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The dynamics of the decline of the cereal cyst nematode, *Heterodera avenae*, in four soils under intensive cereal production

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Summary – Changes in the population density of the cereal cyst nematode, *Heterodera avenae*, were monitored in four soils in microplots for 10 years. For the first 4 years, half of the microplots were treated each year with drenches of formalin (38 % formaldehyde) prior to sowing with susceptible cereals. Thereafter, plots were sown with susceptible or resistant spring barley or ryegrass to manipulate the numbers of female nematodes and eggs produced each year. After the fourth application of formalin, the densities of the nematophagous fungi, *Nematophthora gynophila* and *Verticillium chlamydosporium*, were estimated under each crop in untreated soil and soil previously treated with the partial soil sterilant. Formalin significantly increased nematode populations and reduced the densities of the nematophagous fungi in all soils. By the second year of formalin application, nematode population densities in all soils had increased to levels which caused crop failure in winter wheat; thereafter, populations declined under spring barley unless applications of formalin were continued. Despite continuous cropping with susceptible cereals, populations of the nematode were often not detectable in plots after the eighth year. Changes in the densities of spores of the fungi in soil were closely related to changes in the numbers of nematodes. In soils previously treated with formalin, the fungi had increased to levels similar to those found in naturally suppressive soils within 3 years of discontinuing formalin application and population densities of the nematode had declined to the same levels as in untreated soils. © Orstom/ Elsevier, Paris

Résumé – ***Dynamique du déclin du nématode à kystes des céréales,* Heterodera avenae, *sur quatre sols mis en culture intensive de céréales*** – Les changements affectant la densité de population du nématode à kystes des céréales, *Heterodera avenae*, ont été observés pendant 10 années dans des micro-parcelles contenant quatre types de sol. Pendant les 4 premières années, le moitié des micro-parcelles ont été traitées annuellement par un arrosage à l'aide de formol (38 % d'aldhéhyde formique) précédant un semis de céréales sensibles. Les parcelles ont été ensuite ensemencées en orge de printemps, sensible ou résistant, ou en rye-grass de façon à estimer le nombre de femelles et d'oeufs produits chaque année. Après la quatrième année de traitement au formol, la densité des champignons nématophages *Nematophthora gynophila* et *Verticillium chlamydosporium* a été estimée pour chaque culture dans les sols non traités et dans ceux traités auparavant avec le produit partiellement stérilisant. Le formol augmente les populations du nématode et diminue la densité des champignons nématophages. A la deuxième année d'application de formol, la densité des nématodes s'est accrue dans tous les sols pour atteindre un niveau provoquant une diminution de récolte chez le blé d'hiver ; les populations déclinent ensuite sous orge de printemps même si les applications de formol se poursuivent. Après la huitième année, malgré une culture continue de céréales sensibles, les populations du nématode n'étaient souvent plus détectables. Les modification dans la densité des spores de champignon présentes dans le sol sont étroitement liées aux changements dans le nombre de nématodes. Dans le sols traités auparavant au formol, le niveau des champignons s'est accru pour atteindre des valeurs similaires à celles observées dans des sols naturellement intolérants (= suppressive soils) au cours de 3 années sans application de formol, et les densités de population du nématode ont diminué pour atteindre des niveaux similaires à ceux des sols non traités. © Orstom/Elsevier, Paris

Keywords: *Heterodera avenae*, nematophagous fungi, *Nematophthora gynophila*, population dynamics, suppressive soils, *Verticillium chlamydosporium*.

In several countries, soils have been identified in which the population densities of specific plant parasitic nematodes have declined under monocultures of susceptible crops because nematophagous parasites and antagonists have increased, in some cases, to densities which limit nematode multiplication (Stirling, 1991). The causal agents of this natural control have been identified and include several species of nematophagous fungi and the bacterium *Pasteuria penetrans*. These parasites increased sufficiently to prevent the multiplication of cyst and root-knot nematodes, respectively, within 3-5 years of continuous cropping. Few detailed studies have been made on the population dynamics of nematodes and their natural enemies in suppressive soils and there is a dearth of quantitative methods for estimating the numbers of nematophagous microbial parasites. In the laboratory, Jaffee *et al.* (1992, 1993) have done pioneering research on the quantitative relationships between both endoparasitic and trapping fungi and their nematode hosts, and

have provided much valuable information on the density dependence of these interactions. In Florida, the impact of *P. penetrans* on the population dynamics of *Meloidogyne arenaria* has also been studied on a range of crops (Oostendorp *et al.*, 1991; Chen *et al.*, 1997). This paper describes a 10-year study done in small plots out-of-doors in which the population dynamics of the cereal cyst nematode, *Heterodera avenae*, were investigated in four soils containing nematophagous fungi.

The decline of populations of *H. avenae* under monocultures of susceptible cereals was first reported in the UK (Collingwood, 1962) and is now a widespread phenomenon throughout northern Europe (Kerry, 1982). Kerry *et al.* (1982*a*,*b*) demonstrated that the suppression of nematode multiplication was caused by the nematophagous fungi *Nematophthora gynophila* and *Verticillium chlamydosporium* which parasitised female nematodes and their eggs in the rhizosphere. *N. gynophila* prevented the formation of nematode cysts, which are the survival stage of the nematode between crops, and *V. chlamydosporium* reduced fecundity and the numbers of healthy eggs. The partial soil sterilant formalin (38 % formaldehyde) applied as a soil drench resulted in increased nematode multiplication (Williams, 1969) and was demonstrated to reduce parasitism of cereal cyst nematodes by parasitic fungi (Kerry *et al.*, 1980, 1982*b*). Applications of formalin and the fungicide Captafol have been used in the field to estimate the level of natural control exerted by these fungi (Stein, 1993).

Natural control of cereal cyst nematodes has proved the most sustainable method of nematode management in intensive agriculture but it is slow to establish in soils and difficult to exploit. However, little is known of the dynamics of the decline phenomenon. The study described in this paper uses different crops and formalin soil drenches to investigate the relationship between the population densities of the cereal cyst nematode and its fungal parasites under intensive cereal cropping in microplots.

Materials and methods

Soil from four sites (Table 1) was used at IACR-Rothamsted to fill 96 microplots (24 for each soil) made from polypropylene cold water tanks (Osma, Hayes, UK) which were 625 × 488 × 510 mm (length x width x depth) in size and had five drainage holes (diam. 20 mm) drilled in their bases. The tanks were buried in sand and ballast on a gravel base and the area surrounded by a retaining wall. Each site had been selected for its history of intensive cereal cropping and all had soils which were thin and free-draining, lying over chalk; each soil was naturally infested with *H. avenae* and contained both *N. gynophila* and *V. chlamydosporium*. Three of the soils were calcareous silt loams and the soil from the site at Sutton Veny contained significant amounts of organic matter; all soils were alkaline (Table 1).

For the first 4 years, 94 ml of formalin (38 % formaldehyde) was applied to twelve of the plots of each soil (48 plots in total) 3-4 weeks before the cereal crop was sown (Table 2). The application of formalin was equivalent to 3000 L ha^{-1}, a rate demonstrated to cause significant increases in *H. avenae* population densities (Williams, 1969) and decreases in the density of parasitic fungi (Kerry *et al.*, 1980). The formalin was applied in 3.5 L of water as a soil drench to each plot. Before each spring barley crop, the equivalent of 376.5 kg ha^{-1} 0:20:20 fertilizer with 188.3 kg ha^{-1} Nitrochalk and 188.3 kg ha^{-1} $MgSO_4$, were applied to the seed bed; a second similar rate of Nitrochalk was applied 6 weeks later. In the second year of the experiment, winter wheat cv. Maris Huntsman was sown and received 376.5 kg ha^{-1} 13:13:20 fertilizer in the seed bed and 100 kg ha^{-1} Nitrochalk in the spring. The perennial ryegrass received fertilizer applications in the spring at rates similar to that applied to the barley crops. All cereal crops were sown

Table 1. *Characteristics of the four soils used in the microplots (all shallow soils over chalk).*

Site	pH	Soil type	Soil texture (%)			*H. avenae* (eggs g^{-1} soil)	Fungal density (spores g^{-1} soil)	
			sand 2000-63 µm	silt 63-2 µm	clay < 2 µm		Ng *	Vc**
Crux Easton	7.5	Silt Loam	6.7	73.3	20.0	37	41	39
South Tidworth	8.1	Calcareous silt	31.5	54.8	13.7	20	5	218
Sutton Veny	7.9	Calcareous humose silt	29.1	58.3	12.6	41	140	434
Devizes	8.1	Calcareous silt	37.1	53.2	9.7	50	27	621

* Ng = *Nematophthora gynophila*
** Vc = *Verticillium chlamydosporium*

Table 2. *Management of the four soils.*

Year	Crop*	Treatment	Date of formalin application
1	s. barley (S) cv. Athos	formalin**	2 March
1	w. wheat (S) cv. Maris Huntsman	"	16 September
3	s. barley (S) cv. Athos	"	13 March
4	s. barley (S) cv. Athos	"	15 February
5-8	s. barley (S) cv. Triumph; s. barley (R) cv. Tyra; Perennial ryegrass	No formalin	–
9	s. barley (S) cv. Triumph; s. barley (R) cv. Vista; Perennial ryegrass	"	–
10	s. barley cv. Klaxon	"	–

* Spring (s) barley and winter (w) wheat, susceptible (S) or resistant (R) to *H. avenae.*
** Formalin (38% formaldehyde) applied cumulatively to half the plots of each soil. Years 1-4 and 10, all plots sown with the same cereal. Years 5-9 three crops were sown each to one-third of the plots containing either untreated soil or soil previously treated with formalin.

by hand in four rows 15 cm apart at seed rates equivalent to 181.5 kg ha^{-1}; the ryegrass was broadcast at 33 kg ha^{-1}. Pesticide applications were made as required in accordance with manufacturer's instructions. The microplots were grouped in fours with one of each being filled at random with one of the four soils; all plots within each group received the same treatment (formalin or untreated) and were sown with the same crop. Although treatments were assigned at random, each soil - crop - treatment combination was represented within each block of 24 plots when three crops were sown; in the first 4 years of the experiment, when a single crop was sown, each block contained three replicates of each soil – treatment combination. At each harvest, the two central rows of each plot of cereals were cut at ground level and removed for drying at 80 °C overnight; the grain was removed and straw and grain weights at 15 % moisture content were estimated separately. The ryegrass was cut each year but yields were not recorded.

Ten soil cores (2.5 × 15 cm) were removed at random from each plot at the time of preparing a seed bed but prior to the application of formalin or, after this treatment ceased, samples were taken from the seed bed or the ryegrass sward in spring at the time the barley crop was sown. The cores were bulked, screened through a 4 mm aperture sieve, thoroughly mixed and a 50 g subsample removed to determine the moisture content. Soil was stored in sealed polythene bags at 4 °C for up to 4 weeks before they were further processed for the extraction of nematode cysts and fungal propagules. Cysts were extracted from 100 ml sub-samples of soil from each plot. The subsamples were weighed and then washed through a fluidising column (Trudgill *et al.*, 1972) as modified by Kerry (1975). The population densities of nematodes were estimated in terms of the number of healthy eggs g^{-1} air-dried soil using standard methods (Southey, 1970). At the beginning of the fourth year and thereafter, the resting spores of *N. gynophila* and *V. chlamydosporium* were extracted from a 25 g sub-sample of soil by the wet sieving – centrifugation method described by Crump and Kerry (1981) and the numbers of spores g^{-1} air-dried soil estimated. As this method was very time consuming, soil samples from only half the experiment (two replicates from each soil, crop and treatment) were processed; the same two blocks of the experiment were used for the estimation of fungal population densities each year. At the end of the experiment, estimates of the densities of chlamydospores of *V. chlamydosporium* were compared with those of the densities of colony forming units (cfu) of the fungus as assessed on dilution plates of a semi selective medium (Kerry *et al.*, 1993), a method not available when the experiment was initiated.

Results

In both treated and untreated soil, population densities of the nematode increased in the first year of spring barley cv. Athos but decreased between year 2 and 3 when winter wheat cv. Maris Huntsman was grown (Fig. 1). Population densities in untreated soil were > 100 eggs g^{-1} soil after the first year, but they declined after the second year to densities of < ten eggs g^{-1} soil by the seventh year despite the continuous cropping of susceptible barley cultivars (Fig. 1). After the first year of the experiment, nematode population densities were significantly greater ($P \leq 0.001$) in all soils treated with formalin than in untreated soils, and this effect persisted throughout the experiment. However, after year 7, population densities in all soils were < two eggs g^{-1} soil and differences between treated and untreated soils were probably of little biological significance. Nematode population densities declined in all plots after the fourth year, when applications of formalin ceased; by the eighth year, there was little difference between densities of the nematode in untreated and previously treated soil under all crops. After year 8, the nematode was unde-

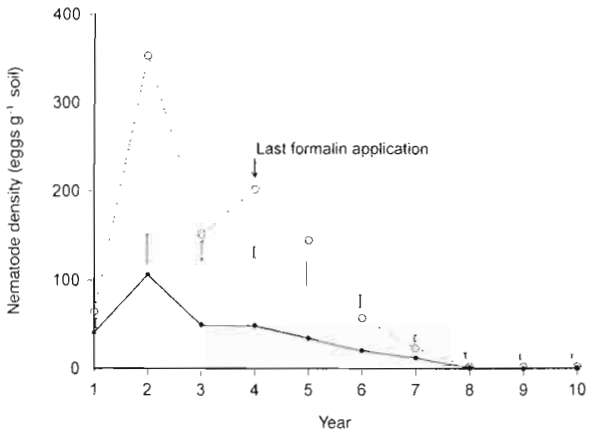

Fig. 1. *Changes in the mean density (eggs g^{-1} soil) of* Heterodera avenae *on susceptible cereals in untreated soils and those treated with formalin (○-----○ soil treated with formalin; ●——● untreated soil).*

tectable in more than 60 % of the plots, even in plots where susceptible cereals had been grown continuously, so statistical analysis was not used on data from these last two years of the experiment. The population trends were similar in all soils but nematode densities were significantly smaller in the soil from the Tidworth site and were greatest in formalin treated soils from Sutton Veny and Devizes (Table 3). At the time of the second application of formalin, and again in year 4, the differences in nematode population densities in treated and untreated soil were significantly smaller in soil from Crux Easton than from the other sites (Table 3). Rates of decline under susceptible barley were significantly slower than under resistant barley in both treated and untreated soil (Table 4); the rate of decline under grass was similar to that under resistant barley in untreated soil but similar to that under susceptible barley in soil previously treated with formalin. Populations of the nematode decreased most rapidly in untreated soils under resistant barley.

In the first 2 years, yields of neither spring barley nor winter wheat (year 2) were affected by the soil type or its treatment with formalin despite considerable differences in the densities of the nematode (Table 5). However, in the second year, when nematodes were most numerous, the yields of winter wheat were very low in all plots and the crop failed. There was a significant interaction ($P \leq 0.001$) between the effects of treatment with formalin and soil type on spring barley yields in years 3 and 4; in soil from Devizes, the application of formalin tended to decrease yields whereas in soil from Tidworth, yields were increased by the treatment. Spring barley yields between the third and seventh years tended to be

Table 3. *Effect of annual pre-planting applications of formalin on the population densities (eggs g^{-1} soil) of cereal cyst nematode in four soils.*

Soil	Year							
	1		2		3		4	
	–°	+	–	+	–	+	–	+
Crux Easton	42	33	183	252	53	140	78	196
South Tidworth	12	27	39	154	19	90	7	90
Sutton Veny	28	53	69	335	47	192	41	287
Devizes	50	49	91	274	71	200	61	261
Mean	33	41	96	254	48	156	47	208
$\pm SE_{DIFF}$	5.7		19.9		14.6		11.1	
	NS		***		***		***	
$\pm SE_{DIFF}$ interactions	11.4		39.8		29.2		22.2	
	NS		**		NS		***	

° Untreated soil (–) and soil treated with formalin (+).

Table 4. *Decline of* Heterodera avenae *population densities (eggs g^{-1} soil) under three crops in untreated soil (–) and soil treated (+) with formalin for the previous four years.*

Crop	Treatment	Year			
		5	6	7	8
Susceptible barley	–	34	20	12	1
	+	144	57	23	2
Resistant barley	–	26	3	1	0
	+	119	26	6	2
Ryegrass	–	34	8	3	1
	+	175	66	22	2
$\pm SE_{DIFF}$		29.4	10.5	3.7	0.6

Means of sixteen replicates; four soils.

significantly larger in soil from Tidworth than from the other sites and often smaller in soil from Devizes (Tables 5, 6). For the first two years following the last formalin application, yields of barley from treated soil remained significantly smaller than those from untreated soil but thereafter there was no effect of treatment (Fig. 2); there were no significant interactions between this previous treatment and soil type or cereal cultivar. Resistant barley produced greater yields than those from the susceptible cultivar in all soils only in years 7 and 8 (Table 6). After year 8 when nematode populations were small, yields of resistant and susceptible barley were similar in all plots. Grain yields were similar in all plots in the final year of the experiment and the data are not presented; yields of straw showed similar trends to those for grain and are not included.

Table 5. *Yields of spring barley cv. Athos and winter wheat cv. Maris Huntsman (year 2) in four soils infested with cereal cyst nematode and treated with formalin or left untreated.*

Soil	Year											
	1			2			3			4		
	–	+	Mean	–	+	Mean	–	+	Mean	–	+	Mean
Crux Easton	4.45	4.25	4.35	2.50	2.12	2.31	4.24	4.03	4.14	3.63	3.21	3.42
South Tidworth	4.98	4.75	4.87	2.52	1.86	2.19	3.87	5.52	4.70	4.77	5.57	5.17
Sutton Veny	4.07	4.69	4.38	2.67	2.75	2.71	4.02	4.36	4.19	4.26	4.56	4.41
Devizes	4.43	4.72	4.58	2.71	2.57	2.64	3.89	3.69	3.79	4.80	3.63	4.42
Mean	4/48	4.60		2.60	2.33		4.01	4.40		4.36	4.24	
$\pm SE_{DIFF}$	0.22		0.31	0.17		0.24	0.16		0.22	NS		0.22
	NS		NS	NS		NS	*		**			***
$\pm SE_{DIFF}$ interaction	0.44			0.34			0.32			0.31		
	NS			NS			***			***		

Means of twelve replicates.

Table 6. *Yields of susceptible (S) and resistant (R) cultivars of spring barley grown continuously in four soils infested with cereal cyst nematode.*

Soil	Year														
	5			6			7			8			9		
	S°	R°°	Mean	S	R	Mean	S	R	Mean	S	R	Mean	S	R	Mean
Crux Easton	2.47	2.99	2.73	5.41	5.41	5.41	2.57	5.00	3.79	3.71	4.63	4.17	4.62	5.03	4.82
South Tidworth	3.94	3.51	3.72	5.53	4.15	4.84	4.04	4.79	4.41	4.26	4.49	4.38	4.93	5.00	4.96
Sutton Veny	2.63	2.74	2.68	5.67	4.46	5.06	2.37	3.52	2.94	4.25	4.62	4.44	4.91	5.38	5.15
Devizes	2.51	2.81	2.66	4.38	4.21	4.30	2.89	4.59	3.74	4.42	4.90	4.66	4.60	4.59	4.59
Mean	2.89	3.01		5.25	4.56		2.97	4.47		4.16	4.66		4.77	5.00	
$\pm SE_{DIFF}$	0.25		0.35	0.34		0.47	0.30		0.42	0.20		0.29	0.26		0.37
	NS		**	*		NS	***		*	*		NS	NS		NS

° cv. Triumph except year 7 when cv. Georgie was grown.
°° cv. Tyra except year 9 when cv. Vista was grown.
Means of four replicates.

At the beginning of the fourth year, the numbers of spores of both *N. gynophila* and *V. chlamydosporium* were significantly fewer ($P \leq 0.001$) in soils treated with formalin for four years than in untreated soil (Table 7). *N. gynophila* and *V. chlamydosporium* were most abundant in untreated soils from Sutton Veny and Devizes, and least common in soils from Tidworth and Crux Easton. In general, as nematode populations declined under susceptible barley both in soils previously treated with formalin and in untreated soils, there was a tendency for spore numbers in soil to increase (Fig. 3). However, the spore densities in soil previously treated with formalin appeared to increase erratically, especially those of *N. gynophila*, although in years 7 and 9 even in these plots the densities were similar to or greater than those in untreated soil in year 5 (Fig. 3). Between years 5 and 9, spore densities of both fungi increased under susceptible barley both in soils previously treated with formalin and in untreated soils (Table 8). However, under resistant barley and ryegrass, which supported few nematodes, the spore densities declined in untreated soil and failed to increase in treated soil. Although there were significant differences ($P \leq 0.001$) between the densities of spores in the different soils, the effects of crop and treatment with formalin were similar in each and the data are not presented separately. The relationship between estimates of the density of

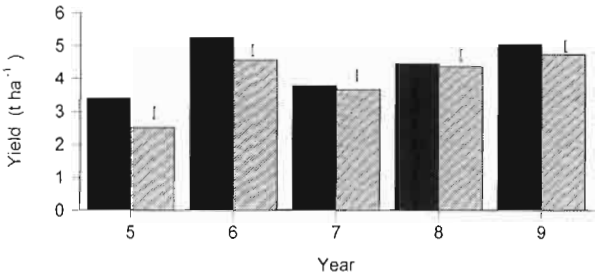

Fig. 2. *Mean yields (t ha^{-1}) of spring barley in soils previously treated with formalin (grey) and untreated soil (black).*

Table 7. *Densities (spores g^{-1} soil) of* Nematophthora gynophila *(Ng) and* Verticillium chlamydosporium *(Vc) at the beginning of the fourth year in four untreated soils (–) and those drenched each year for four years with formalin (+).*

Soil	Treatment	Fungus	
		Ng	*Vc*
Crux Easton	–	41	39
	+	2	9
South Tidworth	–	5	218
	+	3	78
Sutton Veny	–	140	434
	+	46	231
Devizes	–	27	621
	+	2	85
Mean	–	53	324
	+	13	100
SE_{DIFF}		8.7	29.7
		***	

Means of six replicates.

V. chlamydosporium based on the physical extraction of chlamydospores and that derived from the numbers of cfu developing on the selective medium was examined for the last sampling only. The effect of formalin on the density of cfu in soil was similar to that for spores and data for this comparison are not presented. However, chlamydospores formed a significantly greater proportion of the total number of cfu in soil from Devizes than in soil from Crux Easton and differences in the density of the fungus in each soil based on estimates of the numbers of spores are not consistent with those based on cfu (Table 9); soil from Devizes, which consistently contained the greatest densities of chlamydospores, often had the smallest numbers of cfu. Although there were similar numbers of cfu under the three crops, chlamydospores were most

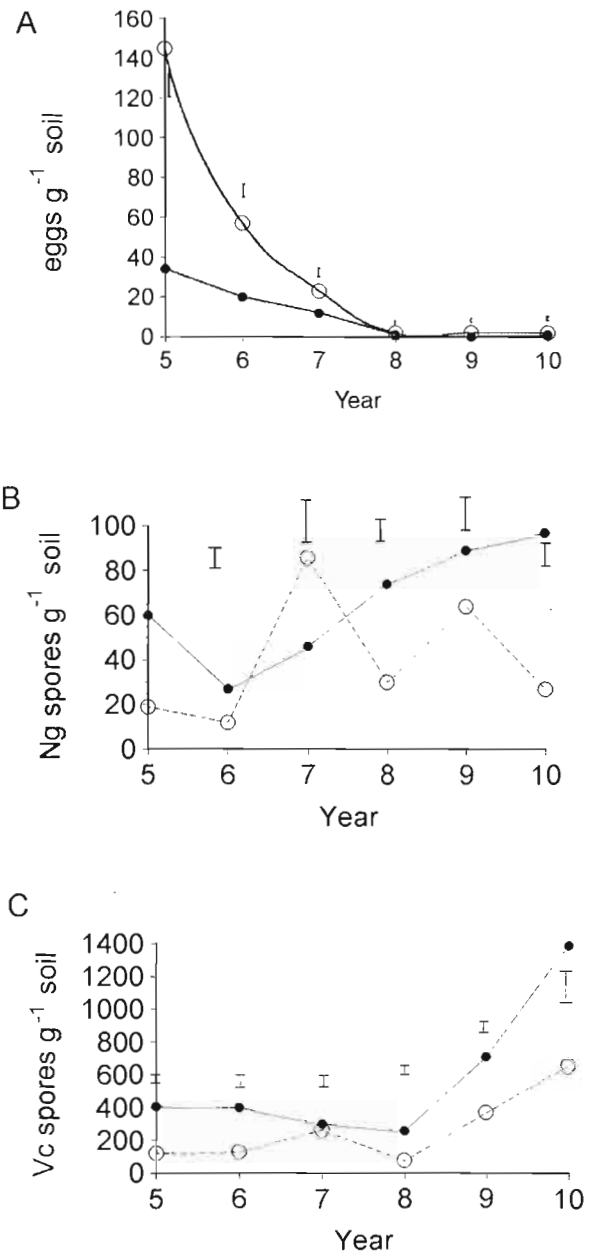

Fig. 3. *Changes in the mean numbers (eggs g^{-1} soil) of cereal cyst nematode (A) on susceptible barley and densities of resting spores of* Nematophthora gynophila *(B) and* Verticillium chlamydosporium *(C) in soil previously treated with formalin and untreated soil (Mean nematode counts from sixteen replicates; mean spore counts from eight replicates; ○-----○ soil previously treated with formalin; ●——● untreated soil).*

abundant ($P \leq 0.05$) in soil under susceptible barley (Table 9).

Table 8. *Densities (spores g^{-1} soil) of* Nematophthora gynophila *and* Verticillium chlamydosporium *after one and four years under different crops in soil previously treated with formalin and in untreated soil.*

Crop	Treatment	*Nematophthora gynophila*				*Verticillium chlamydosporium*			
		Year 5		Year 9		Year 5		Year 9	
		Count	Mean	Count	Mean	Count	Mean	Count	Mean
Barley - S	–	27		63		401		511	
			20		54		266		386
	+	12		44		131		260	
Barley - R	–	59		29		374		157	
			38		23		224		119
	+	16		16		73		81	
Ryegrass	–	54		20		204		147	
			34		15		133		103
	+	14		10		61		58	
SE_{DIFF}		7.6	5.4	13.3	9.4	66.6	47.1	52.9	37.4
		***	**	NS	***	***	*	***	***

Means of eight replicates.

Table 9. *Differences in the numbers of colony forming units (cfu) and chlamydospores (chlam.) of* Verticillium chlamydosporium *in four soils after five years of three crops.*

Soil*	Susceptible barley		Resistant barley		Ryegrass	
	cfu	chlam.	cfu	chlam.	cfu	chlam.
Crux Easton	2242*	247	1054	37	2333	119
South Tidworth	1504	979	2596	270	4879	191
Sutton Veny	5496	1208	4963	371	6500	446
Devizes	1583	1620	1488	373	1257	830
Mean	2706	1013	2525	263	3742	396
SE_{DIFF}		(crop *vs* propagule)		± 493 ($P \leq 0.05$)		
SE_{DIFF} interaction		(soil *vs* propagule)		± 427 ($P \leq 0.01$)		

Means of four replicates.

* Numbers of propagules g^{-1} soil.

Discussion

After partial soil sterilisation with formalin, populations of the cereal cyst nematode built up to very large densities in the four soils, and in individual plots populations > 600 eggs g^{-1} soil were recorded. However, there were differences between the soils in their ability to support the multiplication of the nematode, and populations in soil from the Tidworth site were significantly smaller than those in soil from elsewhere. In the second year, when nematode populations were at a peak in all soils, the winter wheat crop failed and a mean yield of only 2.47 t ha^{-1} was obtained. Wheat is more susceptible to damage than barley (Gair, 1965) but winter wheat is a relatively poor host for the nematode and post-crop population densities were smaller than those observed following barley. In the third year of the experiment, yields of spring barley were larger in formalin treated soil than in untreated soil despite the differences in the numbers of nematodes. This effect was dependent on the soil and may indicate that in soil from Tidworth and Sutton Veny formalin was affecting other factors than the nematode, such as soil nutrient availability or soil borne fungal pathogens, which were limiting crop yields. However, by the fifth year, yields of barley were on average 27 % smaller in formalin treated soil in which the nematodes were more numerous, than in untreated soil; this effect was reduced to 3 % by year 8 when populations of the nematode were similar and very small in both treated and untreated soil. The differences in mean yield observed between treated and untreated soil may also be related to the change of barley cultivar after year 4; cv. Athos may be more tolerant of nematode attack than the other cultivars used.

In untreated soil from each of the four sites, the nematode populations declined, even under continuous susceptible cereal crops and the nematode was often undetectable in many plots after the eighth crop, despite the intensive soil sampling procedure used. However, when these same soils were treated with a partial soil sterilant, the nematode was capable of multiplying to densities which severely reduced yields and these damaging populations were maintained when repeated applications of the formalin sterilant were

made. It is clear that the soils and susceptible cereal cultivars used were capable of supporting large nematode populations and that a soil factor(s) removed by the formalin was limiting nematode populations. These results confirm earlier observations in which the decline of *H. avenae* populations under intensive cereal production has been reported (Kerry, 1982). Several workers have associated this decline with the parasitism of adult female and egg stages of the nematode (Kerry, 1982; Schonhammer & Fischbeck, 1985).

Estimations of the numbers of nematodes parasitised by fungi and the impact of these natural enemies on the population dynamics require intensive, destructive sampling (Kerry *et al.*, 1982*b,c*) which was inappropriate for the small plots used in this long term study. Hence, the activities of the fungi were estimated by measuring changes in the densities of the resting spores of the two main causal agents of the decline phenomenon, *N. gynophila* and *V. chlamydosporium*. Although it had proved difficult to relate changes in densities of resting spores of these fungi with the levels of parasitism of the nematodes in a single growing season in field trials at three sites (Kerry *et al.*, 1982*c*), it was possible to detect significant differences between the numbers of spores found in suppressive soils and those in which the nematode multiplied (Crump & Kerry, 1981). In this study, in which plots were sampled only annually but more intensively than in previous field trials, it was possible to demonstrate that applications of formalin significantly reduced the numbers of spores of both *N. gynophila* and *V. chlamydosporium* in each of the four soils and that large nematode infestations were associated with small spore densities and small nematode populations with large spore densities. Also, in the absence of further formalin applications over a 4-year period, the numbers of spores of both fungal species increased in all soils where there were nematode females and eggs on susceptible barley crops, but the numbers of spores decreased under resistant barley or ryegrass on which these nematode stages were scarce or absent. Thus, it was possible to relate changes in the densities of the nematode with those of the fungi as estimated by the numbers of spores in soil. The densities of spores found in the untreated soils of year 4 and in the previously formalin treated soils by year 7 were similar to those reported from known suppressive soils (Crump & Kerry, 1981; Kerry *et al.*, 1982*a*). However, the size of soil sample (25 g) and the processing method used appeared not to be sufficiently sensitive to estimate reliably changes in the annual density of these fungi. Although general increases in the numbers of spores in soil under susceptible barley were observed as nematode populations declined, there was much variation in the estimates of fungal densities in samples taken from the same soil in the same year and in estimates between years. Also, in the case of *V. chlamydosporium*, the use of the selective medium demonstrated that the fungus was much more abundant in some soils than was indicated by counts of the chlamydospores alone. Isolates of *V. chlamydosporium* differ in their ability to produce chlamydospores *in vitro* (Kerry *et al.*, 1986) and may also differ in this ability in soil. Also, the selective medium may differ in its efficacy in estimating the abundance of the fungus in different soils (Kerry *et al.*, 1993); although there is a general positive and linear relationship between the abundance of chlamydospores in soil and the densities of propagules of the fungus, there is much variation between soils (Kerry *et al.*, 1993), which makes it difficult to interpret detailed observations on changes in fungal abundance. Interestingly, chlamydospores not only formed a greater proportion of the total numbers of propagules in some soils, such as that from Devizes compared to that from Crux Easton, but they were also significantly more abundant in soils under susceptible barley than under resistant or poor hosts for the nematode. The fungus may depend on the nematode to produce its resting structures in large numbers and ensure its long term survival in soil.

Changes in the densities of resting spores of both the obligate parasite, *N. gynophila*, and the facultative parasite, *V. chlamydosporium*, were closely associated with changes in the densities of the nematode in soil not treated with formalin. Populations of the nematode declined to densities which did not affect the yield of tolerant crops in 4 years and the two species of nematophagous fungi increased, within 3 years, to levels previously reported to be associated with the decline of the nematode (Crump & Kerry, 1981). The rates of decline of the nematode under resistant barley and ryegrass were similar to those reported elsewhere (Gair, 1965; Gair *et al.*, 1969); but it was not possible to demonstrate a positive density dependent effect of the fungi on nematode populations as has been observed by Jaffee (1992), who found that the weakly saprophytic fungus, *Hirsutella rhossiliensis* parasitised few nematodes at low densities and exerted little population control unless nematode hosts were abundant. In our studies, the estimates of fungal densities may have been too variable for the detection of density dependence; indeed, Jaffee's research was done in small microcosms to avoid such variation in sampling. Although the numbers of spores of both fungi were associated with changes in the density of *H. avenae*, it might be expected that parasitism by the obligate parasite *N. gynophila* would show greater density dependence on the host than *V. chlamydosporium*, which may grow saprophytically in soil. The latter species was the more prevalent in the soils used in this

experiment and populations of *H. avenae* declined more slowly in the years in which the nematode was abundant compared to those when the populations were smaller (< twenty eggs g^{-1} soil). This study has provided further evidence of the decline of populations of *H. avenae* under susceptible cereal crops and, for the first time, has provided information on the associated dynamics of the nematophagous fungi which cause this phenomenon. Changes in the densities of the nematophagous fungi to levels which were associated with significant reductions in *H. avenae* populations appeared to take 3-4 years, even in soils where the fungi were established. It remains to be seen whether the speed of such changes can be increased by applications of selected isolates of these fungi to provide a more easily managed strategy for biological control.

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