL crop growth

Example 13. Growth reduction.

The assumptions on plant growth reducing effects of potato cyst nematodes, conveniently chosen by Van Oijen *et al.* (1995*a*) from a multitude of reports in the literature, are:
1. Accelerated leaf senescence
2. Root death
3. Allocation of assimilates in favour of roots
4. Decreased Light Use Efficiency
but all are demonstrably untrue for (economically important) small and medium nematode densities (Been & Schomaker, 1986; Seinhorst, 1986b; Schomaker *et al.*, 1995).
The validity of the model and its predictive ability at the explanatory level (farmer's field) was checked by comparing simulated results with observations in a field experiment (without control) with two cultivars on fumigated and unfumigated plots. The same observations (biassed by questionable methodological methods, especially with respect to the estimation of nematode densities) were used to describe the nematode-plant relations and to estimate the parameters in the model. Aberrant observations in a second field experiment (Van Oijen *et al.*, 1995*b*) were ignored. Differences in growth between the two cultivars could not be explained by the model as it attributes one (linear) mechanism of growth reduction to all nematode densities. Seinhorst's theory on growth reduction by nematodes, confirmed for 29 different nematode/plant combinations in 36 experiments, explain the observations in both experiments well as it discriminates between two mechanism of growth reduction: one operating at small and medium nematode densities (P/T<100) and one at large (P/T>30-100) nematode densities.

model (Spitters & Schapendonk, 1990) and still obtained, in hindsight and thanks to a convenient number of adjustable parameters and - because of their arbitrariness - adjustable mathematical equations describing nematode-plant relations, some resemblance between simulations and observations in one experiment. Their approach is described in Example 13 as an illustration of 'incomprehensive' and incomprehensible modelling.
The drawbacks of the 'Deterministic Dynamic Simulation Models' could be eliminated if these models paid more attention to empirical methodology and if synthesis alternated with pattern analysis of phenomena at the explanatory level to trace and describe processes and causes of relevant processes. Arbitrary equations must be replaced by comprehensive equations describing biological processes. Neither the 'Deterministic Dynamic Simulation Models' nor the 'Dynamic Energy Budget Model' are necessarily incompatible with Seinhorst's models. It is obvious that models inducing causes from their effects, guided by (mathematical) properties of the latter, are useful to models deducing effects from principles and *vice versa*. The two types of models could meet half-way on the hierarchic ladder if the 'believers' in the various models (including the authors of this paper) were able to overcome their inclination to worship the one and only true 'model' and to strive for

more consistency of experimental methods and biological theories between different schools of thought.

2. CAUSES AT LOW HIERARCHIC LEVELS

Patterns and theories in the 'Seinhorst Research Program' indicate properties of nematological processes at lower hierarchic levels. In Example 1 the causes at a lower hierarchic level of the 'first mechanism of growth reduction' at small and medium nematode densities are biochemical or physiological processes and their properties are indicated by the theory that an individual nematode affects plant growth for only a limited period of time. Seinhorst (1998) confirmed that this 'first mechanism' applies to many - if not all - plant-parasitic nematodes and their hosts. To find new, broadly applicable, nematological control strategies, these processes should be investigated under the guidance of their properties derived from the mathematical patterns. Other processes considered suitable for investigation in Seinhorst's theory on growth reduction at small and medium nematode densities are the mechanism that neutralizes or inhibits the effects of small nematode densities and the mechanism inducing differences in plant growth reducing effects between the first and the second generation (Seinhorst 1986*b*, 1995, 1998). The causes to the 'second mechanism of growth reduction', which becomes noticeable at $P/T>100$ (Seinhorst, 1998) and is accompanied by diagnostic characteristics (Seinhorst 1986*b*), and the relation of this mechanism with plant age should be investigated as well.

In population dynamics, much work at molecular and genetic level has already been done, but unfortunately it is not or little (only by establishing a multiplication factor) related to quantitative nematological work at higher hierarchic levels. Therefore, this research cannot be used to make predictions on population dynamics and noxiousness of nematode populations, nor to establish similarities in relevant agricultural properties between nematode populations. For the sake of consistency in theory building the relationship between the research at higher and lower hierarchic levels must be established in the years to come.

3. CAUSES OF DISTRIBUTION PATTERNS

A new simulation model would be that of distribution patterns of sedentary nematodes, based on distribution patterns of potato cyst nematodes. The small and medium scale distribution patterns of potato cyst nematodes are extensively described (Seinhorst, 1988; Been & Schomaker, 1996; Schomaker & Been, unpubl.). The small scale distribution of these and other nematodes is well described by the Negative Binomial Distribution (Seinhorst, 1988). For potato cyst nematodes the dispersing forces causing small and medium scale patterns proved to be constant and independent of time, place (external conditions) and population density (Schomaker & Been, unpub.). As the mobility of potato cyst nematodes is only a few centimetres per year - which is negligible - their distribution patterns depend on their population dynamics and on the activities of farmers, who disperse cysts within

and between fields and horizontally and vertically through the soil with their machinery - including soil fumigation equipment. It would be relatively simple to use vector analysis of the dispersal forces to explain the distribution patterns of these nematodes. More complex distribution patterns of other nematodes could then be deduced by means of simulation models of the vectors using sub-models of the population dynamics and mobility of these nematodes. The relevant external conditions for mobility should not be suggested by preconceived ideas, *e.g.*, on effects of organic matter or biodiversity, but by mathematical properties of distributions patterns of nematodes under natural conditions, following the empirical cycle (Figure 11.1.) in a consequent manner. If the presence or absence of micro-organisms and their metabolic products are critical for nematode mobility then the distribution patterns of these micro-organisms must be studied too, for instance by means of DNA extraction from soils (Van Elsas *et al.*, 1997), and related to that of the nematode species under study and to (small and medium scale) geographic patterns.

4. GROWTH REDUCTION BY ROOT NEMATODES IN PERENNIAL PLANTS

During the first year after planting, effects of nematode attack on perennial plants can be investigated in the same way as that on annual plants. We cannot yet answer the question whether a reduction in growth and productivity should be expected in the second and following years, depending on nematode densities at planting, especially small densities. It is known from studies on *Radopholus similis* in citrus that nematodes spread from older to new roots at the periphery, thus rapidly increasing in numbers. The same tendency to rapidly increase in numbers and to migrate is observed for stem nematodes in red clover and lucerne - here via moist surfaces of plant leaves. We know that nematodes do not generally cause specific disease symptoms (see Examples 1, 2, 4, 9, 11 and 12 on growth reduction) and that annuals are more tolerant to second and later generations of nematodes than to a first generation present at planting (Seinhorst, 1995). Therefore, it is by no means certain that the presence of large nematode numbers in orchards with old trees will cause substantial reductions in productivity. An increase in productivity, in some but not all cases, after treatment with non-fumigant nematicides is not proof of nematode damage if the effect of these chemicals on the yield of trees without nematodes under the given conditions is not known.

To investigate growth reduction of perennial plants by increasing nematode populations, patterns of weight of the total plant and its fruits must be studied at sufficient wide ranges of nematode densities and at regular time intervals. To produce a clear pattern, a simple system with external conditions as constant as possible must be studied first. Later, more complex systems can be studied and their patterns compared with the patterns from the simple system.

5. FUNGI AND INSECTS

Extension of the Research Program with research on infestation focus development

and distribution patterns of fungi and insects living in the soil is worth consideration. The model for foci developed by Van den Bosch (1990) has similarities with our model. It contains a gamma density function for the relative number of biological entities produced per time unit and an exponential function for the spatial distribution. For nematodes the spatial distribution function is the same as in the model of Van den Bosch (1990). The parameters of the function must be estimated for each separate species. For nematodes, we are planning (see Future Research) to deduce comprehensive temporal model from spreading forces and the population dynamics of nematodes. We might do the same for other organisms.

6. POTATO CYST NEMATODES

Of all plant parasitic nematodes, the methodological and nematological theories in the 'Seinhorst Research Program' are the most advanced for potato cyst nematodes. The reason is that potato cyst nematodes are economically important and relative simple research objects because they have only one generation per year and they are easily manageable. Seinhorst (1998) has demonstrated that many causes to phenomena at the explanatory level, especially those with respect to growth reduction, apply to all nematodes. Therefore, we want to choose potato cyst nematodes and their hosts as model organisms to induce theories on the causes at low hierarchic levels, causes of distribution patterns, and molecular aspects of population dynamics. From these theories and supplementary observations similar theories can be deduced for other nematodes.

11.8. Final considerations

Seinhorst and the authors of this paper worked together in close collaboration for almost thirteen years. To us, his pupils, Seinhorst's approach to science is a sensible one, adopted naturally. We cannot imagine working in a different manner. During these years of co-operation, the research program was developed further and our influence gradually increased. At the present stage, it is difficult to discriminate between Seinhorst's and our own contribution in the development of the research program during the last decade. Either contribution is reflected in both Seinhorst's and our publications, but we always discussed and criticized each others' work and we wrote some papers together. Our contribution consists mainly in the development of computer programs for analyses and in the synthesis or composition part of the program. This synthesis resulted in simulation models for both distribution patterns of nematodes in farmers' fields (from which sampling methods can be developed) and expert systems for potato cyst nematodes (from which control scenarios can be chosen). Much time and energy were invested in the implementation of scientific results in agricultural practice, *e.g.*, Seinhorst's concept of partial resistance of potato cultivars to pathotypes of *Globodera pallida,* and in the introduction of new sampling methods for the detection of small foci in fields of ware and seed potatoes. This made

it possible to reduce nematicide use and improve financial returns.

During the period of our cooperation with Seinhorst, we had many discussions on methods, theories and philosophy, not because we disagreed but because a relative small Research Program is 'subject to offence' (Lakatos, 1978) and must develop strategies for its defence. We feel we succeeded in establishing and defending the 'Seinhorst Research Program' so that, in our opinion, it has a fair position and good prospects in comparison with other Research Programs. If the foregoing paragraphs gave the impression that we reject all concepts on which other Research Programs are based, then this image wants nuance. We mainly object against the way these concepts were interpreted and applied for nematode/plant relations, but we know from experience that any Research Program can be put in an unfavourable light if amateurs tamper with its models (Anon., 1991). Therefore, we will not hesitate to adopt approaches or theories from other Research Programs if patterns from observations should guide us in that direction.

Many foreign colleagues co-operated with Seinhorst for long periods. Without suggesting to be complete, we want to mention here our Scandinavian colleagues who appointed Seinhorst as an Honorary Doctor at the Agricultural University of Uppsala and our Italian colleagues from the 'Instituto di Nematologia Agraria' in Bari, where Seinhorst was a consultant for many years, who made a large contribution to the confirmation of Seinhorst's theories (Seinhorst, 1998). They all greatly influenced the course the 'Seinhorst Research Program' has taken in the past, and we hope that they and other colleagues will continue to do so in the future.

Chapter 12

Conclusions

12.1. Introduction

The results presented in this thesis are part of a research effort to control potato cyst nematodes in the seed, ware and starch potato areas of The Netherlands. Although this thesis contains 10 papers it hardly can be considered as presenting a comprehensive overview of the elements required and actually investigated to complete an integrated pest management system for potato cyst nematodes. Instead, we decided to present a sample of different parts of the research program which were not or incompletely published before. The chapters represent samples of successive steps of the research philosophy, described in Chapter 11. Consequently, this thesis contains papers about methodology, models concerning chemical control, soil sampling for detection of potato cyst nematodes, and plant growth affected by nematodes and an example of the synthesis of models; the advisory system for starch potato areas.

12.2. Fumigants

Although the investigations concerning soil fumigants did not result in dosage response relations which could easily be applied to concentration time (*CT*) products measured in the field, and therefore did not lead to a quick method to access the efficiency of soil fumigation, several interesting results were obtained. The research into the efficiency of 1,3-dichloropropene (Chapter 6) provided evidence that only the (Z)-isomer of 1,3-dichloropropene was lethal to potato cyst nematodes. The (E)-isomer merely inhibited hatching for a period less than six months, about the period between soil fumigation in autumn and planting of the potato crop in spring. These findings prompted further research on the effect of both isomers under field conditions, resulting in a confirmation of the results described in Chapter 6. The producers of the fumigant were induced to develop new nematicides like 'Nematrap' and 'Teleone-cis' which consist mainly of the (Z)-isomer. When the Dutch government initiated the Multi-Year Crop Protection Plan to reduce the use of pesticides in The Netherlands the applications of the old formulations of 1,3-dichloropropene had to be terminated as soon as possible, at the latest on January the first, 1995 (Anonymous, 1991). As these new fumigants are applied at dosages of 75 l/ha, a reduction of 50% of 1,3-dichloropropene use could be obtained. As the volume of 1,3-dichloropropene applied in agriculture still amounted to approximately 38% of the total volume of fumigants used when the Multi-Year Crop rotation plan was instigated in 1991, a reduction by 19% of the total volume of fumigants could be achieved.
As hardly anyone ever used an intermediate period of six month between application of a nematicide and testing the potato cyst nematode population on survivability (the period required for the effect of the (E)-isomer to wear out) or anticipated the prolonged hatching process of exposed nematodes (Chapter 4), once described by Fenwick (1957), all information in the literature on the effect of 1,3-dichloropropene

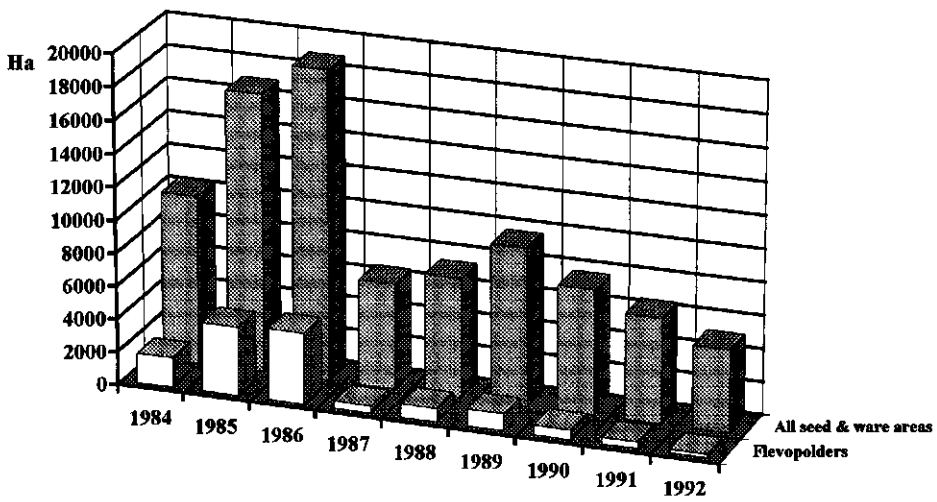


Figure 12.1. - The area (ha) fumigated in the Flevopolders (white bars, front) and in the total seed and ware potato growing area (grey bars, back) during the years 1984 to 1992. In 1987, the disappointing results concerning the efficiency of soil fumigation on marine clay soils were published which resulted in a prompt decline of preventive soil fumigation. Then, application increased coinciding with the increasing number of actual infestations detected. Starting in 1989 the prototype sampling methods combined with a preliminary advisory system became available. Abolition of statutory soil fumigation for farmers growing susceptible potatoes in a 1:3 rotation became possible when growers used the new sampling method.

can be considered to overestimate mortality of potato cyst nematodes after soil fumigation.

The research concerning the effect of 1,3-dichloropropene (Chapter 6; unpublished data) in field tests provided ample evidence that potato cyst nematode control on heavy marine clay soils, where seed and ware potatoes are grown in The Netherlands, is insufficient. Mortalities ranged from 50 to 60% throughout the upper 30 cm of the tilth. In most cases this was caused by an instantaneous breakdown of the active ingredient by microorganisms.

In fact, soil fumigation on these soils can be regarded as the primary factor leading to new infestations as proper cleaning of the fumigation equipment is extremely difficult and time consuming and some parts of the machinery cannot be reached at all. In commercial situations no such cleaning efforts will be made. As with most other agricultural machinery (Hofmeester, 1991), the adhering soil is constantly being refreshed in the course of the work. Soil fumigation equipment will therefore be one of the major contributors to the dispersal of potato cyst nematodes between fields.

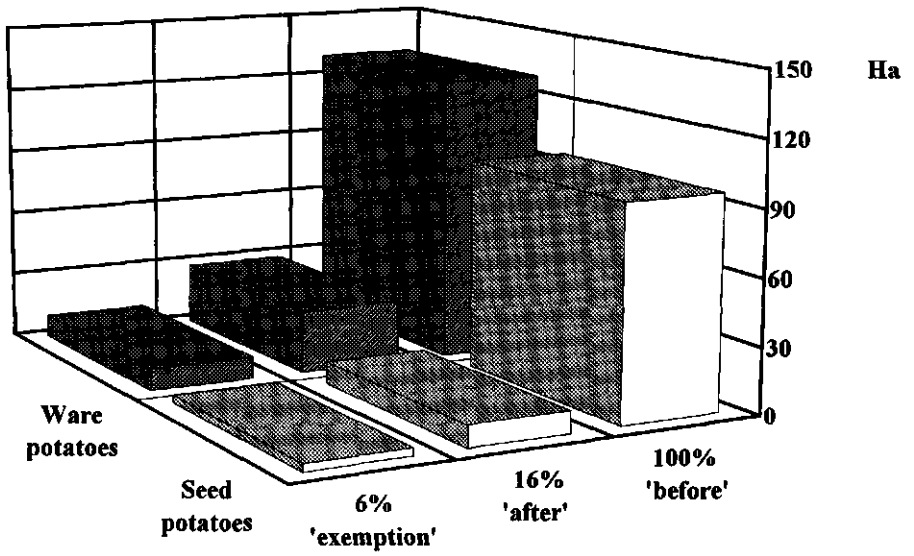


Figure 12.2. Results of a demo project in 1989 executed by the Groene Vlieg Ltd./Ministry of Housing, Physical planning and the Environment. The graph displays the number of ha planned to be fumigated by the participants of the project 'before', the number of ha actually fumigated after they received their sampling results 'after' and the number of ha which would have been fumigated if exemption of statuary soil fumigation was possible.

Publication of these results caused a major decrease in the use of nematicides in these areas (Figure 12.1) and prompted the need for improved detection methods and the application of partially resistant potato cultivars as an alternative for soil fumigation.

12.3. Sampling

The research on new sampling methods for the detection of potato cyst nematodes (Chapters 7 and 8) resulted in a prototype detection method for small infestation foci with a central population density of 50 cysts/kg of soil to be detected with an average probability of 90%. Using this detection method foci with a central population density of 150 cysts/kg soil have an almost 100% probability of being detected. If no cysts are found, but a small infestation is present, the probability of visible damage in the following potato crop is nil and the detection probability by statutory soil sampling methods almost zero. If cysts are found the size of the focus can be estimated using the spatial distribution of the cysts obtained from the seven samples/ha (Been *et al*, 1996). In 1989, a demonstration project was carried out by the Groene Vlieg Ltd, Nieuwe Tonge, a Dutch private company, with financial aid of the Ministry of Housing,

Physical planning and the Environment (VROM). About 200 hectares were sampled using the prototype method. Farmers were asked before and after they received the results of the sampling method (which was combined with an ELISA test for species identification) about their intentions with respect to soil fumigation. A 84% reduction of soil fumigation could be achieved by the participants. The reduction was a result of establishing the presence/absence of potato cyst nematodes, thereby avoiding preventive soil fumigation, the possibility to choose the proper resistant potato cultivar as the species per individual infestation was determined and the reduction of the area that had to be treated to the area of the actual infestation including bufferzones. If exemption of statuary soil fumigation for farmers who grow susceptible potato varieties in a 1:3 rotation had been possible this reduction could have amounted to 94% (NOVEM, 1995). In 1990, in reaction to these results, government regulation concerning the statuary soil fumigation was changed and farmers applying the new sampling method could gain exemption (Figure 12.2). In 1990, 82% of all detected infestations were *G. rostochiensis* against which resistant potato cultivars are available. As the new infestations are detected when they were still small, resistant potato varieties could be cropped without yield loss. They caused an 80% reduction of population densities throughout the complete root zone down to 1.2 m thereby greatly surpassing the effect of a nematicide treatment. In the case of a *G. pallida* infestation, against which no completely resistant cultivars were available, the use of partially resistant cultivars was recommended. In 1991 the profit made by the farmers using the prototype sampling system to obtain exemption was estimated to be 1.400.000 guilders; in 1992 twice as much. In that year approximately 13.000 ha were sampled using the new detection method. As from 1993, the statutory soil fumigation was cancelled by the Dutch government and application of the new sampling method stabilized at approximately 10.000 ha annually.

However, since 1989, research on detection methods has continued and new methods have been developed based on an extended data set (Chapter 8). Different methods for ware and seed potato growers have been developed, the former differentiating in the size of the focus to be detected with the crop rotation frequency applied. As a result of the insight acquired, the new methods require less soil and will be cheaper than the prototype sampling method introduced in 1989. The methods were handed over to the Dutch Regional Inspection Services for Agricultural Seeds and Seed Potatoes (NAK) and offered to the farmers in 1998. The vertical distribution of potato cyst nematodes throughout the tilth proved to be quite uniform. Therefore, differences in sampling depth will not have a large effect on the reliability of sampling results.

The new sampling methods generate a cyst count for each 5 or 6 m strip of a field. A typical sampling result therefore consists of several successive strips where cysts are detected. The number of infested strips (1), the number of cysts/strip (2) and the number of cysts per focus (3) are indices for the probable size of the infestation. By using the bivariate distribution of both the length and width gradient of the focus

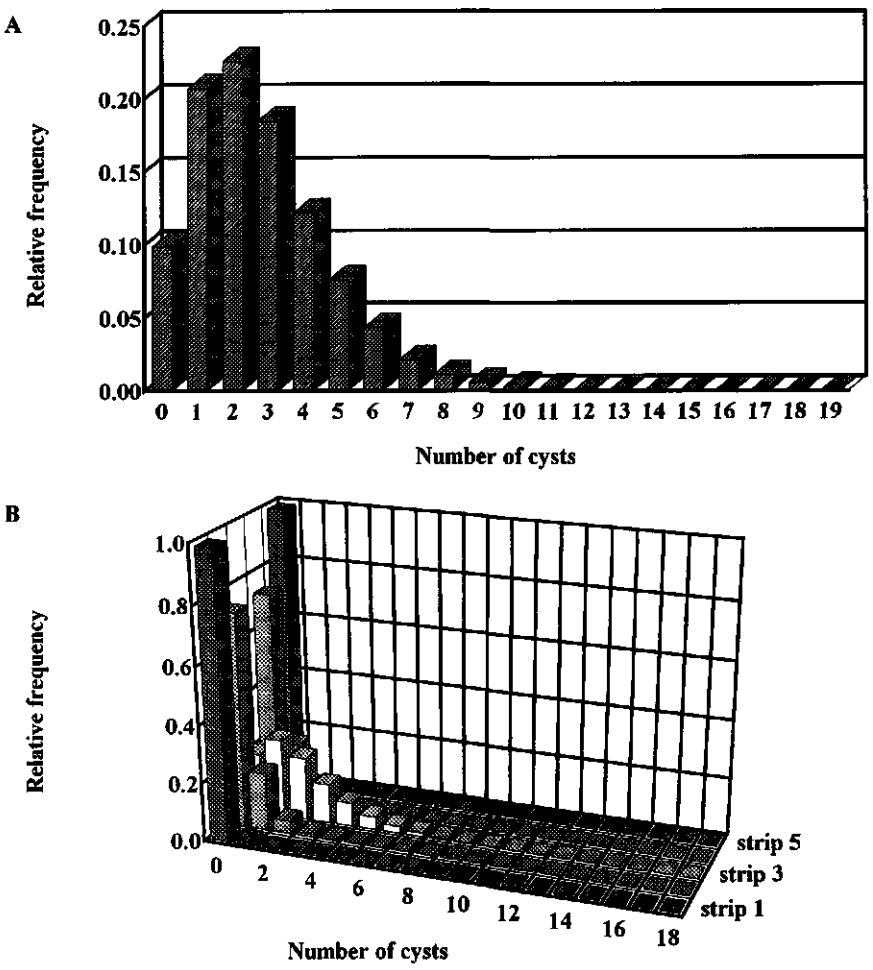


Figure 12.3. A: Probabilities of finding a certain total number of cysts in soil samples originating from a focus with a central population density of 50 cysts per kg soil and sampled with the prototype detection method (strip = 5 m wide and 300 m long). B: Probabilities of finding a certain number of cysts in soil samples originating from the sampled strips containing same focus as above using the prototype detection method.

model (Chapter 7) the range of possible foci sizes can be narrowed considerably. For the remaining foci these indices can be related to focus size.

For indica 1, the number of infested strips, this has already been done (Been *et al.*, 1996) and a preliminary advisory system was introduced in 1992. Only crude

differences in focus size could be made. However, by applying a Monte Carlo approach, it is possible to calculate estimates of the number of cysts per focus and the number of cysts/strip (indices 2 and 3). In Fig 12.3.A the frequency distribution of the number of cysts detected per focus is depicted while in Fig 12.3.B. cyst counts per infested strip are displayed. By using all three indices more accurate information about the probable size of an infestation focus can be provided and even the presence of more than one focus can be recognized. In combination with Seinhorst's relation between pre-plant densities of potato cyst nematodes and yield reduction, the information can be provided needed for decision making. As expected crop losses can be predicted, cost/benefit analysis can be made for all possible control measures - growing cultivars of (partially) resistant potatoes, crop rotation and application of nematicides. In this way, maximum profit for the farmers can be combined with minimum input of nematicides. In fact, calculations indicate that controlling infestation foci of potato cyst nematodes by nematicides is almost always a waste of money compared to the use of (partially) resistant potato cultivars.
The new sampling methods would also provide the Dutch government with the tools required to apply fumigants only by prescription as it was one of the objectives in the Multi-Year Crop Protection Plan to reduce the use of pesticides in The Netherlands (Anonymus, 1991).

12.4. Partially resistant potato cultivars

Research by Phillips (1984), Seinhorst (1984) and Seinhorst & Oostrom (1984) showed that potato cultivars resistant to *G. pallida* pathotype 2 (Pa 2, biotype D) have also some resistance to Pa 3 (biotype E). Several cultivars were tested at the IPO-DLO and this association proved to be a general one. The degree of partial resistance can range from low to very high. Figure 10.3. displays the concept visually by comparing the population dynamics of a susceptible and a partially resistant potato variety. The parameters a (maximum multiplication rate at low initial nematode density) and M (maximum population density per unit of soil) are lower for the resistant cultivar. For the ware potato cultivar Santé the relative susceptibility is 30%. This means that, whatever the actual multiplication rate in a certain field may be on a susceptible cultivar, it will be only 30% of that when the partially resistant cultivar Santé is grown. The maximum population density (M), the maximum number of larvae produced per g of soil, will be reduced by the same percentage and stay at that level even if the cultivar is grown annually. Molendijk (1997; pers. com.) demonstrated this concept for the heavy marine clay soils where our prime seed and ware potatoes are grown. By choosing the proper partial resistance and cropping frequency, low population densities can be kept low whereas high ones will decrease. This result can be achieved without any significant crop losses in those areas where infestations occur in foci. Only in the centre of the focus, in a limited area, population densities will reach magnitudes where

crop yield may be reduced. In the seed and ware potato areas partially resistant potato cultivars can be used to keep *G. pallida* infestations in check - keeping them small and under the detection level of the statutory soil sampling methods - until breeders have had the time to breed new and more resistant potato cultivars. In cropping areas where the presence of potato cyst nematodes is tolerated, e.g. the starch potato areas, these varieties can decrease the need for fumigation significantly (Chapter 10) by keeping nematode densities below the financial threshold for nematicide application.

In the Netherlands, the possibilities offered by the application of partially resistant potato cultivars were quickly recognized. They resulted in a combined effort of the Research Station for Arable Farming and Field Production (PAV), the DLO-Centre for Plant Breeding and Reproduction Research (CPRO-DLO), the H.L. Hilbrands Laboratory for soil-borne pests (HLB) and the DLO-Institute for Plant Protection (IPO-DLO) to introduce this concept into agricultural practice. A test program was set up to screen all economically important cultivars for their degree of partial resistance, and to downscale the research method used at the IPO-DLO into a test method suitable for testing new breeding lines and to perform risk calculations (Chapter 10). The research concerning optimum pot size for breeders' tests is completed; the new methodology was translated into new protocols which will be introduced by the Variety Registration Board in 1999. Since 1994, information on partial resistance of cultivars is published in the Dutch Cultivar List; the 'Rassenlijst'. Farmers can choose the degree of partial resistance suitable to them.

12.5 Advisory systems

In 1992 the IPO-DLO started a large field test (20 fields throughout the starch potato production area; each of 1 ha size) to validate the use of partially resistant potato varieties (Chapter 11). Dependent on the initial population density of the potato cyst nematode - *G. pallida* in all fields - a soil fumigation was carried out to obtain acceptable population densities before planting of tubers. No granulates were used when the partially resistant cultivar Ellis was planted in 1993. In 1995 or 1996, according to the local cropping frequency, Ellis was grown for a second time. No fumigation or granulates were used in that year as population densities were reduced both by the partially resistant cultivar and 1 or 2 non-host crops.
Although Ellis does not have a high partial resistance (approximately 70% for the most virulent Pa 3 pathotype known in the Netherlands), this cultivar was chosen to maintain population densities in the field high enough to be measured accurately. In spite of the low partial resistance, average yields were high: 50 tons per ha and, after correction for the starch content, 60 tons of payable weight. The results were better than on normal fields where nematicide application, fumigant and non-fumigant, continued. In Figure 12.4. the decrease of population densities in all 20 test fields (F01-

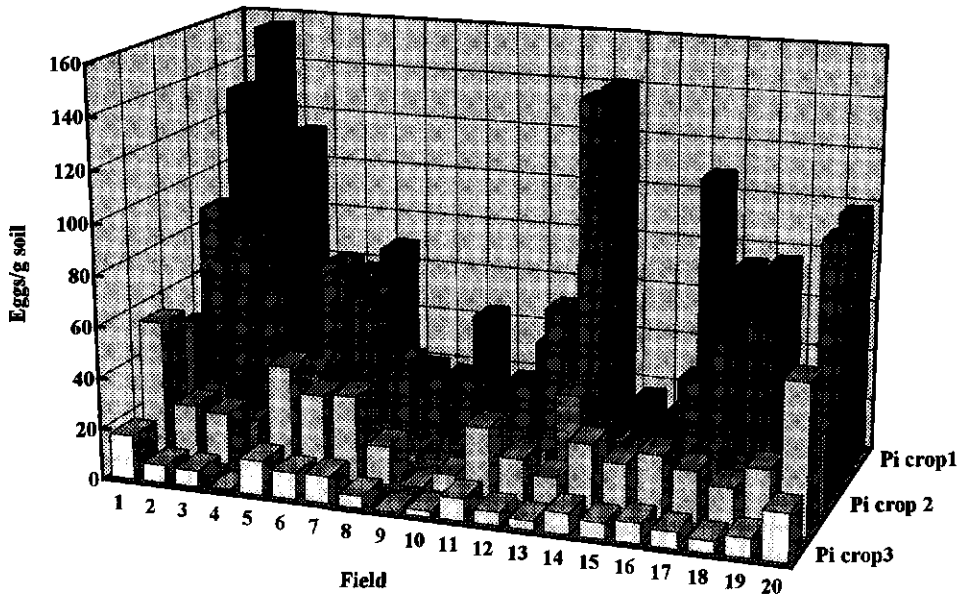


Figure 12.4. - Decline of population densities in 20 fields of 1 ha, in the Dutch starch potato growing area before and after cropping the partially resistant potato cultivar Ellis in 1:3 and 1:4 crop rotations for two subsequent rotations.

F20) are visualized. At the beginning of the field experiment population densities in some of the test fields were extremely high, in fact higher then ever reported after non-host crops. Non-fumigant nematicides, which were applied routinely, are suspected to be the cause, interfering with the normal population dynamics of the nematode. At the start of the third potato crop, densities of the potato cyst nematode, will have decreased in almost all fields to acceptable levels (20 eggs/g soil). At this density the financial loss caused by yield reduction equals the cost of soil fumigation.
One of the exiting new bits of information obtained was that the average decline of the population density the first year following a potato crop is much higher than was believed before. Instead of the expected 35% reduction the average population decline was 68% (based on 60 soil samples of 800 g per ha per field), confirming the results of Andersson (1987, 1989).

These results and developments were solid enough in the early 1990's to envisage a rapid decline of the use of nematicides in the following years (Been & Schomaker, 1992). Undoubtedly, this information stimulated the decision makers designing the Multi-Year Crop Protection Plan to reduce the frequency of soil fumigation from 1 in 2 years to 1 in 4 years in 1993. In the year 2001 fumigation will be restricted to 1 in 5

years. Thanks to the increasing utilization of partially resistant cultivars and, more recently, the development of new potato cultivars with high partial resistance (>95% e.g. Seresta, Sjamiro) no problems with potato cyst nematodes are reported at present although the use of nematicides has been reduced by 85% in 1997 compared to the reference years 1984-1988 (Figure 12.5).

The research presented so far resulted in a rapid reduction of the volume of nematicides used in the Netherlands. In addition, application of the results reduced the dependence of farmers on nematicides. The effect of nematicides was certainly overrated, especially on marine clay soils, and most of the time their use was questionably. Because of the adaptation of micro-organisms to rapidly break down the active compound their usefulness will decline even more. Partially resistant potato cultivars provide a possibility to limit the use of nematicides and granulates on a permanent base and to ensure farmers of higher financial returns. Combined with a soil sampling method, used periodically to detect or measure population densities, Dutch potato growers should be able to manage the potato cyst nematode without major problems. Thus, the dependence on nematicides virtually vanishes.

12.6. Present status

The advisory systems envisaged at the beginning of this research program are not yet completed, although all sub-models are now available, parameterized and even integrated in computer programs for scientific use. The major problem faced at the moment lies in the stunning decrease of nematicide use in The Netherlands which resulted in a stop of all funding of research on potato cyst nematodes in 1995. A simplified prototype advisory system exists for farmers which use the new detection methods in the seed and ware potato areas. Farmers receive advise based on pre-calculated tables of yield damage for partially resistant cultivars and are only advised to apply nematicides when the financial loss of the estimated yield reduction lies above the cost and impact of the nematicide application. In the starch potato areas which still lack soil sampling methods for population density estimation - they are not finished because of lack of funding - partially resistant varieties are now widely grown.

Indeed, the impression could take hold of the non-initiate that the potato cyst nematode problem is disappearing. However, the absence of adequate legislation concerning potato cyst nematodes after abolishment of the old rules in 1993 resulted in a stagnation of the use of the new sampling systems. Seed potato growers (40.000 ha) are still obliged to sample prior to growing potatoes, but the detection probability is low (Chapter 8) when the EPPO prescriptions for statutory sampling are followed. Irresponsible seed potato farmers, having a potato cyst nematode infestation in the field, will have this infestation resampled repeatedly by a third party using the old

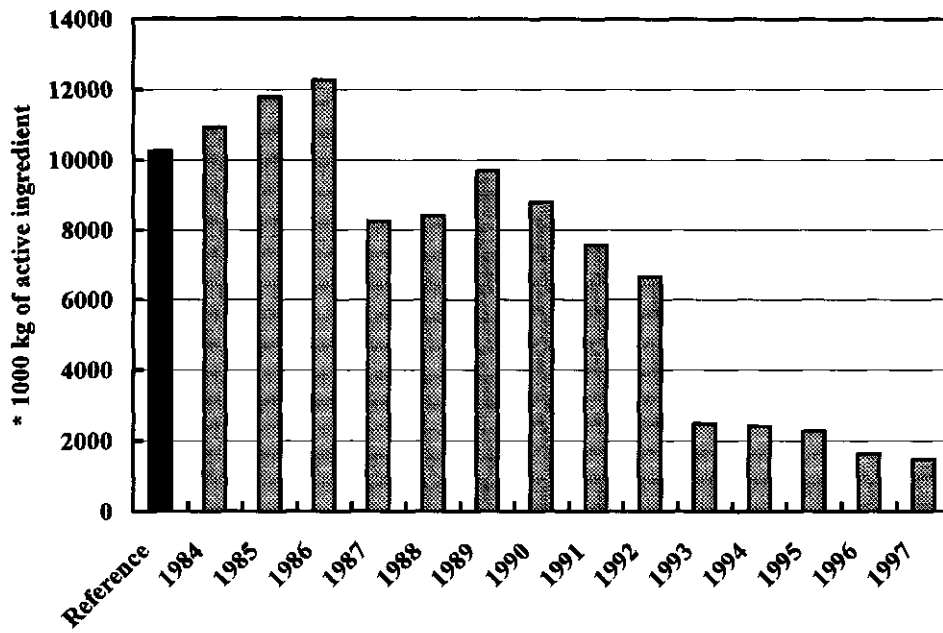


Figure 12.5. - The volume of fumigants used annually in The Netherlands (source Nefyto). Black bar: reference volume (years 1984-1988) used by the Multi-Year Crop Protection Plan for measuring the reduction of fumigant use. In 1995 a 45% reduction was aimed for (77% achieved); in the year 2000 a 68% reduction was aimed for (now, 1998, an 85% reduction has been attained.

statutory (EPPO prescribed) sampling method until a 'no cysts' result appears. With that result they will be permitted to grow potatoes. Ware potato growers (80.000 ha) have no longer an obligation to sample; instead aerial surveillance is carried out to detect infestation foci. Fifteen infestations have been detected in the last four years using this method. The actual number of infestations will grow though they have been declining in the official records during the last years as hardly any new ones are added and the old ones decline. In 1998 all official infestations originating from soil sampling and dating before 1994, the year that aerial surveillance of infestations was introduced, were declared non-existing because of legal reasons. Ware potato growers can become seed potato growers any time.

The sudden termination of research and the non-utilization of research results in an advisory system will certainly cause major problems in the near future.

As research on potato cyst nematodes is 'out of fashion', the main research effort at the moment is directed towards free-living plant parasitic nematodes such as root knot nematodes, *Pratylenchus* spp and trichodoridae. An approach is chosen consistent with

the described research program. Main research items consist of spatial distribution patters (small scale) in an attempt to develop sampling methods for scientific research, population dynamics and damage relations, of course preceded by a thorough investigation on methods, to finally quantify and model nematode-plant interactions and devise proper control schemes.

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Summary

Potatoes are among the most profitable agricultural crops in arable farming in the Netherlands and consequently are grown as frequently as conditions allow. As a result Dutch farmers experienced huge problems with potato cyst nematodes during the last 50 years. In **Chapter 1** an outline is presented of the situation of potato cyst nematode control in the 1980's, the problems, possible solutions and the research initiated, of which a part is presented in this thesis.

In 1984 emphasis was put into research concerning the efficiency of soil fumigation on heavy marine clay soils where the majority of Dutch seed and consumption potatoes are grown. Both laboratory and field experiments were carried out to investigate the efficiency of 1,3 dichloropropene and metam-natrium. This required the processing of thousands of larvae suspensions. One of the most labourious and tedious occupations in nematological research is the counting of individuals of the pathogen. In **Chapter 2** A GOP-302 image analysis system - Context Vision, Sweden - was used to automate the counting of large numbers of larvae-suspensions of *Globodera rostochiensis* and *G. pallida*. These suspensions originated from hatching tests, which were conducted to estimate percentage mortality in field and lab experiments of nematodes exposed to nematicides. The result is called ANECS (Automatic **NE**matode Counting System), a software program that can count up to 64 compartments with larvae suspensions successively without the aid of an operator. A special object carrier was developed. Images of up to eight object carriers (512 larvae suspensions) can be stored and image analysis can be suspended to off-office hours. The time needed to count one compartment was reduced by 80% to one minute compared to 'manual' labour while the time for probe preparation remained the same. The percentile error is highest at very low larvae densities (<20 per suspension) and is caused by pollution with small fibres carried by air during the handling of the larvae suspensions. This problem can be minimised by setting up clean-laboratory procedures. At least 95% of the larvae originating from hatching tests were recognized and counted. The program can and has been be adapted to count other nematode species or to suit more complicated problems like counting both larvae and eggs in one suspension.

In **Chapter 3** errors due to subsampling and laboratory procedures affecting the expected value and variance of cyst counts were investigated. Several fields, infested with potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) were sampled by collecting bulk samples of approximately 70 cores amounting to 1.8 or 2.5 kg soil from a number of square metre plots located in a regular grid pattern over a 0.33 ha

area. Bulk samples from five fields, I-V, were thoroughly mixed and from one field, VI, lightly mixed, and subsequently divided into three subsamples of approximately equal weights. Two, sometimes three, subsamples were elutriated separately. Cysts were elutriated by two commercial laboratories, 1 and 2, and separated from the debris and counted at two research laboratories, 0 and 3. Random bulk samples from five fields, I-V, were divided into three portion after thoroughly mixing and taken to Laboratory 0, to compare elutriation precision and accuracy of commercial and scientific laboratories and to check the quality of mixing. To this purpose, pairs or triples were divided into classes. The expected value of the variance within pairs was estimated per class and could be described by a distribution function analogue to a negative binomial distribution, but with three in stead of two parameters. Cysts appeared to be randomly distributed in the well mixed samples, resulting in a binomial or trinomial distribution between pairs or triples. The expected values of the coefficient of variation associated with elutriation were 3.6, 9.6 and 5.5% in the Laboratories 0, 1 and 2, respectively. The upper 95% confidence limit, $\delta_{0.95}$, of coefficients of variation associated with elutriation in Laboratories 1 and 2, were estimated by the differences in 95% upper limits of coefficients of variation between the Laboratories 1 and 2 on the one hand and Laboratory 0 conversely. This difference, $\delta_{0.95}$, ranged from 73% to 42% for Laboratory 1 and from 43% to 19% for Laboratory 2 if 10 to 100 cysts were counted in samples. The consequences of these laboratory errors for the accuracy of sampling methods for both research and extension purposes are discussed.

Nematicide trials require reliable results concerning the effect caused by the fumigant. The percentage mortality of potato cyst nematodes can be estimated by comparing the hatchability of untreated larvae with that of nematicide treated larvae. In **Chapter 4**, research is described to improve the quality of hatching tests. Hatching tests using potato root diffusate are labourious and yield quite variable results. Sources of variability were identified and analysed, and solutions were presented. A method was developed to conduct hatching tests using inert materials so that the total variation at the end of the test is minimized. A number of hatching tests was carried out to increase reliability, optimize the method and limit the amount of work. Thus, it was possible to obtain a coefficient of variation (*cv*) of the hatching process which is in accordance with the combined errors expected when a certain number of cysts is treated and eggs are used in a hatching test. An Appendix is provided listing the different errors and ways to calculate and cope with them. The results indicate that the hatching process is no longer an important source of variation for the end result. All variation higher than expected could be explained by variation between replications of batches with the same treatment, indicating that small differences in nematicide application cause major differences in the end result. The treatment effect was more important in field experiments than in laboratory experiments.
The hatching curve could be described adequately by a log-logistic curve with 3 parameters (λ final number of hatched larvae, α time, β slope parameter). Addition of

a fourth parameter (γ, incubation time) improved the fit of the hatching curve significantly. Using the log-logistic model, final hatch can be predicted with a certain error before the actual hatching test ended, but in general final hatch is underestimated. When an error of 5% is accepted, the length of time required to perform a hatching test of a laboratory experiment can be reduced by 80% for untreated batches and by 40 to 80% for batches treated with nematicides. Acceptable reduction is negatively correlated with the concentration of the fumigant used.
Hatching tests with cysts originating from field experiments are unsuitable for prediction using a time limited data set. In cyst batches from the field compound hatching curves could be distinguished in 4 out of 6 fields, indicating that the soil samples contained at least two fractions of cysts with different hatching responses. Prediction would cause a significant underestimation of final hatch and consequently an overestimation of mortality.

Because of its high vapour pressure, 1,3-dichloropropene is primarily used on marine clay soils. In **Chapter 5** a laboratory experiment is described investigating the two stereo-isomeres of 1,3-dichloropropene for their efficiency in killing nematodes. Batches of increasing numbers of *Globodera rostochiensis* cysts were exposed to a range of concentrations of the (E)- and (Z)-isomers of 1,3-dichloropropene. The cysts were of identical origin. Temperature during treatment was 10 °C, humidity 100% and time of exposure 8 days. The integrals of concentration time products (*CT*) created were 0, 3, 7, 14, 31, 60, 125, 242, and 437 µg/ml•day for the (E)-isomer and 0, 3, 16, 59, 240, and 419 µg/ml•day for the (Z)-isomer. Survival was estimated with hatching tests 1.5, 3, and 7 months after treatment. The relationship between dosage of (E)-isomer and numbers of hatchable nematodes followed a log-logistic equation at all hatching dates. Hatchability, and therefore lethal dosages, increased as hatching tests were more delayed. Seven months after treatment, practically all treated nematodes had recovered and hatchability of treated and untreated nematodes was the same. A log-logistic relationship was also found for dosage (Z)-isomer and numbers of hatchable nematodes 1.5 month after treatment. When hatching tests of nematodes treated with the (Z)-isomer were delayed till 3 and 7 months after treatment, the results were better explained by a compound model, assuming two independent log-logistic effects, one stimulating hatch at low dosages and one reducing hatch at all dosages. Only the (Z)-isomer of 1,3-dichloropropene was effective as a nematicide.

Chapter 6 presents research concerning the efficiency of standard doses of 1,3 dichloropropene in fields with a high silt content. Three fields of marine clay soil were fumigated with 150 l/ha 1,3-dichloropropene (DD) (Teleone II ™, Shell 95™). On three dates after application, concentrations of Z- and E- 1,3-dichloropropene were measured per 5 cm layer of soil to a depth of 40 cm and integrals of concentration time products were calculated. When the fumigant was no longer detectable, a top soil treatment with either 150 l/ha metam-sodium or 180 kg/ha dazomet (active compound methyl isothiocyanate) was applied, followed immediately by autumn ploughing. Soil

samples were taken before and after fumigation and after the top soil treatment to extract potato cyst nematodes (PCN). Survival was determined by means of hatching tests. Mortalities after the DD treatment, defined as 100 - % survival, were estimated per 5 cm layer of soil to a depth of 30 cm to construct dosage response curves. Fumigation with DD killed 48, 48 and 72% of the PCN per field, respectively. Accelerated breakdown of DD by microorganisms accounted for the two lower mortality rates. The additional top soil treatment with metam-sodium increased mortality to 90% or more. Dazomet, however, was less effective (53 and 80%) considering that twice as much of the active compound was applied as in the metam-sodium treatment. Multiplication of hatched larvae originating from the injection layer after the DD treatment was 25% less than that from untreated plots. This was caused by a lower fraction of larvae developing into cysts. PCN could be retrieved from soil layers as deep as 80 cm below the surface. Fumigation reached only a fraction of the infested soil, down to 25-30 cm. The infestation foci were so small compared to the standard minimum area fumigated (1 ha) that 90% of the active compound would be wasted on non-infested soil. Soil fumigation, whether or not combined with an additional top soil treatment, will seldom be profitable. Monitoring for infestation foci is recommended.

As soil fumigation was not a viable option to keep potato cyst nematodes in check on heavy marine clay soils, another way of control had to be found. Research was focussed at the development of sampling methods for the detection of small infestation foci with high reliability (≥90% probability of detection). Precautionary soil fumigation can be avoided, the area where a control measure has to be applied can be minimized to the actual infestation, and detection occurs so early that (partially) resistant potato cultivars can be grown without significant yield reduction as population densities are still low. Research in the Flevopolders yielded promising results. Therefore, in 1990, a research program was initiated to develop new sampling methods for the detection of patchy infestations of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) with known accuracy in all potato cropping areas of The Netherlands. Patchy infestations in cropping areas of the provinces of Zeeland, Friesland, Groningen and Drente were sampled to validate a model based on data from cropping areas in Flevoland and to determine whether one detection method could meet the requirements of all cropping areas in The Netherlands. The results are presented in **Chapter 7**. Eighty two fields were presampled to locate patchy infestations using a coarse sampling grid (8 · 3 m). Parts of thirty seven fields, containing one or more foci, were sampled intensively by extracting at least 1.5 kg of soil per square metre (1.33 · 0.75 m). Forty foci were analysed for spatial distribution characteristics of cysts using Generalized Linear Models (GLM's) and classical Multiple Linear Regression Analysis, differing in assumptions about the distribution of the input variable (number of cysts per kg of soil). The results showed that the data from all investigated cropping areas fit well to an exponential model with two parameters, the length and width gradient parameters. Significant differences in these parameter values between cropping areas could not be demonstrated. As both parameters follow

a normal distribution, the probability of any combination of these parameters can be described by a bivariate normal distribution. Gradient parameters were correlated but significant correlations between these parameters and certain variables, such as the nematode species involved (*G. pallida* or *G. rostochiensis*), the time interval between sampling and the last potato crop, soil type, cropping frequency and cyst density in the focus centre could not be demonstrated. It can be concluded that one detection method for small infestation foci suffices for all investigated cropping areas. Its expected accuracy is independent of soil type, potato cyst nematode species, cropping frequency or time interval between sampling and last potato crop.

In **Chapter 8** the model for infestation foci developed in the previous chapter was applied for practical usage. A computer program called SAMPLE was developed to evaluate existing and create new sampling methods for the detection of patchy infestations or 'foci' of the potato cyst nematode (*Globodera* spp.). By combining a model for the medium scale distribution of cysts, which provides the expected population densities at each position within the focus, and a model for the small scale distribution within square metres (negative binomial distribution) SAMPLE allows to simulate sampling procedures. The importance of the parameters of the two distribution models - the length and width gradient parameters for the medium scale distribution and the aggregation factor k of the negative binomial distribution for the small scale distribution - was investigated by sensitivity analyses. The aggregation factor k proved to be less important when calculating the average detection probability of a focus than the length and width gradient parameters. Several existing versions of the statutory sampling method used in The Netherlands were tested for their performance on a standard infestation focus with a central population density of 50 cysts/kg soil. The standard focus is small enough to use resistant potato varieties as a control measure without noticeable yield reductions in a 1:3 potato crop rotation. As the statutory soil sampling methods did not perform with the desired average detection probability, set at 90%, the program was used to develop several new sampling methods for focus detection and to investigate their performance. SAMPLE is a tool to develop sampling methods on demand for every possible combination of characteristics required for use by seed and ware potato growers (recommendations for optimum control measures leading to maximum returns, Integrated Pest Management) and by governments (legislation, quarantine and export protection).

For advisory purposes a model is required describing the relation between the number of potato cyst nematodes and tuber yield. A stochastic model with biologically relevant parameters was available. In **Chapter 9** the direct relation between the number of potato cyst nematodes and plant growth is described and used to deduce the relation between nematode density and yield reduction of total plant weight and tuber yield. The relation between small and medium initial population densities and the relative total plant weight was derived as cross sections at right angles to the time axis of a growth model with three dimensions: time after planting t, relative total plant

weight Y and relative growth rate r_p/r_0. The relative growth rate is the (constant) ratio between the growth rate r_p of plants of a certain weight at a nematode density P and the growth rate r_0 of (younger) plants of the same weight without nematodes. Therefore, the ratio between the time after planting that plants need to reach a certain weight in the absence of nematodes and at nematode density P, t_0/t_p equals the ratio r_p/r_o (2). The relative growth rate $r_p/r_o = k + (1-k)0.95^{P/T-1}$ for $P > T$ and = 1 for $P \leq T$ (3). Formally, k is the minimum relative growth rate as $P \rightarrow \infty$. As a result the arbitrary equation $y = m + (1-m)0.95^{P/T-1}$ for $P > T$ and = 1 for $P \leq T$ (6) also applies to the relation between small and medium initial population densities and relative total plant weight. T is the tolerance limit, below which growth and yield are not reduced by nematodes; m is the relative minimum yield.

The relations between small and medium initial population densities of potato cyst nematodes and relative tuber weight of potatoes can be derived from the growth model in an analogous way. However, there is one complication: tuber initiation does not start at the same haulm weight in plants with and without nematodes, but at the smaller haulm weight the larger the nematode density. As a consequence, tuber weights of plants with a certain total weight at nematode density P are not equal to those of plants with the same total weight without nematodes, but $r_p\Delta t$ units of weight larger, Δt being the difference between the actual time of tuber initiation and the time total plant weight becomes the same as that of plants without nematodes at the initiation of tuber formation.

Relative total and tuber weights of plants with 'early senescence' and at large nematode densities are smaller than estimated by the model and equation (2). This indicates that at large initial population densities growth reducing mechanism(s) become active that were not operating at smaller densities.

In **Chapter 10** an advisory system is presented for the management of potato cyst nematodes (*Globodera pallida)*. It emphasizes the use of partially resistant potato cultivars, which provide the possibility of keeping population densities of potato cyst nematodes at a low level in short fixed rotations. Using stochastic models based on the population dynamics of potato cyst nematodes and the relation between pre-plant nematode densities and relative yield it is possible to calculate the probabilities of population development and the reductions in yield caused by these population densities. A simulation model is developed which integrates both models, using the frequency distributions of some of the most variable parameters relevant to a particular combination of potato cultivar and nematode population. Also, the natural decline in population density when non-hosts are grown is incorporated in the model. The model makes it possible to calculate the probability of a certain yield reduction, given a certain potato cultivar, nematode population and rotation. Therefore, it becomes feasible for a farmer to evaluate risks and the costs of different control measures in fixed rotations. The application of this model in the starch potato growing areas could lead to significant improvements in financial returns and a major reduction of the use of nematicides.

In **Chapter 11** we describe the 'Seinhorst research program' initiated by Dr J.W. Seinhorst, former head of the Nematology Department of the IPO-DLO. It consists of an empiric philosophy, the scientific methods applied, and the models developed at the IPO-DLO during the last 45 years of nematological research, including the 13 years in which the research described in this thesis was carried out. All theories of the Seinhorst research program are developed by searching for recurring regularities (patterns) in a collection of observations, named 'the empirical base'. To prevent "*ghost theories from sloppy data*" all assumptions underlying the empirical base are carefully described in theories with respect to methodology and technology, including statistics. The patterns to be recognized are summarized by mathematical equations, which must be connected with biological processes to bridge the gap between 'normal' language and mathematical language for the description of biological theories. Often, the patterns result from more than one biological process. If so, the basic patterns are disentangled from one another using a method of pattern analysis. The procedure is best carried out when only a limited number of more or less congruent patterns are involved. Therefore, attention must be given to the choice of the hierarchic level and the complexity of the investigated system. Investigations proceed from simple experimental systems to complex natural systems at a hierarchic level that is neither so high that manifesting processes are very dissimilar nor so low that one runs the risk of describing processes irrelevant for the purpose of the investigation. In the 'Seinhorst Research Program' this purpose is finding methods for improvement of financial returns of host crops attacked by plant-parasitic nematodes through calculating risks of nematode population development and subsequent yield reduction. Pattern analysis yields theories about causes of phenomena observed at the investigated hierarchic level and about properties of processes at the nearest lower hierarchic level. Predictions at the next higher hierarchic level are made by synthesizing several patterns in (stochastic) simulation models. Synthesis is also applied to compound patterns of processes in simple experimental systems, with the objective to explain complicated patterns in complex systems.

In the Conclusions (**Chapter 12**) an overview is presented of the practical results and aspects of the research effort described in this thesis. Some comments are made on the present state of affairs concerning potato cyst nematode control in the Dutch seed- and ware potato growing areas.

Samenvatting

Aardappelen behoren tot de meest winstgevende gewassen in de Nederlandse landbouw en worden daarom zo vaak als mogelijk verbouwd. Als gevolg hiervan ondervonden Nederlandse telers grote problemen met het aardappelcysteaaltje gedurende de laatste 50 jaar. In **Hoofdstuk 1** wordt de situatie rond de bestrijding van het aardappelcysteaaltje in de tachtiger jaren geschetst, de problemen, mogelijke oplossingen en het geïnitieerde onderzoek, waarvan in dit proefschrift een gedeelte is opgenomen.

In 1984 lag de nadruk op onderzoek naar de efficiëntie van grondontsmettingsmiddelen op zware mariene kleigronden waar het merendeel van de Nederlandse poot- en consumptieaardappelen worden geteeld. Zowel laboratorium- als veldproeven werden uitgevoerd om de efficiëntie van 1,3-dichloropropeen en metamnatrium te onderzoeken. Dit vereiste de verwerking van duizenden larvesuspensies. Een van de meest tijdrovende en vervelende bezigheden in het nematologische onderzoek is het tellen van individuen van de ziekteverwekker. In **Hoofdstuk 2** wordt een GOP-302 beeldanalyse systeem - Context Vision, Zweden - gebruikt om het tellen van grote hoeveelheden larvesuspensies van *Globodera rostochiensis* en *G. pallida* te automatiseren. Deze suspensies waren afkomstig van lokproeven, die werden uitgevoerd om het percentage doding te bepalen van nematoden die in veld- en laboratoriumproeven waren blootgesteld aan nematiciden . Het resultaat heet ANECS (**A**utomatic **NE**matode **C**ounting **S**ystem), een software programma dat tot 64, met larvesuspensies gevulde, compartimenten achtereenvolgens kan tellen zonder tussenkomst van menselijke hulp. Een speciale objectdrager moest worden ontwikkeld. Beelden van maximaal acht objectdragers (512 larvesuspensies) kunnen worden opgeslagen en de beeldanalyse kan worden uitgesteld tot na kantooruren. De tijd, benodigd om een compartiment te tellen, kon vergeleken met 'handmatig' tellen worden gereduceerd met 80% tot 1 minuut, terwijl de tijd om een suspensie voor te bereiden hetzelfde bleef. De procentuele fout was het hoogst bij zeer lage larven dichtheden (<20 per suspensie) en werd veroorzaakt door verontreiniging met kleine vezels meegedragen door de lucht bij de verwerking van de larvesuspensies. Dit probleem kan worden geminimaliseerd door het opzetten van "clean laboratory procedures". Op zijn minst 95% van de larven afkomstig van lokproeven werden herkend en geteld. Het programma kan worden en is aangepast om andere nematoden soorten te tellen of om meer ingewikkelde problemen aan te kunnen zoals het tellen van zowel larven als eieren in een suspensie.

In **Hoofdstuk 3** worden de fouten, veroorzaakt door subbemonsteren van bulkmonsters en laboratorium-activiteiten, onderzocht die de cysten tellingen en hun variantie beïnvloeden. Verschillende velden besmet met het aardappelcysteaaltje (*Globodera rostochiensis* en *G. pallida*) werden bemonsterd door van een aantal vierkante meter plots, gelegen in een regelmatig patroon over een oppervlak van 0.33 ha, bulkmonsters te verzamelen bestaande uit ongeveer 70 steken van totaal 1.5 of 2.5 kg grond. Bulkmonsters van vijf velden, I-V, werden grondig gemengd en van één veld, VI, licht gemengd en verdeeld in drie submonsters van ongeveer hetzelfde gewicht. Twee, soms drie, submonsters (paren of tripletten) werden apart opgespoeld. Cysten werden opgespoeld door twee commerciële laboratoria, 1 en 2, en uit de debris gescheiden en geteld door twee onderzoekslaboratoria, 0 en 3. Willekeurige bulkmonsters van vijf velden werden, na zorgvuldig mengen, opgesplitst in drie porties en gebracht naar laboratorium 0 om de spoelfout met de commerciële en onderzoekslaboratoria te vergelijken en de kwaliteit van het mengen te controleren. Hiervoor werden paren en tripletten verdeeld in klassen. De verwachtingswaarde van de variantie binnen paren werd per klasse geschat en kon worden beschreven met een verdelingsfunctie analoog aan een negatief binomiale verdeling, maar met drie in plaats van twee parameters. Cysten bleken een toevals-verdeling te hebben in goed gemengde grond wat resulteerde in een bivariate of trivariate verdeling tussen paren en tripletten. De verwachtingswaarden van de variatiecoëfficiënt van het opspoelen waren 3.6, 9.6 en 5.5% voor laboratoria 0, 1 en 2. De bovenste 95% betrouwbaarheidslimiet, $\delta_{0,95}$, voor de variatiecoëfficiënt voor het opspoelen van laboratoria 1 en 2 werd afgeleid uit de verschillen van de variatiecoëfficiënten tussen laboratorium 1 en 2 aan de ene kant en laboratorium 0 aan de andere. Het verschil, $\delta_{0,95}$, reikte van 73% tot 42% voor laboratorium 1 en van 43% tot 19% voor laboratorium 2 als er 10 tot 100 cysten werden geteld in de monsters. De consequenties van deze laboratoriumfouten voor de nauwkeurigheid van bemonsteringsmethoden voor onderzoeks- en voorlichtingsdoeleinden wordt besproken.

Nematicide proeven vereisen betrouwbare resultaten betreffende het effect veroorzaakt door het grondontsmettingsmiddel. Het percentage doding van aardappelcysteaaltjes kan worden bepaald door de lokking van onbehandelde larven te vergelijken met die van met een nematicide behandelde larven. In **Hoofdstuk 4** word onderzoek beschreven dat ten doel heeft de kwaliteit van lokproeven te verbeteren. Lokproeven met gebruik van aardappeldiffusaat zijn tijdrovend en hebben vaak variabele uitkomsten. Bronnen van variabiliteit werden geïdentificeerd en geanalyseerd, en oplossingen werden aangereikt. Een methode voor de uitvoering van lokproeven werd ontwikkeld die gebruik maakt van inerte materialen zodat de totale variatie aan het eind van de proef kon worden geminimaliseerd. Een aantal lokproeven werd uitgevoerd om de betrouwbaarheid te verhogen, de methode te optimaliseren en de hoeveelheid werk te minimaliseren. Op deze manier was het mogelijk voor het lokproces een variatiecoëfficiënt (*cv*) te bereiken die overeenkomt met de gecombineerde fouten

die kunnen worden verwacht wanneer een gegeven aantal cysten wordt behandeld en eieren worden gebruikt in de lokproef. Een appendix somt de verschillende fouten op, met een methode voor hun berekening en beheersing. De resultaten geven aan dat het lokproces niet langer een belangrijke bron van variabiliteit is voor het eindresultaat. Alle variatie hoger dan verwacht kon worden toegeschreven aan verschillen tussen herhalingen van eenzelfde behandeling, wat aanduidt dat kleine verschillen in het aanbrengen van een nematicide grote verschillen in het eindresultaat veroorzaken. Het behandelingseffect was beduidend groter in veldproeven dan in laboratoriumproeven. De lokcurve kon goed worden beschreven met een log-logistische curve met drie parameters (λ het uiteindelijke aantal gelokte larven, α de tijd en β de hoekparameter). De toevoeging van een vierde parameter (γ, de incubatietijd) verbeterde de fit van de lokcurve significant. Met behulp van het log-logistische model kan de uiteindelijke lokking met een bepaalde fout worden voorspelt voordat de lokproef is geëindigd, maar in het algemeen wordt de uiteindelijke lokking onderschat. Als een fout van 5% wordt geaccepteerd, kan de tijd die een lokproef duurt met 80% worden gereduceerd in laboratoriumproeven met onbehandelde cysten en met 40 - 80% voor cysten behandeld met nematiciden. Aanvaardbare reductie is negatief gecorreleerd met de concentratie van het gebruikte grondontsmettingsmiddel. Lokproeven met cysten afkomstig van veldproeven zijn niet geschikt voor voorspelling gebaseerd op een in de tijd gereduceerde data set. In cysten afkomstig uit het veld konden in 4 van de 6 velden samengestelde lokcurven aangetoond worden, wat erop duidt dat deze grondmonsters op zijn minst twee fracties cysten bevatten met een verschillende lokrespons. Voorspelling zou een significante onderschatting van de uiteindelijke lokbaarheid veroorzaken en een overschatting van de doding.

Vanwege zijn hoge dampdruk wordt 1,3-dichloropropeen voornamelijk op mariene kleigronden gebruikt. In **Hoofdstuk 5** wordt een laboratoriumproef beschreven waarin de twee stereoisomeren van 1,3-dichloropropeen worden onderzocht op hun effectiviteit om nematoden te doden. Groepen *Globodera rostochiensis* cysten van toenemende aantallen werden blootgesteld aan een reeks van concentraties van de (E)- en (Z)-isomeer van 1,3-dichloropropeen. De cysten waren van identieke afkomst. De temperatuur tijdens de blootstelling was 10°C, de vochtigheid 100% en de tijd van blootstelling 8 dagen. De integralen van de concentratie tijd producten (*CT*) waren 0, 3, 7, 14, 31, 60, 125, 242, and 437 µg/ml•dag voor de (E)-isomeer en 0, 3, 16, 59, 240, and 419 µg/ml•dag voor de (Z)-isomeer. Overleving werd bepaald met lokproeven 1.5, 3 en 7 maanden na behandeling. De relatie tussen dosering van de (E)-isomeer en de aantallen lokbare larven volgde een log-logistische curve op alle lokdata. Lokbaarheid, en dus ook de dodelijke doseringen, stegen naarmate de lokproeven werden uitgesteld. Zeven maanden na de behandeling waren praktisch alle behandelde nematoden hersteld en was de lokbaarheid van behandelde en onbehandelde nematoden gelijk. Een log-logistische relatie gold ook voor de dosering van de (Z)-isomeer en het aantal lokbare larven 1.5 maanden na de behandeling. Wanneer de lok-

proeven van nematoden behandeld met de (Z)-isomeer werden uitgesteld tot 3 en 7 maanden na behandeling, konden de resultaten beter worden verklaard met een samengesteld model, met veronderstelling van twee onafhankelijke log-logistische effecten waarvan een de lokking stimuleert bij lage doseringen en de andere de lokking reduceert bij alle doseringen. Alleen de (Z)-isomeer van 1,3-dichloropropeen vertoonde een nematicide werking.

Hoofdstuk 6 beschrijft onderzoek naar de effectiviteit van standaard-doseringen van 1,3-dichloropropeen in velden met een hoog afslibbaarheidspercentage. Drie velden op zeeklei werden ontsmet met 150 l/ha 1,3-dichloropropeen (DD) (Teleone II™, Shell 95™). Op drie tijdstippen na de ontsmetting werden concentraties van (Z)- en (E)-1,3-dichloropropeen bepaald per 5 cm laag grond tot een diepte van 40 cm en de integralen van de concentratie tijd producten berekend. Zodra het grondontsmettingsmiddel niet meer aantoonbaar was werd een toplaagbehandeling uitgevoerd met of 150 l/ha metam-natrium of 180 kg/ha dazomet (actieve stof methyl isothiocyanaat) waarna onmiddellijk op wintervoor werd geploegd. Grondmonsters werden genomen voor en na de grondontsmetting en na de toplaagbehandeling om aardappelcysteaaltjes te verzamelen. Overleving werd bepaald met behulp van lokproeven. Mortaliteit na de DD behandeling, gedefinieerd als 100 - % overleving werd bepaald per 5 cm laag grond tot en diepte van 30 cm om dosis-responscurven te construeren. Grondontsmetting met DD doodde 48, 48 en 72% van de aardappelcysteaaltjes in de respectievelijke velden. Versnelde afbraak van DD door micro-organismen verklaarde de twee lage dodingspercentages. De additionele toplaagbehandeling met metam-natrium verhoogde de doding tot 90% en meer. Dazomet echter was minder effectief (53 en 80%) hoewel twee keer zoveel actieve stof werd toegevoegd als bij de metam-natrium behandeling. De vermenigvuldiging van gelokte larven afkomstig van de injectielaag na de DD-behandeling was 25% lager dan die van larven van onbehandelde plots. Dit werd veroorzaakt door een lager aantal larven dat zich tot cysten ontwikkelde. Aardappelcysteaaltjes konden worden aangetroffen in bodemlagen tot 80 cm onder het oppervlak. Grondontsmetting bereikte enkel een fractie van de besmette grond tot 25-30 cm diepte. De besmettingshaarden waren zo klein vergeleken met de standaard minimale oppervlakte die werd ontsmet (1 ha) dat 90% van de actieve stof verspild werd op aaltjesvrije grond. Grondontsmetting, al dan niet gecombineerd met een additionele toplaag behandeling, zal zelden profijtelijk zijn. Het opsporen van besmettingshaarden wordt aanbevolen.

Daar grondontsmetting geen zinvolle oplossing is om het aardappelcysteaaltje op zware mariene kleigronden in bedwang te houden ontstond de noodzaak een andere bestrijdingswijze te vinden. Het onderzoek richtte zich op de ontwikkeling van bemonsteringsmethoden voor de detectie van kleine besmettingshaarden met een hoge betrouwbaarheid (> 90% waarschijnlijkheid van detectie). Preventieve grondontsmetting kan worden voorkomen, het oppervlak waarop een teeltmaatregel moet

worden toegepast kan worden beperkt tot de echte besmetting en detectie vind zo vroeg plaats dat (partieel) resistente aardappelrassen kunnen worden verbouwd zonder significante opbrengstderving, daar de populatiedichtheden nog laag zijn. Onderzoek in de Flevopolders leverde veelbelovende resultaten op. Daarom werd in 1990 een onderzoeksprogramma opgezet om bemonsteringsmethoden met bekende betrouwbaarheid te ontwikkelen voor pleksgewijze besmettingen van aardappelcysteaaltjes (*Globodera rostochiensis* en *G pallida*) voor alle teeltgebieden in Nederland. Pleksgewijze besmettingen in de provincies Zeeland, Friesland, Groningen en Drente werden bemonsterd om het model gebaseerd op de gegevens uit de teeltgebieden in de Flevopolders te valideren en te bepalen of één bemonsteringsmethode voor detectie kon voldoen aan de eisen van alle teeltgebieden in Nederland. De resultaten zijn gegeven in **Hoofdstuk 7**. Tweeëntachtig velden werden, gebruik makend van een grof bemonsteringsraster (8 · 3m), voor-bemonsterd om de besmettingshaarden te lokaliseren. Gedeelten van zevenendertig velden, met één of meer besmettingshaarden, werden intensief bemonsterd door op zijn minst 1.5 kg grond per vierkante meter (1.33 · 0.75m) te verzamelen. Van veertig haarden werd de ruimtelijke verdeling van cysten geanalyseerd met behulp van Generalized Linear Models (GLM's) en klassieke Multiple Lineaire Regressie, die onderling verschillen in de aannames betreffende de verdeling van de invoer-variabele (aantal cysten per kg grond). De resultaten toonden aan dat de data van alle onderzochte teeltgebieden goed pasten bij een exponentieel model met twee parameters, de lengte- en breedtegradiënt parameter. Significante verschillen van deze parameters tussen de verschillende teeltgebieden konden niet worden aangetoond. Daar beide parameters een normaalverdeling volgen, kan de waarschijnlijkheid van elke combinatie van deze parameters worden beschreven met een bivariate normaalverdeling. De gradiëntparameters waren gecorreleerd, maar significante correlaties tussen deze parameters en bepaalde variabelen zoals de nematoden soort (*G. pallida* of *G. rostochiensis*), het tijdsinterval tussen bemonstering en het laatste aardappelgewas, de teeltfrequentie en de populatiedichtheid in het centrum van de focus konden niet aangetoond worden. Er kan worden geconcludeerd dat met één detectiemethode voor kleine besmettingshaarden in alle teeltgebieden kon worden voldaan. De te verwachten betrouwbaarheid is onafhankelijk van bodemsoort, teeltfrequentie en het tijdsinterval tussen bemonstering en het laatste aardappelgewas.

In **Hoofdstuk 8** wordt het in het vorige hoofdstuk ontwikkelde model van een besmettingshaard voor de praktijk toegepast. Een computer programma met de naam SAMPLE werd ontwikkeld om bestaande bemonsteringsmethoden voor de detectie van pleksgewijze besmettingen of 'besmettingshaarden' van het aardappelcysteaaltje (*Globodera* spp) te evalueren en nieuwe te ontwikkelen. Door het model van de middelschalige verdeling van cysten, dat de verwachte populatiedichtheid op elke positie binnen een haard oplevert, te combineren met het model voor de kleinschalige verdeling binnen vierkante meters (negatief binomiale verdeling) is het met SAMPLE

mogelijk bemonsteringsprocedures te simuleren. Het belang van de parameters van de twee verdelingen - de lengte- en breedtegradiënt parameters voor de middelschalige verdeling en de aggregatiefactor k van de negatief binomiale verdeling voor de kleinschalige verdeling - werden onderzocht met behulp van gevoeligheidsanalyses. De aggregatiefactor k bleek minder belangrijk voor de berekening van de gemiddelde detectiekans van een focus dan de lengte- en breedtegradiënt parameters. Verschillende bestaande versies van de voorgeschreven Nederlandse bemonsteringsmethode werden getoetst op hun prestaties op een standaard besmettingshaard met een populatiedichtheid van 50 cysten/kg grond in het centrum. Deze standaard-haard is klein genoeg om een resistent aardappelras in een 1:3 teeltfrequentie als beheersmaatregel te gebruiken zonder dat noemenswaardige opbrengstreducties te verwachten zijn. Daar deze voorgeschreven bemonsteringsmethoden niet voldeden aan de vereiste gemiddelde detectiekans van 90% werd het computerprogramma gebruikt om verschillende nieuwe bemonsteringsmethoden voor detectie van besmettingshaarden te ontwikkelen en hun prestaties te onderzoeken. SAMPLE is een gereedschap voor het ontwikkelen van bemonsteringsmethoden op verzoek voor elke mogelijke combinatie van vereiste karakteristieken voor gebruik door poot- en consumptieaardappeltelers (aanbevelingen voor optimale beheersmaatregelen die tot maximale opbrengsten leiden, geïntegreerde bestrijding) en door overheden (wetgeving, quarantaine en het veiligstellen van de export).

Om adviezen te geven is een model noodzakelijk dat het verband beschrijft tussen aantallen aardappelcysteaaltjes en aardappelopbrengst. Een stochastisch model met biologisch relevante parameters was beschikbaar. In **Hoofdstuk 9** wordt een directe relatie beschreven tussen aantallen aaltjes en plantengroei. Deze relatie wordt gebruikt om een relatie tussen aantallen aaltjes en opbrengstdervingen van het totale plantengewicht en de aardappelopbrengst af te leiden. De relatie tussen lage en middelhoge begindichtheden en het relatieve totale plantengewicht werd verkregen door dwarsdoorsneden te nemen loodrecht op de tijdas van een groeimodel met drie dimensies: tijd na het planten t , relatief totaal plantengewicht Y en de relatieve groeisnelheid r_p/r_0. De relatieve groeisnelheid is de (constante) verhouding tussen de groeisnelheid r_p van planten bij een gegeven dichtheid nematoden P en de groeisnelheid r_0 van (jongere) planten van hetzelfde gewicht zonder nematoden. Daarom is de verhouding tussen de tijd na het planten die een plant nodig heeft om een bepaald gewicht te bereiken in de afwezigheid van nematoden en bij nematodendichtheid P, t_0/t_p gelijk aan de verhouding r_p/r_0 (2). De relatieve groeisnelheid $r_p/r_0 = k + (1\text{-}k)0.95^{P/T\text{-}1}$ voor $P > T$ en $= 1$ voor $P \leq T$ (3). Formeel is k de minimale groeisnelheid als $P \rightarrow \infty$. Ten gevolge hiervan voldoet de arbitraire vergelijking $y = m + (1\text{-}m)0.95^{P/T\text{-}1}$ voor $P > T$ en $= 1$ voor $P \leq T$ (6) ook aan de relatie tussen lage en middelhoge begindichtheden en het relatieve totale plantengewicht. T is de tolerantielimiet, waaronder groei en opbrengst niet worden gereduceerd door nematoden; m is de relatieve minimum-opbrengst. De relatie tussen lage en middelhoge begindichtheden van aardappelcysteaaltjes en het relatieve

aardappelgewicht kan uit het groeimodel worden afgeleid op een analoge wijze. Echter, er is een complicatie: knolinitiatie vindt niet plaats bij hetzelfde spruitgewicht van planten met en zonder nematoden, maar bij een lager spruitgewicht naarmate de nematodendichtheden groter zijn. Hierdoor zijn aardappelgewichten van planten van een bepaald totaal gewicht bij nematodendichtheid P niet gelijk aan die van planten met hetzelfde totaalgewicht zonder nematoden, maar $r_p \Delta t$ eenheden gewicht groter, Δt zijnde het verschil tussen de actuele tijd van knolinitiatie en de tijd dat het totale plantengewicht gelijk wordt aan dat van planten zonder nematoden bij het begin van knolaanleg. Relatieve totale- en knolgewichten van planten met 'vroege veroudering' en bij hoge nematoden dichtheden zijn kleiner dan worden voorspeld door het model en vergelijking (2). Dit duidt aan dat bij hoge populatiedichtheden mechanismen voor groeireductie actief worden die bij lage dichtheden niet werkzaam zijn.

In **Hoofdstuk 10** wordt een adviessysteem beschreven voor de beheersing van het aardappelcysteaaltje (*Globodera pallida*). Het legt de nadruk op het gebruik van partieel resistente aardappelrassen, die de mogelijkheid bieden om lage populatiedichtheden van het aardappelcysteaaltje laag te houden in korte vaste teeltfrequenties. Door het gebruik van stochastische modellen gebaseerd op de populatiedynamica van aardappelcysteaaltjes en de relatie tussen de populatiedichtheid vóór het planten en de relatieve opbrengst is het mogelijk de waarschijnlijkheid van populatieontwikkeling en opbrengstderving te berekenen bij de gegeven populatiedichtheid. Een simulatiemodel integreert beide modellen, gebruik makend van de frequentieverdeling van sommige van de meest variabele parameters relevant voor een gegeven combinatie van aardappelras en nematodenpopulatie. Ook de natuurlijke afname van de populatiedichtheid onder niet-waardgewassen is in het model opgenomen. Het model maakt het mogelijk om de waarschijnlijkheid van een gegeven opbrengstreductie voor een gegeven aardappelras, nematodenpopulatie en teeltfrequentie te berekenen. Hierdoor wordt het voor de teler mogelijk om risico's en kosten van verschillende beheersmaatregelen te evalueren. De toepassing van dit model in de zetmeelaardappelteelt kan leiden tot een significante verbetering van de financiële opbrengsten en een belangrijke reductie van het gebruik van grondontsmettingsmiddelen.

In **Hoofdstuk 11** wordt het 'Seinhorst Research Program' beschreven, geïnitieerd door Dr. J.W. Seinhorst, voormalig hoofd van de afdeling Nematologie van het IPO-DLO. Het bestaat uit de empirische filosofie, de onderzoeksmethoden en de modellen die gedurende de afgelopen 45 jaar in het nematologisch onderzoek op het IPO-DLO zijn ontwikkeld. Deze omvat ook de dertien jaar waarin het onderzoek, beschreven in dit proefschrift, uitgevoerd is. Alle theorieën in het Seinhorst Research Program zijn ontwikkeld door het speuren naar zich herhalende regelmatigheden (patronen) in een verzameling van waarnemingen, de 'empirische basis'. Om '*spook-theorieën op grond van slordige gegevens*' te vermijden worden alle veronderstellingen genoemd die dienen als grondslag van de empirische basis en zorgvuldig beschreven in theorieën

betreffende methodologie en technologie, statistiek inbegrepen. De herkende patronen worden samengevat in wiskundige vergelijkingen die gerelateerd zijn aan biologische processen om de kloof te overbruggen tussen 'normale' taal en wiskundige taal waarmee biologische theorieën worden beschreven. Vaak zijn patronen het resultaat van meer dan één biologisch proces. Is dat het geval dan worden de basale patronen ontward door middel van patroon-analyse. Deze procedure kan het eenvoudigst worden uitgevoerd als het gaat om een beperkt aantal, min of meer identieke, patronen. Daarom moet aandacht worden geschonken aan de keuze van het hiërarchisch niveau en de complexiteit van het onderzochte systeem. Onderzoek begint met eenvoudige experimentele systemen en breidt zich uit naar complexe natuurlijke systemen. Het hiërarchisch niveau mag niet zo hoog zijn dat zich manifesterende processen zeer ongelijkvormig zijn, maar ook niet zo laag dat men het risico loopt processen te beschrijven die irrelevant zijn voor het doel van het onderzoek. In het 'Seinhorst Research Program' bestaat dit doel uit het vinden van methoden voor de verbetering van de financiële opbrengsten van gewassen, belaagd door plantenparasitaire aaltjes, door risico's op populatieontwikkeling en hiervan afhangende opbrengst-reducties te schatten. Patroon-analyse kan theorieën over oorzaken van verschijnselen op het onderzochte hiërarchische niveau en over eigenschappen van processen op een lager niveau genereren. Voorspellingen op een hoger hiërarchisch niveau worden gedaan middels synthese van verschillende patronen in (stochastische) simulatiemodellen. Synthese wordt ook toegepast op samengestelde patronen in eenvoudige experimentele systemen met als doel gecompliceerde patronen in een complex systeem te verklaren.

In de conclusies (**Hoofdstuk 12**) wordt een overzicht gegeven van de praktische resultaten en aspecten van het onderzoek beschreven in dit proefschrift. Er worden enkele kanttekeningen geplaatst bij de huidige situatie rond de aardappelmoeheid-beheersing in de Nederlandse poot- en consumptieaardappelgebieden.

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At the beginning, as far back as 1981, stood Dr. J. W. Seinhorst†, head of the former Nematology Department of the IPO-DLO. Under his guidance we both, being at that time students in biology at the State University of Groningen, investigated the effect of potato cyst nematodes on the mineral composition of potato plants. Every day, during coffee, lunch and tea, we were exposed to his theories regarding tylenchid nematodes. Although, at that time, we did not comprehended everything he was lecturing, we somehow managed to make the impression that we would become useful nematologists in due time. He encouraged us to apply for jobs at the IPO-DLO and to continue his research after his retirement in 1984. During more than 12 years, during which he was still active as a scientist, he proved to be a valuable mentor and colleague with an unlimited supply of patience and a phenomenal knowledge about the facts and theories of nematology.

At the end of this period the most important man for us was without any doubt our supervisor Professor J.C. Zadoks. He stimulated us with both his criticism on the papers we drafted (mostly several pages long on top of the remarks in the text) and his enthusiasm of what was accomplished and put into writing. His interaction improved the quality of the papers produced far more than we had hoped for. Professor Zadoks, being a phytopathologist, questioned every item of nematological knowledge that we took for granted. His patience with us, who at the same time had to continue our regular research and were almost predictably late with every new draft, was admirable. We will miss the frank criticism he gave and the open dialogues we had.

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Curriculum vitae

Curriculum vitae van C.H. Schomaker

Cornelia Helena Schomaker werd op 3 maart 1949 geboren te Jipsingboermussel in de provincie Groningen. In 1968 behaalde zij haar HBS-B diploma aan het Katholiek Drents College te Emmen. Zeven jaar bekleedde zij de functie van directie-secretaresse bij het aardappelzetmeel en -derivatenconcern AVEBE in Veendam voordat zij begon aan een studie Biologie aan de Rijksuniversiteit Groningen. Daar behaalde zij in 1980 haar kandidaatsexamen en in 1984 haar doctoraal Biologie B1, *cum laude*. Tijdens de doctoraalfase onderzocht zij, samen met partner Thomas Hans Been, het effect van aardappelcysteaaltjes op de minerale samenstelling van de aardappelplant. Bij de vakgroep Zintuigfysiologie van de RUG werd, eveneens in samenwerking met Thomas Been, bijna twee jaar onderzoek uitgevoerd naar de geur- en smaakzintuigen op de antennes en het gedrag van *Hydrotaea irritans*. Vanaf 1984 is zij op het Instituut voor Plantenziektenkundig Onderzoek in Wageningen, IPO-DLO, te werk gesteld als theoretisch nematoloog. In de periode van 1984 tot 1989 werden veld- en *in vitro* experimenten verricht om de overleving en activiteit van aardappelcysteaaltjes na fumigantia-behandelingen te modelleren. Ook werden in deze periode distributiepatronen op verschillende schaalgrootte onderzocht en gemodelleerd om bemonsteringsmethoden te ontwikkelen waarmee kleine besmettingshaarden van deze aaltjes met bekende betrouwbaarheid kunnen worden opgespoord. Van 1989-1996 werd gewerkt aan het ontwerpen van (praktijk)toetsen om resistentie in aardappelcultivars tegen aardappelcysteaaltjes te schatten en aan adviessystemen voor telers van poot- consumptie- en fabrieksaardappelen, die risico's op ongewenste gebeurtenissen en de mate waarin deze optreden kunnen berekenen bij verschillende bestrijdingsscenario's. Sinds 1997 is het zwaartepunt van het onderzoek verlegd naar wortelknobbelaaltjes en naar Pratylenchus- en Trichodorussoorten.

Curriculum vitae of C.H. Schomaker

Cornelia Helena Schomaker was born March the 3rd, 1949, in Jipsingboermussel in The Netherlands. In 1968 she received her HBS-B diploma at the Katholiek Drents College in Emmen. After seven years as a secretary at the head office of AVEBE in Veendam, she began her study in Biology at the State University Groningen in 1976. In 1984 she obtained her MSc in Biology B1, with honours. She investigated, with her partner Thomas Hans Been, the effect of potato cyst nematodes on the mineral composition of potato plants. This research was carried out in cooperation with the Department of Plant Physiology of the State University Groningen and the Nematology Department of the Research Institute for Plant Protection in Wageningen. In cooperation with Thomas Hans Been two years of research were dedicated investigating the olfactory sensilla and behaviour of the sheep head fly *Hydrotea irritans* at the Sensory Physiology Unit at the Department of Zoology of the State University of Groningen. In 1984 she was employed at the Research Institute for Plant Protection in Wageningen (IPO-DLO) as a theoretical nematologist. Between 1984 en 1989 field and laboratory experiments were carried out to model survival and activity of potato cyst nematodes after treatment with nematicides. Distribution patterns at different scales were investigated and modelled to develop sampling strategies for the early detection of infestation foci with high and known probability. From 1989 to 1996 research was aimed at the development of methods to measure resistance of potato cultivars to potato cyst nematodes and at advisory systems for seed, ware and starch potatoes, which calculate risks and their probability for different control scenarios. Since 1997, the main effort of the research is directed towards free living plant parasitic nematodes such as root knot nematodes, *Pratylenchus* spp and trichodoridae.

Curriculum vitae van T.H. Been

Thomas Hans Been werd geboren op 24 januari 1955 in de Amerikaanse zone van West-Berlijn in Duitsland. In 1965 verhuisde hij naar Den Haag. In 1975 verwierf hij aan het Boerhave College te Leiden het Atheneum-B diploma. Na een jaar dienstplicht studeerde hij vanaf 1976 Biologie aan de Rijks Universiteit te Groningen. In 1980 behaalde hij zijn kandidaatsexamen, in 1984 gevolgd door het doctoraalexamen Biologie B5b, *cum laude*. Tijdens de doctoraalfase onderzocht hij, samen met partner Cornelia Helena Schomaker, het effect van aardappelcysteaaltjes op de minerale samenstelling van de aardappelplant. Bij de vakgroep Zintuigfysiologie van de RUG werd, eveneens in samenwerking met Corrie Schomaker, bijna twee jaar onderzoek uitgevoerd naar de geur- en smaakzintuigen op de antennes en het gedrag van *Hydrotaea irritans*. Vanaf 1984 is hij op het Instituut voor Plantenziektenkundig Onderzoek in Wageningen, IPO-DLO, te werk gesteld als theoretisch nematoloog. In de periode van 1984 tot 1989 werden veld- en *in vitro* experimenten verricht om de overleving en activiteit van aardappelcysteaaltjes na fumigantia-behandelingen te modelleren. Ook werden in deze periode distributiepatronen op verschillende schaalgrootte onderzocht en gemodelleerd om bemonsteringsmethoden te ontwikkelen waarmee kleine besmettingshaarden van deze aaltjes met bekende betrouwbaarheid kunnen worden opgespoord. Van 1989-1996 werd gewerkt aan het ontwerpen van (praktijk)toetsen om resistentie in aardappelcultivars tegen aardappelcysteaaltjes te schatten en aan adviessystemen voor telers van poot- consumptie- en fabrieksaardappelen, die risico's op ongewenste gebeurtenissen en de mate waarin deze optreden kunnen berekenen bij verschillende bestrijdingsscenario's. Sinds 1997 is het zwaartepunt van het onderzoek verlegd naar wortelknobbelaaltjes en naar Pratylenchus- en Trichodorussoorten.

Curriculum vitae of T.H. Been

Thomas Hans Been was born on January, 24th in 1955, in the American zone of West-Berlin, Germany. In 1965 he moved to the Netherlands and lived in The Hague. At the Boerhave college in Leiden the Atheneum-B diploma was obtained in 1975. After completion of his military service he went to study Biology at the State University Groningen in 1976, where he received his MSc in Biology B5b (including geology), with honours in 1984. As a research project he investigated, with his partner Cornelia Helena Schomaker, the effect of potato cyst nematodes on the mineral composition of potato plants. This research was carried out in cooperation with the Department of Plant Physiology of the State University Groningen and the Nematology Department of the Research Institute for Plant Protection in Wageningen. In cooperation with Cornelia Helena Schomaker two years of research were dedicated investigating the olfactory sensilla and behaviour of the sheep head fly *Hydrotea irritans* at the Sensory Physiology Unit at the Department of Zoology of the State Univerity of Groningen. In 1984 he was employed at the Research Institute for Plant Protection in Wageningen (IPO-DLO) as a theoretical nematologist. Between 1984 en 1989 field and lab experiments were carried out to model survival and activity of potato cyst nematodes after treatment with nematicides. Distribution patterns at different scales were investigated and modelled to develop sampling strategies for the early detection of infestation foci with high and known probability. From 1989 to 1996 research was aimed at the development of methods to measure resistance of potato cultivars to potato cyst nematodes and at advisory systems for seed, ware and starch potatoes, which calculate risks and their probability of occurrence for different control scenarios. Since 1997, the main effort of the research is directed towards free living plant parasitic nematodes such as root knot nematodes, *Pratylenchus* spp and trichodoridae.

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