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THE POPULATION DENSITY OF *HETERODERA TRIFOLII* IN PASTURES IN THE NETHERLANDS AND ITS IMPORTANCE FOR THE GROWTH OF WHITE CLOVER[1]

De bevolkingsdichtheid van Heterodera trifolii *in weilanden in Nederland en de betekenis ervan voor de groei van witte klaver*

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In a pot experiment the tolerance limit of white clover seedlings to *Heterodera trifolii* was 50 eggs per g of soil. In other pot experiments *H. trifolii* increased to about 1400 eggs per g of soil without apparent damage to the growth of white clover. White clover maintained a relative density of almost 20 percent in a mixture with *Lolium perenne* at a density of *H. trifolii* of 80 to 200 eggs per g of soil. Densities of this nematode in 216 samples from 74 pastures were mostly below 1 egg per g of soil and only very seldom higher than 32 eggs per g of soil. There was a slightly higher frequency of densities over 32 eggs per g of soil in samples from places with a good than from those with a poor stand of white clover. The differences between samples from pastures with good and poor stands and between samples from places with and without clover were very small. The frequency distribution of cysts and eggs in samples from a four year old pasture with a very good stand of white clover was the same as that in the samples from all other pastures. It is therefore concluded that the influence of attack by *H. trifolii* on the stand of the white clover in the sampled pastures was negligible.

INTRODUCTION

WARDOJO, HIJINK & OOSTENBRINK (1963) and HIDDING, HIJINK & OOSTENBRINK (1963) found that in pot experiments with densities of *Heterodera trifolii* of up to 50 larvae or eggs per g of soil, white clover was not noticeably damaged during the first four to eight weeks after sowing. There was damage, however, after the clover had been cut one or more times. By then the nematode had probably multiplied. HIDDING, HIJINK & OOSTENBRINK (1963) concluded from this that they had reproduced experimentally the disappearance of white clover from newly made pastures, because the nematode is widely spread in such pastures and multiplies rapidly on white clover. Their contention that this plant is damaged by *H. trifolii* after multiplication seems to rest only on the tacit assumption that this nematode will keep increasing on white clover in pastures until the plant is seriously damaged, as it seemed to have done in the pot experiments. However, this assumption is premature in view of the evidence presented. Damage by a nematode can occur only when its density exceeds the tolerance limit of the attacked plant (SEINHORST, 1963, 1965a). When the initial density of the nematode is lower than this tolerance limit it will become damaging only if its equilibrium density on the attacked plant and under the given ecological conditions exceeds this tolerance limit (SEINHORST, 1963, 1964, 1965b). Neither the tolerance limit of white clover for *H. trifolii* nor the equilibrium density of the nematode on white clover in Dutch pastures are discussed or were investigated

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by WARDOJO, HIJINK & OOSTENBRINK (1963) or HIDDING, HIJINK & OOSTENBRINK (1963).

From the experiments of these workers, however, we may derive that the tolerance limit of young wihte clover plants is probably 50 or more eggs of *H. trifolii* per g of soil. On the other hand OOSTENBRINK (1957) found that densities of *H. trifolii* in all 661 pastures he sampled were very low. This suggests that in pastures the equilibrium density of this nematode could generally be well below 50 eggs per g of soil.

The tolerance limit of white clover for *H. trifolii* was investigated in pot experiments. Further, densities of this nematode were determined in over 100 samples from 50 pastures in 1963 and in 214 samples from 74 pastures in 1964.

POT EXPERIMENT ON DAMAGE TO WHITE CLOVER SEEDLINGS BY *HETERODERA TRIFOLII*

Steam sterilized sandy soil was inoculated with so many cysts of *H. trifolii* that a density of 400 eggs per g of soil was obtained. Portions of this inoculated soil were diluted with non-inoculated soil to obtain densities of 200, 100, 50, 25, 12.5, 6, 3 and 1.5 eggs per g of soil. Five 2 kg cylindrical plastic pots were filled with each of these soil mixtures and with non-infected soil. Ten seedlings of white clover were planted in each pot at the end of August. The pots were placed on a glasshouse bench in randomized fashion. They were watered twice a week, their weight being then corrected to a fixed value, at which the water content was 14% of the dry-weight of the soil. Tops and roots of the plant in each

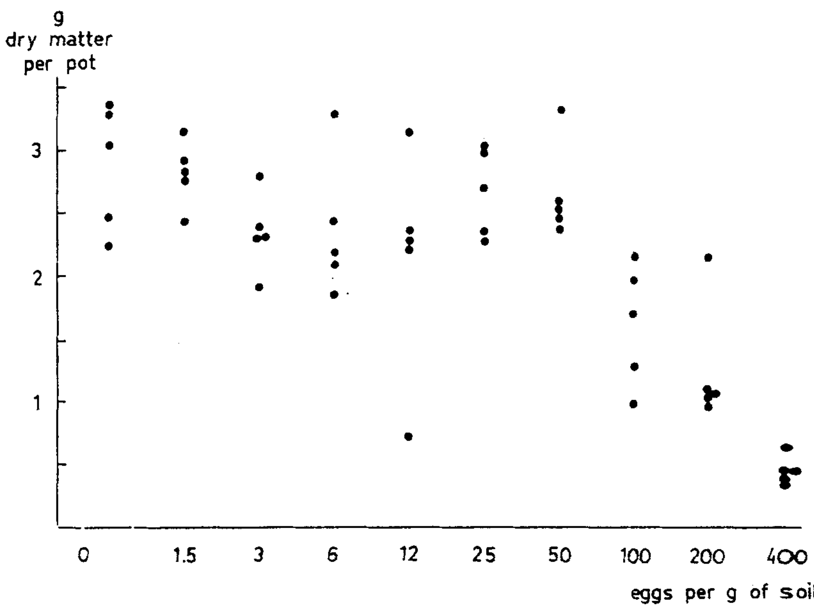

FIG. 1. The relation between the density of *Heterodera trifolii* and the yield of white clover eight weeks after sowing in a pot experiment.
De betrekking in een potproef tussen de dichtheid van Heterodera trifolii *en de opbrengst van witte klaver acht weken na het zaaien.*

pot were collected on November 5 (about eight weeks after planting). Growth conditions had apparently been different in different places on the bench. The arrangement of the pots enabled some correction to be made for the influence of this on the weights of the plants but the variability remained fairly large after this correction. The corrected weights of dry matter harvested per pot are given in Fig. 1. Despite the great variation they show that noticeable reduction in growth did not occur at initial densities of up to 50 eggs per g of soil. This is in accordance with the results of the experiments of WARDOJO, HIJINK & OOSTENBRINK (1963) and HIDDING, HIJINK & OOSTENBRINK (1963). The temperature in the glasshouse (20°–22°C) was higher than the average maximum soil temperatures during the summer months. In the glasshouse *H. trifolii* developed slowly at 18°C or less, but rather quickly at 20°C or more. The temperature during the experiment was therefore more favourable for the nematodes than in normal field conditions. From September onwards the decreasing daylight was unfavourable for development of the clover. As the tolerance limits found in pot experiments are in reasonable agreement with those found in the field (SEINHORST, 1965) we may conclude that the tolerance limit of white clover seedlings to *H. trifolii* in the field will be of the same order of magnitude (50 eggs per g of soil) as in the pot experiments and that it is unlikely that densities below 10 eggs per g of soil will damage white clover. This conclusion is corroborated by the results of the following experiment.

INFLUENCE OF THE CLOVER-GRASS RATIO ON THE DENSITY OF *HETERODERA TRIFOLII*

The experiment was started the 19th of June 1963 with eight clover-grass ratios: 0, 10, 26, 40, 56, 70, 86 and 100% white clover. Pots were filled with 5 kg of unsterilized soil containing about two eggs in cysts of *H. trifolii* per g of soil. In each pot 50 plants of perennial rye grass and/or white clover were planted in the ratios mentioned above. There were seven replications of each ratio and therefore 56 pots in total. The lower part of Fig. 2 gives the yields of white clover (dry matter) at different dates in percentages of the yields of the pots with white clover alone. The lowest initial density of white clover (10%) in the pots with clover-grass mixtures increased during the experiment to 18% of the density in the pots with white clover alone, the higher initial densities of white clover decreased and the more so the higher they were at the start of the experiment. The upper part of Fig. 2 gives the densities of *H. trifolii* in the pots at three dates. At the first sampling date there was little influence of the density of the white clover on nematode densities, but at the second and third sampling dates nematode densities at the different initial clover densities reflected more or less clover yields in the period just before the sampling date. The nematode densities at the last sampling date were proportional to the yield of the clover. Although nematode densities in the pots with clover alone were over 400 eggs per g of soil after March 1964, the yields in June and July 1964 were higher than those in the pots with grass alone, despite liberal dressing with fertilizer (Table 1). This suggests that the clover was not seriously damaged by the clover cyst nematode in the pots with clover alone. As final nematode densities in the pots with clover and with clover-grass mixtures were more or less proportional to their clover content, the degree of attack on the clover must have been the same

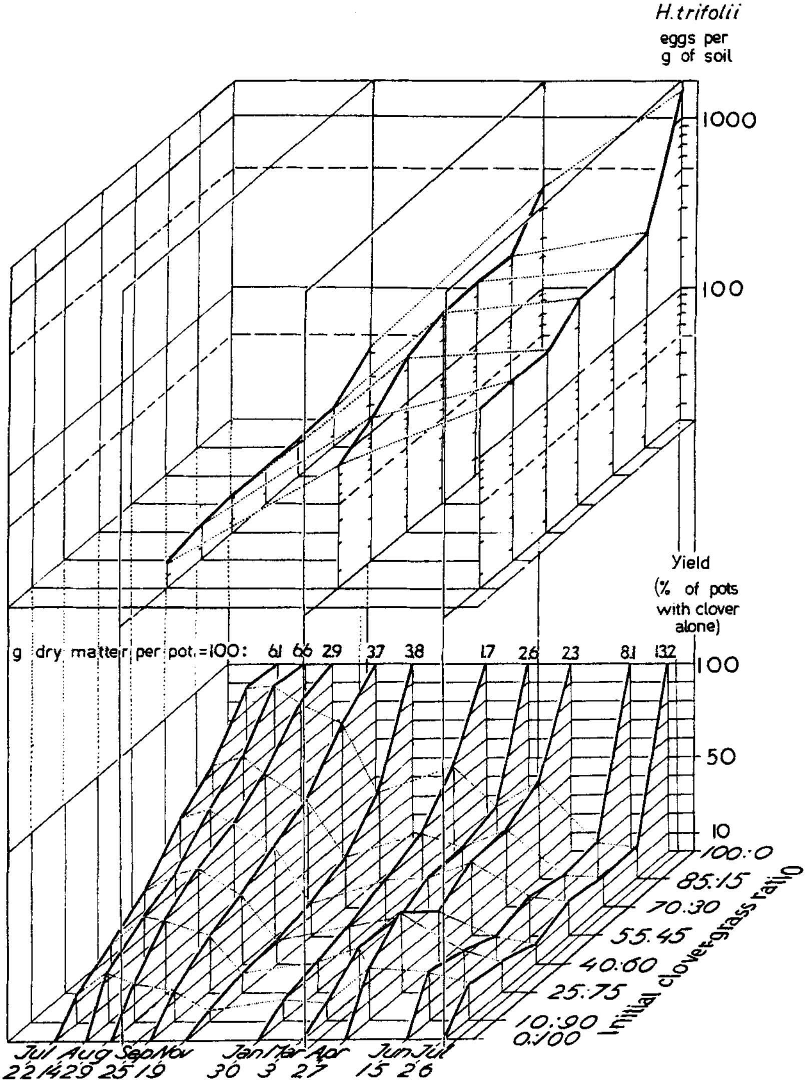

FIG. 2. Yield of white clover in pots planted with white clover and perennial rye grass in eight different ratios, and increase of *H. trifolii* in these pots.
Opbrengsten van witte klaver in potten beplant met witte klaver en Engels raaigras in acht verschillende verhoudingen en de vermeerdering van Heterodera trifolii *in deze potten.*

in both unless the shoot-root ratios of the white clover were different with and without grass. It is therefore unlikely that the clover in the pots with clover-grass mixtures was seriously damaged.

From the data in Fig. 2 it may be concluded that white clover could maintain a relative density of about 18% in a clover-grass mixture in the presence of a

TABLE 1. Yields of pots with white clover alone and with perennial rye grass alone in g of dry matter per 5 kg pot.
Opbrengsten van potten met alleen witte klaver en alleen Engels raaigras in g droge stof per 5 kg pot.

	White clover	Perennial rye grass
June 15	8.1 ± 0.5	6.7 ± 0.25
July 27	13.2 ± 0.8	9.6 ± 0.1
	Witte klaver	*Engels raaigras*

density of *H. trifolii* of about 200 eggs per g of soil. There is no reason to assume that the conditions in the pots were considerably more favourable to the white clover than those outdoors. On the contrary, the combination of high temperature and low light intensity during the winter months acted adversely on the competitive power of the white clover in the clover-grass mixtures (ENNIK, 1960). However, it did not check nematode increase. Despite this increase the clover resumed growth in the beginning of March. It may therefore be concluded that *H. trifolii* will only damage well developed white clover plants in pasture at densities of the order of 200 eggs per g of soil or higher.

The results of this experiment are not quite in accordance with those of HIDDING, HIJINK & OOSTENBRINK (1963). In their experiment the white clover finally disappeared at all initial densities of *H. trifolii*. However, they do not supply information on the rate of increase of the nematode and the densities at which damage began. The rate of increase and the nematode densities in the experiment of HIDDING, HIJINK & OOSTENBRINK (1963) may still have been higher than those in the experiment described above.

In another experiment a density of 1400 eggs per g of soil was obtained six months after sowing white clover in soil inoculated with 20 eggs per g. The clover kept growing well until the and of the experiment.

DENSITIES OF *HETERODERA TRIFOLII* IN PERMANENT PASTURES IN 1963 AND 1964

OOSTENBRINK (1957) found *H. trifolii* in 439 of the 661 pastures he sampled in the Netherlands but the densities were very low. ENNIK, KORT & LUESINK (1964) found fewer than 10 eggs per g of soil in pastures at Wageningen. To obtain more information about the densities of *H. trifolii* in pastures about a hundred soil samples were taken in the summer and autumn of 1963 in 50 permanent pastures at Wageningen, Bennekom, Ottersum, Siebengewald, Kilder, Gendringen and Hoeven, and another 216 in 74 permanent pastures (partly the same as those mentioned above) in the late autumn of 1964.

Stand of white clover in the sampled pastures

The stand of the white clover was good to very good in some, patchy to fair in many and poor to very poor in some of the pastures sampled. Poorly developed plants with small leaves were rare in the first group of pastures, more frequent in the second group and the common type in the pastures with a poor to very poor stand of white clover. Patchiness of the stand had almost always a random pattern but in a few cases more or less round, sometimes expanding patches of well developed white clover were surrounded by grass without any clover at all.

Methods of sampling, extracting cysts from soil and determining cyst contents

In each pasture one or more pairs of samples were taken. Each sample consisted of one 10 to 15 cm wide cylinder of soil taken to a depth of 10–15 cm in 1963 and 10 cm in 1964. One sample of each pair was taken in a place with white clover and the other in a place without, as near to the first sampling site as possible. By investigating single cylinders of soil and not combining several cores from a larger area, the actual densities of *H. trifolii* with which the clover plants had to cope could be determined. Where white clover has disappeared recently because of attack by *H. trifolii,* it would be expected that densities of this nematode and especially the numbers of cysts would be high. There are two possible situations in pastures: 1. the clover shifting from places where the nematode has built up high densities to places where densities are low because white clover has not been growing there for some time; 2. some time after the pasture was sown the white clover occurring only in places where equilibrium densities of *H. trifolii* are low. In both cases high densities of the nematode can be expected just beyond the places where the clover is still growing – in the first instance because it is the place where the white clover has disappeared most recently, and in the second because it is a transition area where disappearance and reinvasion of the white clover has kept densities higher than in places where this plant was absent for longer periods. Therefore the areas just outside the places with white clover provide the best chances for finding high densities of *H. trifolii.*

In the samples, numbers of cysts, "full eggs" and "empty eggs" were determined. "Full eggs" means eggs with a larva. The term is used in contrast to "empty eggs" which are eggs from which the larva has hatched. "Total eggs" means the sum of full and empty eggs. As the samples were taken while plants were growing on the soil it must be assumed that part of the *Heterodera trifolii* population exists as larvae in the soil as wel as specimens in different stages of development in the roots. All these escape detection when cysts only are collected from the soil. However, all these nematodes must have hatched fairly recently from eggs. The numbers of empty egg shells in the cysts give an impression of this part of the population. Counts of cysts, full eggs and empty eggs during eight months in samples from a well mixed mass of soil indicated that none of them diminished very much in number during this period. Empty eggs thus remain recognizable a long time after the larvae have hatched from them.

Some technical difficulties had to be solved before the cyst and egg content of the samples could be determined accurately. The elutriator described by Seinhorst (1964) was used. The generally large quantities of organic debris obtained in this way were dried at 50° to 60°C. Cysts were separated from this debris as described by Seinhorst (1964), first using water as a separating medium but later a mixture of acetone and carbontetrachloride, which gave much better results. Before crushing the cysts according to Bijloo's (1959) method they were fixed in 4% formaldehyde. Eggs that are not fixed in this way (the drying has killed the larvae) are easily destroyed by slight pressure. Later an improved version of this method (Seinhorst & den Ouden, 1966) was used.

TABLE 2. Frequencies of different cyst and egg densities of *Heterodera trifolii* in samples from pastures.
Frequenties van verschillende dichtheden van cysten en eieren van Heterodera trifolii *in monsters uit weilanden.*

Cysts per 50 g of soil or eggs per g of soil	Wageningen, Bennekom				Wychen, Mook, Ottersum				Other regions			
	With clover		Without clover		With clover		Without clover		With clover		Without clover	
	Cysts	Eggs (total)	Cysts	Eggs (total)	Cysts	Eggs (total)	Cysts	Eggs (total)	Cysts	Eggs[1] (full)	Cysts	Eggs (full)
0– 1	10	18	9	18	25	27	24	34	16	21	10	14
1– 2	6	1	3	4	7	9	13	8	4	3	4	1
2– 4	4	2	6	1	5	5	5	5	2	1	0	0
4– 8	2	2	2	2	5	4	4	5	5	3	1	6
8–16	5	1	5	1	6	5	7	2	4	3	4	0
16–32	0	2	2	0	6	2	0	0	2	2	2	0
32–64	0	1	0	1	0	2	1	0	0	0	0	0
>64	0	0	0	0	0	0	0	0	0	0	0	0
Totals	27	27	27	27	54	54	54	54	33[2]	33	21[2]	21
Cysten per 50 g grond of eieren per g grond	*Cysten*	*Eieren (totaal)*	*Cysten*	*Eieren (totaal)*	*Cysten*	*Eieren (totaal)*	*Cysten*	*Eieren (totaal)*	*Cysten*	*Eieren*	*Cysten*	*Eieren*
	Met klaver		*Zonder klaver*		*Met klaver*		*Zonder klaver*		*Met klaver*		*Zonder klaver*	

[1] Empty eggs were not counted in these samples.
Lege eieren werden in deze monsters niet geteld.
[2] More samples were taken from places with clover than from places without.
Er werden meer monsters genomen op plaatsen met klaver dan op plaatsen zonder klaver.

Densities of H. trifolii *observed*

The densities of *H. trifolii* in the samples taken in 1963 did not exceed 20 full eggs per g of soil and there was little difference between the densities in places with and without clover. However, since the samples were taken in different seasons in the different regions, and since the initial difficulties in determining cyst and egg contents may have influenced the figures obtained and furthermore, in some cases other *Heterodera* species may have been included in the counts, the results of the 1963 samplings are not further analyzed here.

The frequencies of different densities of *H. trifolii* cysts and eggs in the samples collected in the autumn of 1964 are given in Table 2 of different numbers of eggs per cyst in Table 3 and of different percentages of full eggs in Table 4. There was little difference in densities of *H. trifolii* between the different regions. The average density in the samples from places with clover was 41 cysts per 500 g of soil and 3.6 (full + empty) eggs per g of soil, and in the samples from places without clover 25 cysts per 500 g of soil and 2 eggs (full + empty) per g of soil. The difference in egg content is largely due to a difference in the frequencies of samples with more than 8 eggs per g of soil.

TABLE 3. Frequencies of different numbers of eggs (full + empty) per cyst of *H. trifolii* in samples from Wageningen, Bennekom, Wychen, Mook and Ottersum.
Frequenties van verschillende aantallen eieren (vol + leeg) per cyste van H. trifolii *in monsters uit Wageningen, Bennekom, Wychen, Mook en Ottersum.*

Numbers of eggs per cyst	Frequencies	
	With clover	Without clover
0– 20	23	34
20– 40	22	21
40– 80	16	14
80–160	13	7
<160	1	0
Aantallen eieren per cyste	*Met klaver*	*Zonder klaver*
	Frequenties	

There were 10 samples without *H. trifolii* cysts.
In 10 monsters werden geen cysten van H. trifolii *gevonden.*

H. trifolii *in places without clover*

The frequency distributions of cysts and eggs in places without clover were almost the same as in those with clover (Table 2), but average and maximum numbers of eggs per cyst were lower than in places with clover (Tables 5,6). The difference was about that which one would expect of a population at the beginning and the end of a period of one to two years without a host plant. This suggests that on the average the places without white clover had been without this plant for not much longer than this period. In almost all cases it must therefore have disappeared while densities of *H. trifolli* were low.

Cysts disappear more slowly than eggs from the cysts. If in a large proportion of the sampled places without white clover this plant had disappeared because of damage by *H. trifolii*, it would be expected that both cyst densities and numbers of eggs per cyst would be lower the longer ago this had happened. As numbers of eggs per cyst are independent of cyst density (Table 5) damage by *H. trifolii* can have been the cause of absence of white clover in only a small proportion of the cases.

TABLE 4. Frequencies of different percentages of full eggs in cysts of *H. trifolii* in samples from Wageningen, Bennekom, Wychen, Mook and Ottersum.
Frequenties van verschillende percentages volle eieren in cysten van H. trifolii *in monsters uit Wageningen, Bennekom, Wychen, Mook en Ottersum.*

Percentage of full eggs	Frequencies	
	With clover	Without clover
0– 20	6	5
20– 40	5	7
40– 60	8	11
60– 80	23	22
80–100	29	32
Percentage volle eieren	*Met klaver*	*Zonder klaver*
	Frequenties	

In 2 samples all cysts were empty.
In 2 monsters waren alle cysten leeg.

Increase and decrease of H. trifolii *in the sampled pastures*

According to Tables 5 and 6 the number of (full + empty) eggs per cyst was larger in places with clover than in those without, except at very low cyst densities, where it was rather low in both cases. They were on the average (53 and 35 per cyst) much smaller than in two pot experiments. In the experiment on the influence of the clover-grass ratio on the density of *H. trifolii* 90 full and 112 full + empty eggs were found per cyst after the nematodes had increased from 2 eggs per g of soil to 1400 eggs per g of soil in twelve months. In another experiment 120 full and 190 full + empty eggs were found per cyst after the nematodes had increased from 20 eggs per g of soil to 1400 eggs per g of soil in six months. The fairly small average number of eggs per cyst and the small difference in densities and numbers of eggs per cyst between places with and without clover suggest that on the average *H. trifolii* did not increase very much on white clover in the summer prior to the sampling. According to Tables 3, 5 and 6 very small to large numbers of eggs per cyst were found at all cyst densities. Large numbers of eggs per cyst occurred at all egg densities. This indicates that some of the cyst and egg densities in each class were the result of population increase in the previous year. The highest egg densities found (32–64 eggs per g of soil, Table 6) were probably all the result of a strong

TABLE 5. Numbers of eggs (full + empty) per cyst (sum of average numbers of eggs per cyst in each sample divided by number of samples) at different cyst densities of *H. trifolii*.
Aantallen eieren (vol + leeg) per cyste (som van gemiddelde aantallen eieren per cyste in elk monster gedeeld door aantal monsters) bij verschillende dichtheden van cysten van H. trifolii.

Cysts per 50 g of soil	Eggs per cyst	
	With clover	Without clover
0– 2	39 (0–138)	36 (0–130)
2– 4	65 (2–140)	27 (0–110)
4– 8	44 (4–170)	17 (8–38)
8–16	57 (2–154)	23 (4–56)
16–32	57 (3–146)	36 (3–95)
32–64		82
Cysten per 50 g grond	*Met klaver*	*Zonder klaver*
	Eieren per cyste	

population increase in 1964, as in all three cases numbers of eggs per cyst were large. Smaller numbers of eggs per cyst indicate that a certain proportion of the cysts is so old that many or all of the eggs they originally contained have hatched. The smaller the numbers of eggs per cyst, the smaller is the proportion of new cysts and therefore the smaller the population increase during the previous year(s). The small numbers of eggs per cyst found in the majority of the samples indicate therefore that the population did not increase very much in the pastures sampled or that increase and decrease alternated in the recent past.

TABLE 6. Numbers of eggs (full + empty) per cyst (calculated as in Table 5) at different egg densities of *H. trifolii*.
Aantallen eieren (vol + leeg) per cyste (berekend als in Tabel 5) bij verschillende dichtheden van eieren van H. trifolii.

Eggs (full + empty) per g of soil	Eggs per cyst	
	With clover	Without clover
0– 2	33 (0–138)	26 (0– 85)
2– 4	35 (11–132)	42 (10–125)
4– 8	94 (34–140)	66 (20–130)
8–16	59 (44– 74)	59 (16–110)
16–32	83 (47–170)	
32–64	130 (91–154)	95
Eieren (volle + lege) per g grond	*Met klaver*	*Zonder klaver*
	Eieren per cyste	

Comparison of pastures containing much and little clover

According to Tables 7 and 8 there was little difference in cyst densities, egg densities and numbers of eggs per cyst between pastures with much and with

TABLE 7. Frequency distributions of numbers of cysts per 50 g of soil and eggs per g of soil of *Heterodera trifolii* in pastures with much and with little white clover at Wageningen, Bennekom, Wychen, Mook and Ottersum.
Frequentieverdelingen van aantallen cysten per 50 g grond en eieren per g grond bij H. trifolii *in weiden met veel en met weinig witte klaver te Wageningen, Bennekom, Wychen, Mook en Ottersum.*

Cysts per 50 g of soil or eggs (full + empty) per g of soil	Frequencies in pastures with			
	much white clover		little white clover	
	Cysts	Eggs (full + empty)	Cysts	Eggs (full + empty)
0– 2	45	49	41	49
2– 4	5	9	13	7
4– 8	7	6	5	6
8–16	12	5	8	5
16–32	5	3	1	1
32–64	1	3	0	0
Cysten per 50 g grond of eieren (volle + lege) per g grond	*Cysten*	*Eieren (volle + lege)*	*Cysten*	*Eieren (volle + lege)*
	veel witte klaver		*weinig witte klaver*	
	Frequenties in weilanden met			

TABLE 8. Frequency distributions of numbers of eggs per cyst of *H. trifolii* in pastures with much and with little white clover at Wageningen, Bennekom, Wychen, Mook and Ottersum.
Frequentieverdelingen van aantallen eieren per cyste van H. trifolii *in weilanden met veel en met weinig klaver te Wageningen, Bennekom, Wychen, Mook en Ottersum.*

Eggs (full + empty) per cyst	Frequencies in pastures with	
	much clover	little clover
0– 20	25	32
20– 40	23	21
40– 80	17	12
80–160	12	9
160–320	1	0
Eieren (volle + lege) per cyste	*veel klaver*	*weinig klaver*
	Frequenties in weilanden met	

little white clover, except perhaps for a slight tendency of the pastures with much clover to have slightly higher frequencies of samples with the higher densities and the higher numbers of eggs per cyst. This indicates that conditions for increase of *H. trifolii* may have been slightly better in pastures with a good, than in those with a poor stand of white clover. The same trend was found by comparing the samples from places with a fairly dense or dense stand of white clover with those from places with a poor stand of this plant. The difference was mainly that in the first group three samples contained more than 32 full + empty eggs per g of soil against none of the second group. Poor growth of white clover was therefore always associated with low to very low densities of *H. trifolii*.

The low densities and small numbers of eggs per cyst in the pastures with a poor stand of white clover could perhaps be explained partly by the shifting of this plant from one place to another. In pastures with a good stand this explanation is quite insufficient. Here rates of increase must have been very low or increase and decrease must have alternated in places with white clover. Equilibrium densities were therefore generally low in these pastures. There may have been increase of *H. trifolii* when conditions were more favourable than average and decrease when they were less favourable.

Population densities in a pasture at Bennekom

All investigated pastures had been sown three years or longer before they were sampled. There was little difference between younger and older pastures except in one case. This pasture had been sown in 1962 and had in 1963 and 1964 the best stand of white clover of all pastures sampled. The stand of clover was still very good in the autumn of 1965. Places without clover were hard to find and the plants were well developed almost everywhere. The densities of *H. trifolii* in ten samples taken in December 1964 ranged from 9 to 213 (average 83) cysts per 500 g of soil with 2 to 170 (average 75) eggs per cyst and 0.06 to 40.7 (average 12.6) eggs per g of soil. This is considerably higher than the average of all other samples (33 cysts per 500 g of soil, 2.8 eggs (full + empty) per g of soil). The average density in 78 samples taken in the same pasture between May and September 1965 was 36 cysts per 500 g of soil and 3.2 eggs per g of soil. There were no indications that the cyst and egg densities or the percentages of full eggs increased or decreased during this period. The frequency distributions of the densities of cysts and eggs in this pasture were the same as those found in all the other pastures together (Table 9). As the stand of the white clover was generally very good, this plant must have been present from 1962 onward in practically all places with clover in 1965 and lack of food cannot have been the cause of the low densities of the nematode there. Population increase was therefore limited by unfavourable ecological conditions, among which soil conditions and temperature might be the most important. Breaking up the old vegetation and sowing a new grass-clover mixture could only have resulted in a more rapid multiplication and higher densities of *H. trifolii* than in the old pasture if it had improved these ecological conditions for the nematode. More favourable soil conditions and temperatures than outdoors may have caused the high rates of multiplication and the high equilibrium densities in the pot experiments.

Densities were also low in a large proportion of the samples from places without white clover in this pasture. As the clover had either disappeared very recently or had not been there since the old pasture was broken up, *H. trifolii* cannot have been the cause of the absence of this plant.

CONCLUSIONS

Decline and poor growth of white clover occurred at least locally in most of the pastures sampled in 1964. However, densities of *H. trifolii* on white clover

TABLE 9. Frequency distributions in 1965 of cyst and egg (full + empty) densities and of numbers of eggs (full + empty) per cyst of *H. trifolii* in a pasture resown in 1962, compared with those in the pastures investigated in 1964. Frequencies in % of total number of samples investigated.

Frequentieverdelingen van dichtheden van cysten en eieren en van aantallen eieren (vol + leeg) per cyste van H. trifolii *in een in 1962 nieuw ingezaaide weide vergeleken met die in de in 1964 onderzochte weiden. Frequenties in % van totaal aantal onderzochte monsters.*

Cysts per 50 g of soil, eggs per g of soil, eggs per cyst × 0.1	Pasture at Bennekom sampled in 1965			Pastures sampled in 1964		
	Cysts	Eggs	Eggs per cyst	Cysts	Eggs	Eggs per cyst
0– 1	32	61	49 (0–2)	42	59	36 (0–2)
1– 2	23	12		18	14	
2– 4	18	12	26	12	8	28
4– 8	14	7	19	8	8	20
8–16	11	7	7	14	6	14
16–32	2	0	0	4	4	1
32–64	2	0		0.5	2	
>64	0	2		0	0	
Cysten per 50 g grond, eieren per g grond, eieren per cyste × 0,1	*Cysten*	*Eieren*	*Eieren per cyste*	*Cysten*	*Eieren*	*Eieren per cyste*
	Weide te Bennekom, bemonsterd in 1965			*Weilanden, bemonsterd in 1964*		

were lower than 2 eggs per g of soil in most and lower than 30 eggs per g of soil in almost all samples from these pastures. They were possibly slightly higher in pastures with a generally good stand of white clover than in those with little white clover and also possibly higher on well developed plants than on small plants. Poor growth of white clover was therefore not associated with higher densities of *H. trifolii* than was good growth. The highest egg densities of the nematode occurred more often (though still fairly rarely) in places with white clover than in those without. Numbers of eggs per cyst were about twice as high at the same cyst densities in places with white clover than in those without, but in the latter places they were the same at low and high cyst densities. White clover had therefore disappeared there on the average some two years before irrespective of cyst or egg density.

Numbers of eggs per cyst ranged from small to large at all cyst densities in places with clover and in pastures with a good as well as in those with a poor stand of this plant. Increase and decrease must therefore have alternated even in the presence of white clover. Consequently equilibrium densities of *H. trifolii* on white clover were low in most pastures sampled and almost always well below the tolerance limit of white clover seedlings of about 50 eggs per g of soil found in pot experiments. There were no indications that breaking up old pastures and sowing a new clover-grass mixture generally resulted in much higher densities than those found in old pastures. On the contrary the frequency distribution of cyst and egg densities in places with and without clover in a four year old pasture with a very good stand of white clover was the same as that in all samples from the other investigated pastures taken together. When samples from the latter pastures were grouped according to good or poor stand of the clover on the sampled spot or in the pasture as a whole, this also resulted in the same frequency distributions of cyst and egg densities. Stand of white clover and densities of *H. trifolii* were almost unrelated. It is therefore unlikely that *H. trifolii* has been or could be the cause of decline or poor growth of white clover in the sampled pasture except on a very small proportion of their area.

Both cyst and egg densities were generally very low in places without clover. If this plant disappeared from these places because of attack by *H. trifolii* this must have happened several years ago. It is difficult to understand then why these places were not reinvaded by white clover. If such a reinvasion is generally impossible, then an equilibrium between white clover and grass would be impossible and the white clover would disappear, even in the absence of *H. trifolii*.

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SAMENVATTING

In een potproef was de tolerantiegrens van witte klaver voor *H. trifolii* gedurende de eerste acht weken na het zaaien ongeveer 50 eieren per g grond. In andere potproeven vermeerderde *H. trifolii* zich tot ongeveer 1400 eieren per g grond zonder zichtbare schade te veroorzaken aan witte klaver. Witte klaver handhaafde een dichtheid van ongeveer 20% in een mengsel met Engels raaigras bij een bevolkingsdichtheid van *H. trifolii* van 80 tot 200 eieren per g grond.

De bevolkingsdichtheden van dit aaltje in ongeveer twee honderd grondmonsters uit 74 weilanden waren meestal minder dan 1 cyste per 50 g grond en 1 ei per g grond en slechts zelden meer dan 32 eieren per g grond. Ze waren wat lager op plaatsen zonder dan op plaatsen met witte klaver en ook op plaatsen met slecht groeiende dan op die met goed groeiende witte klaver. Er was geen verschil tussen weiden met veel en met weinig witte klaver, met uitzondering van een drie jaar oude weide met een zeer dichte stand van witte klaver. Hier werd een wat hogere dichtheid van het aaltje gevonden dan het gemiddelde van alle onderzochte weiden. De stand van de witte klaver was in dit weiland ook vier jaar na het zaaien nog zeer goed. De frequentiedistributie van dichtheden van cysten en eieren op plaatsen met en zonder klaver was toen geheel gelijk aan die van alle andere monsters tezamen. Er was dus geen verband tussen deze distributie en de stand van de klaver.

Uit de verzamelde gegevens wordt de conclusie getrokken, dat de bevolkingsdichtheden van *H. trifolii* in het algemeen te laag zijn om de oorzaak van slechte groei van witte klaver in de onderzochte weilanden te kunnen zijn.

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