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PHYTONEMATOTOLOGY IN WESTERN EUROPE

By

DR. J. W. SEINHORST

Institute Voor Phytopathology

Wageningen, The Netherlands

Prepared and published by the Technical Committee, Southern Regional Nematology Project (S-19), from a lecture series given at the Alabama Polytechnic Institute, January-February, 1957.

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FOREWORD

One of the aims of the Southern Regional Nematology Project (S-19) has been to broaden the scope of the training and knowledge in phytonematology of the participants in the Project and of students majoring in this subject. Recognizing that the workers and teachers in phytonematology in this country are, perhaps, too much "from the same mold", efforts were made to have someone come from abroad as a guest lecturer. It was desired to have someone very well informed and experienced in the field of phytonematology, particularly, as it is being developed and applied in Western Europe. Aided by a grant from the Rockefeller Foundation, it was the good fortune of the Southern Regional Nematology Project to be able to have Dr. J. W. Seinhorst, of the Institute for Phytopathology, Wageningen, Netherlands, come to the southeastern region for a period of six months.

The first half of Dr. Seinhorst's stay was devoted to laboratory work and a lecture series in phytonematology at the Alabama Polytechnic Institute. The remaining three months of his time were spent in visiting each of the Regional Projects member States and Puerto Rico for the presentation of a lecture and demonstrations. Dr. Seinhorst, in exchange, had opportunity to gain a more first-hand concept of the plant nematology problems of this country. The final phase of Dr. Seinhorst's stay under the auspices of the Regional Project was his participation as a leader in several discussion topics at an advanced phytonematology workshop held at the University of Tennessee in the summer of 1957. This workshop was another one of the activities of the Project. The proceedings of that meeting are being printed under a separate title.

To make available to a wider audience the information presented by Dr. Seinhorst in his lectures, this printed version has been prepared with the financial assistance of the Rockefeller Foundation. As indicated and explained by Dr. Seinhorst in the Preface, this printed version represents a condensation of his talks and the discussions. However, much of the information pertaining to techniques has been or is to soon be available in the journals and for those who wish to go further into the other lecture topics, the entire lecture series was recorded on tape and can be borrowed.

Eldon J. Cairns
Chairman, Technical Committee
Southeastern Regional Nematology
Project
(S-19)

PREFACE

The contents of the following pages are largely based on a series of lectures I gave at the Alabama Polytechnic Institute at Auburn, Alabama in the beginning of 1957. The text given here has been restricted as much as possible to what cannot yet be found in comprehensive textbooks or monographs. Instead it tries to give a personal vision of nematode problems in Western Europe. Therefore a discussion of laboratory methods which comprised part of the lectures has not been included here. Without the help of demonstrations no text on this subject would be better than J. B. Goodey: Laboratory Methods for Work with Plant and Soil Nematodes, to which the reader is referred. Also the workshop manuals give a good account of these techniques.

I wish to express my most grateful acknowledgement to those who arranged my six months visit to the Southern States of the U.S. This visit has widened my experience and insight in the general problems of plant nematology both in the United States and Europe tremendously.

I also wish to express my most sincere thanks to all those American colleagues who, by their kind and abundant assistance in my travels and their great hospitality, made this trip a most agreeable and useful one. In particular, I want to mention Dr. Eldon J. Cairns who took off my shoulders almost every burden involved in staying in a foreign country with a family and organizing a traveling schedule.

My family and I wish to express our great appreciation for the kind help in every-day life matters we received from Dr. and Mrs. E. J. Cairns, Mr. and Mrs. Norman Minton, and many other Auburn inhabitants.

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Introduction to the plant nematode problems of Western Europe

Although Western Europe has many important plant parasitic nematode species in common with the U.S.A. the situation in both regions shows some differences.

The cool climate rules out root-knot as an important disease of field crops in most of the region. It has to be controlled in green-houses though, but this is not a major problem.

The stem nematode however is favoured by the climate and is more important than in the U.S.A.

A few cases of attacks by ectoparasites have been reported but, so far, species belonging to this group of nematodes seem to be only of a local importance.

Potato cyst eelworms (golden nematode) and beet eelworm rank first in damage caused by nematodes.

One species has ceased to be of importance in Western and Central Europe: the wheat gall nematode Anguina tritici. Modern methods of cleaning seed eliminate the galls from the seed and so reduce the chance of infestation of young seedlings very considerably. This nematode, which was the first one known as a plant parasite and to which Shakespeare refers in "Love's Labour's Lost", now only occurs in regions with backward agricultural methods.

A second group, the leaf and bud nematodes is losing ground where careful roguing of strawberry planting material has become a general practice and where chrysanthemum plants are sprayed intensively with parathion or related chemicals.

The following nematode species (the groups placed in the order of their importance) cause serious losses in agriculture and horticulture in Western Europe.

1) Cyst forming eelworms

- a. beet eelworm, Heterodera schachtii
- b. potato cyst eelworm (golden nematode) H. rostochiensis
- c. oat cyst nematode, H. major
- d. pea cyst nematode, H. goettingiana
- e. carrot cyst nematode, H. carotae
- f. cabbage cyst nematode, H. cruciferae

2) Stem and bulb eelworms

- a. Ditylenchus dipsaci, in flower bulbs, onions, rye, oat.,

many vegetable crops and ornamental plants.

- b. D. destructor on irises and potatoes
- c. D. myceliophagus on mushroom mycelium.

3) Root lesion nematodes

Pratylenchus penetrans causes root rot in daffodils and other flower bulbs, ornamental plants, strawberries, raspberries, potatoes, and in tree nurseries.

- 4) Root knot nematodes, mainly in greenhouses on tomatoes and cucumbers (Meloidogyne arenaria and M. incognita acrita) and M. hapla in ornamental plants.

5) Bud and leaf nematodes

Aphelenchoides fragariae and A. Ritzemabosi occasionally in ornamental plants.

6) Ectoparasites

- a. Hoplolaimus uniformis is associated with early yellowing of peas and causes damage in carrots.
- b. A Paratylenchus species is of local importance on carrots and celery.

The cyst forming nematodes, Heterodera species

Although many agricultural and a few horticultural crops may be attacked by one of the seven Heterodera species occurring in Europe, the importance of this group is largely due to the tremendous damage done by two species to two important agricultural crops, H. rostochiensis on potatoes and H. schachtii on sugar beets. Damage by these species is always associated with growing their main host crops, potatoes and beets respectively, in short rotations.

The beet cyst nematode became an important parasite in the second half of the 19th century in areas around sugar factories in Germany and the Netherlands, where often sugar beets were grown on the same field for several years in succession.

The potato cyst nematode cropped up in the beginning of this century in the potato growing districts of Lancashire, England, in Scotland and around Rostock in Northern Germany. It has been found since in all European countries always in association with intensive cropping with potatoes, e.g. in allotment gardens, early potato growing areas, areas particularly suited to growing potatoes (Fen lands in England and "Veenkoloniën" in the Netherlands) and land in use by refugees in post-war Western Germany.

H. rostochiensis has been found to occur over a large area in Peru, hence it has been generally accepted that it was brought into Europe from these regions in recent times. It has been spread since with infested seed potatoes and soil clinging to potatoes and other plants.

When the disease was recognized in England and Germany in the beginning of this century, wide areas were already heavily infested in these countries. Other countries such as the Netherlands apparently kept free from the nematode for some twenty years longer. The plant protection services had then become aware of the danger which this disease meant to potato growing and took extensive measures to control it and to prevent further spread or build up of the nematodes. Contrary to the situation in England and Germany in the beginning of the 20th century, the infested area at the time the disease was recognized in the Netherlands in 1941 was still so small there that forbidding the growing of potatoes and tomatoes on or moving plants or soil from infested fields could be considered reasonable measures to prevent further spread. Moreover the farmers are not allowed to grow potatoes in non-infested fields oftener than once in three years.

Control by nematicides is too expensive but the breeding of resistant varieties of the potato shows good promise.

Contrary to the potato cyst eelworm, the beet cyst eelworm seems

to be indigenous in Western Europe. It has a rather wide host-range among wild and cultivated plants. England and Ireland were the last countries in Western Europe where this eelworm became a problem. This is clearly due to the insignificance of sugar beet growing there before the second World War. In England it is legally forbidden now to grow sugar beets on infested land, but there can be some doubt whether this will do more than prevent some fields from becoming so heavily infested that they will not be suitable for sugar beets for a long period. In other European countries legal measures against the beet cyst nematode would be of no use. Here the only possibility is to grow beets in a suitable rotation. This point will be discussed later.

Heterodera major is a serious parasite of oats and barley in Denmark, Sweden, certain parts of England, the Netherlands and Germany. This damage shows a clear relation to the rotation followed, but also other factors seem to be important, e.g. soil type.

Heterodera goettingiana is rather widely spread on peas and broad beans, and very persistent in infested fields.

H. humili on hops, H. carotae on carrots and H. cruciferae on cruciferous plants are of local importance, whereas H. schachtii subsp. trifolii may cause some damage in red clover. Its importance in white clover in pastures in the Netherlands is doubtful.

The stem and bulb nematode, *Ditylenchus dipsaci*

Contrary to Heterodera species, the occurrence of the stem and bulb nematode shows a strong relation to soil type, whereas crop rotation is only of importance in areas where the soil is rather unfavourable to this animal.

It is a serious pest of flower bulbs, ornamental plants, oats and teazel in England, of onions, shallots, vegetable crops, ornamental plants, rye, potatoes and mangolds in the Netherlands and Germany and of red clover, white clover and alfalfa in Denmark and Sweden.

In a number of cases the nematode is spread by seed (red clover, alfalfa, teazel) or by bulbs (flower bulbs, shallots). Then control sometimes is relatively easy, as seed can be treated with methyl bromide, flower bulbs and shallots by hot water treatment.

Where the soil is the source of infestation not very much can be done against it. On light soil a suitable crop rotation may reduce the damage caused by this nematode. On heavy clay soil crop rotation has very little effect. Nematicides are either too expensive or the soil is unsuitable for application.

Contrary to cyst forming nematodes, where damage to plants starts at a degree of infestation of the soil of 2000-5000 eggs per 500 g. of soil, stem eelworm causes considerable damage in many crops at degrees of infestations below 10 specimens per 500 g. of soil.

At least eleven biological races of stem eelworm occur in Europe. These all have a wide host range, which gives ample opportunity for survival on weeds. Breeding resistant red clover, white clover, alfalfa, and rye has been successful.

The Root lesion (meadow) nematodes, *Pratylenchus* species

The root lesion nematodes are represented in Europe by at least six species. However, only two of these cause damage. *Pratylenchus penetrans* causes serious damage to a wide variety of plants, an undescribed species causes root rot in *Convallaria majalis*.

Pratylenchus penetrans

The hosts of *P. penetrans* can be divided into three groups:
(see also SLOOTWEG 1956)

- I. Plants which only suffer at very heavy attacks and show little secondary root rot (cereals, peas, etc.).
- II. Plants which suffer when attacked by medium numbers (potatoes, apples, tulips, hyacinths).
- III. Plants which suffer at low or very low degrees of infestation of the soil (daffodils, strawberries, lilies). In this group the damage is largely done by secondary root rot caused by several fungi to which the roots become susceptible even when attacked by only a few specimens of this nematode.

The third group is the most important one as damage in these plants is found over a much larger area than in the other two groups. Some host plants belonging to the first two groups run a risk of being damaged because either they stay for many years in the same place so that the nematodes can build up a large population (apples) or they are replanted in the same field a few times in succession (nurseries).

Although *P. penetrans* is a dangerous parasite of some plants where it occurs, its distribution in Western Europe seems to be limited. This definitely is not due to lack of host plants in non-infested areas, but most probably to the soil type being unfavorable to this nematode. Also in infested areas there are great differences in the all over degree of infestation. It is low in the sandy soil in the western part of the Netherlands, although in this vegetable growing area there are host plants on the fields most of the time. However, serious damage only occurs in daffodils (root rot results when 1 or more specimens of *P. penetrans* are present in 500 g. of soil) and strawberries which build up a high eelworm population. Tulips and hyacinths are only attacked occasionally.

Fortunately, soils where *P. penetrans* occurs are very suitable for the use of nematicides, and generally the cost of treatment is no objection.

Almost all fields on sandy soil where daffodils are grown are treated regularly.

Pratylenchus pratensis

Although this nematode is far more widely spread than P. penetrans, much less is known about its economic importance. Until now no convincing cases of damage by this nematode have been reported, nor have symptoms of the attack been described. The nematode attacks all cereals and quite a number of other plant species. Many of them are also hosts of P. penetrans. In some of these the latter nematode causes lesions even when present in small numbers, whereas P. pratensis does not cause any visible change of the attacked tissue (cherry, Plantago major, peas).

Most cases of damage, e.g. on cereals, white clover and grasses reported in literature (e.g. OOSTENBRINK 1954), are based on growth improvement after treating the infested soil with a nematicide. Cereals however do not show patchiness which shows close relations to the occurrence of high numbers of this nematode in the soil. The degree of infestation of the soil varies widely even in the same field. So there should either be poor patches where the degree of infestation is highest or the (invisible) damage only occurs in a small part of some fields.

The improvement of growth of white clover and grasses after DD treatment from which Oostenbrink (1954) drew the conclusion that P. pratensis caused damage to these plants, is most probably not due to the killing of parasitic nematodes. Ennik, Kort and van Doorn (in litt.) found that this growth stimulation also occurred where only very low numbers of P. pratensis or other plant parasitic nematodes were present. They could explain the effect of DD treatment on these plants partly by an increase of the amount of nitrogen available to the plant but concluded to the presence of one or more other growth stimulating actions of DD other than killing of eelworms (see also Goffart and Heiling (1958)).

Pratylenchus thornei, P. minyus and P. neglectus are most probably comparable to P. pratensis in their relation to plant growth. No trustworthy reports on damage are known.

Effect of soil type on the occurrence of root lesion nematodes. The distribution of all species of root lesion nematodes in the Netherlands and most probably also in other parts of Western Europe shows a close relation to soil type. P. penetrans seems to find optimum conditions in organic soils or sandy soils rich in organic matter. It also occurs on light sandy soils but was never found in the Netherlands on a true clay soil. P. pratensis is also widely distributed on sandy soils but builds up large populations on loamy sands and even

occurs on clay soils. It is found on a wider range of soil types than P. penetrans.

P. minyus and P. thornei occur on clay soils. The first species is found occasionally in dune sand.

From the present information it is impossible to give one set of soil characteristics defining a favorable soil for each of these nematode species. Some of the soils where P. penetrans is found in England and Australia contain much more fine mineral material than in those soils where the nematode occurs in the Netherlands. However, P. penetrans has never been reported as a serious pest on clay soils.

The ectoparasitic nematodes

Although most genera of the Tylenchida and the plant-parasitic dorylaims are represented by one to several species in Western Europe only a few of these species have been found up to now to be of economic importance. They are Hoplolaimus uniformis and a Paratylenchus species.

Hoplolaimus uniformis

Although this nematode has a wide host range, until now only carrots and lilies (Slootweg 1956) have been shown to suffer from its attack. It seems to make peas susceptible to a secondary attack, most probably by the fungus Fusarium oxysporum var. pisi race 2. This complex causes early yellowing, whereas the nematodes alone even in high concentrations do not damage the peas although they live on its roots (SEINHORST 1954, LABRUYERE and SEINHORST 1954).

In pot experiments some of the other hosts appeared to build up very large populations of the eelworm in the soil e.g. chicory, cauliflower and beets. They were not found to suffer in the field and for the most part they do not even have any particular influence on population numbers there.

This again is most probably due to the influence of the soil. Hoplolaimus uniformis is most abundant in light sandy soils with a rather high humus content. It was never found in clay soils except occasionally in low numbers. Here spiral nematodes of the genus Potytlenchus (Helicotytlenchus, Gottholdsteineria) may be abundant. On arable land either Hoplolaimus uniformis or spiral nematodes may be abundant but never both.

In winter sown carrots damage can be expected at degrees of infestations over 500 specimens per 500 g. of soil. In spring sown carrots this number is higher.

Paratylenchus species

Various species of this genus are widely distributed in all soil types, but only very occasionally they occur in numbers high enough to cause damage (more than 10,000 per 500 g. of soil).

Damage has been described in carrots (WEISCHER, 1957) and celery (COSTENBRINK, 1953) probably caused by the same species. The damage in carrots can be distinguished from that by Hoplolaimus uniformis by the rusty appearance of the roots and the young carrot. When attacked by H. uniformis they look grayish.

Although apparently there are factors which localize the damage by Paratylenchus, nothing is known about the influence of soil type or treatment.

Tylenchorhynchus species

The most abundant genus both in species and numbers is Tylenchorhynchus. In most soils in Europe several hundred to several thousand specimens of one or more species are generally found.

After VOGEL (1958) Tylenchorhynchus claytoni is the cause of root rot and poor growth of azalea in Switzerland. The writer found this species in the Netherlands in soil around roots of Kalmia, a shrub imported from North America, and azalea. Probably T. claytoni was brought in on these shrubs.

No damage by other Tylenchorhynchus species has been reported.

Dorylaimoid species

Of the dorylaimoid plant parasitic genera Trichodorus is found in almost any field in the Netherlands. T. pachydermus occurs most generally, but also T. primitivus is widely spread. So far, no damage by these nematodes which seldom occur in large numbers has been reported.

On the contrary, Xiphinema species are rare.

Estimation of nematode damage

In classical cases of nematode damage the damage was mostly estimated by comparing the yield of the damaged crop with that of the healthy plants. Healthy and diseased plants could be distinguished by specific symptoms of attack caused by the nematodes.

Although all nematodes damage to plants is accompanied by more or less specific symptoms the reactions of plant roots to certain Pratylenchus species and ectoparasites is not so clear cut as, for example, in rootknot, cyst forming nematodes, or sting nematodes. Moreover, as all soils contain one or more ectoparasitic species, the role of nematodes in plant growth can only be evaluated if plants in soil with and without nematodes can be compared. Some nematologists used gentle heat or a treatment with a nematicide, preferably DD, as a means to obtain eelworm free soil. Many estimates of eelworm damage also in Europe are based entirely upon growth improvement found after DD treatment of the soil.

Unfortunately the hope, sometimes an unshakeable belief, of these nematologists that DD or gentle heating would not do anything but kill nematodes has not come true in Europe. Both treatments appeared to result in growth improvement sometimes also under conditions where damage by nematodes in the untreated soil would be a rather far fetched explanation of this phenomenon. (PITCHER, 1957, GOFFART and HELLING, 1958). The author and others also noticed a type of growth improvement by DD treatment in field experiments which was remarkably similar to the effect of a dressing with nitrogen fertilizer.

Moreover some diseases appeared to be reduced considerably by DD treatment which had no relation to nematodes. VOGEL 1958 found that Thielaviopsis infestation of a soil was strongly reduced. The writer found that disease in carrots, which shows no relation to the occurrence of any nematode, was controlled very effectively by DD in the first crop of carrots after treatment but not in the second, whereas the nematodes still were very low in number then.

Therefore, in the preceding pages only those cases have been mentioned where evidence of a more reliable nature than growth improvement after a nematicidal treatment of the soil was available.

Control of Nematode Diseases in Western Europe

Apart from a few special treatments, such as the hot water treatment of flower bulbs and shallots, and the methylbromide fumigation of seed of clovers, teazel and onions, control of nematodes is done in three ways:

- 1) by chemical treatment of the soil
- 2) by using resistant varieties of crops
- 3) by crop rotation.

1. Chemical treatment

The possibilities of chemical treatment of the soil in Western Europe are limited by economical and climatic factors. Chemical treatment comes only into consideration for horticultural crops and ornamental plants and is always relatively expensive in comparison to the same treatment in the United States of America.

Of the climatic factors the overall low temperature limits treatment even by the most favorable fumigant, DD, to the summer months. In the Netherlands treatments with this nematicide before April and later than August carry the risk of damage to the plants. Ethylene dibromide and Nemagon are for this reason practically useless.

Recently Stauffers Vapam and N521 (Crag Mylone) have been tested. On very light sandy soils a very good kill was obtained by treatment in November (SEINHORST, BIJLOO and KLINKENBERG 1956, KLINKENBERG and SEINHORST 1956). Economically this is a very favorable time for treatment. However, on other than these very light soils the effect of Vapam was highly insufficient.

For European conditions a soil fumigant should be active at some 10°C lower than the optimum for DD.

2. Breeding resistant varieties of crop plants

The oldest European work on resistance against nematodes was on the resistance of rye against stem eelworm, but so far the breeding of red and white clover resistant against stem nematodes (BINGEFORS 1957, FRANDSEN 1951, DIJKSTRA 1956, 1957) and of potatoes resistant against Heterodera rostochiensis (ELLENBY, 1948, 1952, TOXOPEUS and DUYSMAN 1952, HUYSMAN 1956) have been more successful.

A. Resistance against stem nematodes

Rye. A few of the many local rye varieties in western

Europe show a certain degree of resistance against the rye race of the stem eelworm. As early as 1910 some breeding had been done by an agronomy teacher in Ottersum, Netherlands. He applied mass selection on an infested field. It seems to be impossible in this way to obtain material with more than 25% fully resistant and 50% somewhat resistant plants. The latter group of plants always yields susceptible plants in the offspring. In the field they cannot be distinguished from the fully resistant plants.

SEINHORST (1953) worked out a method for the investigation of seedlings in the laboratory. Here the fully resistant plants can be distinguished from the slightly susceptible ones, and by this method plants could be selected after three years of breeding work in whose offspring more than 50% fully resistant and no susceptible plants occurred. The breeding work is now done by two professional plant breeders.

Red clover. In South Sweden an eelworm resistant variety was selected from a local variety by mass selection (SYLVEN 1936). BINGEFORS (1957) worked out a method for the investigation of clover seedlings on filter paper strips in the laboratory. He selected plants which looked completely healthy.

SEINHORST (1956) described a difference in reaction to attack of resistant and susceptible red clover by the red clover race of the stem nematode. The susceptible plants show stunting and swelling, the resistant ones some stunting and necrosis.

DIJKSTRA (1957) worked out the selection of resistant red clover on the basis of the findings of SEINHORST (1956). Resistance of this type appeared to occur in all red clover varieties. DIJKSTRA also found a small percentage of plants which showed no symptoms at all after inoculation.

B. Resistance of potatoes against *Heterodera rostochiensis*

In 1945 ELLENBY found that some *Solanum andigenum* lines were resistant against the potato cyst eelworm.

TOXOPEUS and HUYSMAN (1952) used this material as a basis for breeding an eelworm resistant potato variety. The resistance appeared to be dominant and based on one

factor. On resistant plants only a very small number of small mostly empty cysts are formed. However, a good number of males completes development to the adult stage. The testing of the plants can easily be done by growing them in pots with infested soil.

After six years of breeding a few very promising varieties are ready to be released.

Recently a race of the potato cyst eelworm has been found, both in England (JONES 1957) and in the Netherlands, which attacks the originally resistant material.

C. Resistance of beets against *Heterodera schachtii*

Despite repeated efforts, no Beta species resistant against the beet cyst eelworm which can be crossed with Beta vulgaris have been found.

3. Crop rotation

The possibilities of crop rotation are discussed in the following lecture. It is particularly effective against Heterodera species, when the farmer can afford to grow susceptible crops with several years intervals. As with several other nematodes, rotation possibilities depend on soil type, crop, or both and often it has no effect at all.

Population studies of plant parasitic nematodes

Quantitative research on nematode population has for a long time been restricted to Heterodera schachtii and H. rostochiensis. Both the particular importance of crop rotation for the control of these nematodes and the relatively easy recovery of cysts from soil are the causes of this situation.

Population studies on other nematodes are of very recent date. This is mainly due to the fact that no effective extraction methods both as to percentage recovery and economic use of time and man power were known in the past.

Unfortunately the older work on Heterodera species is somewhat less reliable because methods for extraction of cysts from soil were not fully reliable and in both older and some of the newer work the methods for estimating cyst content (number of eggs per cyst or per unit weight or volume of soil) are open to criticism.

1. Research on Heterodera species

The close relation between intensive cropping with host plants and severe damage both in beet cyst eelworm and potato cyst eelworm was very suggestive of suitable crop rotation as a remedy against "beet sickness" and "potato sickness". The farmers ask not only for a safe rotation but, as sugar beets and potatoes are their most profitable crops, they also want to follow the shortest possible safe rotation. Apart from that, the idea of a rotation suitable to prevent the build up of nematode populations to dangerous levels in areas not yet found infested has come up in recent years.

A. Heterodera rostochiensis

Apparently the case is simplest in Heterodera rostochiensis. Here in most cases only one host plant occurs in the rotation, the potato. The number of cases where also the tomato has to be taken into account is small. In H. schachtii the problem is complicated by the existence of more host crops (cruciferous plants) and of susceptible weeds.

In the case of H. rostochiensis two or three questions had to be answered before a rotation could be devised:

1. What is the rate of increase of the nematode when a crop of potatoes is grown?

2. What is the rate of decrease of the population number when no potatoes are grown?

If high degrees of infestation are present, a third question is important:

3. At what level of infestation of the soil does damage occur?

The danger level. The third question has been answered first. Although the amount of damage to the potato also depends on soil fertility, all authors agree fairly well about the maximum level of infestation which the potato can stand without being damaged. CARROLL (1933) found a relation between degree of infestation of the soil and degree of potato sickness when the first was not over 3-4 cysts per gram of soil. At higher degrees of infestation the crop was irregular but always very poor.

The National Agricultural Advisory Service in England found that damage occurs where more than 5 viable cysts per 10 gram of soil are found (equals roughly 15 eggs/g.). This figure also depends on whether soil and weather conditions are favorable to the potato.

Rate of increase. In the early experiments in Europe very variable rates of increase were found. This was due to the fact that the initial degrees of infestation were always high. The first experiments which gave a better understanding of the factors governing the rate of increase were not done in Europe but on Long Island, N.Y., by CHITWOOD and FELDMESSE (1948). These show clearly that the rate of increase depends on initial degree of infestation. At very low levels there may be one ratio between initial degree of infestation and number of eggs formed on a potato plant. At higher levels the increase becomes smaller the higher the degree of infestation, whereas at very high levels there may be a reduction in number of the parasite. An increasing portion of the larvae hatched from the eggs in the cysts cannot complete their life cycle as the potato roots die too soon because of the heavy attack. The decrease in number of eggs per unit of soil when potatoes are grown in heavily infested soil explains

why sometimes a reasonable crop can be grown after a complete failure.

After CHITWOOD and FELDMESSER the increase on one potato crop is about 10-to 20 fold until about 50,000 cysts per plant are formed. If more larvae penetrate into the roots a portion of them die before maturity is reached. Now in the field and even in pots the larvae are not evenly distributed over all root tips. Therefore even at low degrees of infestation the invasion may locally be heavier than the roots can bear, and the maximum reproduction factor is found at very low degrees of infestation.

OOSTENBRINK (1950) came largely to the same conclusions as CHITWOOD and FELDMESSER. He calculated a factor of 10 for increase and assumed that this factor would be applicable from very low initial degrees of infestation to where damage to the plants begins to occur. This picture is too simple however. At very low degrees of infestation the rate of increase may be well over 10 times also in the field (DEN OUDEN 1958).

There is only one generation of the nematode on a potato crop. Cysts formed in summer do not release larvae before the beginning of December (DEN OUDEN 1958).

Decrease of the population in the absence of a host plant. In the absence of a host plant the population decreases gradually. Determinations of numbers of eggs per unit of soil show that this number decreases, whereas only a few dead eggs are found. So, although larvae of Heterodera rostochiensis do not hatch easily in water which does not contain an activating substance diffusing from the roots of host plants, they do gradually leave the eggs in the soil. If these larvae do not find a host plant they die after a short time. This is the main cause of the reduction of the population in the absence of a host plant.

In field experiments GOFFART found a reduction of the population to $1/3$ - $1/6$ in two years. OOSTENBRINK (1950) estimated it at 50% in one year. In pot experiments FENWICK (1952) found the same figure.

OOSTENBRINK'S figure is a mean calculated from figures which vary between reductions of less than 20% to more than 80%. Part of this variation may be due to experimental errors. However, there is a good chance that the actual decreases will differ in different places. No data are known about the reduction in the third and further years. Moreover, these numbers may be different under different climatic conditions. Thus crop rotations can only be based on these figures tentatively.

In the Netherlands the legally enforced rotations with not more than one year of potatoes in 3 years is an optimistic interpretation of the practical effect of a 10 fold increase of the nematode on a crop of potatoes and a decrease of 50% in the absence of a host. It will at least slow down the increase of the heavily infested area in regions where before this compulsory rotation more than 50% of the area might be under potatoes.

In Germany 3 or 4 course rotations have been advised to the farmer or have been compulsory since 1933. On heavily infested land this did not prevent damage. In England 5 to 6 years should elapse between two potato crops on infested land to be reasonably sure that no serious damage will occur. GRAINGER even found a definite increase of the population in these rotations.

It is generally assumed, that the larvae are released from the eggs gradually in the absence of a host crop. There is some evidence that this is not the case, but that most of them hatch in a rather short period in late spring (DEN OUDEN 1957).

B. Heterodera schachtii

In the beet eelworm, population dynamics in infested fields are complicated by the occurrence of more than one generation of the nematode (in the case of H. rostochiensis one generation) and the existence of a number of host plants, both field crops and weeds.

On one beet crop the population may increase up to 40 fold. The reduction in the absence of a host is roughly estimated at 50%. It is generally

assumed that 6-10 years should elapse between two beet crops in case of beet sickness. As it is impossible to predict the incidence of beet sickness so far ahead, no fixed rotation is followed in the Netherlands. Instead of that, the farmer can have his soil investigated by the Institute for Sugar Beet Research at Bergen op Zoom and get advice as to whether or not it is suitable for growing sugar beets.

Danger Level. HELLINGA (1942) found that reduction of yield could be expected at degrees of infestation of 10 cysts or more per 75 grams of soil. This figure agrees fairly well with the danger level found by JONES (1945) which is 10 viable eggs per gram of soil.

Distinguishing Heterodera Species. As this advisory work is based entirely upon cyst and egg counts, it is necessary to distinguish the different Heterodera species by their cyst and larval characters. In Western Europe it is important to distinguish the beet cyst eelworm from H. major, H. schachtii, H. trifolii and H. galeopsidis. HIJNER (HIJNER, OOSTENBRINK and DEN OUDEN 1953) worked out larval characters and (OOSTENBRINK and DEN OUDEN 1954) cyst characteristics, mainly based on differences between the vulvar regions of the different species. COOPER (1956) extended this work and included more species.

Top Level of Infestation. Experiments by JONES showed that with different host plants there is a top level to which population numbers can increase. Generally such a top level is explained as being the maximum number of nematodes which can feed on the plant without serious reduction of the root system. This could be the case in beets. It is not necessarily due to damage also affecting the above ground parts. JONES (1956) found that with cabbage the top level of the nematode population was reached without any signs of damage to the plant. This agrees very well with the impression that cabbage, although heavily attacked by H. schachtii, is not damaged in the field. It is damaged though by H. cruciferae.

Catch Crops. KÜHN (1882) tried to catch and remove the larvae from the soil by growing a sus-

ceptible Brassica species and plowing up the field before cysts had ripened. This appeared to be either useless or too dangerous because cysts ripened before the crop was destroyed.

A refinement of the catch crop technique was investigated by DEN OUDEN (1956). Instead of a host plant he used a plant which produces a hatching agent and into which the larvae penetrate but do not complete their life cycle. A promising plant was Hesperis matronalis. However, the increase of hatching over that with normal non-susceptible crops appeared to be not more than 10% and this is not sufficient to justify the cost and trouble of growing this relatively uneconomic plant.

C. Other Heterodera species

Both H. rostochiensis and H. schachtii are characterized by the fact that their eggs can be stimulated to hatch by a substance which diffuses out of the roots of host plants. This is not the case with all other Heterodera species, and does not affect their population dynamics. These other species do not show fundamental differences with the two most important Heterodera species.

In almost all cases Heterodera spp. host range comprises two or more crop plants and several weeds. However, the effectiveness of these different plant species as hosts varies widely. JONES and MORIARTY (1956) investigated the effectiveness of several host plants of H. schachtii, H. cruciferae and H. goettingiana.

Influence of soil type. In the preceding talks the effect of soil type on the population dynamics of Heterodera species has not been mentioned. The impression gathered from most investigations is that it generally is not of major importance. Both increase and decrease of the populations seem to be largely independent of soil type. However, upon closer examination this might appear not to be quite true. JONES found that on a certain English soil the beet eelworm population was kept low even after growing beets on it repeatedly. Heterodera species differ from the species discussed below in this respect. Results of experiments in

pots of the right size and on microplots and of a relatively small number of field experiments can be generalized. This is impossible for some other nematodes. The reason for this is that population fluctuations in these nematodes are much more subject to influences by local environmental factors than is generally the case in Heterodera species.

2. Research on stem nematodes, root lesion nematodes, and
Hoplolaimus uniformis.

The potential of multiplication of many plant parasitic nematode species is much larger than that of Heterodera species. This is due to a shorter life cycle and the succession of several generations in one season. The offspring of one stem nematode in one year on a suitable host may be several thousands. In the Heterodera species discussed above the reproduction potential is of the same order of magnitude as the actual population increase measured in the field (the latter generally is not lower than 1/2 to 1/4 of the first). In stem nematodes, root lesion nematodes, and Hoplolaimus uniformis the last value often is only a small fraction of the first one. A high death rate of the nematodes in the soil and reduction of the activity of the nematodes while in the soil are the main causes of this. In these species population dynamics can only be studied in the fields where they occur naturally, otherwise the results might not be applicable to agricultural practice at all. Therefore, for his population studies on the species mentioned in the title of this lecture, the author did not follow the practices of research on Heterodera species: pot experiments, microplots and a small number of field experiments with a large number of host and non-host crops. Instead of that, a large number of areas about 3 m² in size, were chosen on different fields and here population numbers were measured mostly two times a year for several years in succession. The advantage of this method is that the normal farming practices are followed on the investigated fields, and that as many soil types can be included in the investigations as made possible by the capacity of investigation of the samples. A weak point is that no influence can be exerted on the crops grown, but if on the other hand the experiments are done during a sufficient number of years many crops will have been grown at least a few times in most of the fields. So, at least, the influence of the main crops on the population can be measured adequately. This can be improved if this method is combined with classical field plot experiments.

3. Population dynamics of the stem nematode

The importance of soil type for the behaviour of stem nematodes has been noticed by several authors (DEWEZ 1940, VAN BEEKOM 1940, NIGON and RITTER 1947, SEINHORST 1950, 1956). Their observations can be summed up as follows:

1. Some regions are infested with stem nematode (clay soils, loamy sand and light sand with low humus content, others are not (sandy soils rich in organic material) irrespective of crop rotation.
2. In the infested areas the eelworm is more persistent on the heavy than on the light soils.

In the south western part of the Netherlands it is well known by the farmers that onions on soils containing more than 30% of clay always run a risk of being attacked and that crop rotation seems to be of no importance. On lighter soil onions can be grown, however, once every four years. A shorter rotation carries the risk of stem nematode attack again.

Population studies on more than 30 fields in different areas showed the relation between population dynamics and the above observations (SEINHORST 1956, 1957).

Growing host plants appeared to be relatively unimportant for population fluctuations. Most host crops have no other effect on the population than non-hosts. Onions and rye may cause a considerable increase of the number of stem nematodes in the soil on all soils infested with the onion race or the rye race. Occasionally also peas cause a marked increase in number of the onion race. With other crops, hosts or non-hosts the fluctuations show the same trends:

1. a. On clay soils there is nearly always an increase of population numbers during summer and a decrease during winter except when the degree of infestation is very high.
- b. On heavy clay soil increase in summer balances decrease in winter at degrees of infestation of 10-50 stem nematodes per 500 g. of soil. Higher degrees of infestation nearly always decrease more in winter than they increase in summer.
2. a. On sandy soils generally degrees of infestation over 10 stem nematodes in 500 g. of soil decrease both in summer and winter if no onions, rye or oats are grown.

- b. Below this degree of infestation there is an increase in summer with all crops and a decrease in winter. Increase and decrease balance each other at a degree of infestation between 0 and 5 stem nematodes per 500 g. of soil.
3. a. The mean decrease in number of stem nematodes during winter on sandy soil is more than on clay soil at the same initial degree of infestation.
- b. On some light sandy soils very low degrees of infestation are reached more rapidly than on more loamy types of soil provided no rye is grown.

Onions and many other host plants of the stem nematode are already seriously damaged when some 5-10 stem eelworms are present in 500 g. of soil. Therefore 1. a. explains why on heavy clay soils there is always a serious risk of damage to these crops, whether host plants of the stem eelworms have been grown in previous years or not.

On more sandy loams the persistence level of the stem eelworms is much lower than on the heavy clay soils. Therefore here onions can be grown in a suitable rotation. Once the onions became moderately infested, the degree of infestation of the soil rises to a few hundred stem nematodes in 500 g. of soil and it takes several years before these have died out sufficiently for safe onion growing.

The damage in rye on sandy soil shows some relation to the crop rotation. On the light sandy soil rye can be grown much oftener than on loamy sands which is in accordance with population data mentioned under 3.b.

Population numbers and their fluctuations do not explain all peculiarities in attacks of crops by stem nematodes. So on clay soils crops are attacked practically in all seasons. On sandy soil susceptible cereals are heavily attacked in winter, but only lightly or not at all in spring and summer. Also volunteer plants of rye growing where rye was attacked heavily in the same year have nearly always been found healthy at degrees of infestation of the soil which may have been 20 to 100 times as high as those causing severe damage to autumn sown rye.

Also the weather has been found important. A dry spring reduces the incidence of bloat in onions in Germany (NOLKE 1957) whereas also in the Netherlands dry springs, as the one which occurred in 1947, have this effect.

In laboratory experiments the water content of the soil appeared to be of major importance for the activity of stem eelworms. At moisture contents below field capacity this activity tends to be low (SEINHORST 1950).

After SEINHORST (1950) the activity of stem eelworms in moist clay soil is generally high. In moist sandy soil it fluctuates.

It is always high in soils which have been partly sterilized by heat (even 50° for 30 minutes is effective), ether, chloroform, ethoxy-ethyl mercury chloride and sublimate. Most probably population numbers are influenced indirectly by this temporary inactivity. The inactivating factors do not kill the nematodes however. They become active again when a soil with inactive stem nematodes is treated with ethoxy-ethyl mercury chloride or sublimate (SEINHORST 1950).

4. Population studies on root lesion nematodes

The importance of soil type for the occurrence of root lesion nematodes has already been mentioned.

OOSTENBRINK (1956) investigated population fluctuation of Pratylenchus pratensis and P. penetrans in field experiments and stresses the importance of population build up on host plants like cereals and consequently advises crop rotation as a general practice on sandy soils. From the author's investigations the conclusion may be drawn that the situation is more complicated for both nematodes and because of that, partly, not alarming.

A. Pratylenchus pratensis

Although this nematode is probably of little economic importance its widespread occurrence made it a good subject for population studies. These were done by the author in the same way and largely on the same fields as the work on stem nematodes.

Importance of host crops. Although cereals are hosts of the nematodes they did not cause a large increase in number in any of the fields investigated. Their effect (comparing autumn degrees of infestation) ranged from a distinct decrease to a 2 fold increase of nematode numbers. The effect of non-hosts like potatoes and mangolds ranged from a marked decrease to a slight increase. In some of the fields even continuous cropping with cereals for three years did not increase the population, which in all these cases (on light sandy soil) fluctuated between 50 and 200 specimens per 500 g. of soil. In other fields there was a fluctuation of about the same magnitude but at higher mean levels.

Occasional observations on other fields have revealed that wider fluctuations both in increase in summer and decrease during winter may occur. On the other hand a sampling of ten small areas in each of ten fields in one area revealed that the means of the ten counts in each field showed less variation than the separate counts in each field. As these fields had only in common that they were destined to carry peas this is not very suggestive of a major importance of crop rotation for levels of infestation with P. pratensis in this area.

It is not very probable that the multiplication

potential of P. pratensis on cereals would not be higher than 1 to 3 or 4. Pot experiments with the closely related P. penetrans showed that here an increase of 1 to 20 was possible in about six weeks and 1-200 in less than a year. So it must be concluded that there are factors in the environment, most probably in the soil, which keep the nematode population in check.

The economical importance of P. pratensis has so far not been demonstrated by reliable experiments. However, as seems to be the rule rather than the exception with plant nematodes, this species will, even when crop rotation is most favorable, only reach dangerous levels in part of its area of distribution. It is therefore misleading to generalize from observations on experiments which are done on fields where damage occurs. These fields may just as well be the only ones in existence. Population research must be done over a much wider range of fields including those where less favorable conditions for the nematodes might prevail.

B. Pratylenchus penetrans

Although this nematode is much more important than P. pratensis no extensive studies on population fluctuations have been done. OOSTENBRINK (1956) did some experiments on fields which showed excessive degrees of infestation and found that cereals were good hosts of the nematode although they did not suffer. Mangolds were poor hosts. As these high degrees of infestation only occur in very small areas it is more important to have information about the persistence and increase of P. penetrans under more normal conditions, especially so because some plants (e.g. daffodils) already suffer at low initial degrees of infestation of the soil. In some areas the nematode seems to be very persistent at low degrees of infestation.

Higher numbers come down rapidly, however. Here a situation seems to exist which is comparable to that of the stem nematode. Only a few of the many host plants raise population numbers. So investigation of soil samples from the light sandy areas in the western part of the Netherlands shows that only where strawberries were growing or had been growing very recently somewhat higher numbers of the nematode were found. On all other fields these numbers were very low. Infestation in strawberries and some other host plants occurs very generally in this area.

Like with stem nematodes there seems to be a persistence level which is probably different for different soils and is independent of cropping practices.

5. Hoplolaimus uniformis

As this nematode occurred in all fields investigated on sandy soils, a good amount of information could be collected about its reactions to different crops. The general impression is that:

- A. The general level of infestation is highly dependent on soil type. The nematode prefers light sandy soils. It finds optimum conditions on other soil types than P. pratensis and P. penetrans.
- B. There was hardly an influence of the crops on the fluctuation of population numbers. In certain areas repeated culture of a host crop did not increase the relatively low population. Occasionally high to very high numbers were found among the roots of garden shrubs, and also in some fields. In the latter cases there were no apparent reasons (crop rotation or such) for the local build up. So we must conclude that locally the soil offers suitable conditions for the nematode.

Pot experiments revealed an increase potential of about 1 to 200 in one year. Increases under field conditions seldom exceeded 1 to 2.

The species is very widely distributed and occurs over a large range of soil types, but in some of these only rarely. Nevertheless damage is very limited.

6. Summary and conclusions

The plant parasitic nematodes can be divided into two groups.

- A. Those showing a very close relation to crops rotation in their population dynamics, e.g. Heterodera species, Anguina species.
- B. Those being relatively independant of host crops for their persistence in the soil at a certain level of infestation. This level depends on soil type. Also top levels of infestation and persistence of the nematodes in the soil at higher levels of infestation is highly dependent on soil type (Ditylenchus dipsaci, Pratylenchus spp., and Hoplolaimus uniformis).

In areas of optimum conditions for the nematode the species of group B. show more or less the same behavior towards the growing of host plants as those of group A. As, however, the species of group B. mostly have a very wide host range they also show a high persistence level under such conditions, and the effect of crop rotation on population numbers and damage is limited.

Demonstration of the damaging nature to plants of certain nematode species combined with knowledge of its area of distribution is an insufficient base for evaluation of its importance. It is also necessary to know at what degree of infestation of the soil damage can be expected and in what part of the distributional area such degrees of infestation are likely to be built up under cropping practices favorable to the nematode.

In some cases these areas coincide. This occurs when damage already begins at degrees of infestation just detectable with the present means of investigation. This is the case with stem nematodes, Belonolaimus gracilis, and Pratylenchus penetrans on daffodils, raspberries and probably some other plants, but not on potatoes and many other host plants.

The higher the number of specimens of a certain species required to cause damage the larger the ratio between distributional area and area where damage occurs will be.

7. Methods for quantitative investigations on Heterodera populations.

Population studies on Heterodera species either in relation to crop rotation or chemical control have for a long time suffered from inadequacy of methods for measuring the populations. It was not until FENWICK (1940) devised a good method for the extraction of cysts from soil that an accuracy of cyst counts up to the standard required for experiments could be reached. The method based on the fact that dry cysts float is adequately described by GOODEY (1957). However, cyst numbers only still do not mean very much. The only reliable measure of degree of infectivity is the number of hatchable eggs in these cysts. Older research workers helped themselves out with dividing the cysts in empty and filled ("viable") cysts. Others estimated the number of eggs in the cysts either directly or after squashing (OOSTENBRINK 1950). The error involved in all these methods was still tremendous. Estimations could be as far off the truth as being only 1/3 of the actual number of eggs present.

The number of viable larvae present in a certain number of cysts is now estimated in two ways:

- A. Counting the number of eggs with larvae present in the cysts.
- B. Determining the number of larvae which can be induced to hatch from the cyst.

Direct counting of eggs. The use of direct counts of eggs as an estimate of infectivity is based on the assumption that the actual number of infective larvae is not much lower than the total number of full eggs. Hatching experiments show that this is the case when no chemical treatment has been applied to the soil.

In order to get a reliable count of eggs, these must be released from the cyst and brought into suspension in water so that their number in aliquots of this suspension can be counted. The best way to do this is to squash and rub the cysts to free the eggs and get them apart, and wash these eggs and the cyst wall debris in a measuring cylinder with water.

The squashing and rubbing can be done in different ways. The simplest is to rub the cysts between two glass slides. For certain species this is quite sufficient. In others the eggs stick together too much. Better results are obtained than by rubbing them with a glass bar on a channelled metal plate (see GOODEY 1957).

A still better method, although it requires a little more machinery was developed by BIJLOO (1955). The axle of a small vertical motor is elongated by a rod to which a 1 cm. wide and about 5 cm. long plastic cylinder is attached. The cysts to be investigated are brought with a little water in a 2 cm. wide thin walled plastic centrifuge tube after having been soaked in water for some hours. The centrifuge tube is then slid over the turning plastic rod (about 900 revs./min.) and gently pressed against it. The cysts are now squashed between the rod and the wall of the tube and the eggs are rubbed apart. The whole operation takes about half a minute.

Hatching tests. In both Heterodera schachtii and H. rostochiensis larvae in the eggs react to substances diffusing from the roots of host plants by hatching, whereas in plain water most larvae remain quiescent in the egg.

The stimulating effect of root diffusates was already detected by BAUNACKE (1922) but the technique of hatching experiments has been worked out mainly by FENWICK (1950, 1951).

The mean hatching time is different for eggs of different origin and the individual hatching times are distributed more or less normally. This means that no fixed hatching times should be used but that the experiment should be continued until no material increase of the total number of larvae hatched from the eggs is found anymore.

The root diffusate. An important element in hatching tests is the root diffusate, which should contain the chemical compound in sufficient concentration. The only way to measure this concentration is by the effect of the solution on eggs of the Heterodera species, which is capable of attacking the plant from which the diffusate was collected.

For the production of root diffusate plants are grown in sand or soil in pots. When roots have been formed all through this soil the diffusate can be extracted by saturating the pots with water and collecting the solution that drips out of the pots.

FENWICK (1952) devised a method to compare the relative concentrations of root diffusates of different origins. This is done by exposing cysts to dilution series of these diffusates. The dilution at which the number of larvae hatching from the cysts does not exceed the number hatching in water is a measure for the strength of the hatching agent in the original solution. If larval emergence is plotted against log dilution a straight line is obtained. The intersection

of this line with the line indicating the level of hatching in water can be determined accurately. FENWICK indicates the concentration of the hatching agent by the so called L.A. (log activity) value. This is the logarithm of the strength of the original solution compared to the strength at which no activity of the hatching agent is noticeable any more. So if the level of the "water hatch" is reached at a concentration of $\frac{1}{1000}$ of the original solution the latter has an L.A. value of 3, which is a very good concentration for experiments.

The actual hatching test is done by immersing a sufficient number of cysts of the population to be tested in root diffusate and renewing the latter once or twice a week. The degree of variation among numbers of larvae hatching per cyst is tremendous. Therefore at least 200 cysts should be investigated at a time.

The test is continued until no material hatching of larvae is found anymore.

Eighty to ninety percent of the larvae in cysts of Heterodera schachtii and H. rostochiensis kept under natural conditions will hatch in root diffusate of sufficient concentration. The remaining 10%-20% are not all dead. They may hatch in due time.

Hatching tests are particularly suitable for comparison of viability of cysts which have undergone chemical treatment.

However, such a treatment may not only have killed the eggs, it can also delay the beginning of hatching for several weeks (FENWICK).

Hatching agents have not been demonstrated for all Heterodera species. No effect of host root diffusates was found with H. major, H. goettingiana and H. glycines. In such cases only amount of root invasion is a reliable measure for numbers of infective larvae in the soil. No stimulating substances may be present in these cases or they may be broken down in the soil so rapidly that extraction is impossible.

Distinguishing living and dead larvae by staining. HOMEYER (1953) and v.d. LAAN and BYLOO (1955) investigated the effectiveness of staining with acridin orange in distinguishing living from dead nematodes. It appeared impossible to distinguish nematodes killed or moribund by the action of a nematicide from untreated living nematodes with a sufficient degree of accuracy.

Determination of viability of Heterodera larvae by direct observation of larvae released from the egg mechanically. STANILAND (1956) found that if larvae were released from well soaked eggs by cutting the egg membrane with a fine knife the shape of the larva after leaving the egg was a good indication of whether it was alive or dead. The method deserves attention for those cases where hatching tests are too troublesome or impossible because of absence of stimulating substances.

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