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IMPROVEMENT OF WHITE CLOVER GROWTH WITH NON-PHYTOTOXIC COMPOUNDS KNOWN TO CONTROL SOIL PARASITES[1]

Verbetering van de groei van witte klaver met niet-fytotoxische bestrijdingsmiddelen van parasieten

G. C. ENNIK and J. KORT
Institute for Biological and Chemical Research on Field Crops and Herbage (I.B.S.), Wageningen
Plant Protection Service (P.D.), Wageningen

In pot experiments under glasshouse conditions production of white clover could be maintained at a high level for several months and growth of diseased clover could be considerably improved by treatment with various non-phytotoxic compounds or compounds in a non-phytotoxic concentration. Most effective were the systemic compounds Nemafos and V-C 13.

INTRODUCTION

In two previous papers (ENNIK *et al.*, 1962, 1964) it was reported that soil disinfection with D-D and some other compounds considerably stimulated the growth of white clover. Due to their phytotoxicity these compounds can only be used before sowing. Experiments were therefore conducted in which a number of non-phytotoxic compounds and compounds at a non-phytotoxic concentration were tested. The results are discussed in this paper.

SUBSTANCES TESTED

Shell D-D. A mixture of mainly 1,3-dichloropropene and 1,2-dichloropropane.

Nemafos. 25% emulsifiable concentrate (e.c.) of 0,0-diethyl-0-2 pyrazinyl phosphorothioate.

Sulphuric acid.

Gramoxone. Containing 200 g paraquat per litre.

AAdibroom. Containing 10% 1,2-dibromoethane (EDB) by volume.

Dimethyl disulphide.

V-C 13 Nemacide. 75% emulsifiable concentrate of 0-2,4-dichlorophenyl 0,0-diethyl phosphorothioate.

SD 4965. An experimental nematicide of Shell containing 0.38 g emulsifiable concentrate of the active ingredient per ml.

AAmylon. 50% dust formulation of tetrahydro-3,5-dimethyl-2H-1,3,5-thiodiazin-2-thione.

Trapex. 20% solution of methylisothiocyanate in xylol.

EXPERIMENTS

First pot experiment

This was a preliminary experiment, started in 1962. The experiment was carried out in triplicate in 5-litre plastic buckets of 21 cm diameter, with a small

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opening near the bottom to drain surplus water. Each bucket was filled with 5.6 kg of sandy soil on which potatoes had been grown in the preceding season.

On 11 October 1962 31 seeds of white clover 'Witte cultuurklaver C.B.' were sown to each bucket and the buckets were then placed in a glasshouse at a temperature of 19° C. From 16 October 1962 till 4 April 1963 and from 14 August 1963 onwards additional light was supplied with HPL-bulbs to give a total daylength of 16 hours in the first and 17 hours in the second winter, but this was not enough to compensate for the shortage of light in winter.

The following substances which according to the literature might have a controlling effect on certain plant inhibiting factors were tested.

D-D solution 1:1 and 1:3. 9 ml of Shell D-D were mixed with 2 l of water. After one day 1 l of the clear water layer (D-D solution) was diluted with an equal quantity of water (1:1 treatment) and 0.5 l was diluted with 1.5 l of water (1:3 treatment). 500 ml of these dilutions were added to the relevant pots. At a solubility of D-D in water of $2^0/_{00}$ (CARTER, 1944) the dosages applied corresponded to 0.5 and 0.25 ml of D-D in 500 ml of water per pot respectively. It is to be expected, however, that the compositon of this "D-D" will be different from that of the basic D-D because the solubility of the different components of D-D will be different.

Nemafos emulsion. 4 ml of Nemafos were mixed with 2 l of water. 500 ml of the mixture were added to the pots.

Nemafos injection. 0.2 ml of Nemafos was injected into each of 5 holes per pot to a depth of about 11 cm.

Sulphuric acid. 20 ml of concentrated sulphuric acid were diluted with water to 2 litres; 400 ml of this solution were added per pot. After 6 hours each pot was leached with 800 ml of water. To compensate for the decrease in pH of these pots 5 g of calcium carbonate per pot were added 3 weeks after sulphuric acid treatment.

Gramoxone. 2.8 ml of Gramoxone were mixed with 53 ml of water and 0.56 ml of 10% Agral LN (a surface active agent). Of this mixture 1 ml was diluted with 9 ml of water. Then 0.5 ml was sprayed on the leaves.

AAdibroom solution. 9 ml of AAdibroom were mixed with 2 litres of water. After 5 hours the upper, immiscible layer was removed and of the remaining aqueous solution 500 ml were added per pot. The dosage of 1,2-dibromoethane which was applied in this way is not known, but cannot be higher than corresponds with 2.25 ml of AAdibroom per pot.

AAdibroom injection. 0.056 ml of AAdibroom was injected into each of 5 holes per pot to a depth of about 11 cm. This is a very low rate due to a misinterpretation as to the percentage of active ingredient.

Dimethyl disulphide. 0.05 ml of dimethyl disulphide was injected into each of 5 holes per pot to a depth of about 11 cm.

After adequate establishment of the clover the compounds were applied on 27 November 1962 as indicated in Table 1, except AAdibroom and dimethyl disulphide which were not available until January 1963.

Sulphuric acid and Gramoxone appeared to be highly phytotoxic. More than half the number of plants were killed. Rather severe damage occurred after treatment with "D-D solution 1:1" and slight damage after treatment with "D-D solution 1:3".

The first cut was taken on 12 December 1962, the second six weeks later and

TABLE 1. Experiment 1. Substances tested and their application rates and dates.
Proef 1. Overzicht van de gebruikte middelen, de toegediende hoeveelheden en de datum van toediening.

Treatment	Dosage per pot (ml)	Date of application	Reference
D-D solution/*oplossing* 1:1	250 aqueous D-D sol./250 water	27 Nov. '62	CARTER (1944)
D-D solution/*oplossing* 1:3	125 aqueous D-D sol./375 water	27 Nov. '62	CARTER (1944)
Nemafos emulsion	1 Nemafos/500 water	27 Nov. '62	
Nemafos injection	1 Nemafos	27 Nov. '62	
Sulphuric acid/*Zwavelzuur* 1%	4 conc. sulph. acid/396 water	27 Nov. '62	MADER (1947)
Gramoxone	0.0025 Gramoxone + 0.00005 Agral LN/0.4975 water	27 Nov. '62	
AAdibroom solution	500 aq. AAdibroom sol.	4 Jan. '63	EIDE (1959), SMITH (1949)
AAdibroom injection	0.28 AAdibroom	4 Jan. '63	
Dimethyl disulphide	0.25 dimethyl disulphide	4 Jan. '63	HOWITT (1959)
Behandeling	*Hoeveelheid per pot (ml)*	*Datum van toediening*	*Literatuur*

thereafter 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 applied. The dry weight yields at the times of cutting are shown in Fig. 1. From this it is apparent that with some of the compounds (Nemafos emulsion, Nemafos injection, D-D solution 1:1) the clover could be kept productive for a considerable time (Fig. 1a). Yields in these pots increased with increasing daylength (= light). The decrease in yield in autumn is related to the decrease in light, but may also be partly due to a decrease in effect of the compounds (*cf.* Fig. 1c). With D-D solution 1:3 and AAdibroom solution the clover remained productive for three cuts, after which yields gradually decreased to the level in the untreated pots. These compounds thus had an intermediate effect (Fig. 1b). The remaining compounds had no favourable influence on clover growth (Fig. 1c). In spite of adequate light and fertilizer supply the clover in these pots failed to reach a high yield. The condition of the plants on 7 May 1963 under some of the treatments is shown in Fig. 2. On 20 June 1963 most of the pots with poorly growing clover were regrouped and treated with one of the compounds which had been most effective in the preceding months (Nemafos emulsion, D-D solution 1:1, D-D solution 1:3 and AAdibroom solution). The amounts applied were the same as indicated in Table 1. As is shown in Fig. 1c a good effect was obtained only with Nemafos emulsion. The failure of the yield to reach the same level as in summer may have been due to shortage of light. As in spring, treatment with AAdibroom solution gave only a short improvement in clover growth. D-D solution 1:1 and D-D solution 1:3 had no effect.

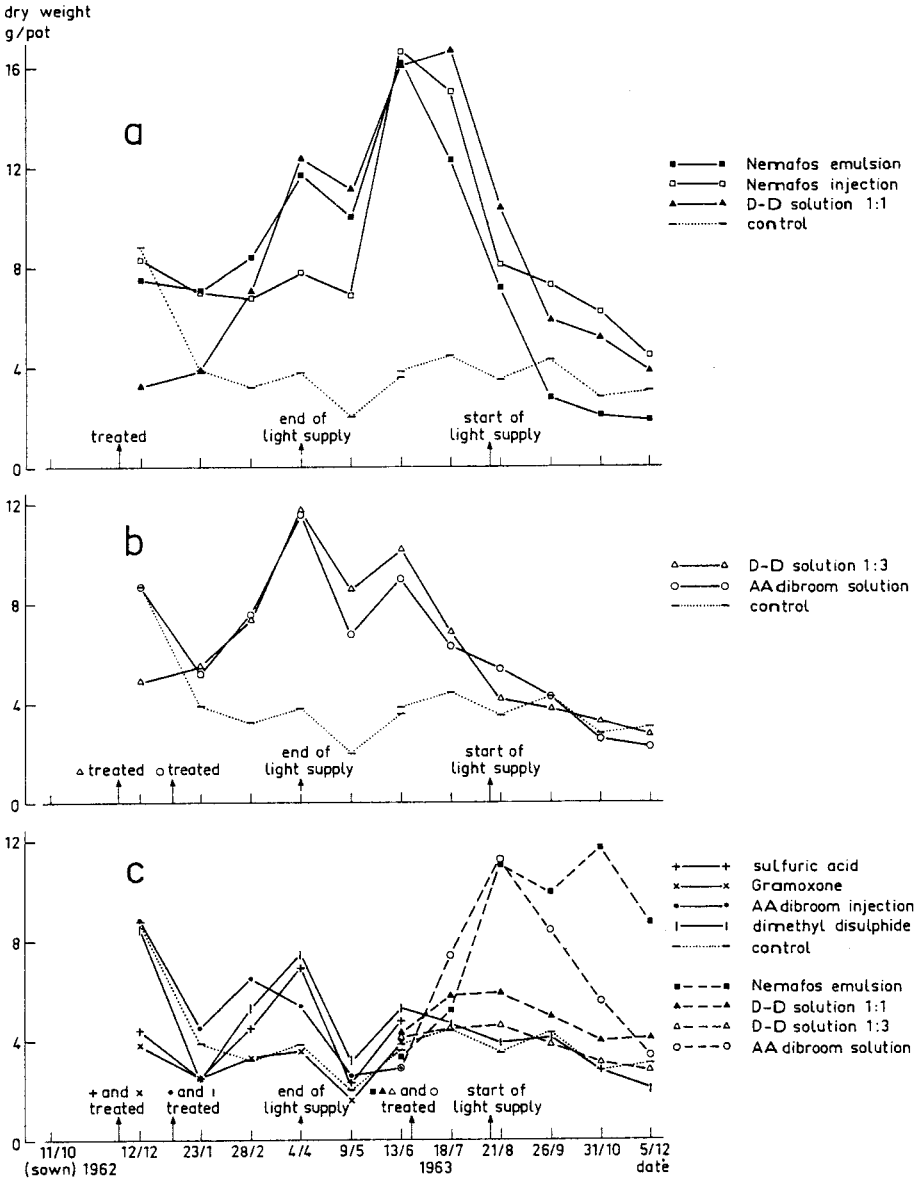

FIG. 1. Experiment 1. Dry weight yield of white clover following disinfection with various compounds. Averages of 3 pots, except for the control, which is an average of 9 pots until 13 June 1963 and of 6 pots after that date.
a. Treatments with good effect; b. treatments with intermediate effect; c. treatments without effect; on 20 June 1963 most pots in this series were regrouped and treated with some compounds of groups a and b.
Proef 1. Opbrengst aan droge stof van witte klaver na behandeling met verschillende middelen. Gemiddelden van 3 potten, behalve het object onbehandeld dat tot 13 juni 1963 een gemiddelde is van 9 potten en daarna van 6 potten.
a. Behandelingen met goed resultaat; b. behandelingen met matig resultaat; c. behandelingen zonder resultaat; op 20 juni 1963 zijn de meeste potten in deze groep gehergroepeerd en behandeld met bepaalde middelen uit de groepen a en b.

Second pot experiment

In this experiment three concentrations of Nemafos were applied to unthrifty white clover growing on vermiculite. The clover was sown in March 1963 in vermiculite which was highly infested with clover cyst nematode and other soil organisms. Clover growth was very poor in these pots. At the fifth cut on 30 October 1963, just preceding Nemafos treatment, the yield varied from 0.3 to 2.2 g dry matter per pot (pot size as in Experiment 1), whereas comparable pots which had been treated with pure D-D or chloropicrin before sowing, yielded 11 to 14 g dry matter per pot. On 1 November 1963 the following dosages of Nemafos were applied: 1 ml, 0.1 ml and 0.02 ml of Nemafos in 100 ml water per pot and the same range in 25 ml water per pot. From January till July 1964 five cuts were taken, but there was no difference in yield between the control pots and any of the Nemafos treated pots.

Third pot experiment

Simultaneously with Experiment 2 a similar experiment was carried out on poorly growing white clover in soil, again infested with various soil organisms. The clover was sown in March 1963 and in most of the pots was resown in May or June. In June 1963 the numbers of clover cyst nematode varied from 1160 to 4225 larvae per 100 ml of fresh soil. Besides Nemafos, the compounds D-D and AAdibroom were also included.

On 19 November 1963 treatments were applied as indicated in Table 2. Except for the aqueous solutions of D-D and AAdibroom which were prepared as described under Experiment 1, the materials were added to the relevant quantity of water, the mixture being then stirred and immediately poured into the pot. Addition of a surface active agent (1 ml of Agral LN per litre of water) was used in some cases to enhance the emulsifiability of D-D and AAdibroom. With AAdibroom a fairly good emulsion was obtained in this way, but with D-D emulsification was still insufficient. From February until August 1964 six cuts were taken. The total dry matter yield per pot during this period is shown in Table 2. The best results were obtained with Nemafos at the highest dosage (1 ml/pot). Compared to the control the yield was tripled. It made no difference whether Nemafos was applied in 100 ml of water or in 25 ml of water. Fairly good results were obtained with both D-D and AAdibroom in aqueous solution and with 2.25 ml of AAdibroom in 500 ml of water plus Agral LN. It is questionable whether the latter result is representative, since no effect occurred when the same dosage of AAdibroom was applied in 100 ml of water or when no Agral LN was added.

Fourth pot experiment

Pots, origin of soil, pot content, and variety of clover were similar to those in the first experiment. On 2 November 1962 the pots were planted with 31 seedlings per pot which had been sown on 11 October. During winter the pots were placed in a glasshouse at a temperature of 17 °C without supplementary light. From April until the end of November 1963 the pots were placed outside in a glasshouse with open sides. The average temperature in this glasshouse was 4 degrees higher than the outside temperature and attained 19 °C for the months June, July and August. The aim of this experiment was to wait till clover growth had diminished to a low level (which according to earlier experiments under our

TABLE 2. Experiment 3. Treatments and dry matter yield per pot from February until August 1964. The concentration of Agral LN added was 1 ml Agral LN per litre of water. The yields from the control and the D-D series are averages of two pots per treatment, the other series were carried out singly.
Proef 3. Overzicht van de behandelingen en de opbrengst aan droge stof van februari tot augustus 1964. De concentratie van de toegediende Agral LN bedroeg 1 ml Agral LN/l water. De opbrengsten van het onbehandelde object en de D-D-objecten zijn gemiddelden van 2 parallellen, de andere series werden in enkelvoud uitgevoerd.

Dosage per pot ml compound/ml water	Yield g dry matter/pot
Control/*Onbehandeld*	22.4
D-D	
500 ml aqueous solution/*waterige oplossing*[1]	47.3
1/100 + Agral LN	23.8
0.5/100 + Agral LN	9.8
0.5/25 + Agral LN	11.3
0.5/100	24.6
AAdibroom (10% 1,2-dibromoethane by volume)	
500 ml aqueous solution/*waterige oplossing*[2]	53.0
2.25/500 + Agral LN	49.2
2.25/100 + Agral LN	23.3
0.5/100 + Agral LN	12.2
2.25/500	16.9
Nemafos (25% emulsifiable concentrate/*emulgeerbare oplossing*)	
1/100	63.4
0.1/100	43.8
0.02/100	24.7
1/25	69.6
0.1/25	35.8
0.02/25	34.5
Hoeveelheid per pot *ml middel/ml water*	*Opbrengst* *g droge stof/pot*

[1] At a solubility of D-D in water of 2 ‰ the dosage applied amounts to 1 ml of D-D per pot.
Bij een oplosbaarheid van D-D in water van 2 ‰ bedraagt de toegediende hoeveelheid 1 ml D-D per pot.

[2] Dosage of 1,2-dibromoethane unknown, but not higher than corresponds with 2.25 ml of AAdibroom per pot.
De toegediende hoeveelheid 1,2-dibroomethaan is niet bekend, doch niet hoger dan overeenkomt met 2,25 ml AAdibroom per pot.

conditions might be expected within one season (ENNIK *et al.*, 1964)) and then to try to recover growth by treating with antiparasitic, non-phytotoxic compounds. Starting on 27 February 1963 the yield of clover was taken every five weeks. After each cut the pots received a dressing with P, K and Mg as a nutrient solution. No nitrogen was given. After 20 August 1963 no more cuts were taken due to poor regrowth. At the end of 1963 clover growth was very poor indeed and far below that of a healthy crop. A soil test for nematodes on 6 November 1963 showed a very high infestation of clover cyst nematode (*Heterodera trifolii* Goffart). The following numbers of this and other nematodes were found per 100 ml of fresh soil: *Heterodera* larvae 1320–7410 (av. 4417), *Meloidogyne* larvae 200–540 (av. 337), *Pratylenchus* sp. 220–660 (av. 418), *Rotylenchus* sp. 400–840 (av. 623), *Paratylenchus* sp. 0–20 (av. 2), *Criconemoides*

sp. 0–20 (av. 2), *Trichodorus* sp. 0–80 (av. 13), other Tylenchida 100–480 (av. 286) and saprophytic nematodes 1120–4900 (av. 2911).

The following three compounds were chosen for treating the diseased clover: Nemafos, V-C 13 Nemacide and SD 4965. As no appropriate data were available concerning the required dosages a wide range of concentrations was tested. On 27 November 1963 the following dosages were applied, each in 100 ml of water per pot: 1, 0.33, 0.11, 0.037 and 0.012 ml of Nemafos; 7.65, 2.55, 0.85, 0.28 and 0.094 g of V-C 13; 5, 1.67, 0.56, 0.19, 0.062 and 0.021 ml of SD 4965. The experiment was carried out in duplicate, except for the control which consisted of three pots. Two days after treatment the pots were transferred from the open glasshouse to an air-conditioned glasshouse at a temperature of 20°C. Until 10 April 1964 and from 2 September 1964 onwards additional light was supplied with HPL-bulbs to a total daylength of 17 hours.

The highest dosage of SD 4965 appeared to be highly toxic to the plants. Rather severe damage occurred after treatment with 1.67 ml of SD 4965 and with the highest dosage of V-C 13. Slight damage was observed after treatment with 2.55 g of V-C 13. At the highest dosages of SD 4965 and V-C 13 the yield was depressed at the first cut (Fig. 3). The plants in the other treatments had already recovered at that time. The relatively high yields on this date are due to the preceding long growing period of over five months. The second cut was taken six weeks after the first, and thereafter the plants were cut every five weeks. After each cut fertilizers were applied as before treatment. As is shown in Fig. 3, treatment of the diseased plants with Nemafos and V-C 13 greatly enhanced the yield. Yields from the Nemafos treatments increased with increasing dosage, while the V-C 13 treatments resulted in good yields at all dosages. The decrease in yield in autumn is associated with the decrease in light, but may also be partly due to a decrease in effect of the compounds. Considering the growth curves for the most effective dosages of both materials it is seen that the total yield over the whole season was almost the same for Nemafos and V-C 13, but production in the Nemafos pots fell off earlier in the season than that in the V-C 13 pots. Though clover growth was slightly improved by treatment with SD 4965, the effect of this material was inferior to that of Nemafos and V-C 13. Fig. 4 shows the condition of the plants on 23 April 1964.

Fifth pot experiment

Four compounds, each at three dosages, were applied to five months old white clover growing in infested soil. Pots, pot content and variety of clover were similar to those in the first experiment. The soil was a mixture of one part of fresh garden soil and one part of "old" soil from Experiments 1 and 3 in equal amounts. During the one month period between the termination of these experiments and the start of Experiment 5 the "old" soil had been stored in large plastic bags. After mixing the soil and filling the pots these were planted with 31 white clover seedlings each on 30 September 1964 and placed in a glasshouse at a temperature of 20°C. An examination of the soil at that time indicated a population of clover cyst nematodes of 2013 larvae per 100 ml of fresh soil. From 5 October 1964 until 21 April 1965 and from 3 September 1965 onwards additional light was supplied with HPL-bulbs to a total daylength of 17 hours. On 19 January 1965 the plants were cut for the first time. The experiment comprised the following compounds: Nemafos, V-C 13, AAmylon and Trapex.

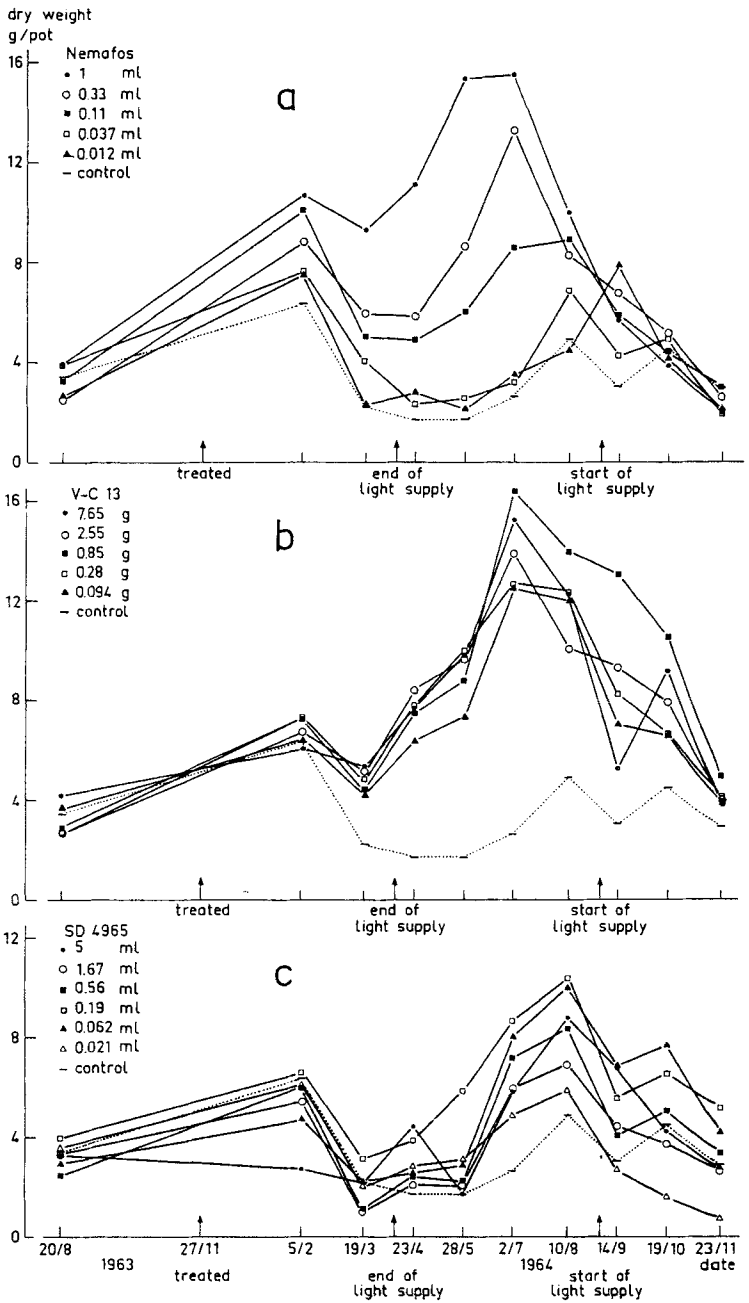

FIG. 3. Experiment 4. Dry weight yield of white clover which was treated on 27 November 1963 with different dosages (ml or g/pot) of a. Nemafos, b. V-C 13, c. SD 4965. Averages of 2 pots, except for the control, which is an average of 3 pots.

Proef 4. Opbrengst aan droge stof van witte klaver die op 27 november 1963 is behandeld met verschillende hoeveelheden (ml of g/pot) van a. Nemafos, b. V-C 13, c. SD 4965. Gemiddelden van 2 potten, behalve het object onbehandeld dat een gemiddelde is van 3 potten.

On 2 March 1965 the following treatments were applied: 2, 1 and 0.5 ml of Nemafos in 100 ml of water per pot; 0.85, 0.094 and 0.047 g of V-C 13 in 100 ml of water per pot; 4, 2 and 1 g of AAmylon mixed with 4 g of sterilized sand per pot; 6, 4 and 2 ml of Trapex in 100 ml of water per pot. The height of the clover was about 7 cm. After application of the compounds the pots were watered by watering-can (about 90 ml per pot), which was repeated on 3, 5, 8 and 9 March. After that date water was given according to need. The experiment was carried out in duplicate, except for the control for which five pots were used. On 11 March the phytotoxic effect of the different treatments was estimated. All dosages of Trapex were lethal to clover. With AAmylon very serious to serious damage was observed at the highest and medium dosages, and even at the lowest dosage there was fairly serious damage. Slight damage occurred after treatment with 2 and 1 ml of Nemafos, and only very slight damage with 0.5 ml of Nemafos and all V-C 13 dosages.

The first cut after treatment was taken on 10 May and then the plants were cut every five weeks. After each cut fertilizers were applied as in the other experiments. The yields are shown in Fig. 5. Whereas the yield of the control pots steadily decreased, yield was considerably increased by all dosages of Nemafos, the highest dosage being the most effective. The lowest dosage of V-C 13 (Fig. 5b) had no effect on growth. In contrast with Experiment 4 (*cf.* Fig. 3b) the effect of 0.094 g of V-C 13 per pot was also insufficient. Treatment with 0.85 g of V-C 13 per pot resulted in a distinctly higher yield, though the effect was less than with Nemafos. In considering the growth curves in Fig. 5 account should be taken of the fact that the yields on 10 May, and also on 19 January, were relatively high, due to the longer preceding growing periods. The effect of the lowest and medium dosage of AAmylon on clover growth was small and unimportant. The highest dosage, although it produced good results, was less suitable because of its high phytotoxic activity immediately after treatment (*cf.* 10 May).

DISCUSSION

As previously described (ENNIK *et al.*, 1962, 1964) soil disinfection before sowing considerably stimulated growth of white clover due to the kill of soil parasites, both in the field and in pot experiments. From the present paper it is evident that under glasshouse conditions similar results may be obtained by treating an established clover crop with certain non-phytotoxic compounds. Most promising in this respect seem to be Nemafos and V-C 13. Only in one case (Experiment 2) did the Nemafos application have no effect. Of Nemafos (25% e.c.) a dosage of at least 1 ml per pot (5.6 kg of soil) is required for an optimal effect. A higher dosage may be still more effective, but the chance of yield depression by phytotoxicity is increased. As to V-C 13 (75% e.c.) a dosage as low as 0.094 g per pot gave a good result in one experiment, but proved to be insufficient in another. In the latter a dosage of 0.85 g per pot gave reasonable results, though the effect was not quite as good as with Nemafos. Both compounds, like most of the other compounds applied, are known as nematicides, but have also some other pathogen killing properties. The numbers of clover cyst nematodes found in Experiments 3, 4 and 5 are high enough to be destructive (ENNIK *et al.*, 1964), but other organisms may also be involved.

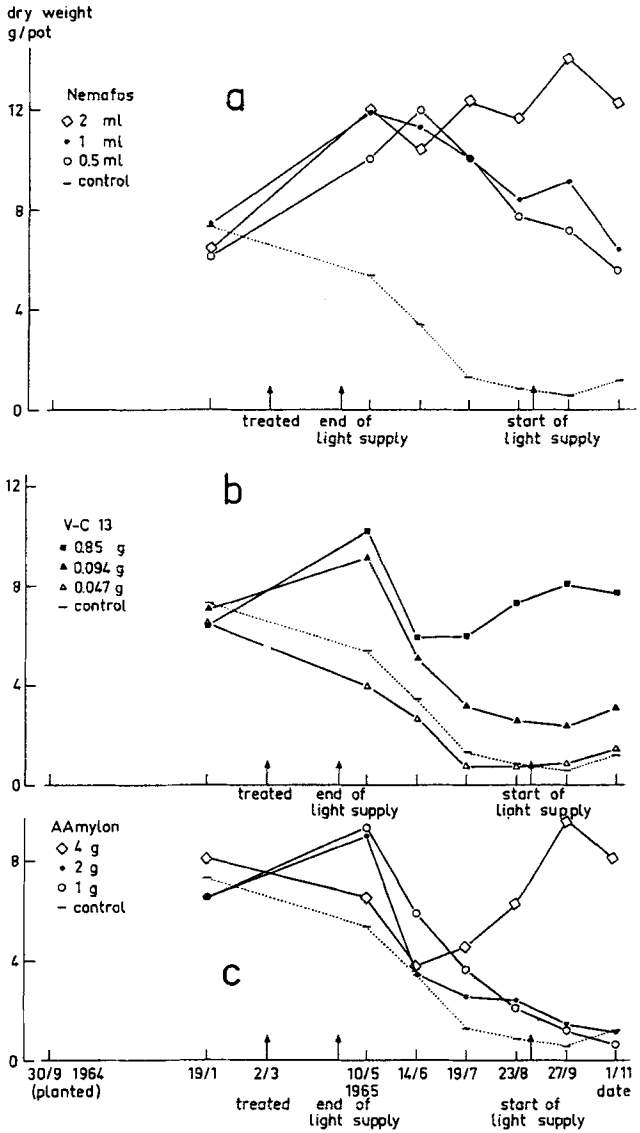

FIG. 5. Experiment 5. Dry weight yield of white clover which was treated on 2 March 1965 with different dosages (ml or g/pot) of a. Nemafos, b. V-C 13, c. AAmylon. Averages of 2 pots, except for the control, which is an average of 5 pots.
Proef 5. Opbrengst aan droge stof van witte klaver die op 2 maart 1965 behandeld is met verschillende hoeveelheden (ml of g/pot) van a. Nemafos, b. V-C 13, c. AAmylon. Gemiddelden van 2 potten, behalve het object onbehandeld dat een gemiddelde is van 5 potten.

Earlier experiments indicated that attack by soil parasites may be an important cause of poor development of white clover in pastures and leys (ENNIK *et al.*, 1962, 1965). If the results obtained in the pot experiments were transferable to the field, this might open new avenues for improvement of grass-clover swards (permanent pastures!). According to the literature both Nemafos and V-C 13 are systemic compounds, which might be especially valuable in applications under field conditions. To obtain a good effect it is necessary that the plants should absorb a sufficiently high amount of the compound. In contrast to a soil treatment before sowing, thorough disinfection of the whole soil may not be necessary. This simplifies application and may also limit the amount of water required for a good distribution of the compound. It may be that some systemic compounds also have a disinfecting effect on the soil. According to our experience (ENNIK *et al.*, 1964) this is the case with Nemafos and it is not unlikely that in the experiments described in the present paper the favourable effect of Nemafos on clover growth is at least partly due to its soil disinfecting capacity. Another advantage of using systemic compounds might be a lesser liberation of nitrogen after treatment, since the contact between compound and soil may be less intensive as compared with disinfection of the whole soil. As reported by ENNIK *et al.* (1964) soil disinfection with D-D under field conditions considerably increases the amount of nitrogen available to the plants, which, in grass-clover swards, biasses the grass-clover ratio unfavourably for clover.

Experiments are now in progress to study the effect of Nemafos and V-C 13 on clover growth under field conditions. However, even if positive results were obtained in these experiments both materials are still too expensive for farm use.

SAMENVATTING

In potproeven in een kas bij een temperatuur van 19–20 °C kon de groei van witte klaver verscheidene maanden op een hoog peil worden gehouden en de groei van zieke klaver aanmerkelijk verbeterd worden door een eenmalige behandeling van de klaver met diverse niet-fytotoxische anti-parasitaire middelen of middelen in een niet-fytotoxische concentratie. De beste resultaten werden verkregen met de systemische middelenNemafos en V-C 13.

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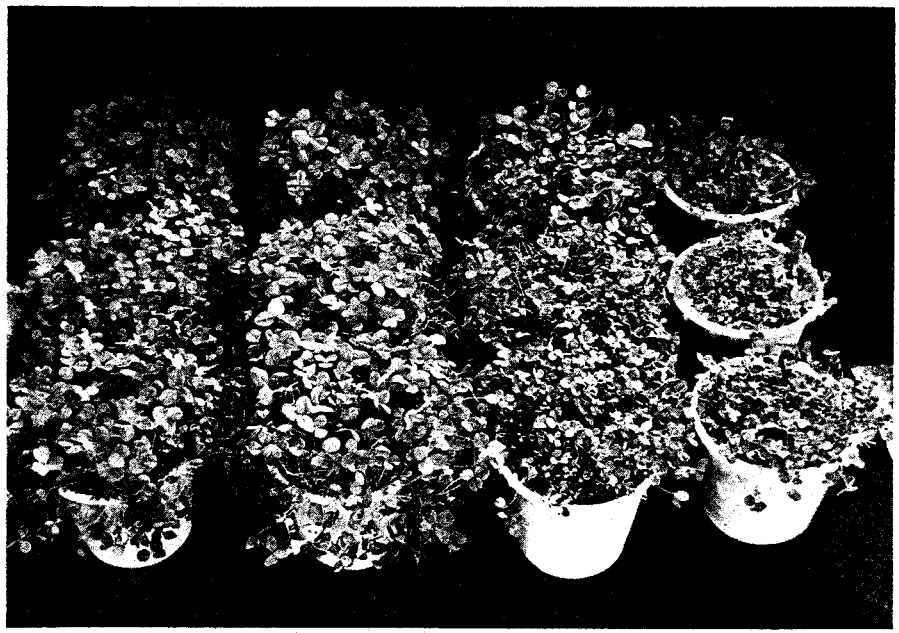

FIG. 2. Experiment 1. Condition of clover on 7 May 1963 under some of the treatments. From left to right (in triplicate) Nemafos emulsion, D-D solution 1:1, D-D solution 1:3, and control.
Proef 1. Stand van de klaver op 7 mei 1963 voor een aantal middelen. Van links naar rechts (in 3-voud) Nemafos-emulsie, D-D oplossing 1:1, D-D oplossing 1:3 en onbehandeld.

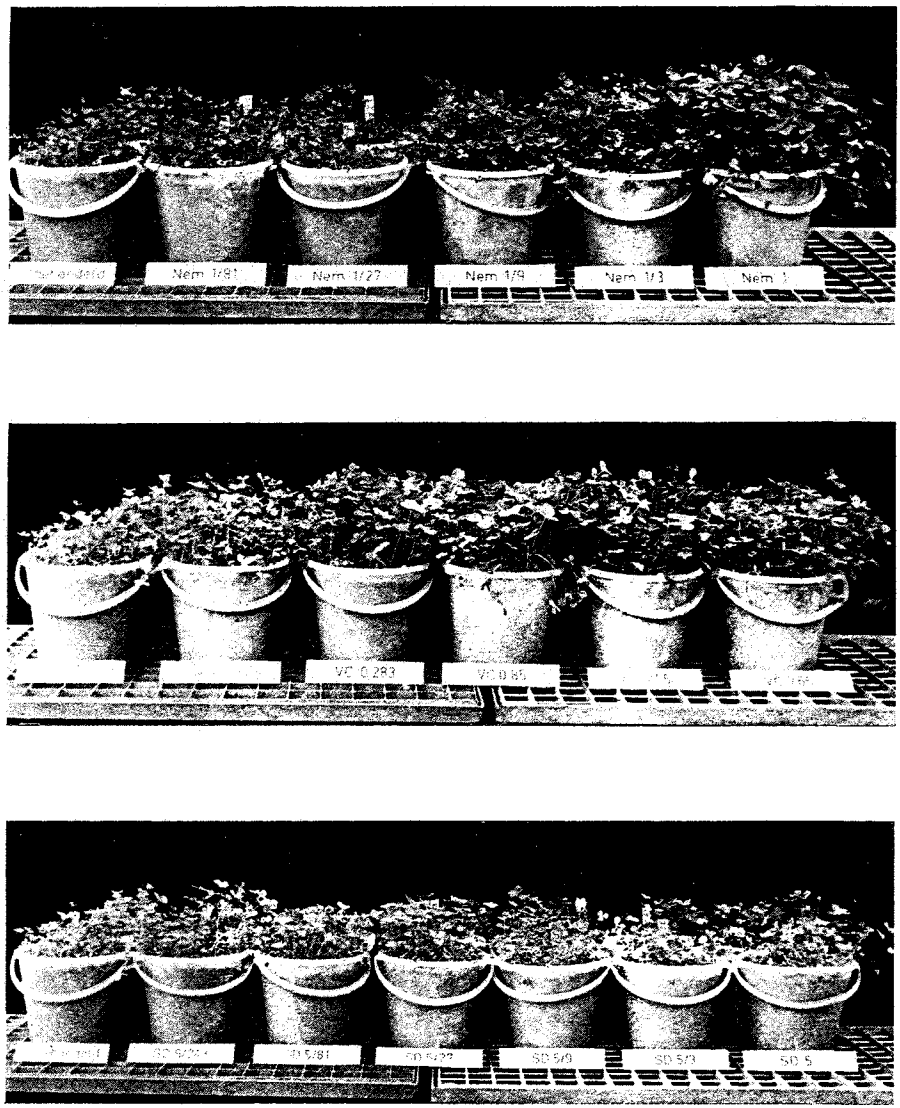

FIG. 4. Experiment 4. Condition of clover on 23 April 1964. From the top downwards: treated with Nemafos, V-C 13 and SD 4965 respectively. From left to right: the control and then increasing dosages of the compounds applied.
Proef 4. Stand van de klaver op 23 april 1964. Van boven naar beneden behandeld met resp. Nemafos, V-C 13 en SD 4965. Van links naar rechts onbehandeld en toenemende hoeveelheden van de toegediende middelen.