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

Stigende mængder kvælstofs indflydelse på havrenematodens opformering. II

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Summary

A further five years of experiments on increasing the amounts of nitro-chalk (0–232.5 kg N per ha) given to oats grown on cereal cyst nematode infested soil have confirmed the results of earlier experiments. Increasing amounts of nitrogen have restrained propagation of the cereal cyst nematode, which was at 232.5 kg N per ha reduced to $^1/_3$ of the propagation at 46.5 kg N per ha.

Hatching of larvae of this nematode in molar concentrations of $Ca(NO_3)_2$ yielded the highest hatching at 0 mM (dest. water), thereafter hatching decreased rapidly at first and more moderately later on. Already at 4 mM, the hatching was reduced by 50 per cent. At low concentrations the mean weekly hatching increased at first and thereafter decreased. At higher concentrations the mean weekly hatching was more uniform throughout the experimental period and at the highest concentrations applied there was an attempt at further increase in the hatching. The hatching intensity was highest early in the spring at the beginning of the experimental period.

Investigations on amino acid contents in resistant (variety no. 640318-40-2-1) and susceptible (Sun II) oats grown under the same conditions showed at increased amounts of applied nitro-chalk (0-232.5 kg N per ha) increasing contents of bound amino acids in the roots of resistant oats – except for cystine and tyrosine, the contents of which decreased. In susceptible oats, on the other hand, the contents of bound amino acids decreased – except for leucine and especially lysine and histidine the contents of which increased.

Except for the two lowest nitrogen levels, the contents of the individual bound amino acids were all over higher in resistant oats than in susceptible oats.

Key words: Cereal cyst nematode, nitrogen, hatching, amino acids.

Resumé

Yderligere fem års forsøg med stigende mængder kalksalpeter (0–232,5 kg N pr. ha) til havrenematod-inficeret jord har bekræftet tidligere forsøg, idet stigende kvælstoftilførsel hæmmede nematodens opformering, som ved 232,5 kg N pr. ha var reduceret til $^1/_3$ af opformeringen ved 46,5 kg N pr. ha.

Klækning af havrenematodens larver i molære koncentrationer af $Ca(NO_3)_2$ gav størst klækning ved 0 mM (dest. vand), derefter aftog klækningen først hurtigt, senere mere moderat. Allerede ved 4 mM var klækningen reduceret med 50 pct. De gennemsnitlige ugentlige klækninger var ved lave koncentrationer først tiltagende, derefter aftagende. Ved højere koncentrationer var den gennemsnitlige ugentli-

ge klækning mere ensartet igennem hele forsøgsperioden, og ved de højest anvendte koncentrationer var der tilløb til stadig tiltagende klækning. Klækningsintensiteten var størst først på foråret, i begyndelsen af forsøgsperioden.

Undersøgelser over aminosyreindholdet i resistent (nummersort 640318-40-2-1) og modtagelig havre (Stål) dyrket under ens betingelser viste ved øget tilførsel af kalksalpeter (0–232,5 kg N pr. ha) stigende indhold af bundne aminosyrer i rødderne af resistent havresort – dog undtaget cystin og tyrosin, hvis indhold var faldende. I modtagelig havresort var indholdet af bundne aminosyrer derimod faldende – undtagen for leucin og især lysin og histidin, hvis indhold var stigende.

Indholdet af de enkelte bundne aminosyrer var, bortset fra de to laveste kvælstofniveauer, overalt højere i resistent end i modtagelig havre.

Nøgleord: Havrenematoder, kvælstof, klækning, aminosyrer.

Introduction

This report is a continuation of Report no. 1243 (*Juhl*, 1975a) and of the article called »Cereal Cyst Nematode and N Fertilization« published in Eppo Bull. 5 (*Juhl*, 1975b). The main result showed that increasing amounts of nitro-chalk (0–232.5 kg N per ha) given to oats grown on cereal cyst nematode infested soil gave maximum propagation of cereal cyst nematodes at 46.5 kg N per ha and that increasing amounts of nitrogen restrained the propagation, reducing it to about $^1/_3$ of the maximum mentioned above.

In the Eppo Bull. 5 the results from two further years of field experiments made in 1973 and 1974 were included. The results from these experiments supported the previous results. This report gives the results from a further 5 years of field experiments which have been carried out in relation to another study.

In Report no. 1243 some experiments were published on the hatching of cereal cyst nematode larvae in molar concentrations of calcium nitrate. The results from these experiments as well as the results from another two years' hatching experiments have, in Eppo Bull. 5, been summarized in Fig. 4, showing the relative percentage hatching at the different concentrations. The hatching experiments of the latterly mentioned 2 years will be commented on further later in this report.

Prasad and *Webster* (1967) found a significant reduction in the number of females of *Heterodera avenae* around wheat plants grown on *Heterodera avenae* infested soil after watering with amino acid solutions. *Evans* and *Trudgill* (1971) found that among several racemic compounds of amino acids used, DL methionine was the most toxic to the potato cyst nematode *Globodera (Heterodera) rostochiensis*. This report contains a statement about an investigation of the content of amino acids in the roots of a susceptible and a resistant variety of oats grown under identical conditions but with application of increasing amounts of nitro-chalk.

Methods and results

Field experiments

These experiments were made on light loam at Lyngby in a locality where oats have been grown for 25 years. The experimental area comprised 3 × 12 plots of 2 × 2 m each at 6 nitrogen levels: a = 0; b = 46.5; c = 93.0; d = 139.5; e = 180.0, and f = 232.5 kg N per ha, the replicates being placed according to the knight's move method. The experiments have been run permanently since 1969. This report gives the results from 1975–79. Each spring except for 1977, 250 kg P-K fertilizer was supplied as basal dressing. Nitrogen was applied in the form of nitro-chalk, the first half after the sowing, the remainder one month later. Up to date and including 1977, the Sun II variety of oats has been used as host plant and after that time the Selma variety. Soil samples were taken in the autumn about 2 weeks after the harvest. The results obtained from the field experiments will be seen from Tables 1 and 2.

Table 1. Field experiments 1975–79. Number of eggs and larvae of *Heterodera avenae* per 150 g of dried soil (60°C) after harvest. Mean of 6 replicates
Markforsøg 1975–79. Antal æg og larver af Heterodera avenae *pr. 150 g tør jord (60°C) efter høst. Gns. af 6 gentagelser*

Year *År*	kg N per ha 0	46.5	93.0	139.5	180.0	232.5
1975	335	460	43	107	86	38
1976	1248*)	329	281	88	2	329
1977	296	224	26	137	48	18
1978	58	82	10	70	8	21
1979	300	205	190	108	167	60
Mean *Gns.*	447	260	110	102	62	93
Pct.	172	100	42	39	24	36

*) In the a_1 plot this year 5595 eggs and larvae were found. 1120 were found in the following year.
I a_1 parcellen fandtes dette år 5595 æg og larver, året efter 1120.

Table 2. Field experiments 1975–79. Yield of grain of oats in g. Mean of 6 replicates
Markforsøg 1975–79. Kerneudbytte af havre i g. Gns. af 6 gentagelser

Year *År*	kg N per ha 0	46.5	93.0	139.5	180.0	232.5
1975	350	728	863	865	944	891
1976	173	490	652	584	736	619
1977	199	624	653	773	756	838
1978	412	1040	1169	1378	1246	1378
1979	337	967	1332	1273	1586	1464
Mean *Gns.*	294	770	934	975	1054	1038
			LSD = 163			

Hatching experiments

In 1973 and 1974, hatching experiments were made in the laboratory with *Heterodera avenae* larvae exposed to different molar concentrations of calcium nitrate. The cyst material was incubated at 15°C in darkness in syracuse watch glasses containing the respective concentrations. The cysts were contained in sand-filled, nylon net-sealed perspex rings provided with a ring of the same material as foot permitting the access of air to the uppermost net. 20 cysts/concentration/hatching series. In 1973, the hatching series consisted of 7 concentrations from 0 (distilled water) to 32 millimol (mM) calcium nitrate, comprising 11 hatching series of race 1, which were initiated at intervals of one week, the first one on 23rd March. The last hatching series was initiated on 1st June. All hatchings were discontinued on 17th August.

In 1974, 13 series of race I and 7 series of race II were used, also initiated at intervals of one week, the first series on 2nd April, the last series on 4th June. These hatchings were discontinued on 23rd August.

In 1974 concentrations of 48 and 64 mM calcium nitrate were also included. The number of hatched larvae was counted about once a week and the solutions were renewed at the same time.

The accumulative hatchings for each concentration and year are shown in Table 3.

Table 3. Accumulative hatchings of larvae from cysts of *Heterodera avenae* in different molar concentrations of calcium nitrate
Akkumulative klækninger af larver fra cyster af Heterodera avenae *i forskellige molære koncentrationer af calciumnitrat*

	Molar concentration of calcium nitrate in mM *Molær koncentration af calciumnitrat i mM* 0	1	2	4	8	16	32	48	64
Race 1, 1973	21477	15540	10882	6997	9266	6640	7188		
Race I, 1974	16953	14708	13781	10927	7801	7853	6851	4237	3068
Race II, 1974	9252	4701	3337	3602	2903	1853	2338	1318	707
Mean, *Gns.*, pct.	100	70	56	45	40	32	32	20	13

The course of the hatchings in relation to concentrations and hatching periods is recorded as accumulated mean weekly values in Figs. 1 and 2 showing the hatchings in 1973 and 1974, respectively. Fig. 2 for 1974 comprises the hatchings of both race I and race II. The background material for Fig. 1 consists of 77.990 and for Fig. 2, 116.190 hatched larvae.

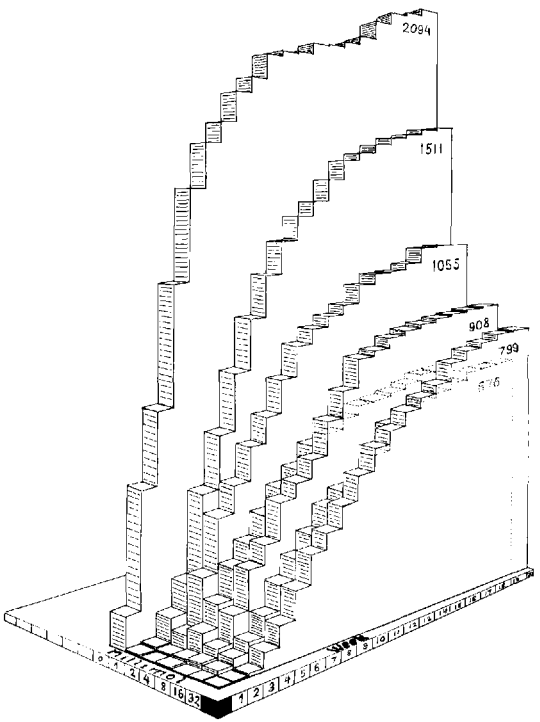

Fig. 1. Accumulated mean weekly hatching of larvae from cysts of *Heterodera avenae* in different molar concentrations of calcium nitrate, 1973.
Akkumuleret gns. ugentlig klækning af larver fra cyster af Heterodera avenae *i forskellige molære koncentrationer af calciumnitrat 1973.*

A tendency towards decreased hatching intensity appeared during the experimental period. Fig. 3 shows the total hatching over a period of 11 weeks of the 13 hatching series of race I and the 7 hatching series of race II for 1974. The values stated against the dates of 21st and 28th May and

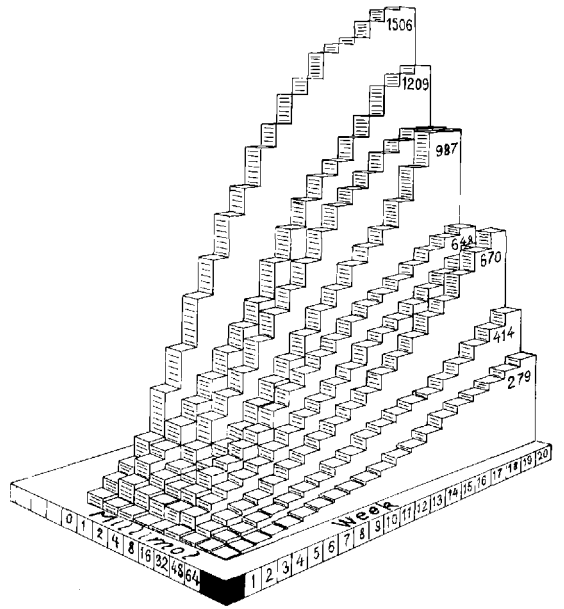

Fig. 2. Accumulated mean weekly hatching of larvae from cysts of *Heterodera avenae* in different molar concentrations of calcium nitrate, 1974.
Akkumuleret gns. ugentlig klækning af larver fra cyster af Heterodera avenae *i forskellige molære koncentrationer af calciumnitrat, 1974.*

4th June represent the mean of two hatching series of race I, which were initiated on the same date.

Investigation of amino acids

In 1974, an investigation was made of the amino acid content in the roots of a susceptible (Sun II) and a resistant oat variety (640318-40-2-1) grown under identical conditions, but with increasing applications of nitro-chalk. The same nitrogen levels were used as with the field experiments. The plants were grown in light loam in concrete pipes, diameter 1 m, dug into the ground. NPK was applied as basal fertilizer (0-4-20) in an amount corresponding to 600 kg per ha and manganese sulphate corresponding to 50 kg per ha. The oats were sown on 1st April in such a way that a susceptible variety was in one half of a pipe and in the other half a resistant one. The amounts of nitro-chalk were applied on 1st April in the case

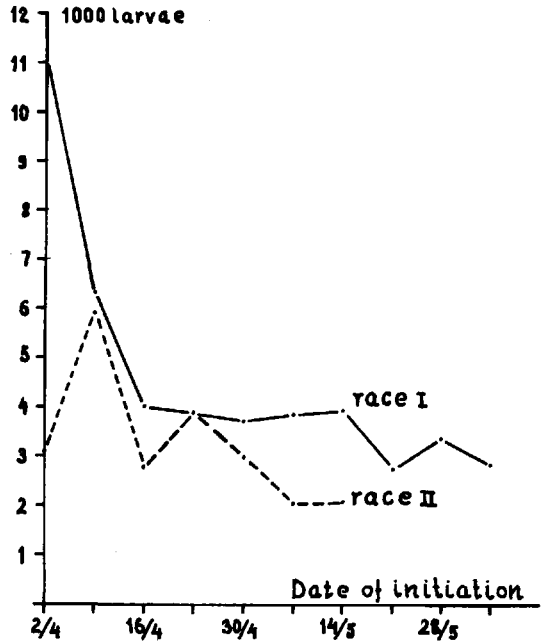

Fig. 3. Total hatch during 11 weeks of race I and II as a function of date of initiation. 180 cysts per hatching series.
Total klækning gennem 11 uger af race I og II som funktion af startdato. 180 cyster pr. klækningsserie.

of the first half and one month later in the case of the second half.

Plants to be examined for amino acid content in their roots were brought in early on 11th and 12th June. The roots were cleaned of the attached humus substances and mineral particles by being brushed with distilled water; they were then frozen down by means of carbon dioxide snow and alcohol in glasses with silica gel followed by freeze drying for 48 hours. The amino acid investigation was carried through by courtesy of the Department of Agricultural Chemistry of the State Laboratory for Soil and Crop Research.

As the results from the investigations of free amino acids showed great variations, this report will only give the results from the investigation of bound amino acids.

The results from the investigation of the content of bound amino acids are shown in Fig. 4 as amino acid N expressed in percentage of total N in the sample in question.

The total content of bound amino acids in relation to the nitrogen level seems to behave differently in the cases of resistant to susceptible oat variety.

Table 4. Total bound amino acid N in roots of resistant and susceptible oats in pct. of total N at increasing amounts of applied nitrogen
Total bundet aminosyre N i rødder af resistent og modtagelig havre i pct. af total N ved stigende kvælstoftilførsel

	Oats variety *Havresort*	kg N per ha 0	46.5	93.0	139.5	186.0	232.5
Total amino acid N+NH_3 *Total aminosyre N+NH_3*	resistant *resistent*	83.01	85.90	83.47	88.50	90.95	92.75
Total amino acid N–NH_3 *Total aminosyre N–NH_3*	resistant *resistent*	72.64	74.59	74.12	78.20	80.28	82.40
Total amino acid N+NH_3 *Total aminosyre N+NH_3*	susceptible *modtagelig*	88.25	82.67	76.50	80.79	81.52	80.72
Total amino acid N–NH_3 *Total aminosyre N–NH_3*	susceptible *modtagelig*	76.60	72.20	67.23	71.04	71.75	71.67

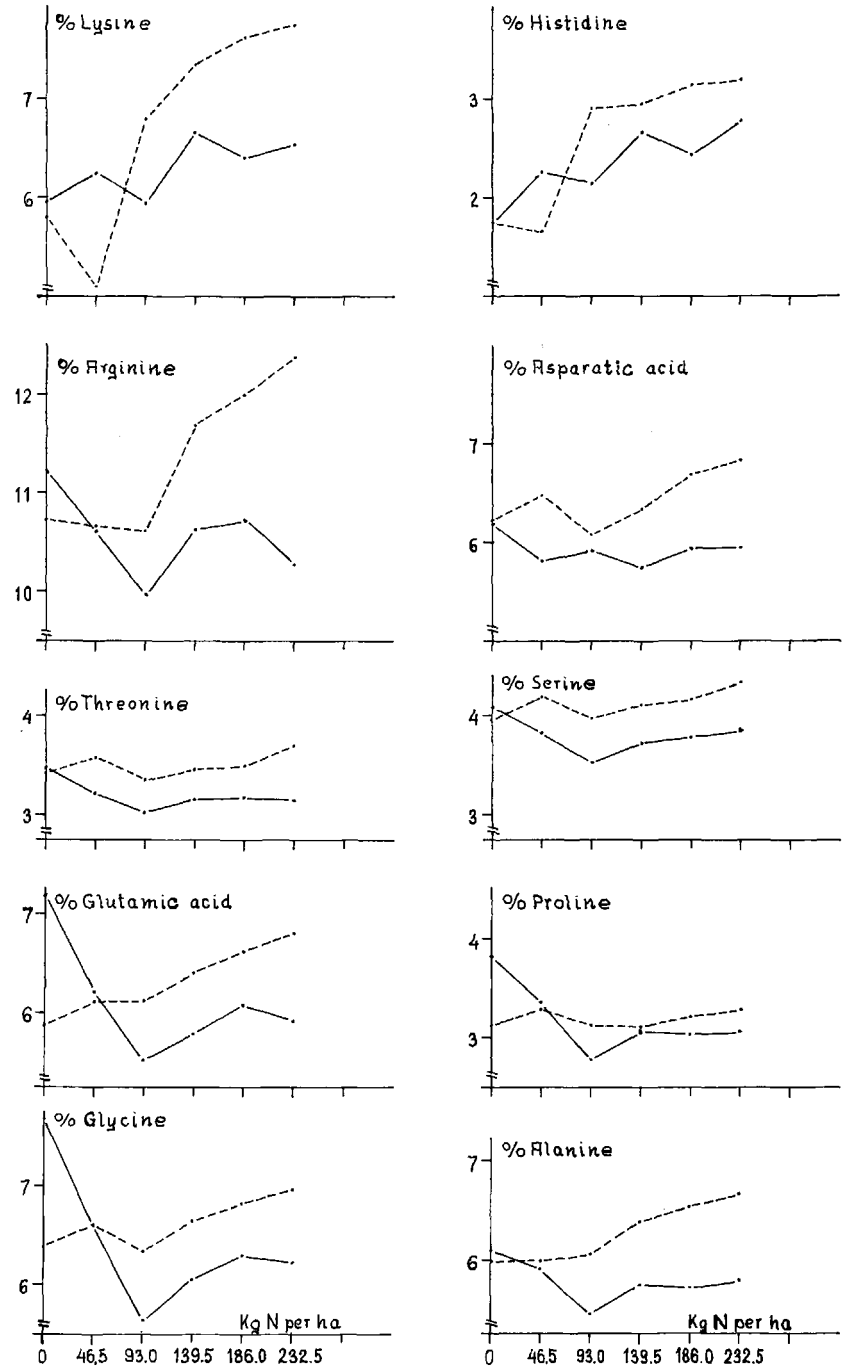

Fig. 4. Contents of bound amino acids in roots of a resistant and a susceptible variety of oats shown as amino acid N in per cent of total N. The oats were grown at 6 different nitrogen levels.
Indhold af bundne aminosyrer i rødder af resistent og modtagelig havre vist som aminosyre N i pct. af total N. Havren dyrket ved 6 forskellige kvælstofniveauer.

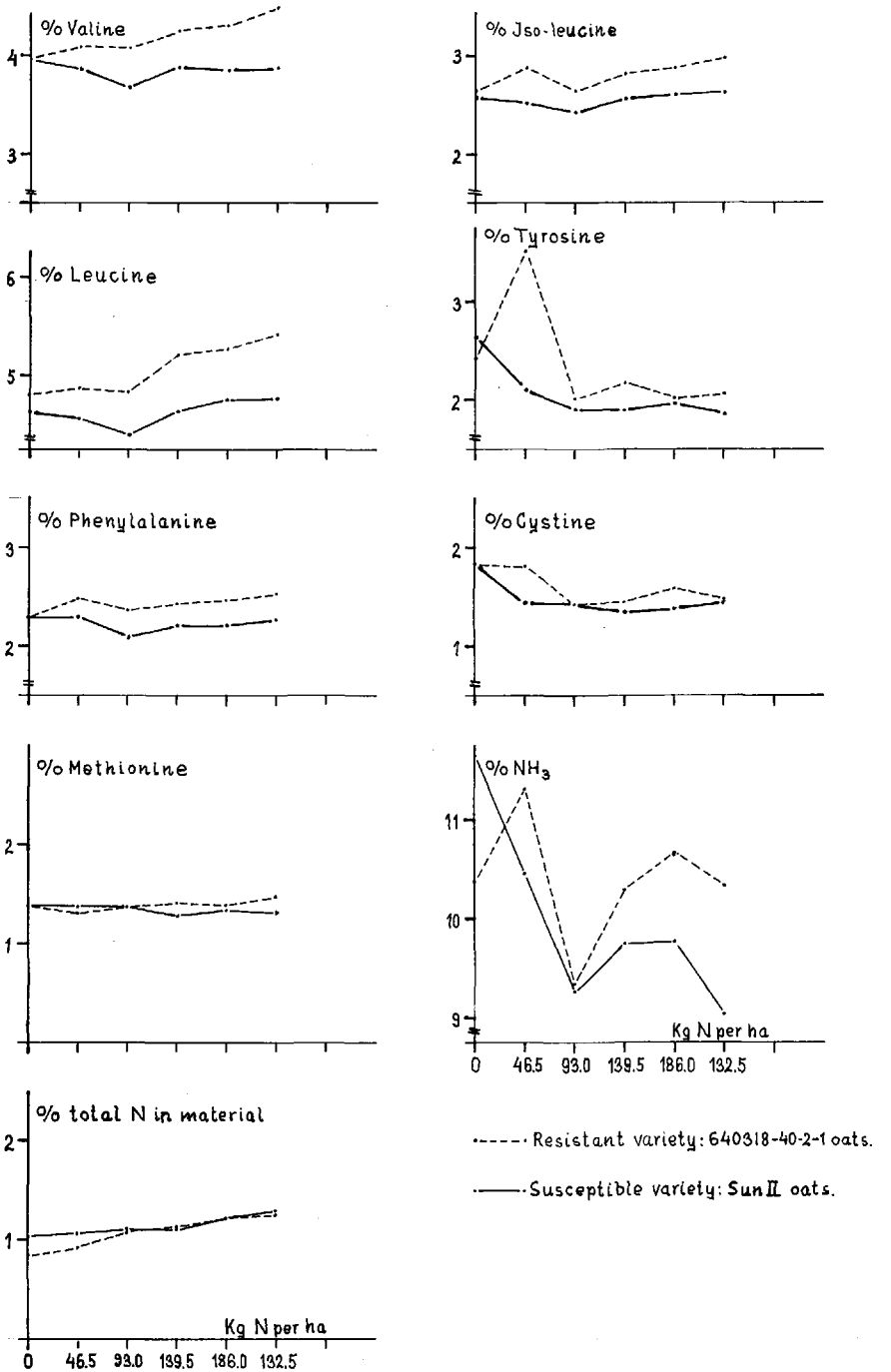

Fig. 4 continued

Discussion and conclusion

The nematode populations in the field experiments during the past five years with applications of increasing amounts of nitro-chalk to oats grown on cereal cyst nematode infested soil have been very small. The finding of a few cysts more or less may have resulted in great variations in the percentage ratio. Thus it may seem (Table 1) that, in the low populations there is a tendency towards maximum propagation at 0 N per ha and not, as seen so far, at 46.5 kg N per ha.

However, the displacement seems, in particular, due to the fact that a single one of the a-plots showed in one single year 5.595 eggs and larvae, and the following year 1.120. Thus, there are reasonable grounds to assume – as previously found – that maximum propagation takes place at 46.5 kg N per ha. If the propagation at 46.5 kg N per ha is put = 100, it will, in accordance with previous results, be found that the propagation at 232.5 kg N per ha has been reduced to 36 per cent, or about $^1/_3$ of the propagation at 46.5 kg N per ha.

The experiments have confirmed previous results, namely, that with increasing amounts of nitrogen a reduced propagation of the cereal cyst nematode is obtained.

The yields (Table 2) showed great variations from one year to another. In 1979, it was twice the amount as that in 1976. For the years with the greatest yields, i.e. 1978 and 1979, Duncan's multiple range test showed, for 1978, significant differences for the lowest four nitrogen levels. For 1979 significant differences were found in the yields from the lowest three nitrogen levels and in these in relation to the second highest N level. On an average for all the years, the yield seemed to be increasing up to and including 180 kg N per ha, but there were only significant differences between the yields at the three lowest nitrogen levels.

Hatchings in the laboratory of cereal cyst nematode larvae in calcium nitrate solutions have always given maximum hatchings at 0 mM (distilled water). Then the hatching rapidly decreased with increasing concentrations, but the greatest reduction was found at the lowest concentrations. Already at 1 mM, the hatchings were, on an average in the experiments (Table 3), reduced by 30 per cent. At 8 mM, the hatching was reduced by 60 per cent: at 64 mM, it was reduced by 87 per cent.

If it is assumed that the amount of nitrate added to a soil is found dissolved in the soil moisture, this means according to *Stendal* (1974) that a solution with a concentration of 1 mM corresponds to an applied amount of fertilizer of 100 kg nitro-chalk (15.5 kg N) per ha.

From Figs. 2 and 3 showing the accumulated mean hatching per week will be seen that the initial hatching is sparse for all concentrations of calcium nitrate, but it very rapidly increases with a subsequent decrease at the lower concentrations. At somewhat higher concentrations, the low initial hatching was followed by a rather higher hatching level, which was more constantly retained through the experimental period. At the highest concentrations there was a tendency towards constantly increased hatching through the whole experimental period; the final result, however, remained low.

The pH has hardly had any influence on the hatching. At 0 mM, pH was found = 6.5. At 1 mM, pH had fallen to 5.6, and then rising through increasing concentrations to 6.7 at 64 mM. Maybe the osmotic pressure could have some influence. According to the equation of state (*Marke*, 1937), the osmotic pressure at 1 mM is 0.07 atm., at 8 mM, 0.56 atm., and at 64 mM, 4.48 atm.

The total hatchings of the hatching series through the experimental period are, as regards the hatching in 1974, shown in Fig. 3. Corresponding developments are found for the hatchings in 1972 (*Juhl*, 1975 a: Fig. 9). At the hatchings in 1973 on the other hand, the total hatchings showed a greater variation, and the decrease of the hatchings as the time passed was less pronounced. In general, it must be pointed out that the hatching of the individual experimental series as a function of the initial date and comprising all concentrations has been largest in the earliest-initiated hatching series. The following decline in the total hatchings was largest in the beginning of the experimental period, later it was more moderate.

It was hoped that an investigation of the amino acid situation in the roots of the Sun II oats variety (susceptible) and the numbered variety: 640318-40-2-1 (resistant oat variety) grown under identical conditions but with increasing application of nitrogen might have helped towards a partial understanding of the restricting influence of increased nitrogen amounts on the propagation of the cereal cyst nematode. However, the results obtained seem to give no such information. Maybe the knowledge of the content of free amino acids could have given certain information. The results obtained for the free amino acids show great variation, however, and are consequently disregarded.

Undoubtedly, the poor result obtained with regard to the content of free amino acids is due to the fact that there was no standard method to work by – there is not found anything in literature about investigations of amino acids in roots – and that they are water-soluble. The daily concentration variations of the plants (*Larsen,* 1973) could be countered by taking out all plants at the same time of the day, whereas the amount of water and the time used for cleaning the roots depended on the size of the roots and the amount of attached mineral and humus particles.

As shown in Fig. 4, the content of bound amino acids was expressed as amino acid N in per cent of total N, everywhere in the resistant oats higher than the content in the susceptible variety – apart from the two lowest nitrogen levels. Further the content of amino acids in the resistant oats increased or was constant at the increased nitrogen level, apart from the content of tyrosine and cystine, which decreased. In the susceptible oats the content of the individual amino acids generally decreased or was constant, apart from the content of leucine and, in particular, lysine and histidine, which increased.

The fact that the total content of bound amino acids in the resistant and the susceptible oats variety reacted differently at increasing nitrogen level will plainly be seen from Table 4, which shows that, while the content of amino acids in the resistant variety rose from 72.64 to 82.40 per cent amino acid N in per cent of total N at an increase of the applied amount of nitrogen from 0 to 232.5 kg N per ha, the content in the susceptible oats variety fell from 76.60 to 71.67 per cent amino acid N. At the same time there has been a slight increase of the percentage content of total N, the largest observed in the resistant oats variety. The percentage content of total nitrogen was a little lower in resistant oats in the lowest two nitrogen levels, but otherwise the same for the two oats varieties.

On the basis of the ascertained contents of amino acids, no explanation can be found of the decreasing effect of increasing amounts of nitrogen on the propagation of cereal cyst nematodes.

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