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The influence of increasing amounts of nitrogen on the propagation of the cereal cyst nematode, *Heterodera avenae* Woll.

Stigende mængder kvælstofs indflydelse på havrenematodens opformering

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Summary

The effect of increasing amounts of nitrogen on the propagation of the cereal cyst nematode was studied in a series of experiments. Nitrogen applications as nitro-chalk ranged from 0-1500 kg per ha. (0-232.5 kg N per ha.). The main results are:

Maximum propagation was obtained at about 46.5 kg of N per ha. Increasing N-amounts reduced it to about ½-⅓ of maximum.

Two factors are involved:

1) reduced fertility of females with increasing N-applications and
2) fewer females were apparently developed with N-applications larger than 46.5 kg per ha. – evident after a year.

Hatching in solutions of $Ca(NO_3)_2$ decreases with increasing concentration. There was an indication that, when removed to N-free solutions, the hatching was equal.

It was further indicated that fewer larvae would invade the roots with increased N-application.

Resumé

Virkningen af stigende mængder kvælstof på havrenematodens opformering er undersøgt i en række forsøg. Der er anvendt kalksalpeter i mængder fra 0-1500 kg pr. ha. (0-232,5 kg N pr. ha.). Følgende hovedresultater er opnået:

Ca. 46,5 kg N pr. ha giver maximal opformering af havrenematoden. Ved øgede N-mængder opnås en reduktion til ½-⅓ af det maximale.

Bl.a. to faktorer spiller ind:

1) Nedsat frugtbarhed hos hunnerne ved stigende N-mængder, samt
2) udvikling af et tilsyneladende færre antal hunner ved N-mængder på over 46,5 kg pr. ha. Denne virkning bliver åbenbar efter 1 års forløb. Klækning af larver i opløsninger af $Ca(NO_3)_2$ aftager med stigende koncentration.

Der er indikation for indtrængen af et færrre antal larver i rødderne ved øget kvælstofgødskning.

Contents

Introduction

The experiments described in this paper were conducted at The State Plant Pathology Institute, Zoology Department, the objective being to elucidate the influence of increasing amounts of nitrogen – especially nitro-chalk – on the propagation of cereal cyst nematodes.

The author wants to express his sincere thanks to the Norwegian Hydro Foundation for the financial support received, contributing to the carrying through of these experiments.

Abstracts from literature

In a five years experiment with oats, barley, wheat, and rye in fertilized as well as unfertilized plots, *Hesling* (1959) found almost invariably the most severe attacks of cereal cyst nematodes in the fertilized plots and concluded that while applications of fertilizers may increase the yield, such applications may, at the same time, give rise to an increase of the nematode population. In the experiment an amount of 35.1 kg N per ha. was applied.

Andersen (1963) states that when cereal nematodes are, for some time, exposed to an ammoniacal atmosphere, their hatching will be affected, the period of treatment as well as the concentration of the ammonia solution being factors of importance. Further, *Andersen* (1967) states that heavy nitrogen applications, eventually combined with irrigation, if any, may to some degree weaken the attacks of cereal cyst nematodes, but he finds that, in case of severe attacks, this procedure is not without side effects as the propagation of the nematodes is increasing with the vigorous growth of the plants. Further, he mentions that liquid ammonia acts partly as a N-fertilizer and partly as a toxic chemical, the nematodes being killed if getting close enough. Andersen concludes that the distance between the injection knives must be reduced to 10 cm and that the amount injected must be increased to 225 kg per ha. If so, there would, in his opinion, be a change of killing 30 per cent of the nematodes.

Further may be mentioned a paper published by *Williams* (1969) on experiments over 3 years, among other things, treatments with formalin and increasing amounts of nitrogen applied to spring wheat on soils infected with cereal cyst nematodes, *Heterodera avenae,* and take-all, *Ophiobolus graminis.*

Through formalin treatments, he obtained, compared with control, a doubling of the total yield over three years, but found that the number of nematodes was 8 times that of control. When N-amounts of 75, 150, and 226 kg per ha. were applied, the yield rose from 66.4 to 94.6 hkg per ha. over three years taken together (the same yield level as that obtained by formalin treatment) whereas the number of nematodes only rose from 17 to 24 when calculated as number per g of soil.

Own investigations

Methods and results

As a preliminary investigation a pot experiment in greenhouse was carried out in 1964 with oats grown in nematode-infested soil, 4 treatments: 300, 600, 900 and 1200 kg nitro-chalk per ha. being given calculated per ccm soil at a depth of 20 cm. Further, all treatments were given superphosphate and potash (50 per cent) corresponding to 300 kg per ha. of each. 5 replicates per treatment. According to the same method, a corresponding experiment was made with increasing amounts of potash. The result will be seen in table 1.

Table 1. Results from preliminary pot experiments in greenhouse 1964.

Treatment	Mean number of cysts per pot	Mean number of cysts per ccm of root volume	Mean root volume per pot, ccm
nitro-chalk			
300 kg/ha	213	343	0.86
600 » »	205	218	0.83
900 » »	96	151	0.88
1200 » »	62	71	0.94
potash			
300 kg/ha	201	269	0.73
600 » »	212	287	0.92
900 » »	36	89	0.40
1200 » »	25	59	0.43

Microplot experiments

In the 1965-72 period, microplot experiments were made with increasing amounts of nitro-chalk applied to oats grown in nematode-infested soil. The experiments were carried out in 6 concrete pipes with an inside diameter of 1 metre dug into the soil. The soil used originated from a locality on the Institute's own grounds formerly infested with cereal cyst nematodes. The thoroughly mixed soil constituted the uppermost 20 cm of soil in the pipes. In all the years, the basal dressing in the microplot experiments was: 600 kg 0-4-20 NPK per ha. and 50 kg manganese sulphate per ha. (in 1965, however, only 25 kg manganese sulphate per ha.). Each year, 15 g of Sun II oats were used per plot (200 kg per ha.).

In 1965, the following N-amounts were applied: 0 – 46.5 – 93.0 – 139.5 – 186.0 and 2 × 93.0 kg per ha. In 1966, the N-amounts were as follows: 0 – 2×23.25 – 2×69.75 – 2×93.0 and 186.0 kg per ha. In cases with two applications, the first half of the amount was applied at sowing time, the second half one month later.

In 1965 as well as in 1966, the nematode propagation was determined by counts of the number of cysts from roots plus adhering soil of 50 plants from the middle of the microplots. It was found that divided applications of nitrogen seemed to have the greatest nematode-reducing effect.

In 1967, the microplot experiment was transferred to six new plots. The soil from the microplots used so far was collected, mixed, and transferred to the new plots. Since then, the experiment has been carried on in these microplots. The initial cereal cyst nematode population in the new microplots was determined on the basis of soil samples of about 250 ccm taken from each microplot in the spring of 1967 (25-30 core samples being taken per microplot). Onwards, soil samples of 250 ccm each per microplot were taken 2 weeks after harvest and immediately after thorough digging. After being dried at 60°C., the soil samples taken weighed 160-200 g. From 1969 and onwards, all samples were dried at 60°C., and 150 g dry soil was weighed out for the determination of the number of cysts therein. The cysts were sorted as empty cysts, including cysts with totally parasitized contents, and sound cysts, the so-called »full« cysts. The number of eggs and larvae from the »full« cysts was determined with due regard to the contents, if any, of parasitized eggs and larvae.

Since 1967, 6 nitrogen levels have constantly been used, namely: 0 – 46.5 – 93.0 – 139.5 – 180.0 and 232.5 kg N per ha. in the form of nitro-chalk. These amounts were divided into halves, the first half being spread at the time of sowing, the second half one month later. The microplots were marked 1-6 in the said

order. For three years, 1967, 1968 and 1969, the order was that stated, but in 1970 it was changed in such a way that from that year and onwards, the largest amount of nitrogen was applied to the microplot to which no nitrogen had been applied before and so on, until the microplot having got the largest application received no nitrogen.

The soil treatment, basal dressing, sowing, and spreading of the first half of the nitrogen amounts were, each year, carried out on the same day.

The influence of the nitrogen amounts on the nematode population in the microplot experiments over the years 1967-72 is shown in fig. 1.

Yield in microplot experiments

From 1968 and onwards, the microplots were covered with net whereby it was possible to ascertain the yield. The yield results from the microplot experiments are given in the below table 2.

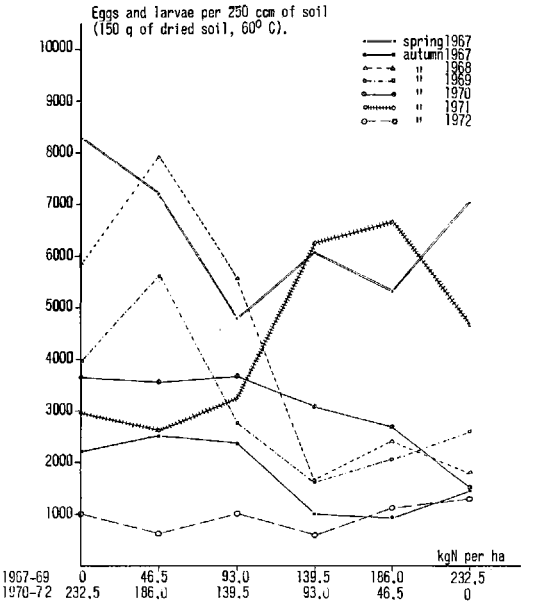

Fig. 1. Microplot experiment with increasing amounts or nitro-chalk to Sun II oats grown on cereal cyst nematode infested soil, 1967-72. Eggs and larvae per 150 g of dried soil (60 °C).

Table 2. Microplot experiments. Yield figures from each of the years 1968-72

Microplot Nos.		1	2	3	4	5	6
kg N/ha.		0	46.5	93.0	139.5	186.0	232.5
Yield of grain	1968*) ...	203.5	229.0	248.0	248.0	282.4	273.5
hkg/ha.	1969	23.6	38.6	47.7	52.6	55.5	52.2
kg N/ha.		232.5	186.0	139.5	93.0	46.5	0
Yield of grain	1970	29.6	39.6	38.3	39.4	30.9	21.3
hkg/ha.	1971	38.6	39.4	34.6	37.7	26.2	12.9
	1972	45.3	64.5	47.6	44.0	33.3	14.4

*) Weight of straw and grain on the date of harvest.

Greenhouse experiments

As a supplement to the microplot experiments, a series of greenhouse experiments was started in 1967, in which oats were grown in river sand in PVC pipes with cysts of cereal cyst nematodes added, increasing amounts of nitro-chalk being applied. The PVC pipes used had a height of 20 cm and a diameter of 5 cm. 100 kg/ha. corresponds to 0.00005 g/ccm – calculated for a depth of 20 cm. The treatments consisting of 10 PVC pipes in 1967 were placed in plastic-lined compartments. All subsequent experiments exclusively comprised treatments consisting of 6 PVC pipes placed in plastic bags. The infection material consisted of 20 cysts per pipe, placed at a depth of 5 cm in vertically placed microchambers: perspex rings filled with sand and sealed with nylon nets, height about 5 mm, inside diameter about 8.5 mm.

Superphosphate, potash, and manganese sulphate were given as basal dressing in all greenhouse experiments. Each treatment in the 1967 experiment was given 0.42 g superphosphate corresponding to 18 kg P per ha., 0.42 g potash, corresponding to 93.4 kg K per ha.,

Photo showing a greenhouse experiment (M.J.).

Microplot experiment on June 27, 1972 (M.J.).

Microplot experiment, harvest on August 12, 1969. Left to right: 0-232.5 kg N/ha. (M.J.).

Field experiment on June 27, 1972 (M.J.).

and 0.04 g manganese sulphate, corresponding to 22 kg $MnSO_4$ per ha. In later experiments, each treatment was given a basal dressing consisting of granulated superphoshate/potash (0-4-20), corresponding to 24 kg P and 120 kg K per ha., and 0.035 g manganese sulphate, corresponding to 30 kg $MnSO_4$ per ha. Nitrogen in the form of nitro-chalk was given in these experiments in amounts from 0.117 g (in 1967: 0.14 g) to 1.404 g (in 1967: 2.10 g) per treatment, corresponding to 23.3 (in 1967: 11.1) and 186.0 (in 1967: 189.7) kg N per ha., respectively. The basal dressing and the varying amounts of nitro-chalk were applied in solutions supplying the same amount of liquid to alle treatments within one experiment.

The results from the greenhouse experiments will be seen from the tables and figures below.

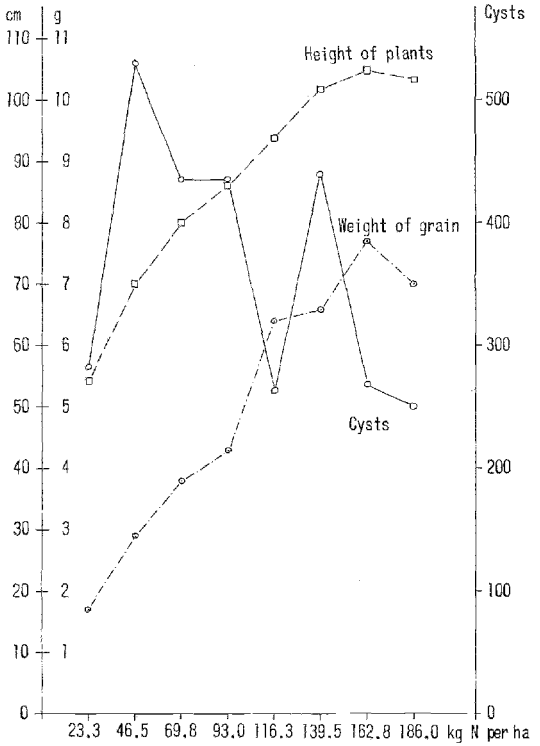

Fig. 2. Greenhouse experiment I, 1969 (23/5-14/8) *Heterodera avenae*. Sun II oats grown in sand in PVC pipes at increasing amounts of nitrogen. Height of plants, weight of grain and number of cysts per 12 plants.

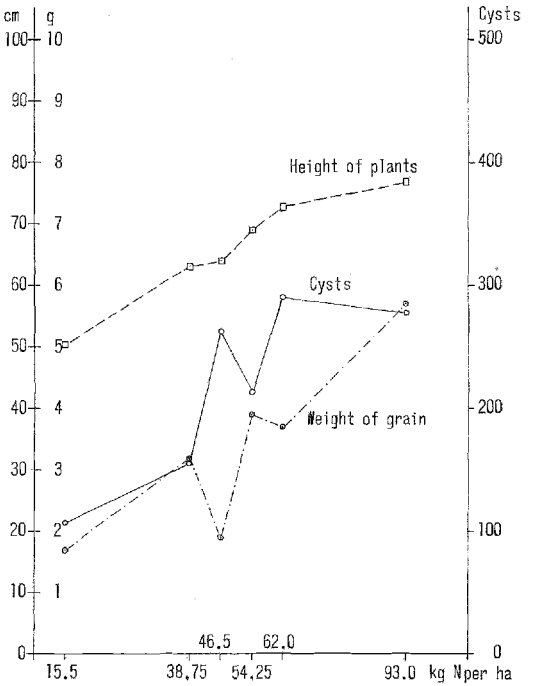

Fig. 3. Greenhouse experiment II, 1969 (29/5-19/8) *Heterodera avenae*. Sun II oats grown in sand in PVC pipes at increasing amounts of nitrogen. Height of plants, weight of grain and number of cysts per 17 plants.

Table 3. Greenhouse experiment 1967 (6/7-22/9) Heterodera avenae. Oat variety 1359 grown in sand in PVC pipes with increasing amounts of nitrogen

Experimental units Nos.	1	2	3	4	5	6
Nitro-chalk, g	0.14	0.42	0.84	1.26	1.68	2.10
kg N/ha.	11.1	34.9	69.8	104.6	139.5	189.7
Mean height of plants, cm ..	42	51	71	93	71	62
Number of cysts per 24 plants	66	152	80	54	40	30
Proportional	43	100	53	36	26	20

Table 4. Greenhouse experiment 1968 (30/10-68 – 5/2-69) Heterodera avenae. Sun II oats grown in sand in PVC pipes at increasing amount of nitrogen

Experimental unit Nos.	1	2	3	4	5	6	7	8
Nitro-chalk, g	0.177	0.351	0.525	0.702	0.879	1.056	1.227	1.404
kg N/ha.	23.3	46.5	69.8	93.0	116.3	139.5	162.8	186.0
Mean height of plants, cm	43	58	61	67	75	59	43	45
Number of cysts per 12 pl.	191	197	192	40	16	5	2	12
Proportional	97	100	97	20	8	3	1	6

Table 5. Greenhouse experiment 1970, Heterodera avenae, Tylstrup populations. Oat variety 1359 grown in sand in PVC pipes at increasing amounts of nitrogen. Race I: 8/6-16/10; race II: 23/6-23/10

Experimental unit Nos.		1	2	3	4	5	6	7	8
Nitro-chalk, g		0.177	0.351	0.525	0.702	0.879	1.056	1.227	1.404
Kg/N/ha		23.3	46.5	69.8	93.0	116.3	139.5	162.8	186.0
Mean height	race I	43.7	53.1	61.8	66.2	72.1	70.7	74.3	71.3
of plants, cm	race II	41.8	48.7	64.4	61.9		71.7		
Number of cysts	race I	45	292*)	430	375	350	266	287	304
per 18 plants	race II	84	922	1194	852		853		

*) The cysts from a single pipe lost. The number found, increased with 1/5.

Field experiments

In the years 1969-72, a permanent field experiment was carried out at Lyngby on a locality with cereal cyst nematodes where oats have been grown for several years. The experiment was carried out on 3 × 12 plots (2 × 2 metres) and comprised 6 nitrogen levels: a = o, b = 46.5, c = 93.0, d = 139.5, e = 180.0, and f = 232.5 kg N/ha. with 6 replicates placed according to the knight's move system.

Each spring, 500 kg P-K fertilizer per ha. was applied as basal dressing. Nitrogen was applied in the form of nitro-chalk, the amount being halved and the first half applied shortly after sowing, the second half one month later. In 1969, however, the first half was only applied 28 days after sowing. Sun II was used as host plant, 200 kg/ha. being sown. Soil samples were taken in spring and in autumn to be subjected to the procedure described under microplot experiments. The experiment was covered with net.

Table 6. Field experiment. Number of eggs and larvae of Heterodera avenae per 150 g of dried soil (60 °C). Mean of 6 replicates

Year	Plots	a	b	c	d	e	f
	Kg N/ha.	0	46.5	93.0	139.5	180.0	232.5
1969	Spring*)	945	728	698	586	704	682
1969	Autumn	474	1338	1798	1574	2054**)	741
1970	Spring	331	473	747	689	435	415
	Autumn	1589	2595	1605	1616	1596	861
1971	Spring	2478	2239	1529	1150	1456	649
	Autumn	4035	4356	3302	2313	2088	1278
1972	Spring	3250	2606	2988	1943	1023	1345
	Autumn	2716	3676	2714	1791	1779	1370

*) Samples taken one month after sowing.

**) The high mean number is caused especially by the e_2-plots with 6915 eggs and larvae. Remainder e-plots: 3210, 620, 890, 390 and 300.

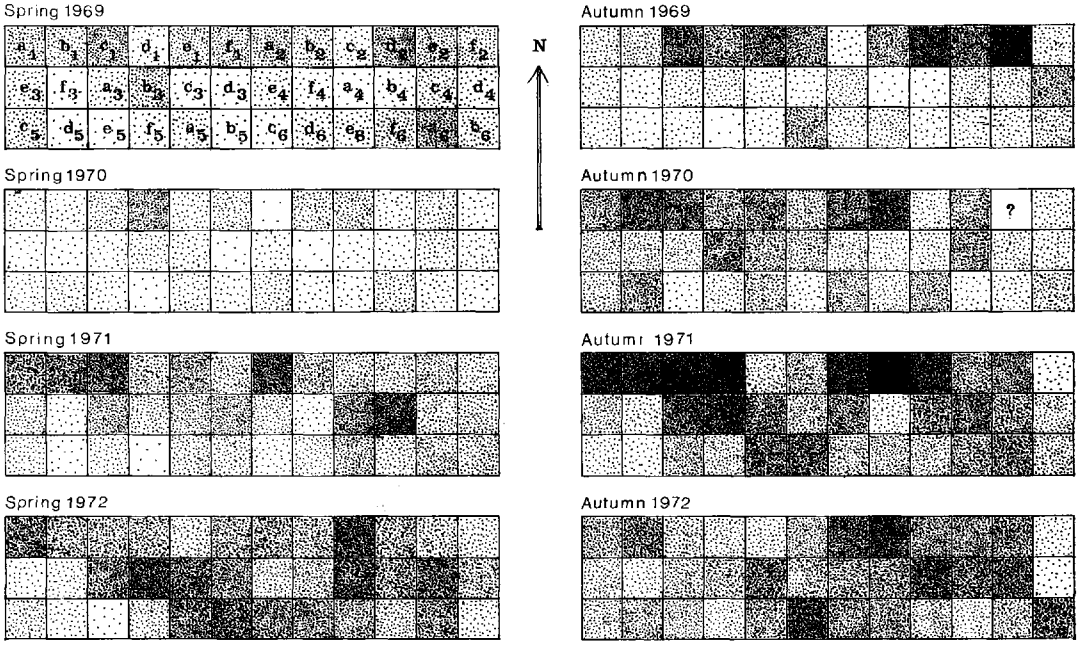

Fig. 4. Field experiment 1969-72 with increasing amounts of nitro-chalk to Sun II oats grown on cereal cyst nematode infested soil.
Plot distribution and number of eggs and larvae for each plot per 150 g of dried soil (60 °C) in spring as well as in autumn. One dot = 10 eggs and larvae. Plot size 2 × 2 m.

The placing of the plots and the degree of soil infestation in the spring and the autumn in each of the four years of experimentation are shown in fig. 4.
The yields of the individual years and the corresponding mean soil infestation per treatment after harvest will be seen from fig. 5 below.

The influence of increasing amounts of nitrogen on the cyst contents

The microplot and field experiments have shown that not only the actual number of eggs and larvae, but also their number per cyst, are being reduced when increasing amounts of nitrogen are being applied.

During the investigations, empty and totally

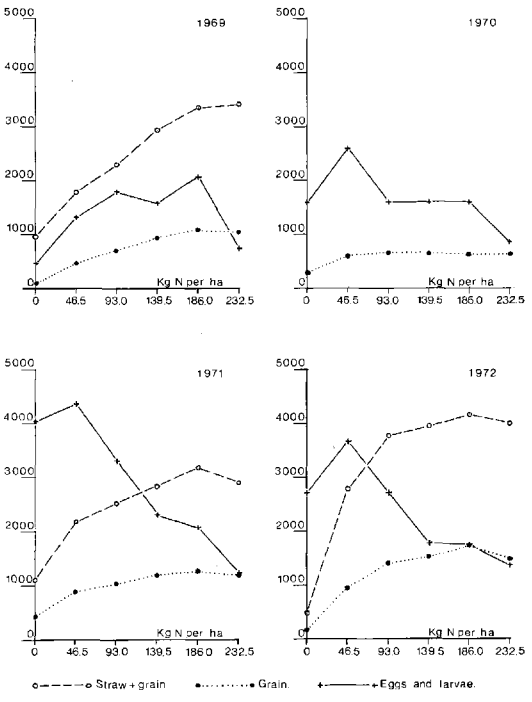

Fig. 5. Field experiment 1969-72 with increasing amounts of nitro-chalk to Sun II oats grown on cereal cyst nematode infested soil.
Yields in grammes and eggs and larvae in autumn per 150 g of dried soil (60 °C). Mean of 6 replicates 2 × 2 m.

parasitized cysts were counted and sorted out. The number of eggs and larvae per cyst was consequently determined as the number per 'full' cyst or, more correctly, per cyst containing potential infestation material.

The number of 'full' cysts per treatment was likewise influenced by the application of increasing amounts of nitrogen; however, this reaction does not seem to be evident within the first year.

The proportional numbers of eggs and larvae in the cysts, the number of 'full' cysts, and the degree of soil infestation after the various treatments in microplot and field experiments are shown in fig. 6 below.

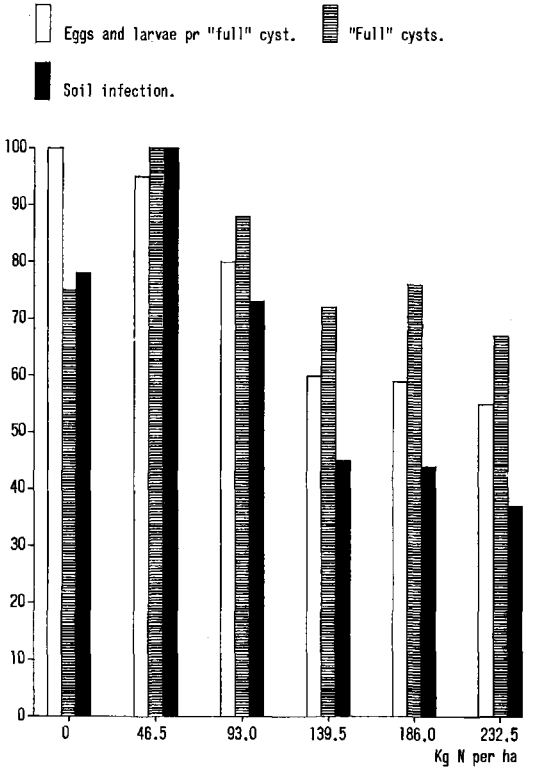

Fig. 6. *Heterodea avenae*. Proportional numbers of 'full' cysts, eggs and larvae per 'full' cyst and soil infestation after growing of Sun II oats at increasing amounts of nitrogen. Mean of microplot experiments 1967-69, 1971-72, and field experiments 1970-72.

Nematode investigation concerning experiments with increasing amounts of nitrogen

At the 'Askov' Experimental Station, experiments have been carried out with application of increasing amounts of nitrogen in a crop rotation comprising barley, oats, broad beans, swedes, and beets. The experimental design prescribed an interval of 6 years between oat crops in the same plot. The amounts of nitrogen used were applied either as nitro-chalk or as liquid ammonia with 30 cm between the chords, placed at a depth of 14 cm.

On account of the occurrence of pronounced symptoms of cereal cyst nematode attacks, 1 plant sample per treatment was taken from the oat plots towards the end of June, 1969, for nematode examination. From the ammonia plots, which showed a distinct difference in the development of plants above and between the ammonia chords, samples were taken above as well as between the chords. Out of each sample, 4 plants were examined for cysts on the roots and for larvae in the roots. The result is given in table 7.

After harvesting, soil samples from 4 oat plots per treatment were examined for contents of cereal cyst nematodes. The result is given in fig. 7.

Hatching of larvae in calcium nitrate solutions

With a view to making a special investigation of the influence of calcium nitrate on the hatching of cereal cyst nematode larvae, a laboratory experiment was initiated in 1971 on the hatching of larvae in a series of molar concentrations of $Ca(NO_3)_2$, H_2O in the form of nitro-chalk dissolved in distilled water. It must be considered to be of the greatest interest if the concentrations used comprise the amounts used in practise. It was found that about 46.5 kg N per ha, corresponds to an 0.003 M solution of calcium nitrate. At the preparation of the stock solution of 0.1 M, 186.6 g nitro-chalk was reckoned with per 1.0 M solution. 10 concentrations ranging from 0.001 to 0.01 M were used. The cyst material originated from 'Tylstrup' populations of cereal cyst nematode races

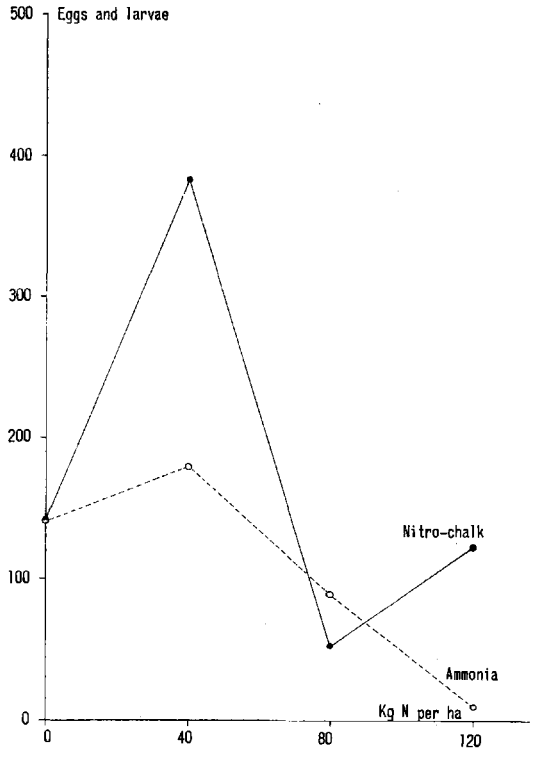

Fig. 7. Number of eggs and larvae of *Heterodera avenae* in 150 g of dried soil (60 °C) after harvest of oat plants from an experiment with increasing amounts of nitrogen. Mean of 4 plots.

I and II. In each treatment, 20 cysts were used, filled into sand-filled microchambers like those described under greenhouse experiments. For this purpose, the rings were provided with yet another perspex ring at the bottom whereby the upper net was raised above the surface of the liquid in the Syracuse watch glasses where they were placed during the whole experimental period. The experiment comprised two series of race I and one series of race II. Hatched larvae were counted at intervals of 3-4 days. At the same time, fresh solution was added. Apart from the periods in which the larvae were counted, the material was kept at 20°C in climate box during the whole of the experimental period.

The experiment carried out in the period from May 28 to August 13 showed a tendency towards reduced hatching with increasing concentration of calcium nitrate.

After the completion of the experiment, the cyst material just used was kept in perspex rings at 4°C for well over 2 weeks and then for 1 week at room temperature (Juhl, 1968); thereafter, hatching was started again but this time in distilled water.

The result reckoned as the sum of the 3 series of race I and II and, further, at the sum of the original 5 lowest and 5 highest concentrations, is shown in table 8.

A minor hatching experiment instituted after the preceding temperature treatment of the cyst material described above and comprising the concentration already mentioned confirmed the tendency towards decreasing hatching with increasing concentrations of calcium nitrate.

Table 7. Numbers of cysts and larvae of Heterodea avenae from the roots of 4 oat plants from a nitrogen experiment at the end of June 1969.
(Ammonia placed in a depth of 14 cm and with 30 cm's between the chords)

N-fertilizer	kg N/ha.	cysts	larvae	larvae/cysts
Control	0	75	98	1.31
	40	23	51	2.22
Nitro-chalk	80	53	12	0.23
	120	21	19	0.90
	40	100	64	0.64
Ammonia – over the chords	80	47	6	0.13
	120	29	6	0.21
	40	77	139	1.81
Ammonia – between the chords	80	71	117	1.65
	120	28	18	0.64

Table 8. Heterodera avenae. Hatching in dist. water during 10/9-8/2 from cysts previously subjected to varying molar concentrations of $Ca(NO_3)_2$

	Molar concentration of $Ca(NO_3)_2$ at previous hatching									
	0.001	0.002	0.003	0.004	0.005	0.006	0.007	0.008	0.009	0.01
Number of hatched larvae	413	206	337	152	188	160	232	286	210	365
Average number			1296					1256		

Experimental period: September 20-February 7.

In 1972, a hatching experiment was set up, comprising 14 series of race I as well as race II started at intervals of one week; the first series was started on March 21, the last one on June 20. The cyst material used was 'Tylstrup' populations, which were kept in climate box at 15°C during the whole experimental period. All hatching series had an experimental period. of 13 weeks. Each series contained 20 cysts per treatment. The result of the aggregate hatchings of alle 14 experimental series of race I and race II respectively as a function of the molar concentration is shown in fig. 8, wheras the total hatching of the same series as a function of the starting time is shown in fig. 9. A total of 121.339 larvae were hatched of race II and 107.591 larvae were hatched of race I, or 88.7 per cent of the number of race II larvae.

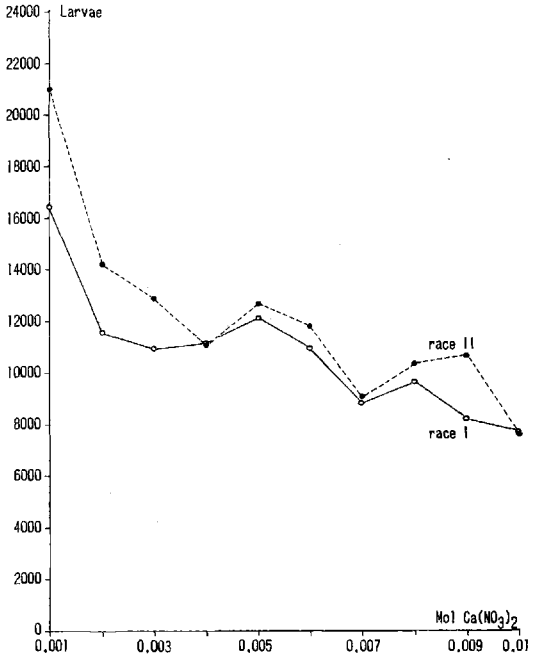

Fig. 8. *Heterodera avenae.* Total hatch through 13 weeks from 14 series of race I and II in different molar concentrations of $Ca(NO_3)_2$.
20 cysts/conc./series.

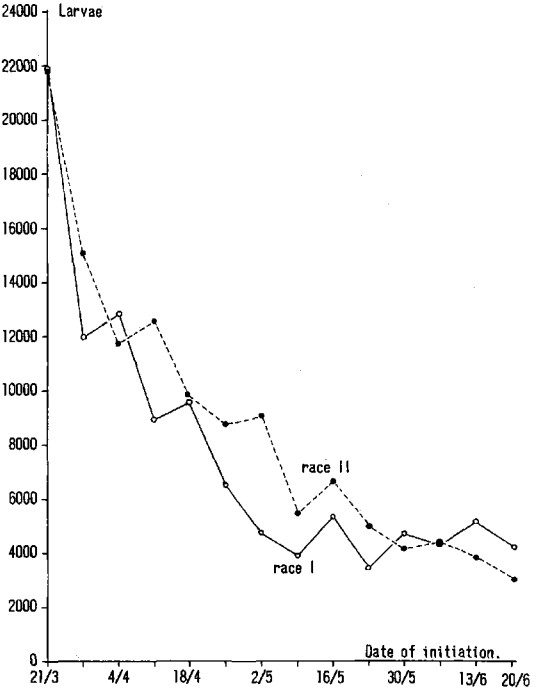

Fig. 9. *Heterodera avenae.* Total hatch during 13 weeks for each of 14 hatching series of race I and II as a function of the date of initiation. 200 cysts per series. Interval: 1 week.

Discussion

The preliminary investigation made in 1964 (table 1) showed so heavy decline in the nematode population with applications of increasing amounts of nitrochalk that research on this problem was continued. Likewise, the potash treatment gave good results, but also an unstable growth of the plants and therefore any further potash treatments were given up.

During the first two years, microplot experiments indicated that a divided application of nitro-chalk was preferable. The method used for the determination of the number of cysts in the microplot experiments made in the said two years was highly unfavourable relative to the results, this method involving that in connection with increasing root amounts it is necessary to examine a larger amount of soil from the rhizosphere as well.

From 1967 and onwards, the number of nematodes in microplot experiments as well as in the field experiments started in 1969 was determined from soil samples of about 250 ccm each or, more precisely, from 150 g of the samples dried at 60°C.

In the microplot experiments, the order of the nematode populations according to numbers was found to have changed after the harvest in 1967. The largest population was found where the application had been 46.5 kg N per ha.; the lowest population figures were found in connection with the largest doses (fig. 1). The same relationship was to appear during the following two years, only to a more pronounced degree.

After 3 years with the same univocal results, which, after all, derived from the same 6 microplots, it was interesting to put the question whether these results could be managed through the application of N-fertilizer. Therefore, from 1970 and onwards, it was decided to apply nitro-chalk to the same microplots in reverse order.

As will be seen from fig. 1, a smoothing out of the cereal cyst nematode population graph after that year's harvest was ascertained, however, so that the minimum figures were still to be found where the largest amounts of nitrochalk had originally been applied. But already after the harvest in 1971, the population graph had become a reflected image of the graph representing the years prior to the reversal of the doses. Once more the maximum population is found in connection with the application of 46.5 kg N per ha., and the size of the populations decreases with increasing N-amounts.

Out of the 4 years of field experiment, the last 3 years have shown the greatest average soil infestation in the treatment given 46.5 kg N per ha. (table 6). In each of the 3 years this has been so in at least 4 out of the 6 replicates in the said treatment, but not the same 4 replicates in each year. In the first year, only 3 of the replicates in treatment b (46.5 kg N per ha.) showed maximum soil infestation. As already mentioned under Methods and results, the e_2-plot of that year caused the results to deviate from the other results obtained. The possibility cannot be ruled out that a 'pocket' with a very great number of nematodes may have been hit. Unfortunately, the e_2-sample taken after the 1970 harvest was lost, but the number counted in the spring of 1970 is not alarmingly high.

As, in the field experiment, soil samples were taken in spring as well as in autumn, it is possible to follow what happens in the period from harvest to sowing. During such periods, the nematodes, deprived of their host plants, are exposed to attacks from their natural enemies and, further, soil treatment may spread and move them. As will be seen from fig. 4, a certain spreading and movement of the nematodes may be perceptible and, likewise, a reduction of the soil infestation in the winter months can be seen. Presumably, this reduction must, to a large extent, be ascribed to the existence of their natural enemies, maybe fungi in particular. Relative to fig. 5, showing yield levels and nematode populations with reference to the field experiment, it should be mentioned that the summer of 1970 vas very dry.

It has been demonstrated that increasing amounts of nitrogen affect the cyst contents

too. The number of eggs and larvae per 'full' cyst has proved to be decreasing with increasing amounts of nitrogen applied. The number of 'full' cysts per treatment reaches maximum figures where 46.5 kg N per ha. has been applied, decreasing with increasing amounts of nitrogen. It seems, however, that it takes about 12 months for the last-mentioned reaction to become evident. The reduction in the number of eggs and larvae per 'full' cyst is not far from that found by Dropkin and Boone (1966), and that found for root knot nematodes by Turligina (1962).

The number of 'full' cysts and the number of eggs and larvae per 'full' cyst constitute the two factors that, taken together, give the immediate picture of total soil infestation. The dependence of these three figures on the nitrogen level is shown in fig. 6. In connection with this graph it should be noted that the sparsely developed roots at 0 kg N per ha. will reduce the number of 'full' cysts and, consequently, the total soil infestation despite the fact that the number of eggs and larvae per 'full' cyst is at a maximum. Where 46.5 kg N per ha. has been applied, the number of eggs and larvae per cyst is decreasing, but the roots have now become so highly developed that they may accomodate a greater number of cysts. This development combined with the fact that the number of eggs and larvae per cyst has as yet been but slightly reduced results in maximum soil infestation. With the application of larger amounts of nitrogen, the number of eggs and larvae per cyst as well as the number of cysts will decline. In total, this results in a soil infestation which, with the application of 186.0 kg N per ha., is reduced to 44 per cent, and with 232.5 kg N per ha., to 37 per cent of the maximum soil infestation at 46.5 kg N per ha. The results found by Hesling (1959) were probably due to his application of only 31.5 kg N per ha., i.e. at a level of maximum propagation.

Hatching experiments with application of calcium nitrate solutions have revealed yet another factor, which may very well be of essential importance to the level of soil infestation in the field. It has been demonstrated that the hatching of larvae decreases with increasing concentration of calcium nitrate (fig. 8) and, further, that the greatest decrease in the number of hatchings is obtained through the application of the lowest concentration of those used. Considered as a function of the time at which the hatchings started, the greatest number of hatchings was observed in the series first started. Especially in the following series started, the decrease was highly pronounced. On the whole, hatchings were halved during the first two weeks, but had not yet stopped at the end of the experiment (fig. 9).

In the greenhouse experiments it was demonstrated that, when oats were grown in pure sand (river sand) infested with cereal cyst nematodes, it is possible, by means of increasing amounts of nitrogen, to reduce the propagation of the cereal cyst nematodes, the result being analogous to those obtained in microplot and field experiments. The greenhouse experiments in 1967 and 1968 gave the most convincing results. With N-amounts not exceeding the amounts applied to the e-treatments in the microplot and field experiments, the number of cysts was reduced to ¼ of, or even less than that, of the maximum of cysts obtained, which, in these experiments as in greenhouse experiment I, 1969, was found at a N-level corresponding to 46.5 kg N per ha. (tables 3 and 4 and fig. 2). On the other hand, it had not been possible, in greenhouse experiment I, 1969, to force the number of cysts below 47 per cent of the maximum number obtained.

Greenhouse experiment II, 1969, was aiming at a more exact definition of the level of maximum cyst formation. The plan did not prove a success. On the other hand, the results of this experiment and of greenhouse experiment I, 1969, showed a marked, negative correlation between cyst numbers and yield (fig. 3 and 2).

The greenhouse experiments in 1970 (table 5) showed a maximum cyst formation at N-levels corresponding to 69.8 kg N per ha. and, further, a slight decrease with increasing

N-amounts. It is probable that the reason for the poor results of the greenhouse experiments during recent years is to be found in the experimental technique used. The failure of reactions to the treatments given coincides with the change of the method of applying nitrogen. The method was changed from top applications to applications through watering at the bottom of the plastic bags. 5 cm columnar samples of the sand columns in the PVC pipes have shown through nitrate tests that, 10 days after the preparation of the treatments, the nitrate content of the samples taken at the bottom was but one half of the nitrate content in the upper 5 cm layer when the nitrogen amounts applied had been poured in at the bottom of the plastic bags.

The results obtained through investigations of plant and soil samples from the 'Askov' experiment (table 7 and fig. 7) have been included because they originate from an experiment that was not designed with a view to nematode research and, further, because it represents a locality in quite a different part of the country and because the results tend to confirm the results obtained at Lyngby. Apart from the results obtained by examining the roots of the nitro-chalk fertilized plants at the end of June, all the other results show the largest nematode number at the level of 40 kg N per ha., decreasing with increasing N-applications, but when the effect of ammonia on the number of nematodes in roots from plants above the chords is compared with that in roots from plants between the chords, it is remarkable how, in the plants between the ammonia chords, an essential effect is not observed until the largest dose used – 120 kg N per ha. – is applied. The larva/cyst ratio, which shows a decreasing tendency with increasing nitrogen amounts, indicates – when related to the fact that the number of larvae shows the greatest decrease – that larvae penetrate into the roots, maybe due to reduced hatchings.

The difference between the number of cysts and hatched larvae in race I and race II of the 'Tylstrup' populations may be ascribed to the effect of natural enemies. Race I was propagated in 2 beds, race II by rotation between 5 beds. Propagation in only 2 beds will probably provide conditions of existence to a far greater amount of predacious fungi than the slower rotation between 5 beds.

It may be objected that there are no comparable results available from similar experiments with other degrees of infestation or with soils free of nematodes. In practice, it is, however, impossible to carry out experiments in which one factor, the soil infestation, is altered without a simultaneous alteration of soil type, structure, microflora etc. Instead, reference is made to the below fig. 10, which, on the basis of figures given by Sigurd Andersen (1961) shows in a highly instructive manner the influence of soil infestation on the yield of straw + grain in oats and barley.

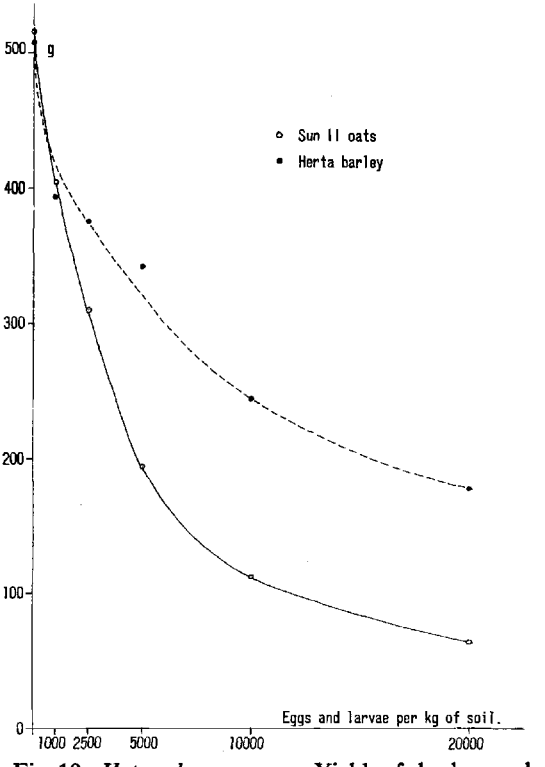

Fig. 10. *Heterodera avenae.* Yield of barley and oats as a function of soil infestation. Total weight of straw and grain from experiments in clay pipes. (By Sigurd Andersen).

Conclusion

A long series of experiments through several years with applications of nitro-chalk against cereal cyst nematodes have been described. All experiments were made to elucidate the influence of increasing amounts of nitrogen on the propagation of the cereal cyst nematode. Nitrogen amounts from 0 to 232.5 kg per ha. have been applied. Statistical analyses have not been worked out, but a high degree of harmony between the results lengthwise as well as across the different kinds of experiments indicates reasonably safe results, which can be summed up as follows:

N-amounts of about 46.5 kg per ha. bring about maximum propagation of cereal cyst nematodes. Increased N-amounts reduce the population to ½-⅓ of the maximum number. With no N-application, intermediate population sizes were obtained.

It has proved possible to manage and reverse the effects by applying the nitrogen amounts in inverse order.

Supposedly, liquid ammonia has a similar effect, but the distance between the injection knives must be less than 30 cm.

Among others, two factors have brought about the effect obtained, namely:

1) The number of eggs and larvae per 'full' cyst is at a maximum at 0 N, decreasing with increasing N-applications, and
2) the number of 'full' cysts is at a maximum with applications of about 46.5 kg N per ha., decreasing with increasing N-applications.

The hatching of larvae in series of calcium nitrate solutions from 0.001 to 0.01 mol shows decreases with increasing concentrations. The largest decrease in the hatching already takes place at low concentrations. There is some indication that the said effect will cease when the nitrogen influence is removed. There is some indication that a lower number of larvae penetrate the roots with increased nitrogen applications, probably due to reduced hatching.

The applications of large amounts of nitrogen does not reduce the biological value of the oat grains (Bengtsson and Eggum, 1969, Bille, 1973), and is apparently in agreement with the measures taken for the control of take-all (Schulz, 1969).

In the experiments – apart from special cases – it has proved economically justifiable to apply up to 186 kg N per ha.

It seems as though predators, the natural enemies, play a more important part than generally attributed to them so far.

The results obtained may be ascribed to physiological effects registered for the nematode at several stages of its development: reduced fertility, development of apparently fewer females, and reduced inclination of the larvae to hatch from the individual cysts.

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