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THE INFLUENCE OF SOIL DISINFECTION WITH DD, CERTAIN COMPONENTS OF DD AND SOME OTHER COMPOUNDS WITH NEMATOCIDAL ACTIVITY ON THE GROWTH OF WHITE CLOVER[1,2]

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In pots containing soil fumigated with DD before planting the growth of white clover was considerably better than in untreated pots. A similar effect was obtained both with cis- or trans-1,3-dichloropropene. Some other components of DD, including 1,2-dichloropropane, had no influence on clover growth in the concentration at which they occur in DD. Of the compounds not associated with DD, EN 18,133 and sugar showed a favourable effect on clover growth, but X 323 had no influence. In the untreated pots with poorly growing clover many roots had died. On the places where dead lateral roots had been attached many fungi were present in the tissue, especially *Rhizoctonia* sp. and *Fusarium* spp. These pots contained also more clover cyst nematodes and more springtails (Collembola). In one of the pot experiments poor growth could probably be attributed to an attack by the clover cyst nematode (*Heterodera trifolii* Goffart). The nematode level above which damage to the plants occurred was calculated at 1000–3000 eggs (larvae inclusive) per 100 ml of soil. In the other pot experiment, however, poor growth could not be explained by cyst nematodes only. In field experiments clover growth was promoted by soil fumigation with DD, also when only a few cyst nematodes were present. These observations increase the evidence that attack of white clover by pathogens is a complex phenomenon in which nematodes as well as fungi and perhaps insects too are involved. Apart from a small increase in growth at the beginning, soil fumigation with DD did not raise the yield of perennial rye grass.

INTRODUCTION

DD, which is a by-product of the oil industry, is a mixture of 1,3-dichloropropene and 1,2-dichloropropane plus small quantities of many other compounds. Though mainly used against nematodes, it also kills many other organisms and causes chemical and physical alterations in the soil which produce several side-effects.

ENNIK *et al.* (1962) concluded that fumigating the soil with DD considerably stimulated the growth of white clover but that the increased growth could not be attributed solely to nematode kill. To obtain more information on the factor responsible for this growth-stimulating effect, two pot experiments were carried out in which the influence of some components of DD were separately investigated. These components were kindly supplied by Shell Nederland Chemische Fabrieken N.V. In the second experiment, some other nematocides were also tested, for comparison. The results of both experiments are discussed in this article.

[1] Accepted for publication 19 November, 1963.
[2] In this article the name DD is used as abbreviation for the trade name "Shell D-D".

Composition of DD

In literature very few details are given about the composition of DD. It is generally described as mainly a mixture of 1,3-dichloropropene and 1,2-dichloropropane, usually in a 1:1 or a 2:1 ratio. Some details are given by CARTER (1945), LANGE (1945) and MOJE *et al.* (1957). According to BESEMER & OOSTENBRINK (1955) DD, as it was brought on the market in the Netherlands in 1955, was a mixture of about 50% dichloropropene, 25% dichloropropane and 25% of other related compounds.

Shell provided us with the following analysis of the present composition of "Shell D-D"[3]:

1,3-dichloropropene	cis	31 %
	trans	29 %
1,2-dichloropropane / 2,3-dichloropropene		24 %
1,2-dichloroethane		4.2%
1,2,3-trichloropropane		1.5%
1,3-dichlorohydrin		1.3%
allylchloride		1.1%
epichlorohydrin		0.6%

Besides these, many other compounds are present in small amounts, *e.g.* 1,1-dichloropropene, cis-1-chloro-1-propene, trans-1-chloro-1-propene, 2-chloropropene, 1,1-dichloroethane, cis-1,2-dichloroethene, trans-1,2-dichloroethene, 1,5-hexadiene, vinylchloride, n-propylchloride, isopropylchloride and 2,3-dichlorohydrin.

Side-effects of soil fumigation with DD against nematodes

Several cases have been reported in which the growth stimulation due to DD fumigation could not or only partly be explained by killing nematodes (CARTER, 1943, 1945; MCFARLANE & MATSUURA, 1947; PETERS, 1948; EDWARDS, 1950; GOFFART & HEILING, 1958). PETERS (1948) and EDWARDS (1950) stress that such a soil-amendment effect commonly occurs with partial soil sterilization, though CARTER (1943) did not obtain the same result with chloropicrin.

In several cases the growth-stimulating effect of DD in addition to nematocidal action may be due to one of the following causes.

Physical and chemical alterations in the soil

OOSTENBRINK (1958) found a slight increase in the pH after soil disinfection with DD, which may have a yield improving effect in cases where the pH is marginal. GOFFART & HEILING (1958) consider the increased growth of fodder beets, which occurred two years after DD treatment, as due to a greater wilt resistance of the plants. They suppose this to be caused by the high amounts of chlorine added to the soil by DD treatment. According to MARTIN & PRATT (1958) it has frequently been shown that soil fumigation with DD and other

[3] The composition of DD was somewhat changed in 1959. The present content of 1,3-dichloropropene is about 57–60%, but the sales specification still guarantees only a minimum content of 50%.

compounds resulted in a temporarily higher availability of certain macro-elements (*e.g.* Ca) and trace elements (*e.g.* Mn).

A commonly occurring side-effect of soil fumigation with DD is an increase in the amount of mineral nitrogen (ammonium plus nitrate) in the soil and of nitrogen which can be liberated by mineralization of organic matter (WOLCOTT *et al.*, 1960; ENNIK *et al.*, 1962), which generally results in a more or less important yield increase. By retardation of the nitrification the ammonium content of the soil after fumigation is higher and the nitrate content lower than normal (TAM, 1945; MARTIN & PRATT, 1958; WOLCOTT *et al.*, 1960). As several plants can absorb and assimilate ammonium more rapidly than nitrate, a retarded nitrification in itself (at a constant level of total mineral nitrogen in the soil) may already result in improved growth (TAM, 1945). In some cases in which very much ammonium is liberated (soils with a high organic matter content), DD treatment may cause yield depression (WOLCOTT *et al.*, 1960).

The amount of nitrogen available for the plants after soil disinfection may be quite important. This has not always been recognized and a part of the secondary action of DD referred to in literature is probably due to this nitrogen effect. Also in cases in which crop growth is stimulated by control of pathogenic organisms it should be taken into account that the response may be partly due to a nitrogen effect. However, in the case of leguminous crops, such as white clover, which have their own nitrogen supply, this effect does not occur or may be less important.

Killing of pathogenic organisms other than nematodes

A DD treatment kills not only nematodes but also most of the insects in the soil, such as wireworms (STONE, 1944; LANGE, 1945) and several harmful weevils or their larvae (CARTER, 1944). As is reported by MOJE *et al.* (1957) DD has a rather high bactericidal activity and also some fungicidal activity. ALTMAN & FITZGERALD (1960) found after fumigation of heavy clay soil with DD (14 gallons/acre) a tendency to reduced frequency of *Fusarium* in isolations from treated soil compared with those from untreated soil. Other investigators, however, at higher rates of DD found little or no influence on *Fusarium* spp. (PARRIS, 1945; STARK & LEAR, 1947; REINKING & NEWHALL, 1950; GILLARD, 1961) or *Rhizoctonia* sp. (PARRIS, 1945). Some cases are reported in which infection of certain crops by *Fusarium* spp. (MCFARLANE & MATSUURA, 1947; MORGAN, 1957) or *Rhizoctonia* (ALTMAN & FITZGERALD, 1960) has been diminished by soil fumigation with DD. However, it is not unlikely that in these cases the fungi act as secondary root invaders, and are controlled by killing the organisms causing the initial root penetration (see next point). SMITH (1947) describes a case in which bacterial wilt on tobacco could be controlled by soil fumigation with DD. Though it is likely that the bacteria in question have been killed by the DD treatment, it is not quite certain as in this case too there is a possibility of a combined attack as described under the next point.

Thus, if DD is used as a nematocide, growth stimulation may also occur as a result of killing other parasites among which there may be parasites of which the presence is unknown.

A diminished attack by secondary acting pathogens which are not killed themselves by DD

In cases in which nematodes act as precursors of certain pathogens (*e.g.* secondary fungi) by wounding roots or weakening the plant, or in cases in which nematodes are carriers of a pathogen (*e.g.* viruses), the associated diseases may be controlled by controlling the nematodes (MEULI & SWEZEY, 1949; ANONYMOUS, 1955; WHITEHEAD, 1956; REP. ROTHAMSTED, 1963; SYMPOSIUM etc., 1963).

Though in case of a combined attack of the plant elimination of the nematodes may have a direct stimulating effect on plant growth after fumigation, an important part of this growth stimulation may be due to eliminating the other organisms. It seems plausible that in such cases the growth stimulation may be disproportionally large in comparison to the number of nematodes that has been killed.

Changes in the non-pathogenic microbiological population in the soil

The changes in the microbiological population of the soil following fumigation may exert a biological control effect on root parasites (MOJE *et al.*, 1957; MARTIN & PRATT, 1958). As to the fungi, *Trichoderma viride* often becomes dominant following partial soil sterilization with chemicals. It has been shown to exert an antagonistic influence on *Phytophthora* spp., *Pythium* spp., *Rhizoctonia* spp., and other parasitic forms. This may lead to a diminished infection of the crop by these fungi.

Influence of different components of DD on various organisms

In the past several investigators have already paid attention to the toxicity of certain components or fractions of DD to pathogenic organisms. As far back as 1912 SALIMBENI referred to the bactericidal activity of 1,2,3-trichloropropane on tubercle bacilli. 1,2-dichloroethane (= ethylene (di)chloride) and/or 1,1-dichloroethane (= ethylidene (di)chloride) are reported to have bactericidal, insecticidal and nematocidal properties (GABBANO, 1928; HOYT, 1928; ROARK, 1928; LEHMAN, 1933; SNAPP, 1945; STARK & LEAR, 1947; LINDGREN *et al.*, 1954). HUTSON (1933), DIBBLE (1933) and SNAPP (1945) referred to the usefulness of 1,2-dichloropropane (= propylene (di)chloride) as an insecticide.

CARTER (1945) investigated the toxicity of some fractions of DD-mixture on rice weevils in fumigation chambers. He concluded that 1,3-dichloropropene (= dichloropropylene) is the most toxic component of the mixture and that 1,2-dichloropropane in a pure state is not very toxic. By adding 1% of 1,3-dichloropropene to the pure 1,2-dichloropropane the toxicity of 1,2-dichloropropane is considerably increased due to synergism between the two compounds. Consequently the toxicity of 1,2-dichloropropane in the DD-mixture is higher than shown by this compound in a pure state. More recently CARTER (1954) has concluded from experiments with pineapple that when soil is fumigated with DD at dosages of 200–400 lbs per acre the ratio between 1,3-dichloropropene and 1,2-dichloropropane within the mixture does not have much influence on the effect if the 1,3-dichloropropene content is not lower than about 40%.

Other investigators have shown that 1,3-dichloropropene is more toxic than 1,2-dichloropropane against other organisms also (garden symphylids: HOWITT,

1959; several fungi: ZENTMYER & KENDRICK, 1949; ZENTMYER & KLOTZ, 1949).

BALOCK & LINDGREN (1951) and MOJE *et al.* (1957) compared the toxicity of several DD components to the oriental fruit fly and to citrus root nematodes, fungi and bacteria, respectively. They found 1,3-dichloropropene to be the most toxic compound of the DD mixture in all cases. As was shown by MOJE *et al.* (1957) and MOJE (1959) the cis-isomer of 1,3-dichloropropene is more toxic than the trans-isomer.

EXPERIMENTS

First pot experiment

This experiment was designed to test the growth stimulating effect on white clover of six components of DD. Together with DD, these components were studied in a pot experiment with white clover 'Witte cultuurklaver C.B.', perennial rye grass, and a mixture of both species as test crops. Details of the components used and the dosages employed are given in Table 1.

TABLE 1. Experiment 1. Survey of the components used and the dosages employed in ml per pot.

Pot mark	Component	Purity of the components (weight %)	Components ml/5.9 kg of soil	Petroleum ether ml/5.9 kg of soil
S	DD		3.0	10
A	1,2-dichloroethane	99.8	0.018	1.782
B	Allylchloride	96.7	0.003	2.997
C	1,2,3-trichloropropane	99.6	0.009	8.991
D	1,2-dichloropropane	96.3	0.35	3.15
E	2,3-dichloropropene	96.4	0.35	3.15
F	"Heavy ends"		0.21	20.79
O	Control			10

The experiment was carried out in quadruplicate in 5-litre plastic buckets of 21 cm diameter, with a small opening near the bottom to drain possible water surplusses. Each bucket contained 5.9 kg of sandy soil from the experimental garden on top of a thin layer of gravel.

As we were interested in the effect of the components as a part of DD, the same rates of the components were used in the experiment as would be applied with a complete DD treatment. To achieve this, 3 ml DD per pot was taken as a basis. The analysis of DD at our disposal at that time, was somewhat different from the present analysis of DD on page 118. The calculation of the dosages of the various components was based on the following contents: 1,2-dichloroethane 0.6%; allylchloride 0.1%; 1,2,3-trichloropropane 0.3%; 1,2-dichloropropane 11.5%; 2,3-dichloropropene 11.5%; "heavy ends" 3.5%. By mistake the "heavy ends" were applied in double dose. In order to add very small quantities of the components, they were diluted with petroleum ether as indicated in Table 1. A corresponding volume of the solvent was added to the DD and to the control pots. After treatment the pots were covered with plastic for one week at a temperature of about 20°C. After that the soil was well-aired. In April 1961, about one month after treatment, the pots were planted with young plants, which had been raised on non-fumigated soil, and were placed in a

greenhouse at a temperature of 20 °C. The number of plants per pot of white clover or perennial ryegrass in monoculture was 31. The pots with a mixture of both species contained 16 white clover plants and 15 rye grass plants. The plants were cut once a month. A total of six cuts were taken. At each cut the dry matter yield was determined and after each cut the pots received a dressing with P, K and Mg as a nutrient solution. No nitrogen was given except for the pots with grass alone which received 0.66 g ammonium nitrate per pot after the second, third and fourth cut. This corresponds with 100 kg N per ha each time.

The dry weight yields in the course of time are shown in Figs. 1 to 3. From these it is apparent that the components tested in the dosages applied did not

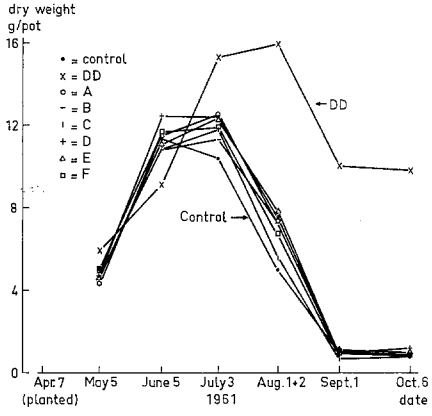


FIG. 1. Experiment 1. Mean dry weight yield of white clover (monoculture) after fumigating the soil with DD or certain components of DD. A–F = various components of DD (see Table 1).

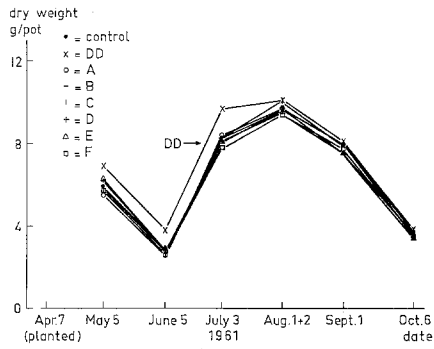


FIG. 2. Experiment 1. Mean dry weight yield of perennial rye grass (monoculture) after fumigating the soil with DD or certain components of DD. A–F = various components of DD (see Table 1).

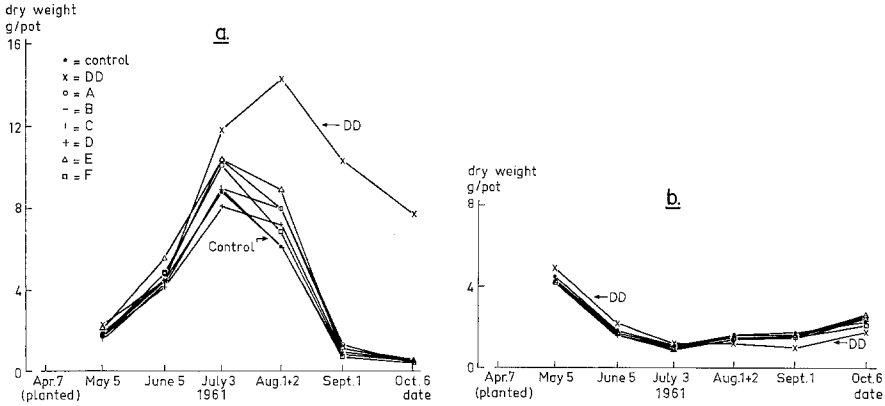


FIG. 3. Experiment 1. Mean dry weight yield of white clover (a) and perennial rye grass (b) from the pots with a mixture of both species, after fumigating the soil with DD or certain components of DD. A–F = various components of DD (see Table 1).

FIG. 4. Experiment 1. Condition of clover and grass 5 months after planting (August 31, 1961). From the top downwards white clover, white clover plus perennial rye grass, and perennial rye grass, respectively. A–F = various components of DD (see Table 1), O = control, S = DD.

FIG. 7. Experiment 2. Condition of clover 5 months after planting (June 14, 1962).
Above: O = control, S = DD. G, H, and G + H = cis-, trans-, and cis- + trans-1,3-dichloropropene, respectively. A t/m H = combination of components A to H inclusive of experiment 1 and 2.
Under: from left to right: sugar, EN 18,133, DD, control, × 323, and Emkal, respectively.

have any influence on the growth of clover or grass. In the pots which had a complete DD treatment, however, a great difference in clover growth occurred compared with the other pots three months after planting. The general decline in clover growth after June corresponds with the shortening day length by which less light is available for the plants. The difference in growth between the clover in the DD pots and the other pots indicates, however, that another factor is involved in the latter as well. Probably, this is damage by parasites which have increased to a critical level in the preceding months. That the decline in growth of the clover in mixed culture (Fig. 3a) started later than that of the clover in monoculture (Fig. 1) may indicate that reproduction of the parasites did not proceed as fast in mixed culture as in monoculture.

The DD treatment had practically no influence on grass growth. Only at the beginning was the yield of grass a little bit higher in the DD pots than in other pots, which is probably due to the nitrogen available after a DD treatment (ENNIK *et al.*, 1962). To avoid that the clover would be overgrown by the grass no nitrogen was applied to the mixed culture of grass and clover. Neither was this done with the first two cuts of grass in monoculture. Consequently nitrogen rather than light was the limiting factor for grass growth. Lest the favourable effect of the DD treatment on grass growth could not manifest itself because of nitrogen deficiency, a rather heavy nitrogen dressing was given to the third, fourth and fifth cut of the grass in monoculture (Fig. 2). But at these cuts DD treatment had not a favourable influence on grass growth either. In the pots with mixed culture grass yields of the DD pots were a little bit lower than those of other pots at the end of the summer (Fig. 3b), which is probably due to the heavy competition by the clover in the DD pots.

Fig. 4 shows the development of clover and grass five months after planting. In Table 2 the total yields of clover and grass over the whole growing period (= half a year) are given for the different treatments. These illustrate once more the great influence of the DD treatment on clover growth.

TABLE 2. Experiment 1. Total yields of clover and grass from the first up to the sixth cut inclusive (g dry matter per pot). Averages of 4 pots.

Pot number	Treatment	Monoculture clover	Monoculture grass	Mixed culture	
				clover	grass
S I-IV	DD	66.0	42.4	51.0	12.4
A I-IV	1,2-dichloroethane	37.9	37.7	26.6	12.5
B I-IV	Allylchloride	36.2	37.3	22.6	12.6
C I-IV	1,2,3-trichloropropane	34.5	38.3	24.5	12.3
D I-IV	1,2-dichloropropane	39.2	37.8	22.6	12.6
E I-IV	2,3-dichloropropene	37.8	37.8	28.8	12.5
F I-IV	"Heavy ends"	37.1	36.9	24.7	12.0
O I-IV	Control	33.4	37.7	22.5	13.0

On October 19, 1961 a part of the experiment was used to investigate the soil and clover roots for the presence of organisms which could possibly be detrimental. The clover in the DD pots appeared to have a much better root system with many fine lateral rootlets. These rootlets missed almost entirely on the clover in the untreated pots. On the roots of this clover many brown spots were

to be seen. These were often the remainder of dead lateral roots. As has been previously described (ENNIK *et al.*, 1962), Ir. J. H. VAN EMDEN (Institute of Phytopathological Research (I.P.O.), Wageningen) isolated several fungi, *e.g. Rhizoctonia* sp., *Fusarium* spp., *Cylindrocarpon* sp., and *Pythium* sp. from these spots. Moreover, the untreated pots contained many springtails (Collembola) as has been proved by Dr. C. J. H. FRANSSEN (I.P.O., Wageningen). In the untreated as well as in the DD treated pots many cysts of the clover cyst nematode were present on the clover roots. The results of an analysis of the nematode population by the Plant Protection Service are shown in Table 3. Of the clover-monoculture series one pot of each treatment has been analysed; for the mixed-culture series this was confined to the treatments control and DD. Of the latter,

TABLE 3. Experiment 1. Numbers of nematodes per 100 ml fresh soil on October 19, 1961, and the yield of the sixth cut (October 6) of some pots of the clover-monoculture and the mixed-culture series.

Pot no.	Free-living nematodes per 100 ml of soil									*Heterodera trifolii* per 100 ml of soil			Yield sixth cut
	P	Pa	T	R	Hl	Ml	Tr	O	S	c	lc	e	g d.m. /pot
White clover monoculture													
A I[1]	825	100	50	100	160	235	5	120	850	848	365	22850	1.52
B III	180	10	125	260	445	415	5	75	1880	783	455	28750	0.61
C III	260	260	190	185	290	100	0	100	2815	508	323	21325	0.63
D III	230	15	140	160	105	1060	0	100	870	585	423	23600	0.77
E III	225	15	105	175	25	725	15	105	975	285	170	11075	1.30
F III	185	0	115	170	55	1190	0	90	1390	465	330	21500	0.54
O III	165	0	165	175	30	3320	0	135	1230	270	203	12150	0.67
S III	40	0	10	25	845	45	0	30	6535	228	175	11350	9.11
Clover-grass mixture													
O III	75	135	220	150	95	1395	0	175	695	413	335	23625	0.52
S III	15	4440	40	5	85	0	0	55	1330	113	95	6375	1.60
S I	30	495	5	10	205	20	0	55	3965	143	95	7175	10.32

Nematode symbols:
P = *Pratylenchus* spp.
Pa = *Paratylenchus* spp.
T = *Tylenchorhynchus* spp.
R = *Rotylenchus* spp.
Hl = *Heterodera* larvae
Ml = *Meloidogyne* larvae (= *M. hapla* Chitwood)
Tr = *Trichodorus* spp.
O = other Tylenchida
S = saprophytic nematodes
c = cysts
lc = cysts with living content
e = eggs plus larvae within the cysts

Pot marks:
A – F = various components of DD (see Table 1)
O = control
S = DD

[1] The Roman numerals refer to the different replicates of one treatment.

two pots were analysed, because after the second cut the clover in pot S III of the mixed-culture series decreased more and more compared with the other DD pots (Fig. 5), so that at the sixth cut it almost resembled the clover in the untreated pots. This is also illustrated by the yields of the separated pots at the sixth cut, which are listed in the last column of Table 3. Only in two of the DD

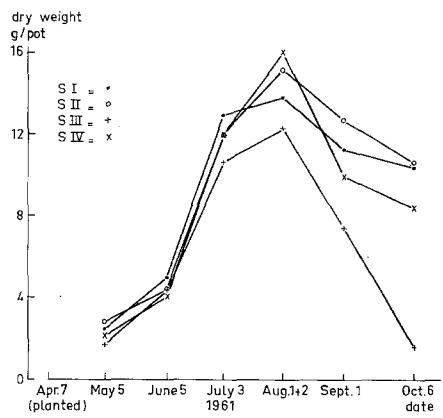


FIG. 5. Experiment 1. Line in Fig. 3a, indicating the average dry weight yield of treatment DD (white clover mixed culture), divided into 4 replicates (S I-IV).

pots (S III-monoculture and S I-mixed culture), the clover yield is still high. Examination of the nematode data shows that the number of cysts and eggs (plus larvae) of the clover cyst nematode (*Heterodera trifolii* Goffart) is high in all pots. According to the norms given by OOSTENBRINK & S'JACOB (1958) there is a heavy attack if the population density is more than 30 living cysts or 2000 eggs (plus larvae) per 100 ml of soil.

Judging from this the clover cyst nematode might quite well be the cause of poor growth of the clover in the untreated pots in this case. The fact that the number of eggs (plus larvae) is also high in the DD pots, is not necessarily in conflict with this. Experience has shown that after DD fumigation the nematodes recover in the course of time, either as a result of incomplete killing or reinfection. The same is true for other organisms so that finally the clover in the DD pots will decline too. It is conceivable that in our case at the time of sampling the cyst nematode population had just reached a harmful level which had not yet manifested itself in the last yield. (This means that a yield depression might be expected in following cuts; as a matter of fact when the yield of the remaining pots was calculated on December 1, 1961 (seventh cut), the difference in clover yield between the DD pots and other pots had almost disappeared.) Then it may be expected, however, that the total number of cysts which has formed during the growing season (including those already left, which stay in the soil for a considerable time) will be noticeably smaller in the treated soil than in the untreated. Information on this is to be found in column c of *Heterodera trifolii* in Table 3. For the series clover-monoculture the number of cysts in the DD pot (S III) is hardly smaller than that in the pots O III and E III. Moreover, as the number of eggs (plus larvae) is almost equally high, the difference in growth between S III on the one side, and O III and E III on the other side is hard to explain by cyst nematodes only. For the same reason the difference in growth between S I and S III of the mixed-culture series can not be explained by cyst nematodes. Examination of the number of other nematodes shows that many larvae of the root-knot nematode (*Meloidogyne hapla* Chitwood) occur in several pots.

OOSTENBRINK & S'JACOB (1958) consider an infestation of this nematode heavy, if the number of free-living larvae exceeds 200 per 100 ml of soil. The number of these larvae in pot S III of the series clover-monoculture is consider-

ably lower than that in the pots O III and E III which might be the cause of the difference in growth between these pots. It is also possible that there is an interaction between the effects of the different nematode species on plants in such a way that damage by cyst nematodes may occur at lower level if a high number of root-knot nematodes is present. In a similar way the difference in growth between S III and S I of the mixed-culture series may perhaps be directly or indirectly explained by the high number of *Paratylenchus* spp. in S III.

The preceding clearly indicates that the decrease in clover growth in our experiment cannot be ascribed to the number of cyst nematodes only. It seems likely that the reduction in clover growth is due to a soil pest complex in which different organisms are involved. The fact that many lateral roots of the poorly growing clover had died and that many fungi were present in the plant tissues on the places where these roots had been attached suggests that fungi are a part of this complex.

Another possibility could be that alterations in the soil inherent to DD treatment make growth conditions for the plants in the DD pots somewhat more favourable in the beginning, which may result in a higher resistance of the plants to pests. In that case a nematode density detrimental in untreated soil, may be harmless in treated soil, implying that the occurrence or not of damage to a crop cannot be predicted on nematode numbers only.

In grass too, the root system of the plants from the DD pots contained more roots and had a better (less brown) colour than that of plants from the untreated pots. However, in spite of this, the yield of the overground parts of the grass was equal in both cases.

On November 8, 1961, pH-water of the soil was determined in the remaining control and DD pots. It appeared to be equal for both treatments with a mean value of 4.9.

Second pot experiment

In the first experiment the main component of DD, 1,3-dichloropropene, was not included because this component could not be obtained in a sufficiently pure form. However, after an improvement in the laboratory equipment of Shell it became possible to start an experiment with this component, split up in the cis- and the trans-isomer. Compared were: control, cis-1,3-dichloropropene, trans-1,3-dichloropropene, 1,3-dichloropropene (cis + trans), DD, and a combination of all separate components of DD which have been used in this and the preceding experiment. Moreover, the following compounds were also included: EN 18,133 (an experimental nematocide of the American Cyanamid Co., now on the market under the trade name Nemafos or Zinophos), sugar (the nematocidal activity of which has been described by FEDER (1960)), and X 323 (a new nematocide against free-living nematodes described by VAN BERKUM (1961), now on the market in mixture with NPK-fertilizer under the trade name Exaal). The compound X 323 has a pH-increasing activity. Therefore the Emkal treatment (= calciumcarbonate) was added to the experiment for comparison, this being a control with a lime supply which increases the pH to a similar extent as X 323. A survey of the compounds used and the dosages employed is given in Table 4.

As in the first experiment DD and its components were diluted with petroleum ether. The same volume of petroleum ether was given to the control. Of the

TABLE 4. Experiment 2. Survey of the compounds used and the dosages employed in ml or g per pot.

Pot mark	Compound	Purity of the compound (weight %)	Compound ml/5.5 kg of soil	Petroleum ether ml/5.5 kg of soil
O	Control			25
S	DD		3.0	25
G	Cis-1,3-dichloropropene	98.5	1.0	25
H	Trans-1,3-dichloropropene	98.7	0.9	25
G + H	1,3-dichloropropene (cis + trans)		1.95	25
A t/m H	Combination of components of DD		2.8	22.4
	EN 18,133 (= Nemafos 25%)		—[1]	—[1]
			Compound g/5.5 kg of soil	
	Sugar		275	
	X 323		3.4	
	Emkal		2.7	

[1] Data concerning application rate lost by error.

other compounds EN 18,133 was supplied as Nemafos-25% diluted with petroleum ether and the remaining ones as solids. They were thoroughly mixed through the soil, after which treatment for these pots was similar to that for other pots.

The experiment was carried out in triplicate. As a test crop only white clover 'Witte cultuurklaver C.B.' was used. The pots and the origin of soil were similar to those in the first experiment, but the amount of soil was 5.5 kg per pot. After treatment with the various compounds the pots were covered with plastic for one week. Next, the soil was spread on plates and exposed to air at a temperature of 5–10 °C for two weeks. After replacement of the soil into the pots these were placed in a greenhouse at 20 °C and planted with clover on January 5, 1962 in the same way as in the first experiment. During winter additional light was supplied with HPL-bulbs to a total daylength of 17 hours but this was not enough to compensate the shortage of light. The first cut was taken on March 5 after which the plants were cut every five weeks. After each cut the pots received a dressing with P, K and Mg as a nutrient solution. No nitrogen was given. A total of eight cuts were taken. The dry weight yields in the course of time are shown in Table 5 and Fig. 6. From these it is apparent that in summer the yields of the pots treated with DD, cis-1,3-dichloropropene, trans-1,3-dichloropropene, 1,3-dichloropropene (cis + trans), and a combination of components from DD, similar to the compounds EN 18,133 and sugar, are high, whereas those of the control, X 323 and Emkal, are much lower. The yields of the pots with the first-mentioned compounds increased with increasing daylength (= light supply) and decreased again after the longest day. The yields of the pots with the three last-mentioned compounds, however, already showed a sharp decrease after mid-May. In spite of adequate light and fertilizer supply the clover in these pots was unable to reach a high yield. In the first cut

TABLE 5. Experiment 2. Yield per cut in g dry matter per pot. Averages of 3 pots.

Date of cutting	Control	DD	Cis	Trans	Cis + trans	Combination of components	EN 18,133	Sugar	X 323	Emkal
1962										
March 5	8.2	3.9	8.0	8.2	5.2	6.5	7.8	0.0	10.1	9.7
April 9	10.2	9.5	10.7	10.6	9.7	10.7	8.8	0.5	11.1	10.3
May 14	9.7	17.7	16.6	15.8	18.0	18.0	15.2	5.6	11.5	8.3
June 18	4.0	24.8	24.7	23.3	22.5	25.7	25.7	21.7	5.0	4.2
July 23	3.9	25.5	23.3	17.5	24.3	22.2	22.9	20.0	3.0	3.7
August 27	3.7	18.4	18.1	11.6	14.7	12.8	15.5	14.9	3.4	5.3
October 1	2.4	9.2	10.4	6.5	6.4	6.6	5.5	5.4	2.2	2.3
November 5	4.2	8.5	10.1	5.7	5.0	5.9	2.6	3.5	3.5	3.1
Total	46.3	117.5	121.9	99.2	105.8	108.4	104.0	71.6	49.8	46.9

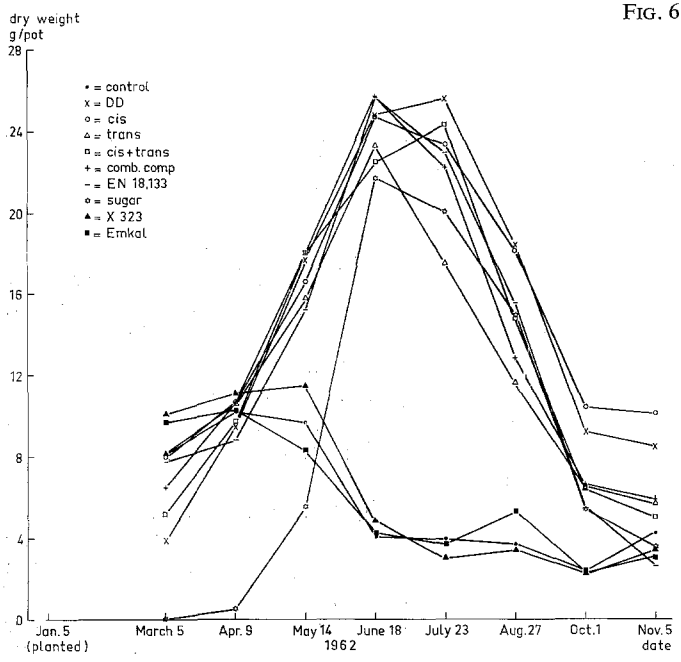


FIG. 6. Experiment 2. Mean dry weight yield of white clover (monoculture) after fumigating the soil with various compounds.

the yield of the DD-treated pots was somewhat retarded, due to the fact that the phytotoxic activity of DD had not yet disappeared completely at that time. On the other hand the yields of the pots treated with X 323 and Emkal were high in the first cut because of the stimulating effect of pH-increase. These effects disappeared entirely, however, in the following cuts. In the pots treated with sugar, plant growth was strongly inhibited at the beginning because heavy fermenta-

tion occurred in the soil some time after treatment, causing many plants to die. Afterwards, the plants which had survived recovered completely. Fig. 7 shows the development of the clover at the different treatments on June 14.

The lines in Fig. 6 are averages of the three replicates. In Fig. 8 these replicates have been separately presented for each treatment. Generally the replicates of one treatment show good resemblance. As to some of the treatments with good plant growth, however, the yield of certain replicates falls more rapidly after the fourth or fifth cut than that of other replicates of the same treatment. As has already been mentioned, following disinfection of the soil detrimental organisms will return to the soil with time, either because killing was not complete, or because of reinfection via the seedlings or spontaneously. As this depends more or less on chance, it may occur sooner in one pot than in another. This may explain why for some treatments the yield of certain replicates falls more rapidly after the fourth or fifth cut than that of others. As is indicated in Table 5 and Fig. 6, the most rapid decrease in clover growth occurred on an average after fumigation of the soil with trans-1,3-dichloropropene, but the differences with some cis-1,3-dichloropropene including treatments (combination of components, cis + trans) are too small to conclude that trans-1,3-dichloropropene is less effective than cis-1,3-dichloropropene.

Because of the great differences in yield between some replicates in the eighth cut on November 5, 1962 (Fig. 8), many pots were examined for clover cyst nematodes on November 8. A few pots with the most extreme yields were also examined for free-living nematodes and fungi. At the beginning of the experiment, just before treatment with the various compounds (December 14, 1961), the soil had also been examined for nematodes, in order to record the initial state. The numbers of nematodes per 100 ml of soil at that time are listed in Table 6.

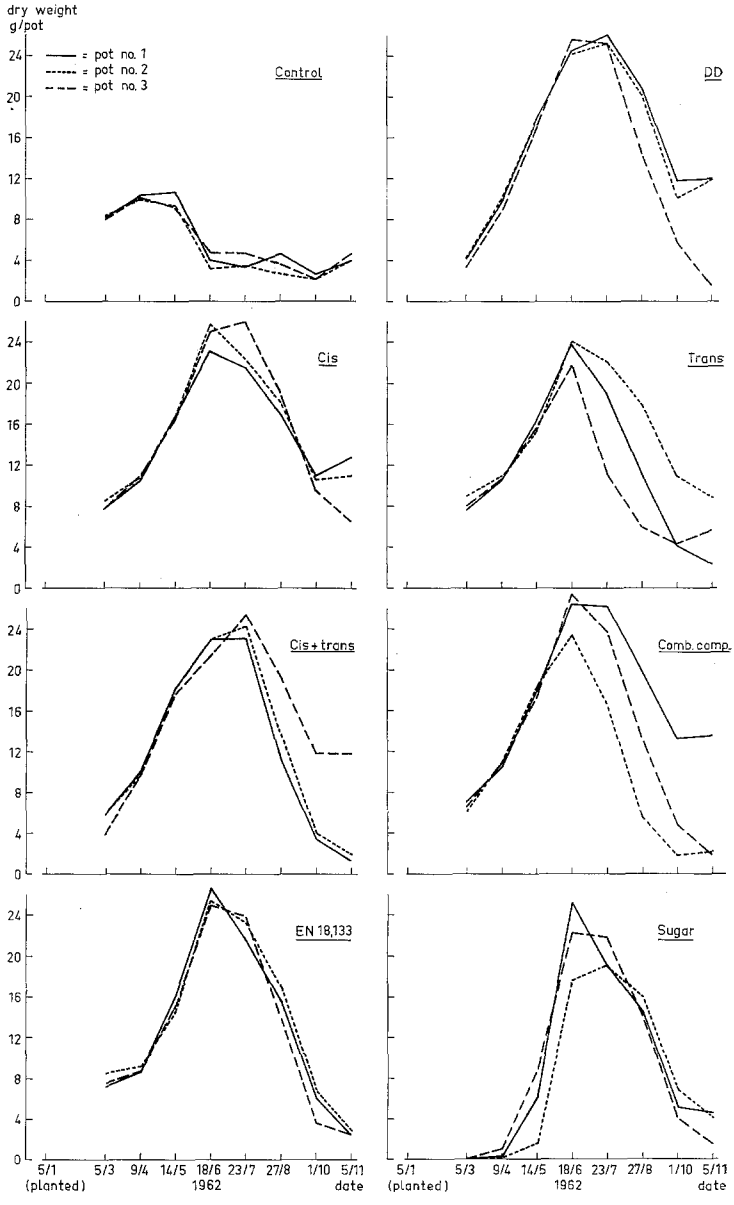
TABLE 6. Experiment 2. Numbers of nematodes per 100 ml of fresh soil at the beginning of the experiment (December 14, 1961).

Pratylenchus crenatus Loof	140	Other Tylenchida	110
Tylenchorhynchus spp.	140	Saprophytic nematodes	755
Rotylenchus spp.	220	Clover cyst nematode: 34 cysts, 4 of which living, containing larvae.	350

Table 7 presents data on the numbers of cysts and eggs in the different pots on November 8, 1962. In the case of EN 18,133, sugar and X 323 a mixed sample from each group of three replicates was taken. The pots containing Emkal were not examined. The yields at the eighth cut are also listed in this table. For the three last-mentioned treatments these are the average values for the three replicates.

The table indicates that in this case there is a close relation between the number of cyst nematodes and the yield of clover. In all cases in which the yield of clover is high, the number of cyst nematodes is low (<200 eggs/100 ml of soil), and in all cases in which the number of cyst nematodes is high (>10000 eggs/100 ml of soil), the yield of clover is low. In a few cases with intermediate numbers of cyst nematodes (4000–8000 eggs/100 ml of soil), the yield of clover is also intermediate.

Very likely, in this case there is a causal relation between the number of cyst


dry weight
g/pot
= pot no. 1
= pot no. 2
= pot no. 3
Control
DD
Cis
Trans
Cis + trans
Comb. comp.
EN 18,133
Sugar
0
4
8
12
16
20
24
5/1 (planted)
5/3
9/4
14/5
18/6
23/7
27/8
1/10
5/11
1962
date

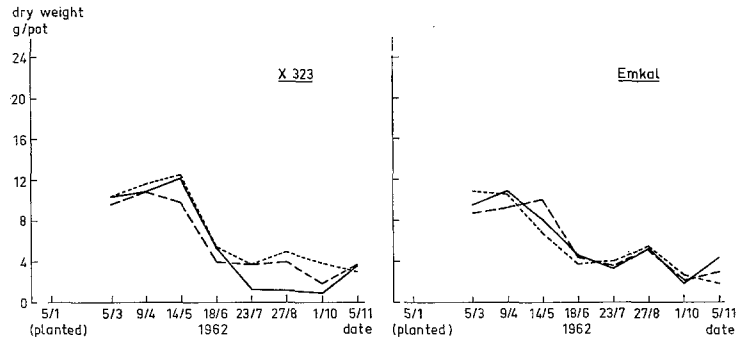


FIG. 8. Experiment 2. Like Fig. 6, but per treatment for the 3 replicates separately.

TABLE 7. Experiment 2. Numbers of cysts and eggs of *Heterodera trifolii* per 100 ml of fresh soil on November 8, 1962, and the yield of clover at the eighth cut (November 5, 1962).

Treatment	Pot no.	Nematode numbers per 100 ml of soil		Yield eighth cut g dry matter/pot
		Cysts	Eggs	
Control	1	324	19505	4.0
	2	597	16746	4.0
	3	598	28889	4.7
DD	1	4	30	12.0
	2	8	20	11.8
	3	399	15741	1.6
Cis	1	30	185	12.8
	2	29	30	11.0
	3	160	5785	6.5
Trans	1	305	14183	2.4
	2	101	4760	8.9
	3	291	8119	5.7
Cis + trans	1	308	19958	1.3
	2	700	48160	1.9
	3	5	0	11.8
Combination of components	1	7	155	13.5
	2	531	13209	2.2
	3	?	15230	1.9
EN 18,133		214	15361	2.6
Sugar		340	16279	3.5
X 323		649	34723	3.5

nematodes and clover yield and not an apparent one, the more so as the phenomenon also occurs *within* the treatments. This does not necessarily mean, however, that (secondary) fungi may not be accessary to destruction of the root system. To investigate this, small pieces of clover root from a few pots were put on agar plates. Due to a heavy contamination with *Trichoderma* spp. it was impossible, however, to isolate pathogenic fungi possibly present.

The results of the determination of free-living nematodes are given in Table

TABLE 8. Experiment 2. Numbers of free-living nematodes per 100 ml of fresh soil and (in 2 samples) per 10 g of roots on November 8, 1962.

Treatment	Pot no.	Nematode numbers									
		per 100 ml of soil							per 10 g of roots		
		P	Pa	T	Hl	Ml	O	S	P	Hl	Ml
DD	1	420	30	45	20	5	125	7360	4430	23	45
	3	45	0	40	255	0	95	4475			
Cis	1	40	0	0	0	0	170	4690	670	0	57
	3	15	0	5	450	0	170	3820			
Combination of components	1	1550	0	20	40	0	45	7510			
	3	75	0	10	165	0	55	7290			

P = *Pratylenchus penetrans* (Cobb)
Pa = *Paratylenchus* spp.
T = *Tylenchorhynchus* spp.
Hl = *Heterodera* larvae
Ml = *Meloidogyne* larvae
O = other Tylenchida
S = saprophytic nematodes

8. The number of free-living larvae of *Heterodera trifolii* is also highest in the pots with poor growing clover (see Table 7 for yield). Of the other nematodes, only *Pratylenchus penetrans* is present to a great extent in two pots. Here the relation is just the reverse, however. The highest numbers are associated with good clover growth. Apparently the population increase of this nematode is promoted by the well-growing clover, without having reached a level detrimental to plant growth.

The yield of some of the remaining pots was also determined in 1963. Though management was the same as in 1962 yield level in summmer 1963 was only half of that of the well-growing clover in 1962. Except for pot DD 2 this decrease in clover growth might well be explained by the high numbers of the clover cyst nematode. The yield of pot DD 2 in 1963 was also only half of that in 1962, though the number of cyst nematodes in this pot was very low in both years. The following numbers were found per 100 ml of soil: on November 8, 1962, 8 cysts and 20 eggs, on March 14, 1963, 26 cysts and 89 eggs, on July 24, 1963, 10 cysts. From this it is evident that at least in this pot the decrease in clover growth cannot be due to clover cyst nematodes.

DISCUSSION

Plotting the yields of the eighth cut in the second experiment against the logarithm of the egg (+ larvae) numbers of clover cyst nematode (both listed in Table 7), will allow an estimation of the nematode level at which plant damage occurred in this experiment (Fig. 9). Taking into account the spreading of the points, this level appears to be 1000–3000 *Heterodera* eggs (larvae) per 100 ml of soil. This corresponds with the number of 2000 eggs (larvae) per 100 ml of soil, given by OOSTENBRINK & s'JACOB (1958). Though the numbers found in the first pot experiment exceed this level considerably, no obvious relation was found in this experiment between yield and number of cyst nematodes. Despite

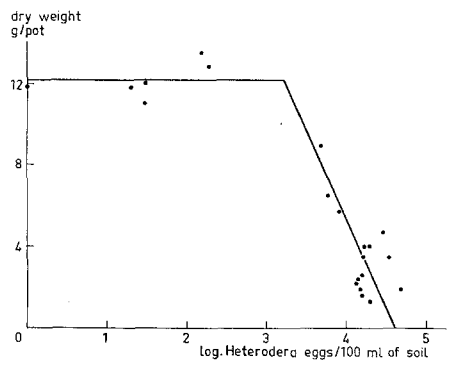


FIG. 9. Experiment 2. Yield of white clover at the eighth cut (November 5, 1962), plotted against the logarithm of the number of eggs (larvae) of the clover cyst nematode on November 8, 1962.

nematode numbers of 7000–11000 per 100 ml of soil two DD pots still gave high yields. As has been reported on page 125 it is conceivable that in these cases the cyst nematode population reached a harmful level only shortly before sampling, so that it had not yet affected yield. Even then, however, the differences in yield in this experiment can not be satisfactory explained by cyst nematodes only. Neither can cyst nematodes explain the decrease of clover growth in pot DD 2 of the second experiment.

An important question is if and how far the symptoms observed in the pots also occur in the field. From 25 field experiments on different sandy soils it appeared that in general the growth of white clover in leys is markedly promoted by soil fumigation with DD (ENNIK *et al.*, 1962). However, there was no relation between the extent of the DD effect and the number of cyst nematodes. As has been proved by a nematode determination at the end of the second year the number of cyst nematodes was low in most cases and always below 1000 eggs (+ larvae) per 100 ml of soil. Though the number of eggs (+ larvae) represents only the situation at the moment of sampling, the small number of *cysts* indicates that the number of cyst nematodes was also low in the preceding season. It is possible, however, that high nematode populations occurred locally in the field, which are not revealed by a random sampling of the whole field. Other possibilities are that in field experiments damage by cyst nematodes occurs at a lower nematode level than in pots or that other organisms have been involved in the decrease of clover growth. In view of the very low number of cyst nematodes in some fields, the last-mentioned possibility seems most probable.

Of the compounds investigated in the second pot experiment, X 323 had no effect on clover growth. This is probably due to the fact that X 323 may kill free-living nematodes, but has little or no effect on eggs and larvae inside cysts (VAN BERKUM, 1961). The fact that there was no difference in yield between the pots treated with X 323 and control pots suggests that free-living nematodes were of no importance in this experiment.

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