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Effect of seed and soil disinfectants on establishment, growth and mutual relations of white clover and grass in leys

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1 Introduction

To see how far fungi and root nematodes inhibit growth of white clover in grassland, we set out two series of field trials with control plots and plots from which such organisms were excluded as far as possible with pesticides. In Series 1 seed disinfection or a single soil disinfection against fungi had no visible effect on emergence, growth or proportion of clover, except for some initial retardation with Brassicol in soil, so Series 2 concentrated on the effect of a single soil fumigation against nematodes. In Series 3, the effect was studied of *Tagetes* (African marigold) on nematodes and on growth of grass-clover mixture.

2 Series 1: fungicides on seed or in soil or nematicide (D-D) in soil

2.1 Design and management of the trials

Series 1 consisted of 16 triplicated trials on sandy fields varying in outlook with plots 8 m × 2.5 m including a border of 40 cm, net area 12.24 m². The grass-clover mixture was sown in 1956 after various arable crops or directly after ploughing up. Seven trials remained under observation into 1957 and 1958.

A mixture of white clover cv. Witte cultuurklaver C.B. 5 kg per ha and grass, mainly perennial ryegrass, 25 kg per ha, was sown either in April (treatments 1-3) or late in June (treatments 4 and 5). The treatments were as follows.

1. Clover seed treated with a mixture of the fungicides thiram (tetramethylthiuram disulphide) 3 g and Voronit (organomercurial + benchinox) 3 g per kg
2. Soil treated a week before sowing with a fungicidal dust, Brassicol (20% pentachloronitrobenzene) 40 g per m² raked in to a depth of 10 or 15 cm
3. Control of treatments 1 and 2
4. Soil injected early in May with the nematicide D-D (main component 1,3-dichloropropene), 60 ml per m², at about 15 or 20 places per m² to a depth of 15 cm. To avoid toxic effects to the seedlings, the soil was forked through twice before sowing
5. Control of Treatment 4, also forked through before sowing.

Pesticides were applied only in the first year. Since most fields had been dressed with phosphate and potassium in 1956 before the trials, no more was supplied until spring 1957: superphosphate supplying 45 kg P_2O_5 and potassium chloride supplying 100 kg K_2O per ha. Also at that time fields poor in magnesium received magnesium sulphate 50 kg MgO per ha. In 1958 all fields received 3 topdressings

supplying in total P_2O_5 85, K_2O 280 and MgO 65 kg.

Nitrogen as ammonium nitrate limestone 15 kg N per ha was supplied to 12 fields before Cut 2 in 1956 and to all fields before each cut in 1957 and 1958.

Treatments 4 and 5 were mown 3 times in 1956 and 5 times in 1957 and 1958. Yield of dry matter per ha was estimated as a whole ('entire yield') and separately for clover and for grass and herbs. Records were not kept for fields damaged by straying cattle (last column of Table 1). As fungicides had no visible effect, yields were not estimated in treatments 1-3 and can be here dismissed from further consideration.

Soil samples taken in June 1956 from each field to a depth of 15 cm had pH-KCl values from 4.7 to 5.8 and humus from 3.2 to 7.4%. Nematodes were counted and classified by elutriation, a method since published by Oostenbrink (1960), in samples to a depth of 20 cm taken before the trial in March 1956 from each field, and in August 1957 from D-D plots and their controls in fields — and × (symbols in figures 2-8). Nematodes were also counted in clover roots from one field (marked ⊥) in November 1956.

Mineral nitrogen and mineralizable nitrogen were estimated in soil samples to a depth of 20 cm from 4 fields taken early in August 1956. Mineralizable nitrogen was taken as the increase in mineral nitrogen in soil kept at 30°C and 60% moisture capacity for 6 weeks.

2.2 Average yields and effect of D-D

Effect of D-D was defined as the difference in yield of dry matter in kg per ha between treated plots (Treatment 4) and controls (Treatment 5); i.e. if D-D decreased yield, the effect was negative. Direct and indirect effects were later distinguished, caused respectively by D-D or by changes in competition from the other crop component.

Right from emergence D-D plots grew considerably better than controls. Numbers of plants were almost the same. The difference was due to vigour and, especially for grass, greenness.

In Cut 1 of 1956 there was more than twice as much grass in D-D plots as controls (Table 1). Clover was more too, though it formed a lower percentage than in controls.

In Cut 2, D-D plots still had more grass but had less clover than controls. The inverse effect on clover proved to be completely due to competition from the luxuriant grass.

In Cut 3, entire yield from D-D plots was no more than from controls but proportion of clover was still slightly lower than in controls.

Average yields and effects in 1956 of the 7 trials continuing into 1957 were in line with average data for all 16 fields, so results for the two years were comparable.

In 1957, D-D had no significant effect on grass but clover proliferated on the

Table 1. Series 1. Average yield components and effect of D-D on them in kg dry matter per ha.

Cut	kg N per ha (av.)	Control		D-D		D-D effect		P% D-D effect		Number of trials
		grass and herbs	clover	grass and herbs	clover	grass and herbs	clover	grass and herbs	clover	
1956										
1	0	686	110	1566	180	+ 880	+ 70	≥99.9	≥99.9	15
2	11	988	258	1347	156	+ 359	—102	≥99.9	≥99.9	14
3	0	671	132	725	77	+ 54	— 55	95-97.5	≥99.9	12
		2345	500	3638	413	+1293	— 87			
1957										
1	15	1129	619	1084	513	— 45	—106	<75	90-95	7
2	15	2204	1723	2166	1896	— 38	+173	<75	75-90	7
3	15	1202	874	1196	1298	— 6	+424	<75	≥99.9	6
4	15	1135	428	1050	653	— 85	+225	75-90	99-99.9	7
5	15	409	94	447	120	+ 38	+ 26	75-90	95-97.5	4
		6079	3738	5943	4480	— 136	+742			
1958										
1	15	1691	363	1855	444	+ 164	+ 81	99-99.9	95-97.5	7
2	15	1506	482	1645	508	+ 139	+ 26	95-97.5	<75	7
3	15	1595	591	1615	494	+ 20	— 97	<75	75-90	7
4	15	1226	387	1238	422	+ 12	+ 35	<75	<75	7
5	15	978	193	963	167	— 15	— 26	<75	<75	5
		6996	2016	7316	2035	+ 320	+ 19			

P% = significance level.

D-D plots, which had 10 percentage units more clover than controls in cuts 3 and 4. As D-D hardly affected grass, soil fumigation must have directly stimulated clover.

In 1958, an effect on clover remained only in the first cut but was accompanied by a temporary positive effect on grass.

2.3 Correlations between yields, effect of D-D and other parameters

Although there was the same general pattern in all trials, values varied considerably between trials. Table 2 shows correlations between 28 parameters. The 1956 data are for Cut 1 or all cuts; 1957 and 1958 data are for all except Cut 5, for which data were too few. The regression lines in Fig. 1 relate yields and effects on grass to those on clover.

Yields of either component in one year were positively related to those in other

Table 2. Series 1. Coefficients of correlation (r). The number of trials is in *italics*. The data for 1957 and 1958 exclude Cut 5.

				Yield control								Yield D-D			
				1956				1957		1958		1956			
				grass		clover		grass	clover	grass	clover	grass		clover	
			Code	Cut 1 a	all cuts b	Cut 1 c	all cuts d	e	f	g	h	Cut 1 i	all cuts j	Cut 1 k	all cuts l
Yield control	1956	grass	Cut 1 a	1											
			all cuts b	0.76 *12*	1										
		clover	Cut 1 c	0.45 *15*		1									
			all cuts d	—0.42 *12*	—0.71 *12*	0.43 *12*	1								
	1957	grass	e	0.66 *6*	0.91 *6*			1							
		clover	f	—0.70 *6*	—0.33 *6*	0.10 *6*	0.69 *6*	—0.45 *6*	1						
	1958	grass	g	0.64 *6*	0.70 *7*			0.95 *6*		1					
		clover	h			0.03 *6*	0.63 *7*	—0.54 *6*	0.96 *6*	—0.58 *7*	1				
Yield D-D	1956	grass	Cut 1 i	0.82 *15*								1			
			all cuts j		0.86 *12*							0.60 *12*	1		
		clover	Cut 1 k			0.79 *15*						0.44 *15*		1	
			all cuts l				0.80 *12*					—0.30 *12*	—0.68 *12*	0.71 *12*	1
	1957	grass	m					0.92 *6*				0.37 *6*	0.63 *6*		
		clover	n						0.96 *6*			0.05 *6*	—0.15 *6*	0.31 *6*	0.65 *6*
	1958	grass	o							0.96 *7*		0.70 *6*	0.84 *7*		
		clover	p								0.95 *7*			0.29 *6*	0.80 *7*
D-D effect	1956	grass	Cut 1 q	—0.27 *15*								0.34 *15*			
			all cuts r	—0.77 *12*	—0.46 *12*							—0.37 *12*	0.06 *12*		
		clover	Cut 1 s	—0.02 *15*		—0.15 *15*						—0.05 *15*		0.48 *15*	
			all cuts t	0.50 *12*	0.49 *12*	—0.09 *12*	—0.46 *12*					0.08 *12*	0.09 *12*	0.22 *12*	0.16 *12*
	1957	grass	u	—0.48 *6*	—0.79 *6*			—0.56 *6*							
		clover	v		0.17 *6*	—0.03 *6*	—0.54 *6*	0.10 *6*	—0.82 *6*						
	1958	grass	w		—0.29 *7*			—0.17 *6*	0.20 *6*	—0.27 *7*					
		clover	x												
	Humus in soil		y	0.68 *14*	0.62 *11*	—0.02 *14*	—0.76 *11*	0.71 *6*	—0.53 *6*	0.56 *7*	—0.47 *7*	0.57 *14*	0.40 *11*	0.03 *14*	—0.59 *11*
No of nematodes	*Pratylenchus*		z	—0.25 *15*	—0.22 *12*										
	saprophagans		aa	—0.24 *14*	—0.01 *11*										
	total free-living		bb	—0.03 *15*	0.05 *12*										

* Analysis of the replicates showed that the high value of r_{aa} was chance.

				D-D effect								Humus in soil	Number of nematodes		
1957		1958		1956				1957		1958					
grass	clover	grass	clover	grass		clover		grass	clover	grass	clover		*Pratylenchus*	sapro-phagans	total free-living
				Cut 1	all cuts	Cut 1	all cuts								
m	n	o	p	q	r	s	t	u	v	w	x	y	z	aa	bb
1															
—0.73	1														
6															
0.85		1													
6															
—0.74	0.92	—0.71	1												
6	*6*	*7*													
				1											
				0.79	1										
				12											
				—0.04		1									
				15											
				—0.76	—0.79	0.48	1								
				12	*12*	*12*									
—0.20					0.19			1							
6					*6*										
0.10	—0.63			—0.70	—0.85		0.87	—0.04	1						
6	*6*			*7*	*7*		*7*	*6*							
	0.06				0.45		—0.23	0.26	—0.44	1					
	6				*7*		*7*	*6*	*6*						
					0.20		—0.39	0.89*	0.15	—0.05	1				
					7		*7*	*6*	*6*	*7*					
0.66	—0.50	0.46	—0.61	—0.15	—0.60	0.04	0.37	—0.41	0.44	—0.42	—0.40	1			
6	*6*	*7*	*7*	*14*	*11*	*14*	*11*	*6*	*6*	*7*	*7*				
				0.40	0.51							0.27	1		
				15	*12*							*14*			
				0.54	0.56							—0.28		1	
				14	*11*							*14*			
				0.73	0.55							0.10			1
				15	*12*							*14*			

Fig. 1. Series 1. Relation between clover and grass (kg dry matter per are). Top, yield on control plots; middle, yield on the D-D treated plots; bottom, D-D effect. A circle or dotted line indicates that the points are not or are hardly grouped around a line. Letters between brackets refer to the code in Table 2.

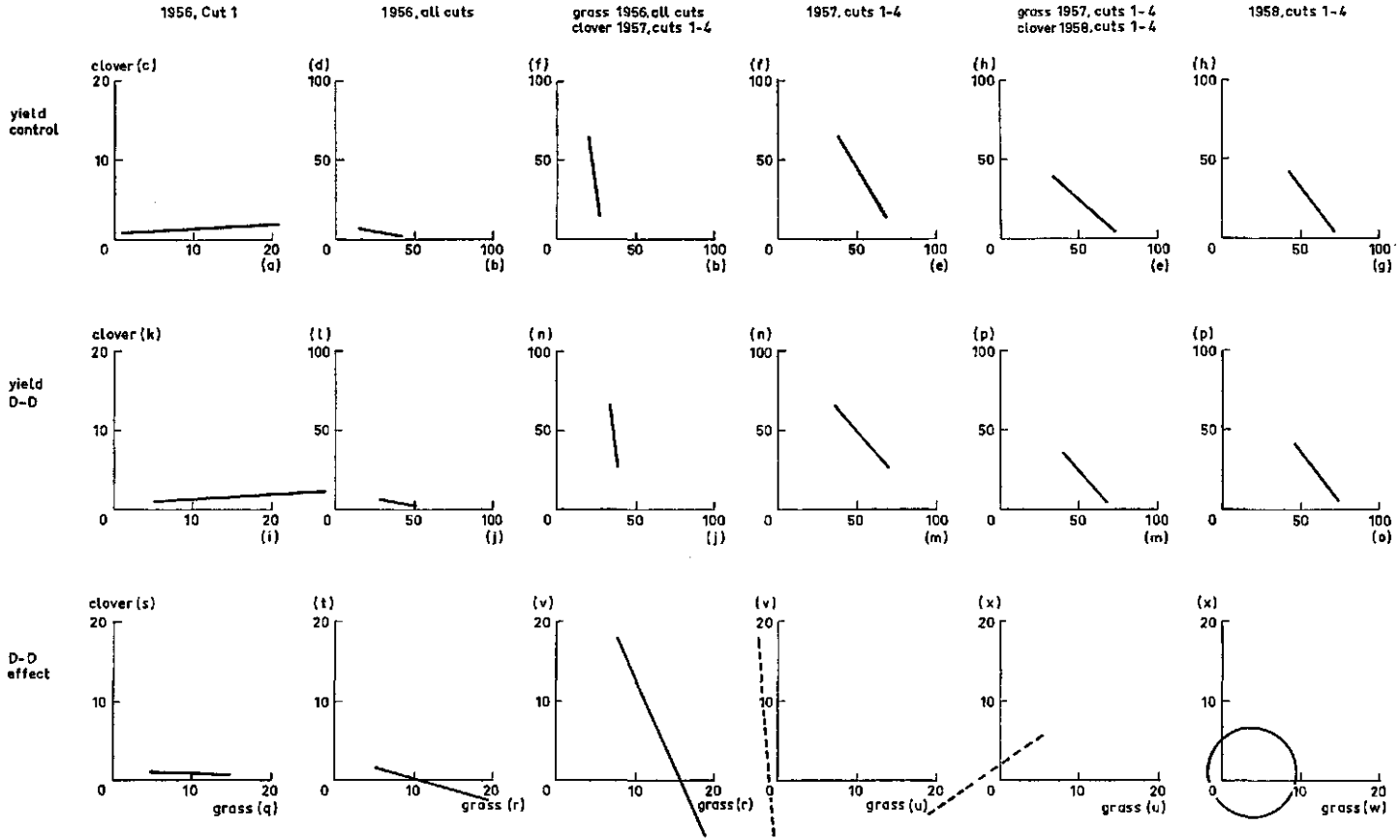


Fig. 2. Series 1. Yield relation between clover and grass (kg dry matter per ha) in 1956 in Cut 1 (left), Cut 2 (middle) and throughout the year (right). Top, control plots; bottom, D-D plots. Average of 3 replicates. The symbols, representing different trials, are the same as in figs 3-8. Expt v had a large yield in Cut 1 because of heavy dressing with N before the trial.

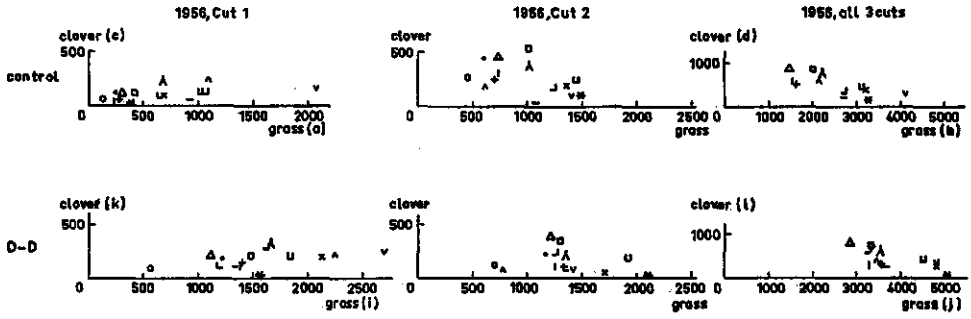
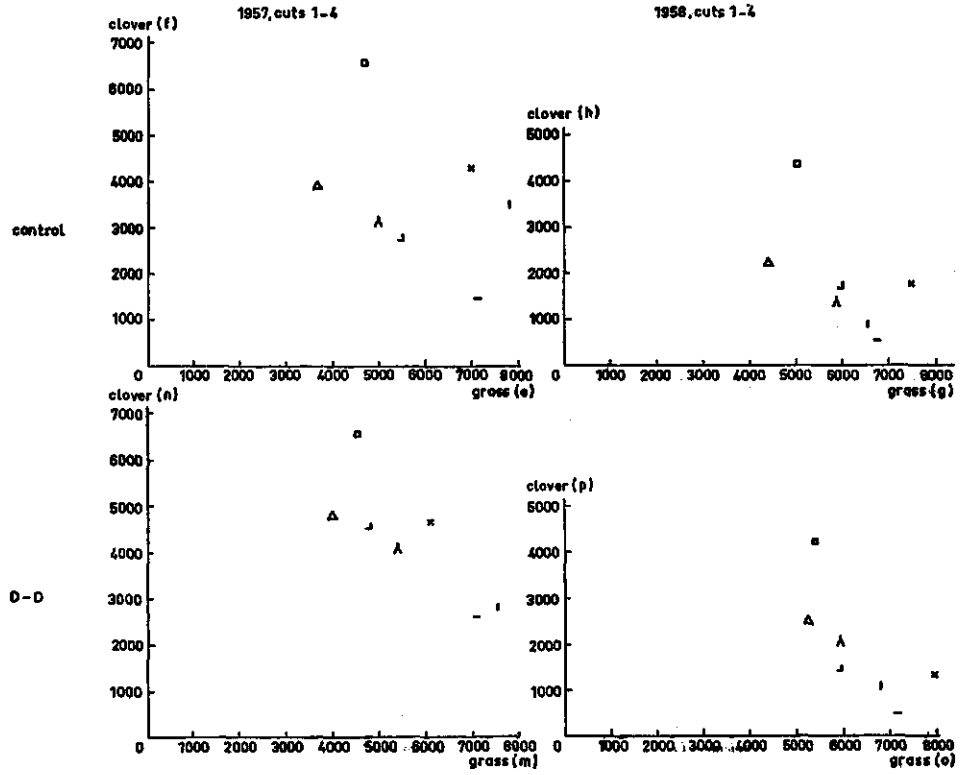


Fig. 3. Series 1. Yield relation between clover and grass (kg dry matter per ha) in 1957 (left) and 1958 (right). Top, control plots; bottom, D-D plots. Average of 3 replicates.



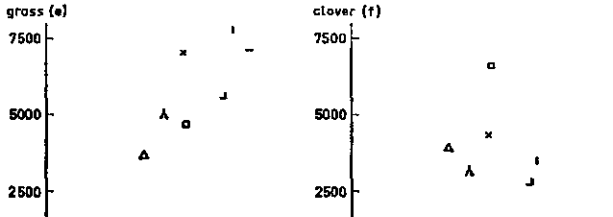
years, e.g. between 1956 and 1958 in controls for grass ($r_{bq} = 0.70$) and for clover ($r_{dh} = 0.63$). Yield of grass in Cut 1 of 1956 was positively related to that of clover in control ($r_{ac} = 0.45$) and in D-D ($r_{ik} = 0.44$) (Fig. 2, top and bottom left) so that soils suitable for grass seem also suitable for clover. Later this effect of soil was masked by competition between grass and clover so that the relations were inverted in Cut 2 (Fig. 2, centre) and became even more so for all cuts (Fig. 2, right; $r_{bd} = -0.71$; $r_{jl} = -0.68$). The inverse relations persisted into 1957 ($r_{ef} = -0.45$; $r_{mn} = -0.73$) and 1958 ($r_{gh} = -0.58$; $r_{op} = -0.71$) (Fig. 3). Fields □ and × had never before grown grass or clover so that absence of pests may cause their yield of clover to be higher in 1957 and 1958 than expected from yield of grass; certainly nematodes could not have caused this effect (Section 2.5).

Yields of grass were positively related to humus in soil (Fig. 4, left). In Cut 1 of 1956 clover was not related to humus (Table 2, r_{cy}) but in later cuts, there was an inverse relation (Fig. 4, right) as others have observed (Sonneveld et al., 1951), presumably by severe competition after Cut 1 from grass whose growth depended on humus. Thus the reverse relation was due to an indirect effect of humus.

The entire effect of D-D in Cut 1 of 1956 ranged from 500 to 1500 kg dry matter per ha, average 950 kg. The variation was due mainly to grass. The effect on either component or on entire yield was independent of yield in controls (i.e. the field's fertility) (Fig. 5), the time since the soil was cropped (5 years to centuries) or the time since a previous crop of grass or clover (Table 3).

After Cut 1 of 1956, the effect of D-D on grass or clover was inversely related to yield of grass or clover, respectively, on control plots (for all cuts, 1956, grass, $r_{br} = -0.46$; for 1957, grass, $r_{eu} = -0.56$; for all cuts, 1956, clover, $r_{dt} = -0.46$; for 1957, clover, $r_{tv} = -0.82$). Between replicates within a trial, there was, however, an inverse relation for grass in Cut 1 also and a positive relation between D-D effect and yield on D-D plots. These relations within trial can be explained by aberrations in either D-D or control plots. Thus if a local factor

Fig. 4. Series 1. Relation of humus in soil with grass yield (left) and clover yield (right) in kg dry matter per ha on control plots in 1957.



fortuitously raises yield in a D-D plot but not in a control plot nearby, the apparent D-D effect is also raised. Replication masks such variation when one trial is compared with another.

In Cut 1 of 1956 before clover and grass were seriously competing, the effect of D-D on clover was constant for different effects on grass (Fig. 1, bottom left; r_{gs} = —0.04) but thereafter effects on grass and clover showed a high inverse correlation in 1956 (Fig. 6; r_{rt} = —0.79). Effect on clover in 1957 depended most on the effect on clover in 1956 (Fig. 7; r_{tv} = 0.87) and hence on grass in

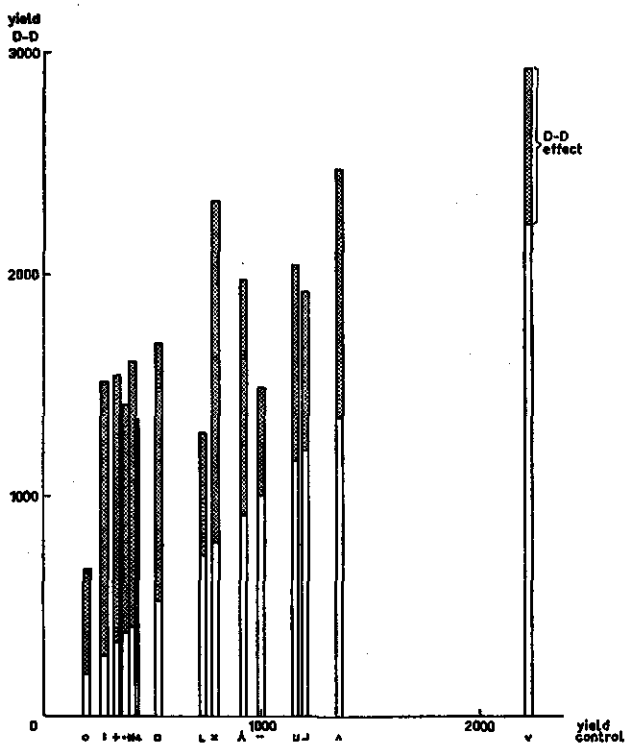


Fig. 5. Series 1, Cut 1, 1956. Relation between entire yield of grass, clover and herbs in D-D and control (kg dry matter per ha). The shaded area is the D-D effect. Average of 3 replicates.

Table 3. Series 1, Cut 1, 1956. Relation between D-D effect (in kg dry matter per ha) and number of years at sowing for the trial since a grass or clover crop.

Years since previous grass or clover crop	Control		D-D		D-D effect		Number of trials
	grass and herbs	clover	grass and herbs	clover	grass and herbs	clover	
0	866	115	1753	146	887	31	4
½-1	675	156	1505	263	830	107	3
4-22	279	67	1302	163	1023	96	3
∞	474	91	1338	154	864	63	4

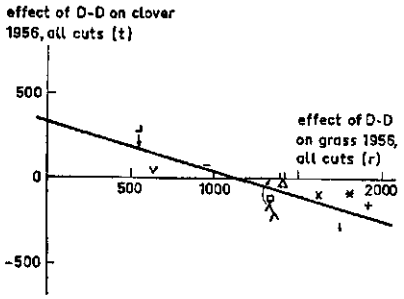


Fig. 6. Series 1, 1956, all cuts. Relation between the effect of D-D on grass and that on clover (kg dry matter per ha). Average of 3 replicates. The values for fields ⌟ and ⅄ are each biased by an extreme value in one replicate. If the extreme values are ignored, values are as indicated by the arrow heads.

Fig. 7. Series 1, clover. Relation between the effect of D-D in 1956 and 1957 (kg dry matter per ha). Average of 3 replicates. For explanation of arrows, see Fig. 6.

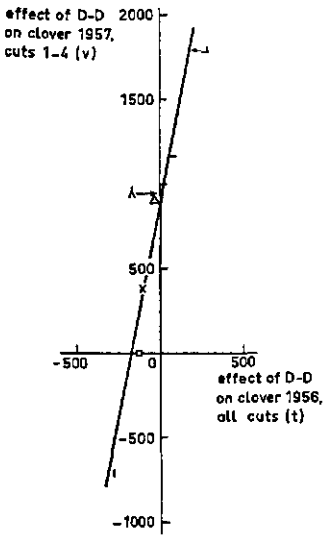
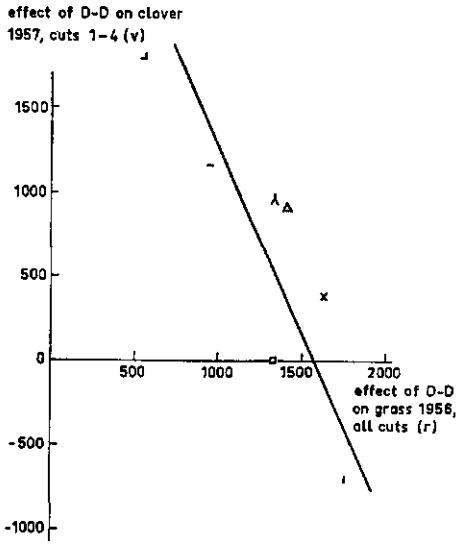


Fig. 8. Series 1. Relation between the effect of D-D on grass in 1956 and the effect of D-D on clover in 1957 (kg dry matter per ha). Average of 3 replicates.



1956 (Fig. 8; r_{rv} = —0.85). The effect on grass in Cut 1 largely determined the effect on grass for all cuts in 1956 (r_{qr} = 0.79) and so also governed the effect on clover for all cuts in 1956 (r_{qt} = —0.76) and 1957 (r_{qv} = —0.70)! The slightly negative effect on grass in 1957 was unrelated to that in 1956 (r_{ru} = 0.19) and so neither was it related to the effect on clover in 1957 (r_{uv} = —0.04).

The effect on clover in Cut 1 of 1958 was positively related to Cut 4 of 1957 but not to all cuts of 1957. For all cuts of 1958 the relation to the previous year was absent. The effect on grass in 1958 (Cut 1 or all cuts) was independent of the effect on clover in 1957 (Cut 4 or all cuts), so that it cannot be explained by nitrogen transfer from clover.

Humus in soil was slightly inversely related to effect on grass but was not related to the effect on clover at any time.

With regression equations relating the effects on grass and clover, direct effect on clover could be distinguished from indirect effect, caused by changes in competition from grass. Thus in 1956 the direct effect on clover was 316 ± 97 kg dry matter per ha (Fig. 6 where the line cuts the ordinate) and in 1957 3490 ± 796 kg (Fig. 8). By allowing for differences in effect on grass in 1956, variation in effect on clover in 1957 was cut from 2500 to 1050 kg.

The positive correlation between direct effect on clover in 1956 and that in 1957 can be seen from the similarity in vertical position of any symbol relative to others between figs 6 and 8. Direct effects on clover in either year were inversely but insignificantly related to humus in soil, but were independent of nematode counts in March 1956 (Section 2.5).

2.4 Influence of available nitrogen

The rank growth of grass on D-D plots in 1956 resembled that after high rates of nitrogen fertilizer. Perhaps more nitrogen became available from nematodes, insects, worms and bacteria killed by D-D and loss of nitrogen as nitrate decreased by slowing down of nitrification.

Soil samples taken in August 1956 from D-D plots of four fields had more mineral and mineralizable nitrogen than controls. The increase in available (mineral + mineralizable) nitrogen represented 18 to 85 kg nitrogen per ha in the top 20 cm, if it is assumed that the dry weight of this layer is 2 500 000 kg per ha. The method was not accurate enough to exclude other causes of the rank growth.

In August 1956 the 20 cm borders of D-D plots in some fields showed no effect of D-D on grass if they adjoined early sown controls. As borders were normal next to late sown controls, the phenomenon could not be due to invasion by parasites from the unfumigated plots. As light interception was not serious in the border, depletion of nutrients by the earlier sown grass seemed most likely. In particular, the soil could be depleted of available nitrogen which would otherwise accumulate as unleachable NH_4^+, thus supporting the supposition of the effect of D-D on grass being a nitrogen effect.

2.5 Influence of nematodes

Although there were positive correlations between D-D effect on grass (Cut 1 or all cuts, 1956) and counts before the trials of *Pratylenchus*, saprophagous nematodes and total free-living nematodes (Table 2), they are unlikely to be due to elimination of parasites, since correlations are as high for saprophagans ($r_{q,aa}$ = 0.54; $r_{r,aa}$ = 0.56) as for the parasite *Pratylenchys* (r_{qz} = 0.40; r_{rz} = 0.51).

More likely nematode count depends on some soil factor which also governs the D-D effect on grass. The low correlations between nematodes and humus (r_{yz} =

0.27; $r_{y,aa}$ = —0.28; $r_{y,bb}$ = 0.10) exclude humus as this factor.

In the trial marked ⊥, clover grew less vigourously than in other fields. Counts per 10 g clover roots in early sown controls in November 1956 were:

Pratylenchus pratensis	145	*Meloidogyne* larvae	25
Paratylenchus	15	Other Tylenchida	25
Tylenchorhynchus	25	Saprophytic nematodes	680
Heterodera larvae	60		

These counts are low (Oostenbrink & s'Jacob, 1959) so that poor growth of clover could hardly be due to nematode damage.

Nematode counts in August 1957 in replicates of two of the trials are given in Table 4 alongside the direct effect of D-D on clover in 1957. In the trial marked —, nematode counts were similar in control and D-D plots but in the trial marked ×, the counts of most of the genera identified were lower on D-D plots. Particularly in Trial × most counts in controls were lower than the critical

Table 4. Series 1. Numbers of free-living nematodes and clover cyst nematode (*Heterodera trifolii*) per 100 ml soil (August 1957) and the direct effect of D-D on clover in 1957 (in kg dry matter per ha) of two trials.

Trial[1]	Replicate	Treatment	Nematode counts per 100 ml soil											Direct effect of D-D on clover in 1957
			free-living nematodes								*Heterodera trifolii*			
			Pratylenchus	*Paratylenchus*	*Tylenchorhynchus*	*Hoplolaimus*/ *Rotylenchus*	free-living *Heterodera* larvae	other Tylenchida	saprophagans	total	cysts	viable cysts	eggs and larvae inside cysts	
—	I	0	490	1480	25	5	380	390	3075	5845	28	19	1510	
	II	0	250	1540	140	0	310	635	4405	7280	15	13	640	
	III	0	245	1915	60	0	380	420	1965	4985	38	28	2070	I 2320
	I	D-D	175	2710	165	5	290	505	2120	5970	28	17	1660	II 4240
	II	D-D	225	1385	20	45	280	350	3415	5720	23	16	1710	III 3240
	III	D-D	35	1820	50	0	240	195	2410	4750	27	15	670	
×	I	0	45	0	95	33	5	205	3950	4333	0	0	0	
	II	0	365	0	30	730	0	415	6390	7930	0	0	0	
	III	0	280	30	25	635	0	585	3860	5415	0	0	0	I 3210
	I	D-D	5	0	10	5	0	0	4035	4055	0	0	0	II 4960
	II	D-D	0	0	0	10	0	50	6675	6735	0	0	0	III 3840
	III	D-D	15	720	10	0	0	125	4140	5010	0	0	0	

[1] Symbols as in figures 2-8.

level for root damage (Oostenbrink & s'Jacob, 1959); the clover cyst nematode (*Heterodera trifolii*) was almost absent from that field. Yet D-D markedly stimulated clover in both fields and so differences between fields and between replicates could not be elucidated by their nematode populations.

As mentioned in Section 2.3 fields □ and × had more clover than expected from yield of grass in 1957 and 1958 (Fig. 3) but not as yet in 1956 (Fig. 2). Those fields were the only ones still then under study which had never before grown grass or clover. This may indicate the introduction or increase of substances or organisms unfavourable to clover growth in other fields after repeated grass and clover crops. If clover growth in other fields were hindered mainly by nematodes, the difference would have disappeared in D-D plots or, at least, the direct effect of D-D on clover (which can be derived from Fig. 8 as the part cut from the ordinate by the regression line plus or minus the vertical distances of the symbols from this line) in fields □ and × would have been smaller than in others but in fact is not. Thus the higher proportion of clover in pasture sown on old arable land must be due to something other than a low nematode population.

3 Series 2: D-D in soil or nitrogen fertilizer

3.1 Design and management of the trials

An attempt was made to allow for differences on clover caused by differences in grass growth. Differences in grass growth were produced by laying out plots with different rates of nitrogen alongside D-D plots. Comparisons between such data should better indicate whether D-D affected clover other than by its effect on grass.

In Series 2, there were 10 trials sown in mid-June 1957 on sandy soils. Plot sizes and seed mixture were as in Series 1. Treatments were as follows.

1, 2, 3 Split nitrogen dressing supplied as ammonium nitrate limestone partly at sowing 22.5, 45 and 67.5 kg N per ha and after Cut 1 11.25, 22.5 and 37.75 kg, repectively

4 D-D (as in Series 1) in the second half of April

5 Control.

In May the fields were worked with a cultivator to allow D-D residues to disperse.

In 1957 no P, K or Mg was supplied as most fields had already been dressed by the farmers. The crop was cut twice on all fields and a third time on one where growth was vigorous.

In 1958 all fields received, per ha, superphosphate, 85 kg P_2O_5, potassium chloride, 280 kg K_2O, and magnesium sulphate, 65 kg MgO, split into 3 dressings, and yielded 5 cuts. During 1958 and 1959 continuing trials were uniformly dressed with 20 kg N per ha as ammonium nitrate limestone for each cut. (The effect of D-D on availability of nitrogen was expected to last only the first year.)

In 1959 5 trials continued but because of severe drought they could be cut only

Table 5. Series 2. Average yields and effects of D-D in kg dry matter per ha.

Cut	Grass and herbs					Clover					D-D effect		P% D-D effect		Number of trials
	treatment in 1957					treatment in 1957					grass and herbs	clover	grass and herbs	clover	
	kg nitrogen per ha				D-D	kg nitrogen per ha				D-D					
	0	33¾	67½	101¼		0	33¾	67½	101¼						
1957															
1 [1]	1601	1709	1861	1983	2040	143	114	91	78	188	+439	+ 45	≧99	≧99	9 [2]
2 [1]	515	711	918	1082	949	115	77	68	50	114	+434	— 1	≧99	<75	7
	2116	2420	2779	3065	2989	258	191	159	128	302	+873	+ 44			
1958															
1	2096	2164	2129	2090	2279	625	428	382	243	659	+183	+ 34	75-90	≧99	10
2	1562	1671	1660	1666	1676	709	605	514	401	784	+114	+ 75	<75	75-99	10
3	1227	1278	1229	1226	1270	741	644	623	509	946	+ 43	+205	<75	≧99	10
4	1167	1253	1227	1247	1312	747	639	598	515	867	+145	+120	95-99	95-99	10
5	909	1024	1029	1079	1016	320	248	234	237	383	+107	+ 63	<75	<75	8
	6961	7390	7274	7308	7553	3142	2564	2351	1905	3639	+592	+497			
1959															
1	1758	1857	1641	1653	2239	647	626	583	581	697	+481	+ 50	95-99	<75	5
2	1254	1130	1162	1193	1231	203	183	206	131	153	— 23	— 50	<75	<75	4
	3012	2987	2803	2846	3470	850	809	789	712	850	+458	0			

[1] One field was cut 3 times, Cut 3 was added to Cut 2 in this table.

[2] The production of one field was excluded because it consisted almost entirely of herbs.

All plots were treated uniformly in 1958 and 1959.

P% = significance level.

twice in May and late in August and one field only once. Of the other fields 3 were dug and resown in the spring without further treatment, and yielded 0, 1 (plus a weed cut) and 3 cuts, respectively; they received no nitrogen until after Cut 1. All 8 fields were dressed in spring with 50 kg P_2O_5, 160 kg K_2O and 25 kg MgO per ha. After Cut 1, the 5 not resown received a further 20 kg P_2O_5, 40 kg K_2O and 10 kg MgO per ha.

As in Series 1, fields damaged by straying cattle were ignored. Yields of dry matter in each cut were estimated for other fields separately for clover, grass and herbs. In the recorded data grass and herbs have been combined.

Nematodes were counted in all fields in spring 1957 before the trials began, in one field with few nematodes in August 1957 and in all plots (50 borings per plot to a depth of 20 cm) from all trials in November or December 1958.

No data were collected on humus contents of the soils.

3.2 Average yields and effect of D-D

During establishment clover grew much better on D-D plots than on others, especially in fields where sward was poorly established, perhaps because of drought, but grass hardly grew any better on D-D plots than others. In Cut 1 yield of grass increased and of clover decreased with increasing nitrogen dressing (Fig. 9). The yield of grass with D-D, 2040 kg dry matter per ha, was equivalent to that obtained with 80 kg nitrogen per ha; corresponding yields of clover were 188 and 75 kg, so that D-D caused a direct increase of 113 kg. In Cut 2 this direct effect on clover was 54 kg. For all cuts of 1957 it was 167 kg, varying from 63 to 267 kg between trials.

The initial effect on yield of grass (Table 5) was a bit smaller than in Series 1 (Table 1) so that the effect on clover remained positive in the first year (cf. Fig. 6).

The uniform dressings in 1958 practically equalized yields of grass from plots

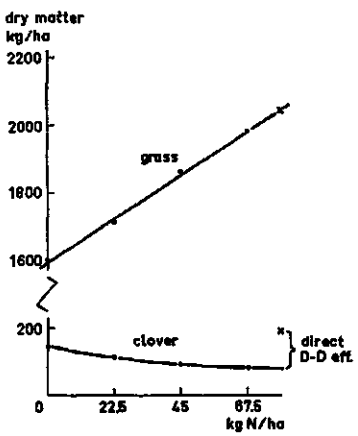


Fig. 9. Series 2, 1957, Cut 1. Effect of nitrogen and D-D on the yield of grass (including herbs) and clover. • = control and nitrogen plots; × = D-D plots. Average of 9 trials.

previously supplied with different rates of nitrogen but yields of clover remained smaller if they had received more nitrogen in 1957.

Unlike Series 1, the positive effect of D-D on grass continued into the second year. Despite this more vigorous growth of grass, there was also a positive effect on clover, D-D plots yielding more than any other.

From the data in Table 5, it can be deduced as was done from Fig. 9 that without the suppression from the extra growth of grass in 1957, the direct effect on clover in 1958 would be 1600 kg dry matter per ha (from the more limited data in Fig. 11, a value of 1100 kg can be deduced). The direct effect on clover in 1958 was positively related to that in 1957.

In 1959 the 5 trials which continued yielded few data because of the drought (Table 5). D-D still had considerable effect on grass in Cut 1 but not in Cut 2. The effect occurred in all trials except 1 and its size was independent of that on either grass or clover in 1958. Despite more competition from grass, clover yield on D-D plots was as high as on controls. As rate of nitrogen dressing in 1957 increased, yield of clover in 1959 still declined, even though yields of grass were not proportional to nitrogen rate.

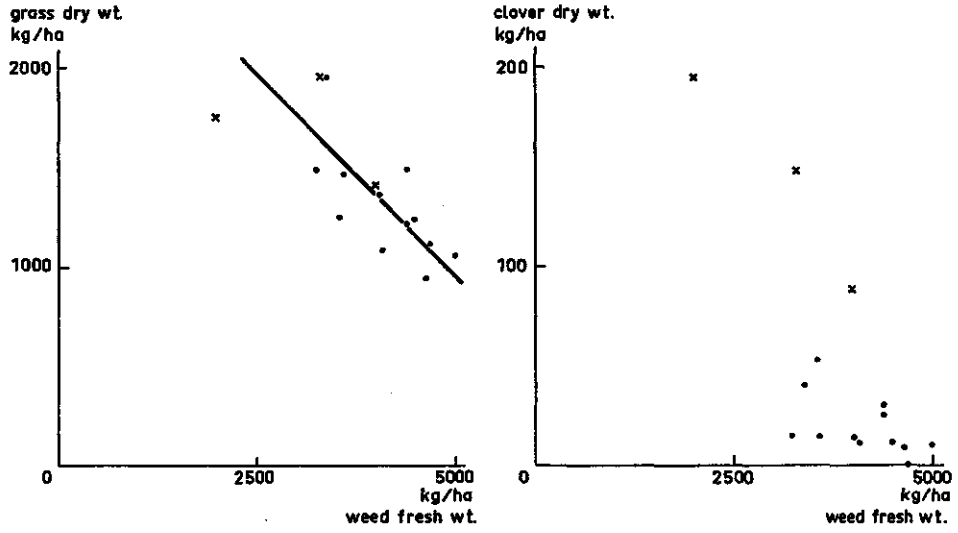
Of the 3 fields resown in 1959, 1 had large reserves of moisture and yielded 3 cuts. On it, former D-D plots yielded a bit more grass than others but were intermediate in yield of clover.

The other 2 fields were hit by the drought. One was mown in May a month after resowing to control weeds, which were slightly fewer on former D-D plots (Table 6), perhaps because D-D had killed weed seeds in 1957. At the cut in August yields of both grass and clover were higher on D-D plots. The higher yields of grass on former D-D plots can be completely ascribed to the low density of weeds (Fig. 10, left). For clover (Fig. 10, right), weed density can explain the difference between D-D replicates but not between D-D and other plots. After 2 years and after resowing, D-D continued to improve growth of clover.

Table 6. Series 2. Yield of weeds in kg per ha in May 1959 and of grass and clover in August 1959 on one of the resown fields (average of 3 replicates).

	Grass and herbs					Clover					D-D effect	
	treatment in 1957					treatment in 1957					grass and herbs	clover
	kg nitrogen per ha				D-D	kg nitrogen per ha				D-D		
	0	33¾	67½	101¼		0	33¾	67½	101¼			
May	mainly weeds (fresh weight)											
	4200	4200	3900	4200	3100							
August	grass and herbs (dry weight)					clover (dry weight)						
	1430	1290	1352	1133	1703	17	13	19	29	144	+273	+127

Fig. 10. Series 2. Relation between yield in May 1959 of weeds and yield in August 1959 of grass and herbs (left) and of clover (right) on a resown field. • = former control and nitrogen plots; × = former D-D plots 1957.



On the third resown field, the crop was too poor to be harvested. Initially there was no visible difference in growth but later grass and clover on D-D plots grew somewhat better and the grass was a darker green.

3.3 Correlations between yields, effect of D-D and other parameters

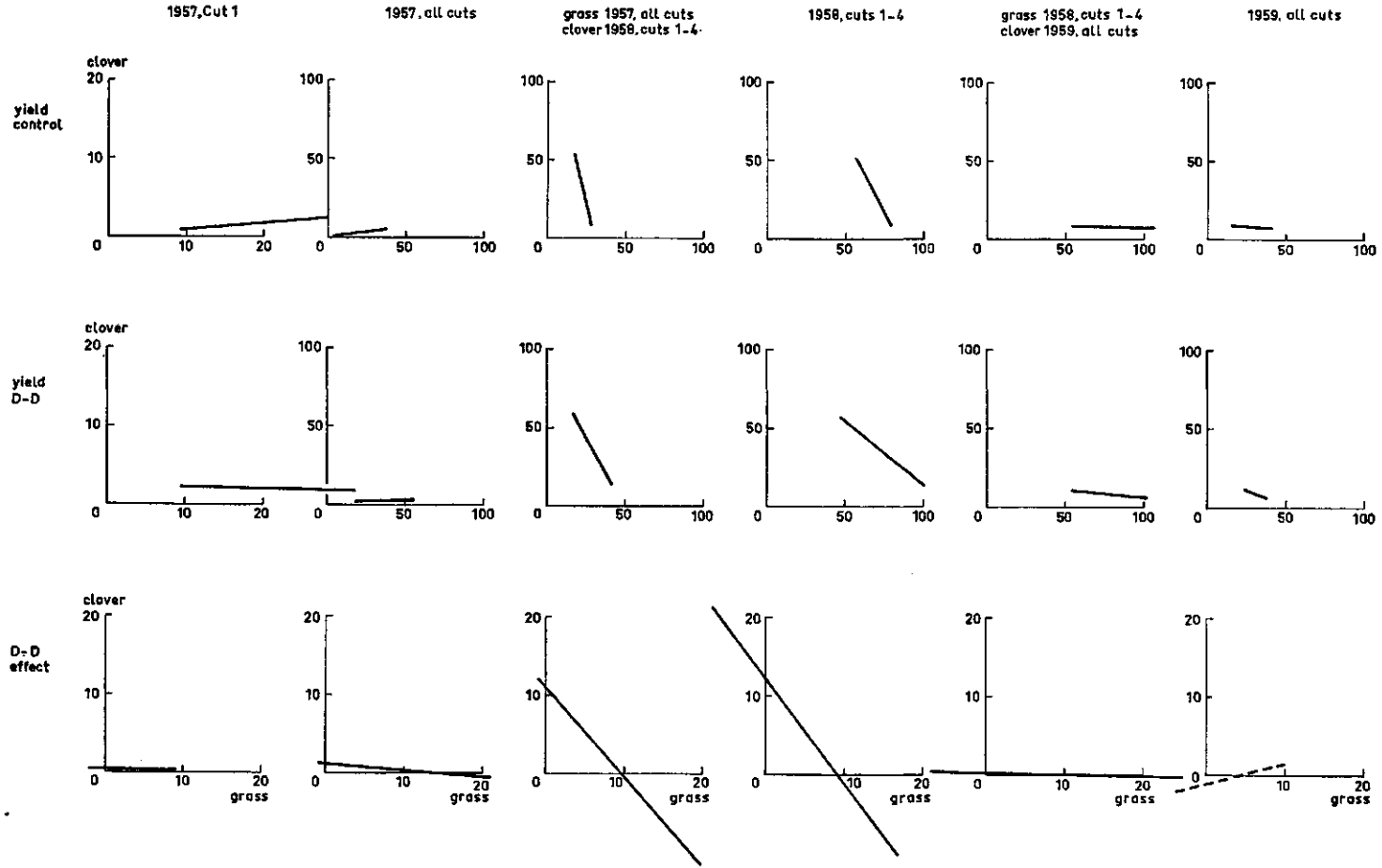
Correlations were tested as in Series 1 but only those between grass and clover are recorded in Fig. 11.

In controls and D-D plots positive correlations from year to year existed for grass yield, although less pronounced initially than in Series 1, but for clover yield only initially, because of a sharp decline in one field from 1958 to 1959. The relation in yields between clover and grass was positive in controls in 1957 (Cut 1 or all cuts) but inverse thereafter in both controls and D-D plots.

For effects of D-D, there were slight positive correlations from year to year for both grass and clover. As in Series 1, the effect on clover was initially independent of that on grass, in 1958 the relation between grass and clover turned negative and in 1959 vanished. Between effect on grass and yield of it in controls there was no correlation in any year; between replicates, there was, however, an inverse relation in 1957 (cf. Section 2.3).

For clover there was a slight inverse relation between yield of controls and effect in each year. Between yield of grass and effect on clover there was no relation.

Fig. 11. Series 2. Relation between clover and grass (kg dry matter per are). Above, yield on the control plots; middle, yield on the D-D treated plots; below, D-D effect.



3.4 Influence of nematodes

Counts of free-living nematodes in spring 1957 bore no relation to either direct effect of D-D on clover or effect on grass in 1957. One field, markedly rich in clover for all treatments (e.g. control, 1957, 24% and 1958, 45%), was similar to others in nematode density. Thus clover growth seemed independent of nematode density. In another field there were too few nematodes to cause any damage but D-D caused a slight direct increase in clover (63 kg dry matter per ha); counts were repeated there in August 1957 to check that the effect could not be due to a sudden increase in nematode population during growth.

Counts of free-living nematodes late in 1958 were unrelated to nitrogen rate in 1957 but counts of nearly all types were still considerably lower after D-D (Table 7). Counts of clover cyst nematode (*Heterodera trifolii*) but not of cereal cyst nematode (*H. avenae*) fell with increasing rate of nitrogen and hence with decreasing amounts of clover (Table 8). D-D still reduced counts of both species of cyst nematode.

Despite the low nematode counts and high clover yields of D-D plots, there proved to be no relation. Counts of free-living types in controls were independent

Table 7. Series 2. Number of free-living nematodes per 100 ml soil in November or December 1958, averaged over all replicates and trials (30 plots per treatment) including those in which the genus could not be established.

Treatment in 1957	Genera of nematodes														Total
	Pratylenchus	*Paratylenchus*	*Tylenchorhynchus*	*Gottholdsteineria*	*Heterodera* larvae	*Criconomoides*	*Trichodorus*	*Meloidogyne* larvae	*Helicotylenchus*	*Rotylenchus*	*Helicotylenchus/ Rotylenchus*	*Hemicycliophora*	other Tylenchida	saprophagous nematodes	
0	154	98	626	26	10	9	43	61	188	223	25	2	635	2475	4576
	10	*6*	*10*	*1*	*4*	*1*	*6*	*3*	*2*	*6*	*1*	*1*	*10*	*10*	
33¾ N	156	108	552	29	13	8	53	29	226	210	48	3	556	2117	4108
	10	*6*	*10*	*1*	*4*	*1*	*6*	*3*	*2*	*6*	*1*	*1*	*10*	*10*	
67½ N	128	76	617	30	11	14	78	65	213	173	68	2	492	2446	4413
	10	*6*	*10*	*1*	*4*	*1*	*6*	*3*	*2*	*6*	*1*	*1*	*10*	*10*	
101¼ N	147	119	622	22	11	11	43	39	216	206	68	1	532	2214	4251
	10	*7*	*10*	*1*	*4*	*1*	*6*	*3*	*2*	*6*	*1*	*1*	*10*	*10*	
D-D	66	152	337	5	8	4	20	0	13	36	8	1	232	2147	3028
	10	*5*	*10*	*1*	*4*	*1*	*5*	*0*	*2*	*6*	*1*	*1*	*10*	*10*	

In *italics:* the number of trials in which the type of nematode was found under the treatment concerned.

Table 8. Series 2. Numbers of encysted nematodes per 100 ml soil in November or December 1958, averaged over all replicates and trials (30 plots per treatment), including those in which the species was not found.

Treatment in 1957	*Heterodera trifolii*			*Heterodera avenae*		
	cysts	viable cysts	eggs and larvae inside cysts	cysts	viable cysts	eggs and larvae inside cysts
0	7 (9)	3 (6)	120 (6)	1 (7)	0 (4)	6 (4)
33¾ N	5 (10)	2 (6)	71 (6)	2 (4)	0 (3)	8 (3)
67½ N	5 (8)	2 (4)	76 (4)	2 (3)	0 (1)	12 (1)
101¼ N	4 (9)	1 (6)	40 (6)	2 (4)	1 (2)	24 (2)
D-D	4 (10)	2 (6)	49 (6)	1 (7)	0 (4)	4 (4)

In brackets: number of experiments in which object was found under the treatment corcerned.

of effect of D-D on grass or direct effect on clover, even among replicates. None of the nematode counts were related to clover yields of controls: trials with high yields did not have few nematodes or those with low ones have many. Counts of clover cyst nematode were below 5 larvae per 100 ml soil in control plots of 7 of the 10 trials; the other 3 had 535, 435 and 225. They were independent of direct effect of D-D on clover or, in controls, of yield or proportion of clover in 1958, even among replicates. Thus D-D can hardly have affected clover by reducing clover cyst nematodes.

Lower yields of clover on former grassland than on former permanent arable were due mainly to the higher yields of grass, but occasionally yield of clover was lower than expected from the yield of grass. This observation applies both to D-D and control plots. One of the 4 fields formerly under grass had a low count of clover cyst nematode late in 1958 but it yielded no more clover than the other three. For these two reasons it is unlikely that the nematode population of the former grassland caused the difference.

Again better growth of clover on D-D plots of 2 of the 3 fields resown in 1959 did not correspond with nematode counts in December 1958. In one of them and in the field without better growth of clover, clover cyst nematode was almost absent and nearly all free-living nematodes were considerably fewer on D-D plots than controls. On the other field, clover cyst nematode and some types of free-living nematode were fewer on the D-D plots.

4 Series 3: preceding crop of Tagetes

4.1 Design and management of the trials

Tagetes (African marigold) reduces counts of many nematodes in soil (e.g. Oostenbrink et al., 1957). So its effect was studied as a preceding crop for clover and grass in 9 trials established in spring 1958, of which 8 were alongside plots of Series 2. The fields were dressed with 50-85 kg P_2O_5, 120-160 kg K_2O per ha. Plots were triplicated and varied between 18 and 21 m^2. There were 2 treatments

1. *Tagetes erecta* sown in May 1958 (7.2 kg seed per ha; rows 20 cm apart) and dressed with 20 kg N per ha as ammonium nitrate limestone
2. An arable crop dressed with 40-150 kg N according to species and soil: in 3 trials winter rye sown in autumn 1957 and in 2 trials each oats, potatoes or fodder beet (which received an extra dressing of 50 kg P_2O_5 and 120 kg K_2O per ha) sown or planted in April 1958.

The arable crops were harvested in normal manner; *Tagetes* was removed in November or December either by pulling up when alongside a root crop or by pulling up or mowing when alongside a cereal. In 1959 all fields were dressed with 50 kg P_2O_5, 160 kg K_2O and 25 kg MgO per ha and in March or April were sown with the mixture of clover and grass used in the other series.

Most trials were mown in May to control weeds (without weighing). Most fields suffered from drought and for the rest of the season 1 trial was cut 3 times and was dressed with 20 kg N after each; 2 trials were cut twice, 3 trials once and 2 trials not at all and received 20 kg N only once in the late summer. Two trials then finished.

Of the 7 remaining, 5 were dug and resown in spring 1960. Of the other 2, one was cut once and the other twice, receiving one dressing of 20 kg N per ha after Cut 1. The resown fields developed poorly and some plots were overrun by twitch (*Elytrigia repens*); only 2 were cut before the trials ended in August 1960.

Soil samples for nematode counts were taken late in 1958 as in Series 2 and again late in 1959 but samples of the 3 replicates of each treatment were mixed before counting.

4.2 Yields, effect of *Tagetes* and nematode counts

Though counts of nearly all nematodes late in 1958 were lower on *Tagetes* plots than with the other crops (tables 9 and 10), there was no visual difference in either clover or grass between plots in 1959. However, yields were on average slightly more after *Tagetes* (Table 11), despite many exceptions among replicates, especially for grass. For all trials the difference ('effect') per cut was 131 kg grass and 88 kg clover per ha. The differences between replicates and the differences between the *Tagetes* effect and the D-D effect in adjoining plots did not correspond with differences in nematode density late in 1958. For instance in one field

Table 9. Series 3. Numbers of free-living nematodes per 100 ml soil in November or December 1958 with and without previous marigold crop, averaged per crop over all replicates.

Crop	Genera of nematodes												Number of trials
	Pratylenchus	*Paratylenchus*	*Tylenchorhynchus*	*Gotholdsteineria*	*Heterodera* larvae	*Criconomoides*	*Trichodorus*	*Meloidogyne* larvae	*Helicotylenchus*	*Rotylenchus*	other Tylenchida	saprophagous nematodes	
marigold	26	15	207	0	1	0	29	1	52	234	364	1970	3
winter rye	84	28	412	0	1	0	19	13	463	198	444	2281	
marigold	30	0	217	0	0	0	17	22	0	238	217	2930	2
oats	212	0	535	0	0	0	12	53	0	338	307	3467	
marigold	44	63	82	58	0	3	3	2	0	26	578	2647	2
potatoes	231	50	105	53	0	5	3	82	0	344	465	3173	
marigold	93	75	325	0	0	0	68	3	0	290	367	3703	2
beets	402	393	280	0	0	0	67	0	0	283	267	4285	
average of all trials:													
marigold	46	36	207	13	0	1	29	6	17	201	380	2719	9
other crops	216	108	342	12	0	1	24	34	154	281	379	3188	

Table 10. Series 3. Numbers of cyst nematodes per 100 ml soil in November or December 1958, averaged per crop over all replicates.

Crop	*Heterodera trifolii*			*Heterodera avenae*			Number of trials
	cysts	viable cysts	eggs and larvae inside cysts	cysts	viable cysts	eggs and larvae inside cysts	
marigold	2.2	0.3	5	1.8	0.1	2	3
winter rye	2.6	0.7	24	1.2	0.2	4	
marigold	1.1	0	0	1.5	0.3	5	2
oats	3.3	0	0	0.8	0.3	8	
marigold	0.8	0.2	4	3.2	0.1	1	2
potatoes	2.2	0.2	2	3.1	0.3	3	
marigold	1.8	0	0	0.4	0	0	2
beets	3.9	0.7	21	0.9	0.3	7	
average of all trials:							
marigold	1.5	0.1	3	1.7	0.1	2	9
other crops	3.0	0.4	13	1.5	0.3	5	

Table 11. Series 3. Average grass and clover yield per cut in 1959 after growing marigold and other crops.

Preceding crop	Average yield in kg dry matter per ha								
	June			July			August		
	grass and herbs	clover	number of trials	grass and herbs	clover	number of trials	grass and herbs	clover	number of trials
marigold							816	67	1
winter rye							908	40	
difference							—92	+27	
marigold							1238	10	2
oats							1167	4	
difference							+71	+6	
marigold	2196	72	2	2779	92	1	1518	254	2
potatoes	1940	37		2596	68		1280	71	
difference	+256	+35		+183	+24		+238	+183	
marigold				2165	2655	1	1196	1095	2
beets				2119	2486		1107	944	
difference				+46	+169		+89	+151	

nematode counts in *Tagetes* plots were similar to those in D-D plots, and those in oat plots similar to those in Series 2 control plots. Even so yields of grass and clover were similar between *Tagetes* plots, oat plots and controls and were much higher on all D-D plots.

In 1960 there was no visible difference in growth between plots. In the few trials which could be cut, yields did not differ significantly between *Tagetes* plots and other crops. There was no relation with nematode counts late in 1959 (Table 12) although counts of some nematodes (*Pratylenchus* and *Tylenchorhynchus*) were still lower on the *Tagetes* plots.

5 Conclusions

The absence of any stimulatory effect of fungicides suggests that fungi were not an important pest of clover or grass. The effect of pentachloronitrobenzene might have been limited, however, by the cold soil in spring (Rich, 1966).

Series 1 and 2 gave similar results (figs 1 and 11). Initially (Series 1, Cut 1 and Series 2, Season 1) yields of grass and clover were positively related, suggesting

Table 12. Series 3, December 1959. Numbers of free-living nematodes in soil and roots of grass and clover, classified in accordance with the preceding crop (in 1958).

Previous crop	Number of nematodes in 100 ml soil										Number of *Pratylenchus* in 10 g grass-clover roots	Number of trials
	Pratylenchus	*Paratylenchus*	*Tylenchorhynchus*	*Heterodera* larvae	*Criconomoides*	*Trichodorus*	*Meloidogyne* larvae	*Rotylenchus*	other Tylenchida	saprophagous nematodes		
marigold	13	20	1490	30	0	0	30	205	255	3343	65	2
winter rye	115	60	2298	0	0	0	243	230	490	3563	163	
marigold	5	20	1525	0	0	5	55	640	200	3540	38	2
oats	35	0	2528	0	0	0	15	295	205	3470	383	
marigold	65	95	243	180	13	0	5	148	693	4728	120	2
potatoes	405	185	383	13	8	0	65	333	223	3713	2068	
marigold	65	7080	555	0	0	0	15	373	265	5432	440	2
beets	178	5193	468	13	0	15	5	180	350	9490	1075	
average of all trials:												
marigold	37	1804	953	53	3	1	26	341	353	4258	166	8
other crops	183	1359	1419	6	2	4	82	259	317	5059	922	

Counts of cyst nematodes were negligible.

that soils suitable for grass were also suitable for clover. Thereafter competition between grass and clover inverted the relation.

In Series 1 the change in yield of grass from Cut 1 to Cut 2 in 1956 was independent of clover (Fig. 2). The increase in yield of clover, however, was greatest in trials with less grass. Thus grass influences clover but clover hardly affects grass; it forms a 'padding plant' as stated by Van den Bergh & De Vries (1955). This is evidenced by dependence of yields on humus in soil: clover yields were inversely related to humus only in fields where grass yields were positively related to humus. The effect on clover of any increased growth of grass lasts a considerable time (e.g. Table 5, nitrogen treatments in 1959); Heddle (1966) found that a nitrogen dressing in spring had no effect on grass yields in cuts 3 to 5 but still caused considerable reduction in clover yield.

Soil fumigation with D-D temporarily affected grass, at least partly by an increase in available nitrogen. Effects after Year 1 were small, except inexplicably in Cut 1 of Year 3 of both series.

The beneficial effect of D-D on clover was remarkably persistent. In Year 1 it was masked by increased competition by grass, especially in Series 1. The 'direct' effect, after allowing for this suppression of clover by grass, was calculated at 316

and 167 kg dry matter per ha in Year 1 and 3490 and 1600 kg in Year 2 for series 1 and 2, respectively.

Nematodes were considerably reduced by D-D and to a lesser extent by *Tagetes*. As in earlier pot trials (Ennik et al., 1964), counts of various types of nematode were not related to the effects of D-D and *Tagetes* on clover or grass. In other pot trials and in a public lawn (Ennik et al., 1965), however, clover yield was inversely related to counts of clover cyst nematode.

Besides being nematicidal, D-D has many other effects (Ennik et al., 1964), but none seems to explain the stimulation of clover in the present field trials. Results with other biocides are equally inexplicable by their effect on nematode counts (Ennik, 1968; Martin, 1966). Thionazin (O,O-diethyl-O-(2-pyrazinyl)-phosphorothioate), a biocide not toxic to plants, increased pasture yield by 15%, affecting grass much more than clover. With repeated treatment, the effect could be maintained for at least 4 years.

The cause of the effects must be sought in side-effects of D-D and other biocides. Altman (1969) suggests that the soil microflora may produce more nitrogen in forms available to plants by using the chlorinated hydrocarbons as energy sources. This could explain the effect of D-D on grass and the indirect effect on clover. The direct effect on clover, however, remains unexplained so far.

Summary

The effect of disinfection of seed and soil on growth of grass and white clover in newly sown grassland on sandy soil was studied in two series of field trials each lasting 3 years. The relation between grass and clover growth was considered. The results of the two series conformed well.

During establishment, grass and clover growth were positively correlated; thereafter they were inversely related, growth of clover being determined by the grass. The adverse effect on clover of an increase in grass continued long after grass yields had dropped to a uniform level. In its turn grass, once adapted to uniform application of nitrogen, depended mainly on the soil humus.

Disinfection of clover seed against fungi with a mixture of thiram and organomercurial + benchinox, or soil treatment against fungi with pentachloronitrobenzene did not promote clover or grass. However soil fumigation with D-D markedly stimulated growth but in different ways for grass and clover. The effect on grass was highest in Year 1 and decreased rapidly in successive cuts. It was at least partly due to increased availability of nitrogen in the soil. The effect on clover was highest in Year 2, even when the suppression from increased grass growth in Year 1 was taken into account. Thus the cause of the D-D effect on clover was different from that on grass. Because of competition, the observed effect (= direct plus indirect effect) on clover was inversely related to the effect on grass, except for the period of establishment. D-D considerably reduced the population density of both free-living root nematodes and clover cyst nematodes for a longish time,

but nematode reduction could not account for the increased growth response of grass or clover. The cause of the beneficial effect of D-D on clover growth is still unknown.

In Series 3 the effect was studied of *Tagetes* as a preceding crop on nematode density and grass-clover growth. Compared with some other crops, marigold reduced the population density of most nematode species and, on average, slightly increased growth of both grass and clover. No relation was found between nematode reduction and growth response.

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