SESSION VII

CONTROL OF FUNGI AND NEMATODES BY SOIL STERILANTS

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Glasshouse Crops Research Institute

EXPERIMENTS WITH SOIL STERILANTS, WITH PARTICULAR REFERENCE TO SODIUM N-METHYLDITHIOCARBAMATE (METHAM - NA)

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I. Introduction

This report deals only with a group of total soil sterilants for the action of which the biocidal properties of methylisothiocyanate have been taken as hypothesis. Methylisothiocyanate (MITC) is either introduced as such into the soil or is formed in the soil by chemical break-down from Metham-Na or 3,5-dimethyl-tetrahydro-1,3-5, 2-H-thiadiazin-2-thione (DMTT).

The three afore-mentioned products are officially approved in Germany by the Federal Biological Institute for Agriculture and Forestry for the control of harmful soil organisms including nematodes. They are used for the control of nematodes, soil fungi, weeds and arthropods living in the soil, but however do not act against soil bacteria. In our country these products are used mainly in horticulture, nurseries, but also in specific agricultural crops as for example in hops and in seed potatoes. The relatively high costs for the product and for the treatment do not allow a broader use in general agriculture.

The substances may be used under glass as well as in the open.

Several reports on the testing and use of these substances have been published in Germany. The following findings are based on these publications and on further unpublished results of our investigations. This report will deal with experimental methods, the mechanism of activity and the practical use of the products mentioned earlier.

II. Experimental Methods

We deal mainly with the fungicidal and herbicidal testing of soil sterilisers, whereas nematicide tests are not carried out in our laboratories. On such pests, however, Pieroh et al. (1959) have reported.

For the determination of the fungicidal potency of a soil sterilant, the use of 'Fungus Discs' which can be variously modified, have proved very suitable. The test fungus is grown through a rather thick filter-paper disc which is soaked with a substrate containing the necessary nutrients. Thereafter the fungus is introduced in this vegetative form to the soil, and we try later to reisolate the fungus from the 'discs'.

This method is described in the paper by Linden and Schicke (1957). The conditions under which the soil sterilant and the test disc are brought together are governed by the nature of the problem posed. If screening is concerned, the soil sterilant is admixed evenly to the soil and several chains of 'Test Discs' threaded on lines, are then put into the soil. The complete chains are then removed from the soil at intervals of 1, 2, 3 and 4 days and are brought to agar slants. The tests can, however, also be carried out by introducing the discs strata-wise into a soil column. By applying the product to the soil surface or to the base of the column, the spontaneous penetration into the soil can be established after removal of the 'Test Discs'. In this way it is possible by model experiments to ascertain the influence of varying soil types, of soil humidity, temperatures, treatment periods, etc. and on soil penetration.

The same method can also be used to examine the fungicidal efficacy of a soil sterilisation in field tests. It goes without saying that some modifications are, however, necessary. Domsch and Schicke (1960) have used foam rubber blocks soaked with nutrient solution which after formation of fungus growth - were placed in open-weave plastic bags and buried in the treated soil. By fixing a cord to each bag with a wooden handle above the ground, they could easily be located for subsequent removal, when they were packed in sterile glass tubes. Re-isolation of the fungus could then be carried out in the laboratory.

As test fungi for this method Fusarium culmorum, Thielaviopsis basicola, Verticillium dahliae, Rhizoctonia solani, Sclerotium rolfsi, Sclerotinia sclerotiorum and Pythium spp. can be used. As there are always bacteria adhering to the buried discs, it is appropriate to add an antibiotic to the nutrient substrate. In order to find out the actual mortality of the test fungus in the 'Test Discs' it is necessary to observe the re-isolation over a period of 15 days because seriously-affected fungi may only germinate after a long period. Naturally, this method also has its limitations. Even by adding several antibiotics, an elimination of all soil bacteria cannot always be achieved, particularly in soils rich in organisms and with long exposure times. In these cases, we have to reckon with contamination, in particular when using slowly growing test fungi. If very resistant test organisms are involved it can happen that when low concentrations of a substance are used, the test fungus is inhibited by some sort of infection. With medium concentrations it grows quite well however, and it is only by the use of high concentrations that a kill can be obtained without these undetermined 'infections'.

Foam rubber has hitherto, been found to be the best substrate carrier. One must, however, observe that the soil sterilants can penetrate more easily into this porous substance than into normal soil and thus the effective 'minimum concentration' of a soil sterilant can be much lower than in soils.

Finally, there can of course be objections regarding the use of infective fungi in these soil tests. In such cases the test fungi carriers should

be 'planted' conveniently for the subsequent excavation and removal of the surrounding soil, should the substance tested fail to achieve control. The herbicidal effect was measured by the introduction of seeds, which can also be placed in layers. We used cress (Lepidum sativum) for this purpose.

The 'waiting period' was checked with different plants.

III Mechanism of Action

We can distinguish between a physical and a chemical mechanism of action.

1. The physical mechanism of action

The first type of mechanism provides that the applied product reaches the point in the soil at which it must act in order to obtain a sufficient soil sterilisation. In this respect, we have been mainly concerned to find generally acceptable conclusions regarding the efficacy of a product, based on the method of application and the type of soil. For good effect, the prerequisite of a soil sterilant is an even penetration of the soil in sufficient concentration. This penetration can be achieved to some degree by the product itself, and assisted physically by the user. Only products with a high vapour pressure are enabled to penetrate spontaneously. The lower the vapour pressure, the more is mechanical distribution in the soil required. The products discussed in this paper possess a more or less high vapour pressure. For this reason Metham-Na and MITC can be injected into the soil or be applied in a trench or plough furrow without the necessity of subsequent mechanical distribution through the whole area. Its penetration of the soil in the vapour phase is now dependent on a number of important factors. Rising temperatures increase the vapour pressure and promote penetration. Due to the fact that penetration is only effected after some time, the period of influence also has an important bearing on the result of the treatment.

This period of influence is curtailed if for the purpose of minimising the waiting period, the ground is ventilated sometime after treatment i.e., if the surface is disturbed or the watershield eliminated. Furthermore, the soil sterilant vapours will be absorbed by the soil surface. Finally, a concentration slope will be formed, falling away from the point of application and a certain - however, constantly changing - balance between adsorption of the product and desorption, vapour pressure, and sterilant content of the soil air will be created. These circumstances are necessarily changing because the soil sterilant is steadily evaporating from the soil surface into the atmosphere thus steadily decreasing the amount of sterilant remaining in the soil. For these reasons, the type of ground and depth of top-soil (from the adsorption point of view) cannot be ignored. For example, the products penetrate much easier into structured silt than into compost soil as shown clearly in Table 1.

Fig. 1: Spontaneous penetration of Metham-Na into the soil in relation to soil temperature, reaction time, dosage (grs per sq. metre) and relative water saturation of the soil.

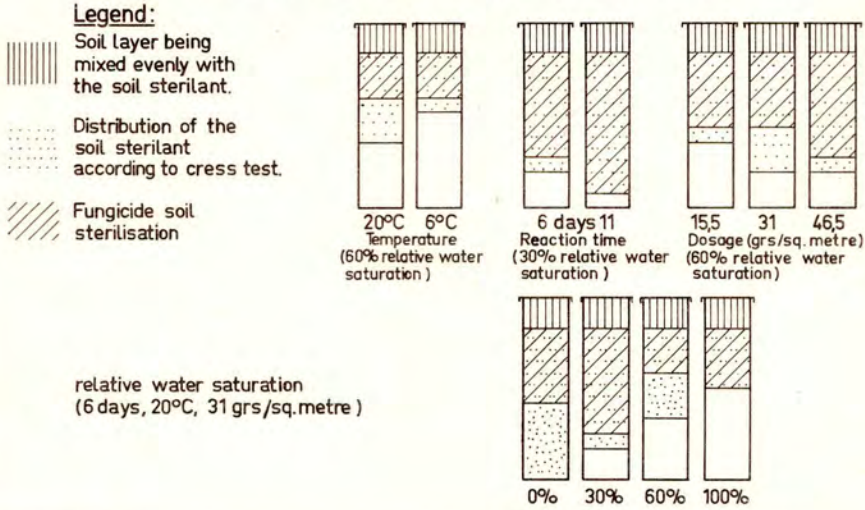


Table 1
Fungus mortality in percent after surface treatment with 31 g. Metham-**Na** (I) and 25 g. Methyl isothiocyanate (II) with subsequent spontaneous penetration of the substances into the soil. Relative water saturation 40%; test fungus Fusarium culmorum

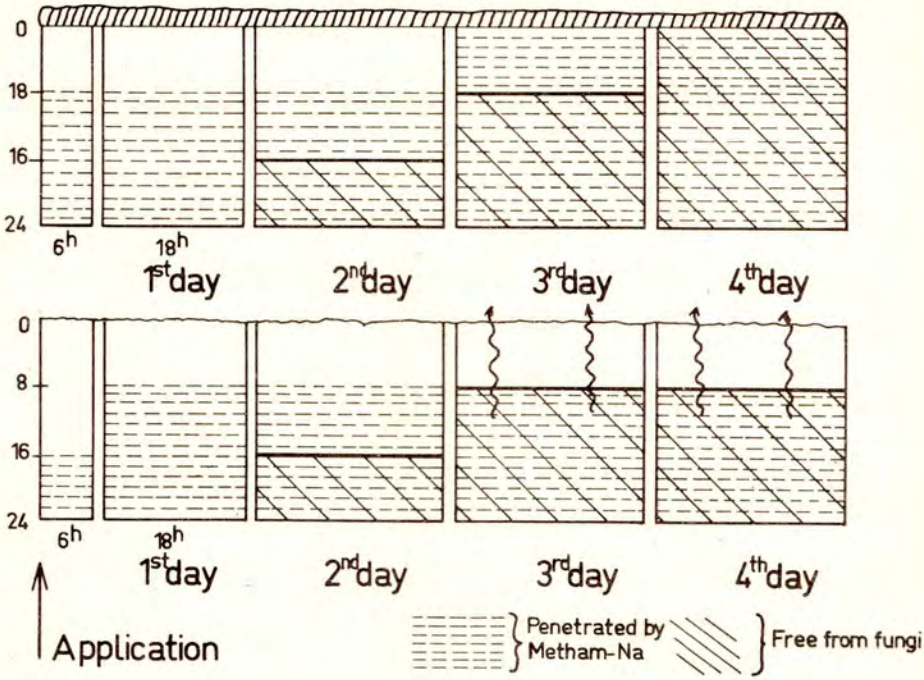
Temperature		6° C						20° C			
Soil type		compost			silt			compost		silt	
Substance		check	I	II	check	I	II	I	II	I	II
Reaction time 3 days											
soil depth in cm.	5	0	17	17	0	100	100	100	100	100	100
	10	0	0	0	0	100	0	83	100	100	100
	15	0	0	0	0	0	0	0	0	100	100
	20	0	0	0	0	0	0	0	0	100	100
	25	0	0	0	0	0	0	0	0	100	100
	30	0	0	0	0	0	0	0	0	100	100
Reaction time 9 days											
soil depth in cm.	5	0	100	100	0	100	100	100	100	100	100
	10	0	83	100	0	100	100	100	100	100	100
	15	0	50	0	0	100	100	100	100	100	100
	20	0	0	0	0	100	100	0	0	100	100
	25	0	0	0	0	100	100	0	0	100	100
	30	0	0	0	0	100	100	0	0	100	100

In this connection also the soil humidity plays an important part because a humid surface adsorbs gases much more readily than a dry one. Penetration is impeded by an increasing water saturation of the soil. Practically, the best penetration effect is obtained in dry soil. This does, however, not mean that the best fungicidal efficacy must also be obtained in completely dry soil because the soil fungi in this case occur in resistant forms (e.g. sclerotiae). It follows naturally that penetration of the soil can be improved by increased dosage (Fig. 1).

The herbicidal effect on cress seeds was in our experiments always found earlier than the fungicidal effect. According to our experience it is necessary that the product acts on the fungus substrate for at least 3 days at a toxic concentration. This condition is particularly important for the sterilisation of the uppermost soil layers when using the plough sole or injection methods. The evaporation of the product at lower levels takes place more slowly than its evaporation into the atmosphere (Fig. 2). On this basis it is possible that in the uppermost well ventilated soil layers the necessary fungitoxic concentrations can at no time be achieved and the sterilisation effect is insufficient. In order to prevent this, one can cover the soil with mats, tarpaulins or foils or by using a water seal during the period of activity. The water seal consists of a soil layer of about 1 inch thickness, being kept humid and in which the ascending sterilant vapours are adsorbed.

Fig:2

The dependency of soil sterilisation with Metham-Na on soil cover and reaction time.

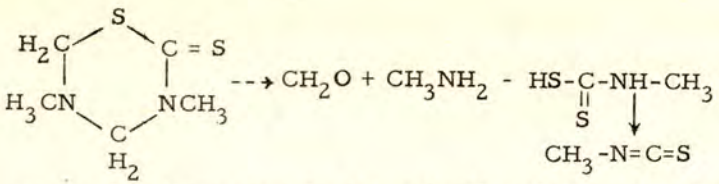


2. Chemical mechanism of action

With the theories of the action of the carbamates, isothiocyanate plays an important part as a fungicidally active break-down product. The effect of Metham-Na too, could easily be explained by a break-down to methyl-isothiocyanate as for example in the following form.

$$CH_3\text{-}\overset{H}{\underset{|}{N}}\text{-}\overset{S}{\overset{\|}{C}}\text{-S-Na} \quad - - \rightarrow \quad CH_3\text{-N=C=S} + NaSH$$

The same final product is also achieved with the break-down of 3,5-dimethyl-tetrahydro-1,3-5,2-H-thiadiazin-2-thione, however, with some intermediate steps as shown in the following formula:



As can be taken from the formulae the addition of water is necessary in the latter case. The break-down of Metham-Na however, based on the given formula would not require any water. Of course, catalytic factors such as soil organisms, mineral components and distribution over a large surface all participate in the break-down processes.

Due to the fact that all these considerations are hypotheses we were interested to find material for their support or refutation. The first foot-hold was gained by an exact comparison of the compounds Metham-Na and MITC. From the molecular weights of both substances, it follows that from one part Metham-Na not more than 0.57 parts MITC can be formed. In a soil sterilisation experiment with the test fungus Fusarium culmorum we tested the efficacy of MITC by using this material as such and in the form of the corresponding quantity of Metham-Na respectively. The graph of the test result shows that the quantity of MITC which possibly is formed by Metham-Na is 2.5 times more active than the 'straight' MITC. If the break-down hypothesis for Metham-Na were correct, the opposite result would be expected, assuming that the supposed active break-down product of Metham-Na had to be formed first. This finding is in conflict with the hypothesis we mentioned earlier. At least this cannot be the only principle of action. Moreover, Metham-Na must have a strong fungicidal efficacy of its own.

This result having been found in a soil sterilisation test, could be confirmed by a spore germination test with Alternaria tenuis. When carrying out the spore germination test, we encountered some difficulty because MITC is not water-soluble and had to be emulsified by addition of Tween 80 (Fig. 4). The ED-values (Effective Dosage) amount to 129 p.p.m. for MITC in direct use, but to 4 p.p.m. in the form of a corresponding quantity of Metham-Na. This astonishingly large difference in Ed-values has probably been caused by the water insolubility of MITC. However, it is still our opinion that this experiment proves that the sterilant action of Metham-Na does not depend only on its break-down to MITC.

These results explain the fact that under practical conditions about 40 per cent more MITC as such must be used, to achieve the biological efficacy of a given quantity of Metham-Na.

Because Metham-Na is marketed in the form of a water solution and usually has to be further diluted with water by the user (except in cases when accurate dispensing equipment is available), many held the view that the addition of water was definitely required to ensure the efficacy of Metham-Na. We have been carrying out additional soil sterilisation experiments to clarify this point. As test fungus Fusarium culmorum was

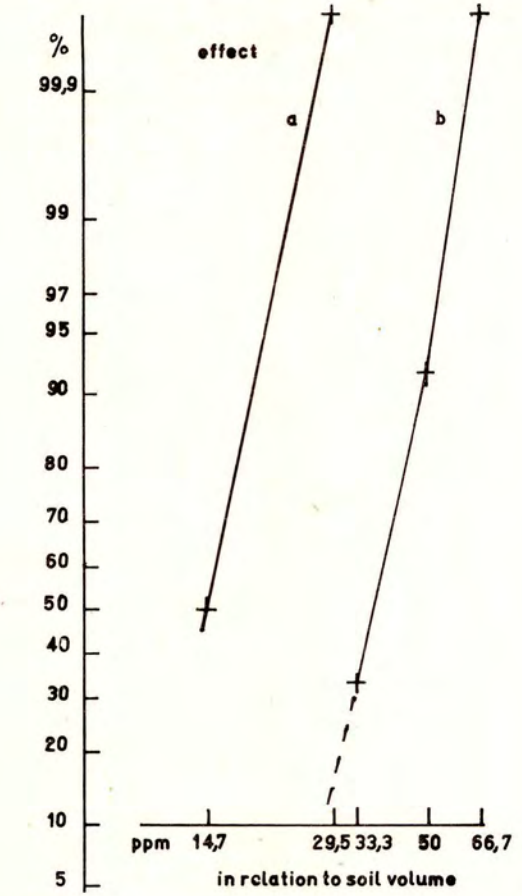


Fig. 3: Dosage-response curves for methyl-isothiocyanate in the soil sterilisation test.

a) in form of indirect use of Metham-Na

b) in form of direct application of methyl-isothiocyanate

again used. Silt was used in dry form and in saturated form to 30% of its relative water capacity, 100 ml. of 31% Metham-Na formulation and 125 ml. of a 20% MITC formulation were applied to the soil. The soil temperature during the experiment amounted to 6° C, the material acted 3 days. The Metham-Na formulation was not additionally diluted with water.

The result of this experiment is shown in Table 2.

Table 2
Fungus mortality and inhibition of seed germination in percent after sole treatment with 31 g. Metham-Na (I) and 25 g. Methyl isothiocyanate (II) with subsequent spontaneous penetration of the soil.

Test fungus: Fusarium culmorum; soil type: loam; soil temperature: 6° C; reaction time: 3 days; seeds: Lepidium sativum; applied undiluted.

		Fungus mortality						Inhibition of seed germination					
Water saturation		0%			30%			0%			30%		
Substance		check	I	II	check	I	II	check	I	II	check	I	II
Soil depth in cm.	5	0	100	0	0	0	0	0	100	100	0	76	28
	10	0	100	33	0	0	0	0	100	100	0	100	96
	15	0	100	100	0	100	17	0	100	100	0	100	100
	20	0	100	100	0	100	100	0	100	100	0	100	100
	25	0	100	100	0	100	100	0	100	100	0	100	100
	30	0	100	100	0	100	100	-	-	-	-	-	-

It can be seen that the action of Metham-Na is in no way hindered in air-dry soil. One can even detect a slight superiority of the formulation over the MITC formulation as far as the speed of soil penetration is concerned. As has already been shown a 30% relative water saturation causes a reduction of the soil penetration with both products. We can consequently state that both Metham-Na and MITC do not need any additional water for their effect. In this respect, these soil sterilants differ considerably from the third mentioned, viz. DMTT which is not broken down in the soil without water. A lack of water in the soil leads not only to an insufficient sterilisation, but also to a considerable prolongation of the waiting time.

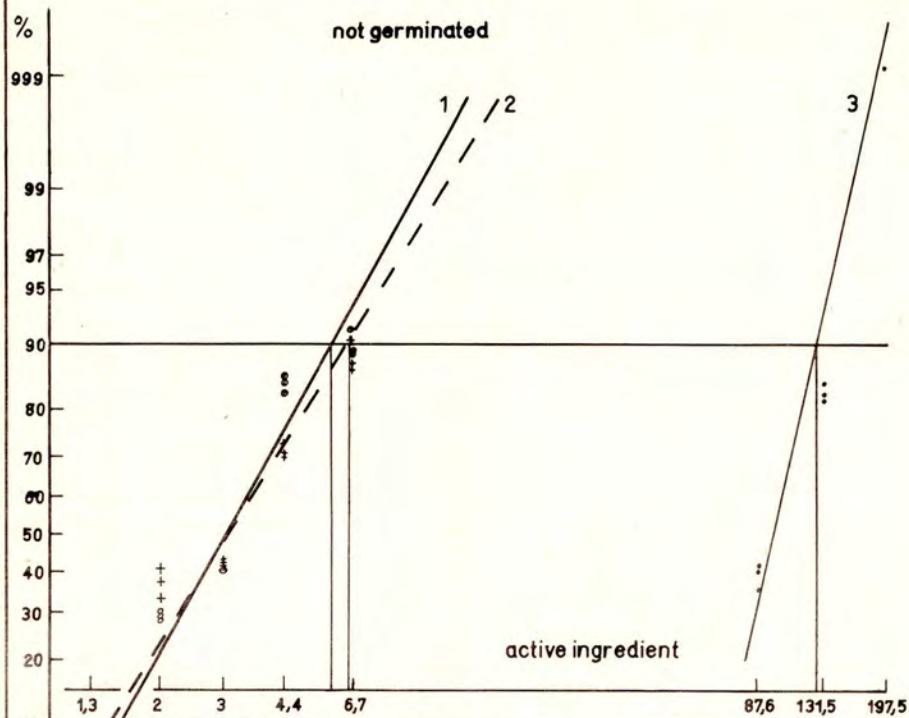
IV Practical Use

1. Methods of application

There are different methods of application available in the use of total soil sterilants.

a) 'Drenching method'

With the 'drenching method' the formulation is applied dissolved in water at about 5 litres wash per square metre of surface area. Thereafter the soil can be watered further. As our experiments have however



curve	active ingredient	ED_{90}	confidential limit p=0,001	b	confidential limit p=0,001
1	Dimethyl tetrahydro-H-thiadiazinethione	6,0	4,6 – 7,7	4,4	3,2 – 5,7
2	Na-N-methyldithio-carbamate	6,7	5,0 – 8,9	3,9	2,8 – 5,1
3	Methylisothio-cyanate	128,7	111,7 – 148,4	10,6	6,0 – 15,3

ED_{90} = effective dosis for 90 % inhibition of germination

Fig.4: Dosis response of the spore germination test

shown, the product is not transported deep into the soil as the water penetrates but is previously absorbed in the upper-most layers. From here it must penetrate on its own by means of its vapour pressure. Subsequent watering can consequently only be of use to provide a water-seal which prevents a quick release of the product into the atmosphere. Mechanical covering of the soil for an appropriate period increases efficacy. The penetration of the soil - as explained earlier - depends on several factors, and for this reason, the 'drenching method' is unreliable. It should only be used when applying a product with a high vapour pressure and which is water-soluble, as in the case of Metham-Na.

b) 'Plough sole' method

As the term indicates, with this method the product is applied either concentrated or diluted with water into the trough of the furrow or trench before the next furrow is turned, covering the treated soil. Here again, the substance must penetrate the soil unassisted. The efficacy of this method therefore depends additionally on the soil structure. On not too heavy, friable and crumbling soils, where the plough fills in the furrows evenly, the 'plough sole' method can be used with good success. On heavy humid soils where the soil does not crumble, but falls unbroken into the furrow, only partial success can be expected. A pre-requisite for this method is again a product with a high vapour pressure. (Metham-Na and MITC).

c) Injection method

The Injection method corresponds in some degree to the 'Plough sole' method as far as the provisions and the mode of action is concerned. Again the substance is brought into the deepest part of the soil to be treated and must penetrate the soil vertically and horizontally.

d) Intermixing method

When using the Intermixing method, the product is applied on the surface and immediately thereafter distributed throughout the layer of soil to be sterilised. Usually a rotatiller is used for this purpose. The rotatiller can be coupled to a Fertiliser scatterer or other dispensing apparatus so that the work can be done in one operation. With this method it is not essential that the substance itself has penetrative powers. It is therefore the surest method as far as effect is concerned, and furthermore products with a lower vapour pressure can also be used. It is however, recommended with this method that the ground be covered by a water-seal or with some kind of sheeting if possible. Suitable products: Metham-Na and DMTT.

2. Characteristics with regard to application of the products

Metham-Na can be used in concentration or diluted with water in all 4 methods of application. The formulations are liquid. An unpleasant irritating smell does under some circumstances make the application difficult.

Methylisothiocyanate is made available as a solution in xylene and must be used undiluted. It is only suitable for two of the described methods of application: 'Plough sole' method and Injection method. This product also has an unpleasant smell.

Dimethyl-tetrahydro-H-thiadiazin-thione: This product must be applied in a water suspension, though under experimental conditions it can also be used dry - however, only by means of the Intermixing method. There is no unpleasant smell. The effect and waiting time are dependent on the prevalence of soil water.

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INVESTIGATIONS ON NEMATICIDAL ACTIVITY AND CROP RESPONSES TO CHEMICAL SOIL STERILANTS

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Soil sterilization by steam or heat is rapidly being replaced by the use of compounds added to the soil which release a fumigant gas. These compounds have broad but varying bands of biocidal activity. They require special methods of application, are sensitive to soil conditions and their lack of specificity makes estimation of kill and crop response difficult to analyse.

Soil sterilant trials against a variety of plant-parasitic nematodes were started in 1959, to compare different compounds and to adapt assay methods. Examples of interim results obtained are given below. Treatments were generally applied at the accepted doses.

FIELD TRIALS

POTATO-ROOT EELWORM (Heterodera rostochiensis)

Trials were laid down on an infested potato field at Woburn Experimental Farm, Bedfordshire. The soil is a sandy loam with 16% clay. Root infestation was used both as an indication of eelworm kill (Goodey, 1957) and as an attempt to evaluate the damage suffered by the potato plant from the invasion of the nematode larvae into the root system.

In the 1960 preliminary trials, 16 different fumigants were used, and of these, only compounds releasing methyl isothiocyanate were promising in terms of decreased root infestation, yield increase and low post-harvest population.

This year the more promising treatments and in addition methyl bromide were tested in replicated plots. A clear relationship emerged from the degree of larval invasion and yield increase. Methyl bromide applied in autumn gave the best overall effect with 98% decrease of root infestation over controls, and a yield of ware potatoes of over 11 tons per acre, compared to only 0.2 tons per acre from control plots, having a root infestation of 6000 larvae per gram of root. Methyl isothiocyanate, sodium methyl dithiocarbamate and dimethyl tetrahydrothiadiazinethione gave decreases in infestation ranging from 91 - 98% and yields varying from 8 tons per acre following autumn treatment to 6 tons per acre following spring treatments. Dichloropropene and dimethyl thiuram disulphide gave 85% infestation decreases and yields of only 3 tons per acre. A substantial decrease in the numbers of eelworm larvae penetrating the root system was required to produce a reasonable yield. Past experience has shown that decreases below 90% are followed by rapid eelworm build-up.

Grainger (1954) has pointed out that effective yield increases can best be obtained by treatment of soils only moderately infested with potato-root eelworm.

The value of these results is in their relevance to tomato growing in infested glasshouse soils, rather than in the outdoor treatment of potato land where cost of treatment is far too high. These trials are therefore, to be repeated in 1961-62 in co-operation with the advisory service, on eight different glasshouse soils and are to involve eelworm assay by all known methods, measurements of soil ammonia-nitrogen after treatment and other disease control estimation.

VIRUS-CARRYING NEMATODE

Trials were laid down in Pembrokeshire on strawberry-growing land against Xiphinema diversicaudatum. The loam soil has 14% clay and is kept in good tilth. In 1960 adequate control of Xiphinema was obtained with the dichloropropene nematicide which gave 50% yield increases and a decrease in the incidence of Arabis mosaic virus. The methyl isothiocyanate liberators failed to give good Xiphinema control probably due to poor penetration.

In 1961, after treatment with the dichloropropene fumigant at 800 lb per acre or methyl bromide at 1 lb per 50 sq.ft, no living Xiphinemas were found. Dichloropropene at 400 lb per acre was 97% effective, but methyl isothiocyanate gave only 54% kill. The methyl bromide was shown to penetrate to 12 in. depth reaching a C. T. P. of 1000 mg./l./hr.

Increase in ammonia-nitrogen resulted from all treatments reaching 63 p.p.m. for methyl bromide, a ten-fold increase over control plots. The direct effect of these values on 1962 yields will have to be considered.

GENERAL OBSERVATIONS

The methyl isothiocyanate liberators have been shown to be highly active against potato-root eelworm but not so effective as dichloropropene against Xiphinema in the two soil types described. Methyl bromide was equally effective and is less sensitive to soil conditions, but its high mammalian toxicity and the need for gas tight seals makes it less attractive than the other nematicides.

The removal of trash following the previous infested crop, the production of a good tilth and careful application at correct temperatures and moistures, followed by rolling and watering, give the normal soil fumigants the best chance of working efficiently. This must be followed by adequate aeration of the soil before planting. The problems of fumigating tremendous volumes of soil by chemical sterilants remain formidable. To help overcome some of these problems these lines of work are indicated:- (a), the mechanisation of the whole application process; (b), further study of the theoretical and practical problems of different fumigants and their performance under different soil conditions; (c), improvement of assay techniques and the understanding of side effects; (d) the development of a new approach to nematode control, which avoids the necessity of treating the soil en masse.

I wish to acknowledge help given by Dr. B. D. Harrison, Mr. J. K. R. Gasser, Mr. G. N. Rao and the manufacturers of EMBAFUME C, TRAPEX, VAPAM, MYLONE, TRIDIPAM and D-D soil fumigants.

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SIX YEARS EXPERIENCE WITH METHAM-NA AS A SOIL FUMIGANT IN THE NETHERLANDS

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After its introduction in 1956, Metham-Na has found a large field of application in several horticultural crops. The following is a general view, seen from a practical standpoint of experience with this fumigant in Dutch horticulture. This experience was obtained from many field tests carried out by several Research Institutes and by our own organisation as well as from results of commercial use.

Brief consideration is given to the effectiveness of the chemical against the principal plant parasitic organisms and to techniques of application. Finally, a summary is given of the recommendations for practical use on various crops.

ACTION AGAINST NEMATODES

Although Metham-Na is effective against several nematode-species, its greatest practical use is for the control of free living species. Dosage rates from 50 ml. per square metre on very light sandy soils to 75-100 ml. for heavier soils give excellent results for at least two seasons. For this purpose the fumigant finds practical application for lilies, strawberries and various outdoor grown vegetables. The action against cyst-forming nematodes lacks residual effect, and may not suffice for one season (carrots).

Although Metham-Na undoubtedly gives a positive effect against the root knot nematode, Meloidogyne marioni, results of practical application vary. As yet there is no uniform opinion about the reasons for those cases where the chemical failed. Up till now for tomatoes in glasshouses Metham-Na has only been recommended when the infestation with root knot eelworm in the foregoing season was light to moderate. In cases of serious infestation more specific nematocides such as DD, Telone or EDB are preferred. Metham-Na is not recommended for use on cucumbers.

ACTION AGAINST SOIL FUNGI

The action of Metham-Na against soil fungi is the most interesting aspect of its use. It has found a wide field of application in the bulb industry against soil borne Fusarium-diseases (hyacinths, iris). Furthermore it has proved to be a usable fumigant for the prevention of damage by the vascular disease Phialophora cinerescens in carnations and "corky root" in tomatoes (Mycelia sterilia). Dosage rates for an economic use against fungi lay in the range of 75-100 ml. per square metre.

In some commercial applications as well as in a detailed field test it was concluded that rates of 75 and 100 ml. per square metre gave a considerable reduction of Verticillium Wilt in tomatoes. However the impression remains that higher rates are desirable for optimal control of

this disease. In a replicated test against this disease in Chrysanthemums, chloropicrin was far superior. Finally, the control of damping off diseases in seed beds (flowercrops, conifers, brassicas, tobacco) coupled with a more vigorous development of young plants has been recognized as a welcome cultural practice.

ACTION AGAINST WEEDS

A long residual action against many annual and some perennial weeds is seen by many growers as an extra advantage of an application with Metham-Na. A good response in weed control, however, depends strongly on the application technique. Too much watering immediately after application often reduces the effect, whereas sometimes failures are shown after mechanical injection. After application in outdoor soils high rainfall may be the cause why weedseeds in the upper soil layers escape from the chemical. Clods and undecayed roots will also prevent an even distribution of the chemical through the soil. The effectiveness of Metham-Na against quack grass, Agropyron repens is worth mentioning. Although for most crops its exclusive use as a herbicide is too costly, Metham-Na has recently found a wide use as a herbicide in seedbeds, especially in those crops where handweeding is impossible or too costly (flowers, cauliflower, lettuce). In these cases a dosage rate of 20 ml. per square metre applied 5 - 7 days before seeding or planting as a drench with reduced amounts of water gives a sufficiently long residual effect against annual weeds.

APPLICATION TECHNIQUES

After much experimental work had been carried out it was concluded that, for most crops, the drenching technique is to be preferred to mechanical injection. The required dosage rate is diluted with a 10 - 15 fold amount of water and evenly sprinkled on the surface of the soil. Immediately after this application the chemical has to be watered in with a relatively large amount of water. In working out a recommendation for this technique it has been found very difficult to indicate precisely an amount of water necessary for a sufficiently deep penetration of the chemical into the soil, because the rate of penetration differs so much in different soil and moisture conditions.

As each grower knows the water capacity of his soil best, we believe that only the grower himself is capable of judging the amounts of water which are needed to reach a penetration of about 12 inches deep.

In some cases the treatment was a complete failure because of the escape of the diluted chemical through the drains. Other cases of failure were caused by a too high water table during application. Attempts to replace watering-in by plastic covers after sprinkling the chemical on the soil surface have not yet led to alteration of the present-day recommendations. Watering-in is with a hose or with an overhead sprinkler irrigation system. Recently some progressive growers started applying Metham-Na by way of their sprinkler irrigation system, but care is necessary for some kinds of paint may be affected by the chemical.

To the statement that the drenching technique is to be preferred for most crops, two exceptions must be mentioned. The first is treatment against a root rot disease in hyacinths, where mechanical injection proved superior to drenching. The second exception applies to the treatment of tomato greenhouses where, for the past two years, both techniques have been practised. Although results seem to indicate a slight superiority of the drenching technique, more experience is needed before a choice of recommendations is made.

PHYTOTOXICITY

Relatively few cases of serious phytotoxicity after commercial application have been reported. With a waiting period of 3 - 4 weeks between application and planting, the method proved to be safe for bulbcrops during August till the beginning of October. For vegetable crops average waiting periods are necessary of 2 - 3 weeks after applications in August, 3 - 4 weeks after applications in September and 4 - 6 weeks after applications in October. Treatments with Metham-Na are only recommended when soil temperature is above 50° F. The above mentioned data mainly refer to the lighter soil types which are poor in organic matter. In soils rich in organic matter a safe waiting period may sometimes be extended to 8 weeks.

Although the fumes of Metham-Na are less harmful than those of chloropicrin, special attention must be given to prevent damage to neighbouring living plants.

PRESENT DAY USE and RECOMMENDATIONS

ORNAMENTAL CROPS

Carnations

After successful trials by the Experiment Station at Aalsmeer, Metham-Na has found a wide use in this crop for control of the vascular disease Phialophora cinerescens, see Table 1. The drenching technique at a rate of 100 ml. per square metre is preferred over mechanical injection. On heavy soils a split treatment is carried out, beginning with a treatment of the subsoil with the upper soil removed; afterwards the upper layer of soil is treated separately. Further tests are needed before a recommendation can be given re Fusarium oxysporum.

Hyacinths

Metham-Na has been widely used during the past 4 years against a root rot disease caused by Fusarium culmorum. At rates of 75 - 100 ml. per square metre only mechanical injection is recommended 3 - 4 weekss before planting. This treatment must be supplemented by a drench of formaldehyde in the seedbed just prior to planting at a depth of 8 inches . (See Table 2). Heavy rains, shortly after treatment, may reduce efficiency.

Bulbous Iris

Metham-Na has proved effective against a root rot disease, probably

caused by a Fusarium sp. Unlike the case of hyacinths, mechamical injection gave inferior results compared to drench applications. (See Table 3). An additional treatment with formaldehyde did not improve the effectiveness. The same difficulties after heavy autumn-rains may be expected as mentioned under hyacinths.

Hippeastrum (Amaryllis) in glasshouses

Against a serious root rot disease in this crop, Metham-Na gave satisfactory results on a number of vineries. Applied on the drenching technique at rates of 100 - 125 ml. per square metre the fumigant gave results comparable to those of steam sterilisation.

Lilies

Metham-Na is used by several growers to prevent a root rot in which free living nematodes play an important part. The drenching technique is used at rates of 50 - 75 ml. per square metre. (See Table 4).

Seedbeds of flowercrops and nurserycrops

Treatment of seedbeds is becoming very popular. Drench applications with relatively small amounts of water are made 7 - 10 days before seeding: (a) for weed control purposes in those crops where mechanical weeding may cause serious damage to the roots. A dosage rate of 20 ml. per square metre gives a sufficiently long residual effect. (b) to prevent damping-off diseases with additional weed control. For this application a rate of 75 ml. per square metre is necessary.

Treatment of potting soil

Metham-Na may be cheaper to use than chloropicrin, and trial applications to layers of soil, 8 inches high, at a rate of 100 ml. per square metre are recommended.

VEGETABLES and FRUIT

Strawberries

Metham-Na proved very effective against black root rot. On very light sandy soils it is applied at a rate of 50 ml. per square metre; on heavier soils or on soils with a higher content of organic matter an increased rate of 75 up to 100 ml. per square metre is desirable. No data are available about the effectiveness against Verticillium Wilt.

Carrots

Striking improvements in growth may be obtained after treatment with Metham-Na, but several cases of failure have been repeated. It is concluded that a recommendation for the use of Metham-Na on carrots can only be given when certain species of free living nematodes are the main

cause of growth reduction, in which case a response to the treatment has been observed for two subsequent years.

Lettuce, endive, radish, beans

Although an exclusive soil fumigation for these crops will seldom be carried out, mention must be made of the favourable responses after a Metham-treatment, especially on light soils (Table 5).

Fumigation often leads to a remarkable acceleration in yield (Table 6), which may be of great importance in catch crops.

Seedbeds

The attractiveness of treating seedbeds with Metham-Na has already been pointed out. A treatment of seedbeds for brassica crops has become an established practice at a rate of 75 ml. per square metre. This year some growers have used 20 ml. per square metre 6 days before planting out lettuce (in soil blocks) for the purpose of weed control.

Tomatoes (and lettuce) in glasshouses

Dutch growers take a great interest in the application of Metham-Na for the following reasons: (a) Metham-Na offers less danger for neighbouring crops than chloropicrin; (b) as contrasted with chloropicrin, Metham-Na has a favourable effect on lettuce when grown as a catch crop for tomatoes; (c) Metham-Na can be applied by the growers themselves.

Table 1

Soil fumigation against Phialophora cinerescens in carnations

Data from G. Scholten, Exp. Station at Aalsmeer.
Treatments made in April, 1956. Planted 3 weeks later.

Number of diseased plants	Oct. 15	Nov. 18	Dec. 31	Jan. 27	Feb. 27
formaldehyde	1	2	4	6	14
mercury compound	8	10	20	32	51
control	14	17	22	33	43
copper-fungicide	4	15	28	41	51
Metham-Na	0	0	0	0	0

Table 2

Soil fumigation against Fusarium culmorum in hyacinths

1. Trial in 4 replicates at van Zanten Bros - Hillegom.
   Application September 17 - 1957. Planted October 18. Var. Pink Pearl.
   Plotsize $\frac{1}{2}$ bed (+ 7 square metres).

Treatment	Yield increase in % per 4 replicates
Control	163
Metham-Na 75 ml/m^2 injection	194
Metham-Na 100 ml/m^2 injection	203
Metham-Na 75 ml/m^2 injection + formaldehyde drench	290

2. Trial in 3 replicates at van Zanten Bros - Hillegom.
   Early treatments with Metham-Na on September 16, 1960
   Late treatments with Metham-Na on October 17, 1960
   Date of planting and treatment with formaldehyde November 23, 1960.

Treatment	Yield increase in % per 3 replicates
Control	99,0
Metham-Na 100 ml/m^2 early injection + formaldehyde	132,6
Metham-Na 100 ml/m^2 drench method, early application	98,9
Metham-Na 100 ml/m^2 late injection	91,5
Metham-Na 100 ml/m^2 late injection + formaldehyde	129,1
Metham-Na 100 ml/m^2 drench method, late application	85,6

Table 3

Soil fumigation against root rot in bulbous Iris

Summary from a trial carried out in cooperation with the Advisory Service at Amsterdam. Var. White Excelsior.

Treatments	Yield in kg.
Control	12,70
Metham-Na injection, 50 ml/m^2	12,75
Metham-Na injection, 75 ml/m^2	14,05
Metham-Na drench, 50 ml/m^2 (+ 10 l. of water)	16,15
Metham-Na drench, 75 ml/m^2 (+ 10 l. of water)	17,90

Table 4

Soil fumigation against free living nematodes in Lilium speciosum rubrum.

Summary from trial carried out in cooperation with the Advisory Service at Heemsker. Treatment on December 10, 1958. Date of planting March 5, 1959.

Treatments	Yield in kg			
	repl. a	repl. b	repl. c	total
Metham-Na, 50 ml + 2 l. of water/m^2	11,8	11,2	11,7	34,7
Metham-Na, 50 ml + 5 l. of water/m^2	12,8	12,0	12,0	36,8
Control	10,6	10,1	10,3	31,0
Metham-Na, 50 ml/m^2 injection	13,1	11,6	12,7	37,4
Metham-Na, 50 ml/m^2 drench + 10 l. of water	12,5	13,5	12,8	38,8

Table 5

Soil fumigation in strawberries, lettuce and carrots

Influence of different amounts of water given after a treatment with Metham-Na. Published in Annual Report 1959 of the Exp. Station at Heemskerk.

	lettuce number of heads	carrots number of branches	strawberries yield in kg/ 100 sq. metres
Control	100	100	159
Intercropping of Tagetes	-	-	171
Metham-Na, 50 ml. + 5 l. of water/m^2	193	153	207
Metham-Na, 50 ml. + 20 l. of water/m^2	203	200	228
Metham-Na, 50 ml. + 40 l. of water/m^2	219	194	234
Metham-Na, 75 ml. + 20 l. of water/m^2	229	197	220
Metham-Na, 75 ml. + 40 l. of water/m^2	235	197	224

Table 6

Acceleration in yield after a treatment with Metham-Na for radish

From a trial carried out by the Advisory Service at Gronigen.
Treatment on November 14, 1959. Radish sown on November 28, 1959.

date	Number of bunches per 100 sq. metres	
	from treated plots	from untreated plots
March, 23	60	28
March, 28	140	101
March, 31	70	82
April, 4	127	140

SOME RECENT INVESTIGATIONS WITH CHEMICAL SOIL STERILANTS

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Introduction

The high cost of sterilising glasshouse soils by steaming has increased the interest in alternative chemical methods. Formaldehyde and certain other chemicals have been used for many years for the treatment of soils which have become less fertile due to the successive growth of like crops. These chemicals have been found, in practice, to give results inferior to those obtained by steaming, even in the absence of soil-borne plant-parasitic nematodes, and often continued use leads to a diminishing response.

Experiments have been made to find if restoration of the fertility of the soil in glasshouses, which has fallen due to cropping with tomatoes for a number of years, can be maintained by annual soil treatments with chloropicrin or metham-sodium (sodium N-methyl-dithiocarbamate).

Tomato plants may become infected with tobacco mosaic virus (TMV) which has persisted in the soil over winter in debris from a previous infected crop. In view of the reduction in crop and fruit quality caused by this virus, knowledge of the effect of the sterilants on the incidence of soil-borne virus is needed.

Sterilisation of many soils by steam causes a considerable increase in the availability of manganese, which may result in manganese toxicity in the tomatoes. Although manganese toxicity can usually be prevented by liming the soil, its possible causation by steaming is a slight disadvantage. Information on the availability of manganese after chloropicrin and metham-sodium treatments has to be sought.

The results of trials commenced in 1958 showed the value of metham-sodium in restoring the fertility of a 'sick' tomato soil, but in view of the incidence of damage on commercial nurseries, experiments were also commenced to obtain information on the distribution of the active chemical methyl isothiocyanate, resulting from the decomposition of metham-sodium in the soil, and the nature of and reason for the occasional, unpredictable injury to crops planted after the application of metham-Na. The role of methyl isothiocyanate in producing certain abnormalities in the growth of tomatoes has been recorded by Hunnam and Waddington (1961).

I. Glasshouse Cropping Trials

Experimental

General design

In these trials the layout was a latin square of the four treatments, steaming, chloropicrin, metham-Na and unsterilised, and was accom-

modated in two contiguous non-partitioned glasshouses.

These glasshouses had been cropped with tomatoes for five successive years without sterilisation of the soil. Hence the condition of the plants in 1958 indicated that sterilisation was required. Examination of the roots at the end of the 1958 crop showed they were extensively rotted and 'corky'. Very little Colletotrichum atramentarium, often present in old tomato soils, was present and there was no acute disease such as Verticillium wilt, or infection by root-knot or potato root eelworm in 1958.

The plots were 25' x 5' and 48 plants were grown in each plot. Plastic sheet barriers were inserted between the plots to a depth of 2 ft. The soil was a fine sandy loam which readily became compacted when watered.

Sterilisation procedures

(1) Steaming

The soil was steamed in the normal manner to maintain a temperature of 110° F throughout the soil to a depth of 12" for 20 mins.

(2) Chloropicrin

3.5 ml. amounts were injected at 12" staggered spacings at a depth of 6", (equivalent to 540 lb/acre), followed immediately by 'sealing' the surface by treading and watering with about ½ gal./sq.yd. This light watering was repeated on two occasions during the ten days following the dates of application: 12/11/1958, 5/11/1959 and 1/11/1960.

(3) Metham-Na

A 1 in 200 dilution of a proprietary concentrate, found on analysis to contain 39% of sodium N-methyldithiocarbamate, was uniformly applied as a drench at the rate of 4½ gals./sq.yd.

Dates of application: 13/12/1958, 14/10/1959, 31/10/1960.

All the plots were thoroughly cultivated before sterilisation, and the chemically treated plots were forked over twice before flooding with water prior to planting.

Planting dates were 6/3/1959, 4/3/1960 and 2/3/1961.

To meet the requirements of other research projects in 1959 each plot was planted with eight plants of six varieties of tomatoes. The varietal yields were recorded for each plot but the figures given in Table 1 are based on the total yields of the plots. Two varieties, Potentate, a non-vigorous variety which is readily affected by adverse soil conditions, and Moneymaker, a vigorous variety more tolerant of soil sickness, were grown in halves of each plot in 1960 and 1961.

Yields and grades of fruit were recorded and the effects of the treatments on root size, and the extent of corky and rotted root were assessed by visual examination. Assessment of the effect of the soil treatments on

root conditions, based on relatively simple physical measurements were found unsatisfactory.

Associated Investigations

(a) Soil-borne virus

The Plant Pathology section of the Institute collaborated in the 1960-61 trial and recorded the number of tomato plants which became infected with tobacco mosaic virus (TMV) via the soil. All plants were infected with TMV in September 1960. Stringent precautions were taken to prevent the plants becoming infected with TMV from sources other than the soil, within 4 weeks from planting. A full account of this work will be published elsewhere.

(b) Availability of manganese

The amounts of available manganese in the soil, and the manganese contents of plant tissue from plants grown in the variously treated soils were determined in 1961 by the Chemistry department of the Institute.

Results

Table 1

Yields of tomatoes - lb/plant

M - var. Moneymaker P - variety Potentate

	1959	1960		1961	
Treatment		M	P	M	P
Unsterilised	7.25	6.57	6.02	4.97	4.57
Steam	9.75	11.79	11.18	9.01	8.25
Chloropicrin	9.63	11.66	10.93	9.04	8.30
Metham-Na	9.48	11.25*	10.27*	8.35	7.81

* Treatments differing significantly from steam at P = 0.05. All the sterilisation treatments in 1959 and 1960 differ significantly from unsterilised at P = 0.01.

It has not been possible to analyse in detail the figures for the 1961 crop since harvesting was not completed until the end of September.

Table 2

Effects of treatments on TMV infection and availability of manganese

	Plants infected with TMV 4 weeks after planting	Manganese content of leaves p.p.m. dry wt.	Water soluble and exchangeable manganese in soil - p.p.m. in dry soil
Unsterilised	2	130	3.3
Steam	0	1160	43.0
Chloropicrin	19	400	8.9
Metham-Na	8	105	2.6

The results (Table 1) show that under the conditions of these trials there was no falling off in response to sterilisation with chloropicrin in comparison with the response to steam. In 1960 the effect on fruit yield of metham-sodium was significantly less than that of steam. The individual plot yields in 1961 suggest that both steam and chloropicrin gave significantly better results than metham-Na.

The response to all sterilisation treatments was most marked in the first half of the season. No treatment resulted in the roots remaining free from corkiness and rotting until their removal at the end of September. There were very wide variations in the size and condition of roots in all the plots, but when classified according to their freedom from corkiness and browned and rotted root, and the amount of clean fibrous root, the roots from the steamed plots were significantly better than those from other treatments.

The marked difference, early in the season, in the growth of plants in all the sterilised plots and those in the unsterilised plots, and the poor condition of the roots at the end of the season indicates re-infection of treated soils from the subsoil.

Studies of the distribution of methyl isothiocyanate in the plots treated with metham-sodium in 1959 and 1960 show that its concentration did not exceed 3 p.p.m. at a depth of 15 - 20 ins. and 10 p.p.m. at 10 - 15 ins. It is apparent from the numbers of plants infected with soil-borne virus (Table 2) that steaming destroys or reduces the amount of virus. The greater incidence of TMV in the chloropicrin and metham-sodium treated plots is probably attributable to the effect of these sterilants on the soil organisms which rot plant residues, and the reduction in the rate of decomposition of plant residues delays the exposure of the virus to biological or other destructive action.

The availability of manganese in the soil (Table 2) is considerably increased by steaming but practically unaffected by Metham-Na. A slight increase resulted from treatment with chloropicrin.

II. Metham-Na - Phytotoxicity Studies with Tomatoes

In view of the many instances of damage following the application of metham-sodium in 1959 the phytotoxicity of this compound, and of the products which are known to be, or might be, produced by its decomposition, were studied. The decomposition of dilute solutions of metham-sodium in contact with soil was found to be so rapid (Hughes, 1960) that the investigations were restricted to an examination of the phytotoxicity of methyl isothiocyanate, N-methylthiourea (which might result from the interaction between the isothiocyanate and ammonia produced in the treated soil) and s -dimethylthiourea (a possible product of the interaction of methyl isothiocyanate and methylamine, which may conceivably be formed by the decomposition of methan-Na).

Tomato plants were grown in pots in sterilised soil to which N-methyl thiourea and s-dimethylthiourea were added. Concentrations of these compounds caused no visible toxicity when present in the soil at 25 p.p.m., and although some temporary marginal leaf scorch and chlorosis on young plants resulted at about 50-100 p.p.m., there was no permanent injury or plant growth modification resembling that which occasionally occurred in commercial houses treated with metham-sodium.

Methyl isothiocyanate, in similar tests, but with complete enclosure of the treated soils and pots to exclude vapour from the plants, caused severe permanent injury to tomato plants at concentrations exceeding 5 p.p.m. The damage from such soil applications was direct by killing the roots or causing the base of the stem to collapse, and growth modifications did not result at concentrations which did not cause direct damage.

There was evidence from cases of damage on commercial nurseries that damage resulted from a volatile substance, and tests were made in which plants were exposed to different concentrations of methyl isothiocyanate vapour in airtight chambers for varying periods.

Concentrations exceeding 0.3 g/m^3 caused severe scorching, wilting and sometimes death of the plants. Exposure for a single period of 17 hrs. to concentrations of 0.2 - 0.1 g/m^3 caused scorching of the lower leaves of tomatoes at the planting-out stage but no subsequent modifications of growth or other abnormalities. On the other hand similar plants exposed to concentrations of 0.05 - 0.01 g/m^3 for 17 hr. periods on 4 or more consecutive days subsequently developed abnormalities ranging from complete blindness due to the termination of the main stem at an abnormally large flower truss and the development of flower trusses instead of side shoots, to the formation of abnormally large flower trusses with long truss stems.

The first one or two trusses which became visible after prolonged exposure to methyl isothiocyanate vapour usually failed to develop, often producing only a few small, seedless fruits. Many of the abnormal flower trusses which presumably were in process of initiation at the time of exposure to methyl isothiocyanate, bore fertile flowers and produced normal fruit.

Tomato seedlings exposed to the low concentrations were distorted, stunted and frequently became blind.

There did not appear to be any differences in the susceptibilities of a large number of tomato varieties.

Conclusion

These results confirm the conclusion of Hunnam and Waddington (1961) that methyl isothiocyanate vapour is responsible for the phytotoxic effects which have occasionally followed the use of metham-Na in tomato houses.

In a glasshouse of average dimensions the release of 1 p.p.m. of methyl isothiocyanate from the soil to a depth of 1 ft. will provide 0.24 g/m^3 in the air assuming there are no air changes.

Studies of the rate of loss of methyl isothiocyanate from the soil in the glasshouses at this Institute have shown that a residual amount of several p.p.m. may be retained in the soil for at least six weeks. Organic and clay soils sorb methyl isothiocyanate more strongly than the sandy loam and residual amounts are almost certainly greater in practice in these soils.

It is necessary to obtain more information on the factors which influence the rate of loss of these strongly absorbed residues in order to devise means whereby the inconveniently long period between the application of methyl isothiocyanate, or substances producing this compound can be reduced.

III. The distribution of methyl isothiocyanate in metham-Na treated soil.

The amount of decayed and swollen roots on plants removed, at the end of the 1959 crop, from the metham-sodium treated plots in the comparative trial of this sterilant with chloropicrin and steam, described above, suggested that re-infection may have resulted from the failure of the methyl isothiocyanate to penetrate to a sufficient depth. The vertical distribution of methyl isothiocyanate following applications of metham-sodium to these plots in the 1960 and 1961 cropping trials was therefore studied.

In some of the trials made in tomato houses by Advisory Officers and others, the injection of metham-sodium concentrates has given better results than its application as a drench; in other trials drenches have proved superior. This may be due to some extent to differences in the pathogenic condition responsible for crop reduction or to differences in soil type and temperature, resulting in differences in the distribution of methyl isothiocyanate. Some experiments have been made at the G.C.R.I. on the distribution of methyl isothiocyanate resulting from alternative methods of applying metham-sodium, but further work is necessary to obtain a comprehensive picture of distribution in other glasshouse soils with alternative application methods, and of the accompanying problem of eliminating hazardous residues from such soils.

A number of workers have investigated the influence of various soil factors and application methods on the biological activity and efficiency of pest and disease control of metham-sodium and certain other compounds which also produce methyl isothiocyanate on decomposition. A comprehensive review of their findings is given by Lambe, who studied the influence of soil temperature and moisture, and the method of application of metham-sodium and 3,5-dimethyl-tetrahydro-1,3,5,2H - thiadiazine-2-thione on fungitoxicity.

Experimental

In all the experiments a commercial concentrate containing 39% of metham-Na was used.

(A) Glasshouse Cropping Trial, 1959-60.

1 pint of concentrate in 25 gallons of water per 50 sq. ft. applied as a drench.

Date of application	14.10.1959
Nature of soil	fine sandy loam
Moisture content 1959 = 18.4%	
Soil temperature at 6 ins. over a 10 day period following application	1959 58° F - 54° F

(B) Experiments with different methods of application

Each treatment was applied to 4 plots

(1) 1 pint of concentrate in $12\frac{1}{2}$ gals. of water per 50 sq. ft., followed immediately by watering with $12\frac{1}{2}$ gals. water per 50 sq. ft.

(2) 1 pint of concentrate in 2 gals. of water per 50 sq. ft., uniformly applied to the soil surface and immediately followed by watering with $12\frac{1}{2}$ gals. water per 50 sq. ft.

(3) 1 pint of concentrate per 50 sq. ft., injected at 11.30 ml. at a depth of 8 ins. at 12 in. rectangular spacings. Injection holes were sealed immediately after each injection with soil and the soil surface was sealed by watering with 5 gals. water per 50 sq. ft.

Samples of soil at 0-5", 5"-10" and 10"-15" depths were removed at random from all plots excepting those in which the metham-sodium was injected, where the samples were taken at points equidistant from four injection points and the sampling holes were firmly filled with soil.

The amounts of methyl isothiocyanate in these samples were determined by a method based on the distillation of the compound into a solution of ammonia and measurement of the absorption of the resulting methylthiourea at 235 mμ(4).

Results

The amounts of methyl isothiocyanate at three different depths is shown graphically in diagrams 1 - 4.

It is apparent that in the soil treated in these experiments, the concentration of methyl isothiocyanate at a depth of 5-15 ins. is higher where the metham-sodium is applied in a relatively large volume of water (Figs. 1 and 2) than where a relatively large amount of water is applied immediately after the application of more concentrated metham-sodium solutions (Fig.3).

It is necessary to stress that current recommendations are that when metham-sodium is applied as the slightly diluted concentrate or by injection it should be thoroughly incorporated into the soil by rotary cultivation. Even so it is possible that such cultivation which will not exceed and may sometimes be less than 8 ins. may not ensure an adequate concentration of methyl isothiocyanate at depths below 8 ins.

The results obtained from the injected plots (Fig. 4) were unexpected. One possible explanation is that the decomposition of the metham-sodium was very slow due to its localised absorption in a small quantity of soil, and loss of methyl isothiocyanate by upwards diffusion or decomposition was sufficiently rapid to prevent the attainment of the expected concentration in the soil most remote from the points of injection. Such an explanation is consistent with the observation that the soil at or very close to the points of injection contained appreciable amounts of methyl isothiocyanate at least 10 weeks after application.

Incorporation of the metham-sodium with the soil by mechanical cultivation should ensure more uniform lateral distribution in the top eight inches but tests are necessary to find whether it appreciably affects the concentration in the soil below 8 - 10 ins.

Conclusions

The paper presented must be regarded as a progress report since much more experimentation is necessary before definite conclusions are possible.

The results of the three years' trial in tomato houses at the G. C. R. I. suggest that in a typical soil which has become less fertile by repeated cropping with tomatoes, annual applications of chloropicrin may be somewhat more effective than metham-sodium and equal to steam in maintaining fertility. Further experiments on similar lines are necessary to see whether this is the case with other soils and where other fungi such as Verticillium spp. Colletotrichum atramentarium are present.

It is known that chloropicrin does not give satisfactory control of root-knot and potato-root eelworms.

The disadvantage of both chloropicrin and metham-sodium is the long period which must elapse between application and planting. The phytotoxic effects of metham-Na on tomatoes result from traces of methyl isothio-

cyanate vapour in the air, and more knowledge of the factors affecting the rate of release of this compound, and its de-activation in soils, might enable this interval to be reduced.

Distribution studies show that with methods of application tried so far, the concentration of methyl isothiocyanate in the soil at depths of 12" or more does not exceed 5 p.p.m., and the condition of the roots at the end of the season suggests that recontamination of the top soil by organisms from the subsoil may be important with each of the three methods of application. The use of larger volumes of more dilute metham-Na than at present advised may increase the depth of effective sterilisation.

Injection of a 40% solution of metham-sodium into a moderately moist soil resulted in unsatisfactory distribution of methyl isothiocyanate. It is almost certain that rotary cultivation immediately after injection, as now advised, will greatly improve lateral distribution but at present it is impossible to say whether penetration beyond a depth of about 8 ins. will be improved.

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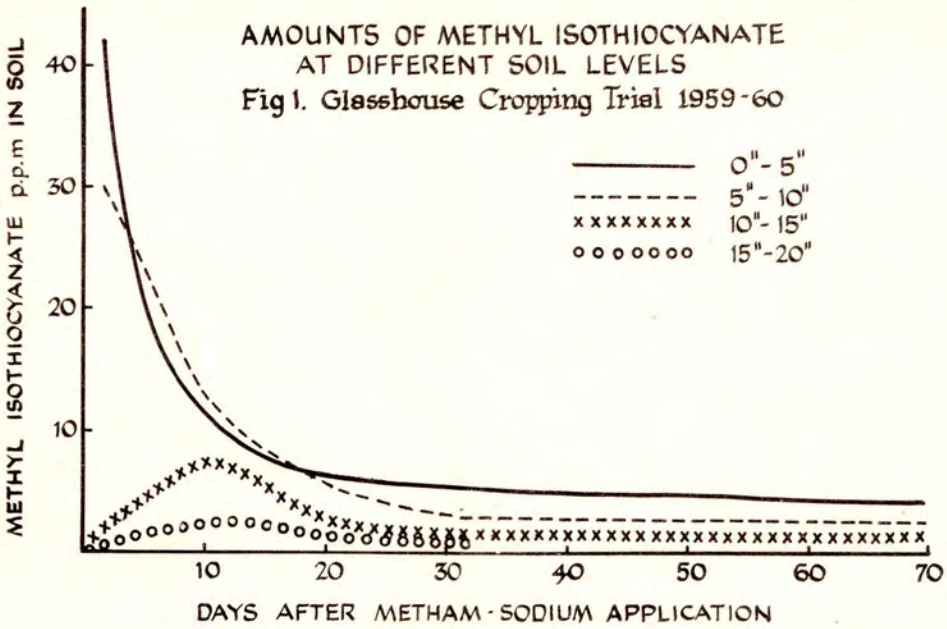
AMOUNTS OF METHYL ISOTHIOCYANATE
AT DIFFERENT SOIL LEVELS
Fig 1. Glasshouse Cropping Trial 1959-60
0"- 5"
5"- 10"
10"- 15"
15"-20"
METHYL ISOTHIOCYANATE p.p.m IN SOIL
40
30
20
10
10
20
30
40
50
60
70
DAYS AFTER METHAM-SODIUM APPLICATION

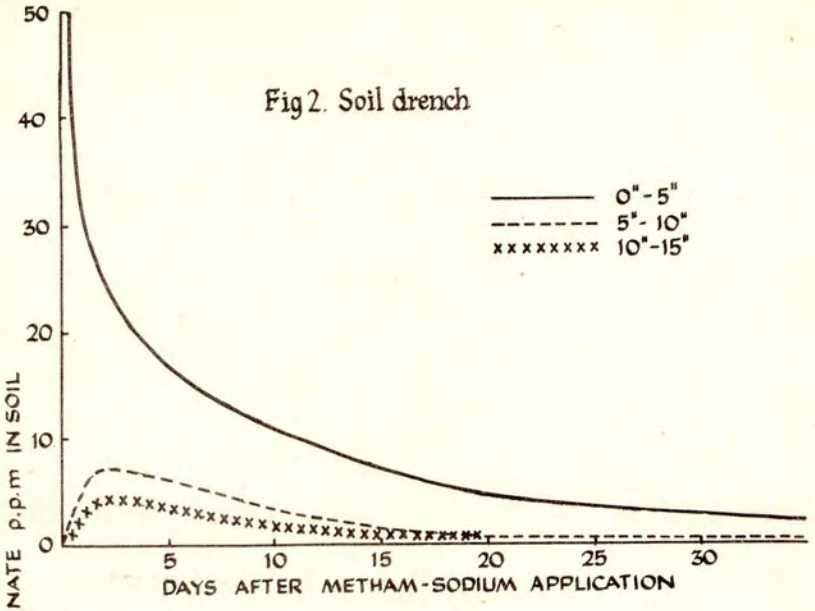

Fig 2. Soil drench

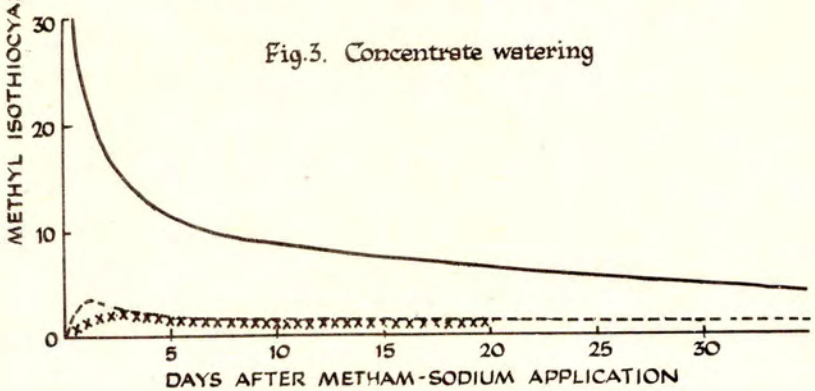

Fig.3. Concentrate watering

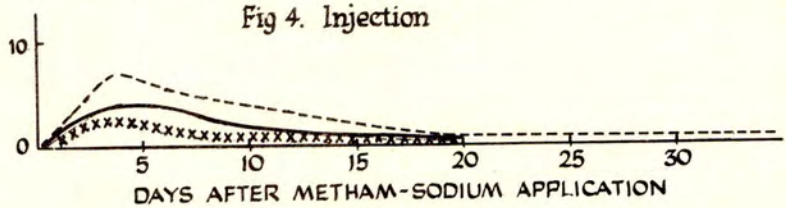

Fig 4. Injection

NABAM, NEW FORMULATION AND NOVEL USES

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Summary

Previous work with nabam as a 19% aqueous solution is reviewed, and the introduction of a new formulation as a 93% soluble powder (code name A. 40) is discussed.

The control of Cladosporium fulvum on glasshouse tomatoes by the use of a mixture of A. 40 and zinc sulphate has been shown to be as good as the approved and established mixture of liquid nabam and zinc sulphate.

Positive action was shown against certain soil pathogenic fungi such as Pythium, Phytophthora spp., and Plasmodiophora brassicae, by the use of A. 40 as a soil drench prior to sowing or planting, or as a powder dressing of seed.

The Brown Root Rot complex on glasshouse tomatoes was reduced and yields increased by the continuous watering of the established tomato plant roots with a solution of sixty parts A. 40 to one million parts water. No phytotoxicity has been noted. The application of a mixture of A. 40 and a liquid fertiliser is possible without any loss of efficiency if applied within one hour of mixing.

Introduction

Hester in 1943 was granted a U.S. patent protecting the use of disodium ethylenebisdithiocarbamate (nabam) as a fungicide, but this compound proved too unstable and too soluble for use on foliage until Heuberger et al. (1947) stabilized it with a mixture of lime and zinc sulphate, a mixture patented in the U.S A. by Heuberger in 1948. Later work showed that the addition of lime was not necessary. In the U.S A. the nabam/zinc sulphate tank mix has been widely used for many years for the control of such pathogenic fungi as Alternaria solani, Helminthosporium turcicum, Phytophthora infestans and Stemphylium solani.

The first work on the control of a foliar disease by means of a nabam/zinc sulphate tank mix in the U.K. was reported by Beaumont (1954). He showed that the control of Cladosporium fulvum on tomatoes with a mixture of liquid nabam containing 19% disodium ethylene-bisdithiocarbamate with zinc sulphate (22.7% zinc) was better than with other materials tested, and he also reported that this tank mix was the only spray to remain efficacious in severe epidemics. Wiggell (1958) continued this work, and his results were in close agreement with Beaumont's findings. At present in the U.K. the nabam/zinc sulphate tank mix is used almost exclusively for control of Cladosporium fulvum on tomatoes under glass. For field crop diseases zineb, the zinc salt of the parent acid, is preferred.

For many years both European and American research workers have been examining the possibility of using nabam with its wide fungicidal spectrum for the control of soil-borne parasitic fungi.

Zentmeyer (1955) reported that nabam was fungicidal to Phytophthora cinnamoni at 25 p.p.m. of soil and to Verticillium alboatrum at 250 p.p.m. He also stated that when nabam was drenched onto the soil the fungicidal effect was more than one hundred times greater than that of zineb. The effects of nabam on soil fungi and bacteria are described by Strecker (1957) and Zanardi (1957).

Messiaen and Lafon (1957) recommended the addition of 0.5% or 1% nabam to hot water for the treatment of maize seed against Gibberella zeae. Sinclair (1957) assessed nabam as a promising chemical for control of Rhizoctonia solani when applied to the soil at sowing time. Whitehead and Brown (1957) showed nabam to give control of Rhizoctonia solani, Pythium and Fusarium spp. when applied to the seed drill.

Grossman (1957) could show no chemotherapeutic effect when a nabam solution was applied direct to roots of tomato plants before or after infection with Fusarium oxysporum f. lycopersici, Alternaria solani and Phytophthora infestans. Very slight root damage was noticed with a concentration of 20 p.p.m., and slight damage with 100 p.p.m. Domsch (1958, 1959) in a series of tests with various soil fungicides showed that nabam gave good results against Pythium spp. and Rhizoctonia solani, although its fungicidal persistence was short. The ED50 value for Pythium was reached in 15 days after application at 100 p.p.m. of soil. In comparison the ED50 value of zineb at 800 p.p.m. was not reached until 80 days after application. He suggested that naban increased the number of soil bacteria and actinomycetes, and markedly reduced the fungal population.

He (1960) further reported that nabam at 100 p.p.m. greatly altered the microflora balance of the compost. Amongst the fungi controlled were Verticillium dahliae var. zonatum, Cephalotrichum medium, Mortierella exigua, Volutella ciliata. Tolerance was shown by three Chaetomium spp. and Penicillium nigricans. Trichoderma viride was reduced by fifty per cent. Corden and Young (1960) reported nabam to be more effective than metham-Na or zineb against Fusarium oxysporum f. cubense chlamydospores when mixed with artificially air-dried soil.

In England studies on nabam as a soil fungicide were started by Wiggell and others on the control of certain root rots on glasshouse tomatoes by constant watering with a weak solution of liquid nabam (300 p.p.m. of the 19% material) after planting in final quarters. This weak solution was used in place of water for normal watering procedure and gave favourable results (Wiggell, 1961).

Up to 1960 all the commercial work involving the use of nabam had been carried out with the 19% aqueous solution of nabam. It had not proved possible to formulate a stable powder form, although the advantages of such a material were well realised. In 1960 trial quantities of a powder form containing 93% disodium ethylene-bisdithiocarbamate became available. This material (code name A.40) is completely soluble and reacts with metal salts in aqueous solution as does the standard liquid formulation of nabam.

Experimental

The aims of the trial work were: (a) To ascertain whether the control of Cladosporium fulvum was as good with powder nabam/zinc sulphate as with the established liquid nabam/zinc sulphate; (b) To provide data on the control of soil-borne parasitic fungi prior to sowing or planting; (c) To determine the effect on the plant and yield of constant watering of tomato plants with a nabam solution using A.40 at 60 p.p.m., and to examine the possibility of applying to the soil a combined liquid nutrient and nabam solution; (d) To determine the effect on Brown Root Rot. Some doubt exists as to the exact nature of this disease which is widespread and found in most glasshouse soils where tomatoes are grown.

Results

1) Nabam as a foliar spray for control of Cladosporium fulvum on tomatoes

Materials:-

Liquid nabam:- disodium ethylene-bisdithiocarbamate as a 22% solution plus zinc sulphate (22.7% zinc). Mixed at the rate of 1 gallon liquid nabam plus 2 lb zinc sulphate in 160 gallons water.

A.40:- disodium ethylene-bisdithiocarbamate as a 93% soluble powder plus zinc sulphate (22.7% zinc). Mixed at the rate of $2\frac{3}{4}$ lb A.40 plus 2 lb zinc sulphate in 160 gallons water.

Both materials applied as a high volume spray at 200-300 gallons per acre.

Key for Tomato Leaf Mould

(Developed by the Disease Assessment Committee of the Conference of Advisory Plant Pathologists.)

Per cent

0.1 Lesions found with difficulty, and on less than one plant in fifty.

1 Lesions on most plants, but only on a few leaves.

5 Lesions on every plant, and on most leaves except the young ones, but only about two to ten spots per leaf.

10 All except the youngest leaves affected, with ten to fifty spots per leaf.

25 All except the youngest leaves affected, but with about three-quarters of the leaf area green although lowest leaves may be severely attacked.

50 All leaves affected. Most of the middle leaves show only half their area green.

75 All leaves affected. Most of the middle leaves show only one-quarter of the leaf green, giving a grey appearance to the crop as a whole.

90 Very little green visible on middle and lower leaves, but youngest leaves show green.

100 All leaves completely covered with lesions.

Leaf Mould Assessment

Site	Material	Date of examination			
		July 12	July 26	Aug. 9	Aug.23
Yorkshire (I) (sprayed June 29 July 12 July 26 Aug. 9 and Aug. 23.)	Liquid nabam	0	0.5	0.1	0.1
	A.40	0	0.5	0.1	0.1

Site	Material	Date of examination			
		June 2	June 15	June 29	July 7
Yorkshire (II) (sprayed June 2 June 15 June 29 and July 13)	Liquid nabam	1.5	3.0	2.0	1.0
	A.40	1.8	3.5	2.5	1.0

Site	Material	Date of examination			
		June 6	June 20	July 7	July 21
Lancashire (I) (sprayed June 7 June 21 July 7 and July 21)	Liquid nabam	1.8	4.0	1.5	1.3
	A.40	1.9	3.8	1.3	1.4

Site	Material	Date of examination			
		June 6	June 20	July 6	July 20
Lancashire (II) (sprayed June 6 June 20 July 6 and July 20)	Liquid nabam	0	0	0.5	0.5
	A.40	0	0	0.5	0.5

Site	Material	Date of examination					
		May 5	May 22	June 6	June 20	July 7	July 18
Lea Valley (sprayed May 9 May 23 June 6 June 20 July 4 and July 18)	Liquid nabam	1.0	1.0	0.5	1.0	1.0	1.0
	A.40	1.0	1.0	0.8	0.8	0.5	0.5

2) Nabam as a soil fungicide

a) Control of damping-off diseases outdoors

The materials used were a commercial seed dressing containing captan, applied dry to seed, and A.40 applied at $\frac{1}{8}$ oz. in 1 gallon of water per 30 ft. of seed drill after sowing.

The layout of 4 replicates of each treatment and untreated control, on a gravel clay site which had been market garden for thirty years. Peas of the variety Onward were sown on 2/3/1961 and the weather, from sowing to germination, was wet and cold. The mean number of emerged codlings per plot were: A.40, 74.25; captan seed dressing, 72.00; untreated, 60.25, with a significant difference of 11.29 ($P = 0.05$).

b) Control of Club root (Plasmodiophora brassicae Woron) on cabbage

The materials used were: A.40 dip, 2 oz in 1 pint water as a preplanting dip; A.40 drench, 4 oz in 100 gallons water. 10 fl. oz of this dilute applied per plant upon planting out; Calomel, 4% commercial preparation used 1 lb in 6.6 fl. oz water, as a preplanting dip.

The layout of randomized plots, 5 replicates of each treatment and untreated control, with 16 plants per plot, on a site with a previous history of club root infection.

Cabbage (Primo) was planted 17/5/61, and the weather during growing period was hot and dry.

The results are assembled in Table 1.

Table 1 Control of club root

Treatment	Mean weight of cut heads of cabbage (oz)	Mean number of infected roots per plot	Notes
A.40 dip	4.18	0.2	Severe scorch noted on roots
A.40 drench	33.19	0	
4% Calomel	25.01	0	Slight scorch noted on young roots
Untreated	21.90	6.4	
	SD (p=0.05)= 6.02 oz	The root infection was in no case more than slight clubbing of laterals	

c) Control of damping-off diseases under glass

TRIAL I

The materials used were: A.40a, 1 oz in 12 gallons water applied to soil as drench before sowing; A.40b, 1 oz in 6 gallons water applied to soil as drench before sowing; A.40 seed dressing, 1 oz in $1\frac{1}{2}$ gallons water, seeds dipped therein before sowing; Cheshunt Compound, a commercial preparation used at 1 oz in 1 gallon water, applied to soil before sowing and again after germination. The layout was of randomized plots, 4 replicates of each treatment and untreated control. Cress (Fine Curled), sown 16/6/1961 at a rate of 180 seeds per replicate, in soil infested with Pythium and Phytophthora spp. The results are given in Table 2.

Table 2 Control of damping-off

Treatment	Mean number of seedlings per plot 8 days after emergence	Notes
A.40a Soil drench	125.75	
A.40b Soil drench	111.75	Slight phytotoxicity noted
A.40 Seed dressing	79.00	Phytotoxicity noted
Cheshunt Compound	159.25	
Untreated	88.00	
	SD(p=0.05) 37.25	

TRIAL II

In this trial the materials tested were: A.40a, 1 oz in 12 gallons water, applied as a soil drench before sowing; A.40e, 1 oz in 18 gallons water, applied as a soil drench before sowing; A.40f, 1 oz in 24 gallons water, applied as a soil drench before sowing, and A.40 seed dressing, seeds dressed with dry powder.

The layout of randomized plots, 4 replicates of each treatment and untreated control. Lettuce (All the Year Round) were sown on 3/7/1961 at the rate of 150 seeds per plot on soil infested with Pythium and Phytophthora spp. The results are given in Table 3.

Table 3 Control of damping-off

Treatment	Mean number of emerged seedlings per plot 10 days after emergence	Notes
A.40a Soil drench	82.75	Phytotoxicity and growth retardation noted
A.40e Soil drench	87.00	Growth retardation noted
A.40f Soil drench	126.50	
A.40 Seed dressing	136.00	
Untreated	91.75	
	SD(p=0.05) 33.80	

TRIAL III

The materials used were: A.40e, 1 oz in 18 gallons water, applied as a soil drench before sowing; A.40 seed dressing, seeds dressed with dry powder; Cheshunt Compound, a commercial preparation, 1 oz in 1 gallon water, applied to soil before sowing and after germination.

The layout of randomized plots, 4 replicates of each treatment and untreated control, planted with tomato (Moneymaker), sown 20/7/1961 at a rate of 10 seeds per plot on soil infested with Pythium and Phytophthora spp. Table 4 gives the results.

Table 4 Control of damping-off

Treatment	Mean number of emerged seedlings per plot 10 days after emergence
A.40e Soil drench	7.25
A.40 Seed dressing	7.00
Cheshunt Compound	7.50
Untreated	3.05
	SD(p=0.05) 2.81

3) Nabam as a soil fungicide on growing crops

Previous work had shown that a continuous application to growing tomato roots of a 300 p.p.m. solution of nabam, as a 19% preparation, had not resulted in phytotoxic effects.

Method:-

A.40, was made into a stock solution of 2 oz to 1 gallon water. This was added to the irrigation water through a dilutor to give a concentration of 60 p.p.m. This weak solution of nabam was used in place of water for the normal crop watering procedure.

To ensure that this application would be suitable for cultural practices entailing constant inclusion of liquid fertilisers with the irrigation water the compatibility of the nabam solution with liquid fertilisers was examined.

a) Compatibility of A.40 solution with a commercial liquid fertiliser

A.40 added to liquid fertiliser as 1.25% or 2.5% solutions.

Liquid Fertiliser mixed with A.40 (w/v analysis) $N : P_2O_5 : K_2O$	Rate of Decomposition of A.40 (% loss of nabam per hour)	
	1.25% solution	2.5% solution
3 6 12	0.40	0.32
6 0 7	0.43	0.30
6 6 7	0.47	0.32
24 6 0	0.52	0.35
Tap water pH 7.0	0.03	0.02

b) Treatment of seedlings and young plants

Tomato seeds were sown in unsterilised soil taken from a glasshouse with a long history of tomato growing. After germination half of the number of seedlings were watered as needed with a 60 p.p.m. aqueous solution A.40. The other half received plain water.

At the time of pricking out into pots the seedlings showed:-

Treatment	Average length of seed leaf	Average length of 1st true leaf
A.40 drench	40 mm.	20 mm.
Plain water	28 mm.	15 mm.

At the time of appearance of the 1st truss the plants showed:-

Treatment	Average length of the leaf immediately below the 1st truss	Average height of plant
A.40 drench	156 mm.	227 mm.
Plain water	131 mm.	203 mm.

c) Treatment of established tomato plants

The application of A.40 as a 60 p.p.m. solution began with the initial ball watering. The same dilution was applied throughout the life of the crop. The amount per plant was the same as the amount of plain water given to each untreated plant.

Assessment of Brown Root Rot on Tomato Roots

This was based upon the following:-

0 = All white root.

1 = Slight corky lesions on some roots.

2 = Corky lesions with some brown rot on a few roots.

3 = 50% roots showing severe corky lesions with brown rot on some roots.

4 = Brown rot on 50% roots with little clean white root to be seen.

5 = All roots diseased.

Performance of nabam

Site L

Soil type:-	Peat soil.
Soil sterilisation prior to planting:-	Carbon disulphide.
Planting data:-	Tomato (Moneymaker) on 23/4/61
Treatment:-	200 plants receiving A.40 solution, 200 plants receiving plain water.

Treatment	Height of plant at 25/7/61	Total weight of crop per plant (21/9/61)	Brown Root Rot assessment on 21/9/61
A.40 solution	73 in.	83 oz.	1.6
None	62½ in.	65 oz.	3.9

Site B

Soil type:- Loam with high clay fraction.

Soil sterilisation prior to planting:- None

Planting data:- Tomato (Ailsa Craig) on 4/4/61

Treatment:- 380 plants receiving A.40 solution
350 plants receiving plain water.

Treatment	Height of plant at 25/7/61	Total weight of crop per plant (25/9/61)	Brown Root Rot assessment on 25/9/61
A.40 solution	87 in.	80½ oz.	2.1
None	81 in.	72 oz.	4.2

Site C

Soil type:- Gravel Loam with gravel and medium clay fraction

Soil sterilisation prior to planting:- None

Planting data:- Tomato (Moneymaker) on 12/4/61

Treatment:- 400 plants receiving A.40 solution
400 plants receiving plain water

Treatment	Height of plant at 22/7/61	Total weight of crop per plant (18/9/61)	Brown Root Rot assessment on 18/9/61
A.40 solution	77 in.	79½ oz.	1.8
None	69 in.	69 oz.	3.8

Site E

Soil type:- Silt loam

Soil sterilisation prior to planting:- None

Planting data:- Tomato (Ware Cross) on 17/4/61

Treatment:- 300 plants receiving A.40 solution
300 plants receiving plain water.

Treatment	Height of plant at 22/7/61	Total weight of crop per plant (22/10/61)	Brown Root Rot assessment on 2/10/61
A.40 solution	72½ in.	97 oz.	1.2
None	65 in.	80½ oz.	3.5

Site W

Soil type:- Sandy loam

Soil sterilisation prior to planting:- D.D. injection

Planting data:- Tomato (Ware Cross) on 2/3/61.

Treatment:- 200 plants receiving A.40 solution
200 plants receiving plain water (This area had received 2% formaldehyde before planting as well as the D.D.)

Treatment	Height of plant at 29/7/61	Total weight of crop per plant (28/9/61)	Brown Root Rot assessment on 28/9/61
A.40 solution	83½ in.	98 oz.	1.8
None	79 in.	90 oz.	3.6

Discussion of Results

1) Nabam as a foliar fungicide

The trial work reported shows that the tank mix of A.40 and zinc sulphate gives equally good control of Cladosporium fulvum as does the established tank mix, when both were applied as a high volume spray.

It proved easier to mix the A.40 and the zinc sulphate together than it did to mix the liquid nabam and zinc sulphate; in both cases the procedure was: - Mix the nabam formulation with one third of the required volume of water, stir in the zinc sulphate and finally mix in the remaining two thirds of water.

The use of A 40 did present a slight disadvantage at mixing time, as the powder was found to be slightly irritant to the nose and throat passages. This could be minimised by careful handling of the powder. During the spraying of tomato crops there was no difference in the effect of A.40 or liquid nabam mixtures upon the nose and throat passages of the operator.

Laboratory results indicate that the present compatibility of liquid nabam and zinc sulphate with 25% miscible D.D.T , kelthane, lindane, magnesium sulphate, malathion and nicotine as a joint high volume spray will also apply to A.40.

2) Nabam as a soil fungicide prior to planting or sowing

The result given of the use of A.40 as a chemical control of pre-emergence damping-off indicates that the use of A.40 gives a significant increase in the number of emerged pea seedlings compared with the untreated plots. The other chemical used, captan, also gave a significant control of pre-emergence damping-off.

A.40 also gave significant control of club root (see Table 1) on cabbage when applied as a soil drench to the base of the seedling immediately after planting. The dipping of seedlings into a strong solution of A.40 led to scorching of the roots with a corresponding drop in weight of the cabbage cut from that treatment. The dipping of seedlings into one of the standard recommended methods of control, namely 4% calomel paste, also showed some scorch on the roots and, while this method did control the attack of club root, the yield was not significantly better than that from the untreated areas. This was possibly due to the very hot, dry weather which continued throughout the growing life of the cabbage, and which undoubtedly reduced the degree of infection by club root on the trial area to the low level reported. The adverse weather together with lack of irrigation affected other trials containing various methods of application of A.40 as a possible control of club root to the point where no significant data was obtained at all. One method of application which holds promise is the rotovation into the affected soil of A.40 7 to 14 days before planting or sowing crucifers.

A drench of A.40 at various dilutions on soil infected with Pythium and Phytophthora spp. before sowing lettuce, cress and tomato, showed a significant increase of emerged seedlings over the untreated sowings (see Tables 2, 3 and 4). Also the dressing of tomato or lettuce seed with the dry A.40 gave a significant increase over the untreated seed. Cheshunt Compound, used in two of the reported trials, was not significantly better than A.40. Cress was not damaged by a pre-sowing drench of 1 oz A.40 dissolved in 12 gallons of water, whereas lettuce growth was definitely retarded and germination impaired by such a solution and by the 1 oz A.40 in 18 gallons. Good results were obtained with 1 oz A.40 in 24 gallons and with a seed dressing of the dry A.40 before sowing. Tomato seedlings tolerated 1 oz A.40 in 18 gallons, good results being obtained with this method and with the dressing of seed with dry A.40. The coating of cress seeds with a strong dilution of A.40 before sowing resulted in fewer germ-

inated seedlings.

It can therefore be seen that the treatment of seeds with the dry A.40 before sowing is promising although more species remain to be treated before this remark can be taken as a general conclusion. The drenching of soil before sowing, while proving satisfactory, showed that the tolerance of certain seeds to a soil treated just before sowing with different strengths of A.40 in aqueous solution varies. As the half-life of nabam is comparatively short it may prove more practical to drench the soil 7 days before sowing thus obtaining control of the pathogens without phytotoxicity.

3) Nabam as a soil fungicide on growing crops

In the U. K. generally the glasshouse tomato is being grown on soils with a long history of tomato culture, and it is therefore not surprising that soil diseases cause a loss of potential crop every year on most holdings even where the partial sterilisation of the soil is practised. One disease which is proving troublesome is the Brown Root Rot complex of which the exact nature is not yet known. Ebben (1949) described the disease as consisting of root lesions varying in colour from pale to dark brown, with the root cortex either split and flaking-off or swollen and "corky" in appearance, girdling the root. On some lesions the whole root cortex rots away leaving a brittle vascular cylinder. The cortex at the base of the stem and below may also be affected. This description agrees with that of McKay (1949), who describes it as Tomato Root Rot, and with that of Noordam, Termohlen and Thung (1957). The causative agent of Brown Root Rot has not yet been agreed upon but McKay (1949) gives Colletotrichum atramentarium as the agent. Work carried out at Kvithamar Experimental Station was reported by Roll-Hansen (1952) who stated that the most prevalent fungus associated with Brown Root Rot was Cylindrocarpon radicicola, and that fungal damage was secondary to some other cause as yet not elucidated. Williams and Ebben (1950-1953) working at Cheshunt Experimental Research Station found Fusarium spp, Colletotrichum atramentarium, Chaetomium cochliodes, Volutella ciliata, Petriella asymetrica and a sterile grey fungus "K" on the root surface and in the affected tissue. They suggested that more than one fungus might well be the causative agent, as the species present on diseased roots varied. Their opinion was that Colletotrichum, Chaetomium, Volutella and Fusarium were the more likely agents. Noordam, Termohlen and Thung (1957) found that a sterile grey fungus taken from diseased roots would, when inoculated into tomato soils, cause symptoms on tomato roots similar to Brown Root Rot. Ebben (1959) found Cephalosporium spp. on the affected root surface and tissue. He (1960) also observed no difference in bacterial population of the rhizosphere of root surface which could be correlated with the development of Brown Root Rot.

Steaming the soil or treatment with metham-Na gives good control of this disease, but for many growers steam is too costly or not available, or the cropping schedule does not allow the use of metham-Na with its necessary 10 week delay between treatment and replanting. In such cases the control, or reduction in severity, of Brown Root Rot by the use of a

non-phytotoxic chemical applied to the growing crop would be welcome. The treatments given in this paper indicate that A.40, when mixed at 60 p.p.m. in the irrigation water and applied continuously throughout the life of the established tomato plant, gives a reduction in amount of Brown Root Rot and an increase in yield.

No phytotoxicity is caused by A.40 at 60 p.p.m. Stronger dilutions of 120 p.p.m. have checked young tomato plants and turned lower leaves bright yellow. Although the plants eventually grew away from this check they never regained their normal growth. A.40 at 240 p.p.m. caused the same effect - but it was more severe in appearance and longer in duration. One application of one quart of A.40 at 400 p.p.m. proved slightly damaging to young roots of mature tomato plants. Tomato leaves sprayed with A.40 at 60 p.p.m. showed no signs of damage or retardation even where they had already received routine sprays of liquid nabam and zinc sulphate for control of Cladosporium fulvum.

The reduction in amount of Brown Root Rot and increase in crop yield, from 9 to 25 per cent, following treatment with A.40 throughout the season was found on various soil types:- sandy loams, clay loams, peat soils etc. and this continuous application in no way affected the pH of any soil type when measured against the untreated areas. On one soil which is normally covered with blue-green algae during the life of the tomato plants, the area treated with A.40 had no such growth.

On many holdings it is the practice to apply liquid nutrients to the tomato crop constantly throughout the season, and here A.40 has the advantage of compatibility with a commercial liquid fertiliser. It has been shown that the breakdown rate of A.40 in tap water is very slight but when mixed with a commercial liquid fertiliser this breakdown rate is accelerated. However, the loss of A.40 is very slight in the first hour after mixing, and no loss of efficiency resulted when a combined A.40, liquid fertiliser is applied within that hour. An added advantage to users of automatic ground or low level irrigation systems is the complete solubility of A.40 and consequent avoidance of nozzle blocking or settling out of the material in the dilutor.

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Contribution by Dr. L. Broadbent

Despite good results which some soil sterilants give, we need to know a lot more about their mode of action and why they sometimes fail. It may interest you to know that we at the Glasshouse Crops Research Institute intend to do more work in the near future on the distribution and activity of soil micro-organisms. I think it is essential that we should know more about the biology of nematodes and all the organisms we are trying to control by soil sterilisation.

Q. Dr. M. Cohen

Mr. Tyson did not make it clear whether he was watering on nabam plus zinc sulphate in his tomato experiment or nabam alone. Would he confirm that it was nabam since zinc toxicity might be a problem to be faced if the mixture was being used?

A. Mr. D. Tyson

The soil watering was carried out with nabam alone.

Q. Mr. J. R. L. Stainton

Can Dr. Flipse explain why it is necessary to employ injection methods for metham-sodium into hyacinth seed beds rather than using the drench method.

A. Dr. L. Flipse

No, we have no good explanation for this. We think that with the drench method the penetration will not go deep enough. Hyacinths are planted rather deeply and they root very deeply. It is only because of our experience over the course of four seasons that we have come to this conclusion. Every year we have observed that the injection method gave superior results to the drench method. A supplementary treatment with formaldehyde is also necessary.

Q. Dr. L. Broadbent

One of the most interesting practices that is being developed in the U. K. at the moment is that of watering dilute fungicides on the soil at frequent intervals during the growth of the crop. Is this done on the Continent?

A. Dr. W. Madel

This practice is widespread in horticulture in Germany. Zineb and sometimes captan are used at intervals of 1 to 2 weeks.

Comment by Dr. L. Flipse

As far as I know the practice of application of fungicides during growth periods as a soil drench is not used in Holland.

Q. Dr. L. Broadbent

Can the people in the U. K. who are using this method comment on whether it is expensive?

Contribution from Mr. D. Wiggell

Nabam costings at the rate used in trials worked out at about £90 per acre. Dilutors at present in use for the application of fungicides to tomato plants are inaccurate and could possibly lead to applications of the fungicide at rates which would be phytotoxic to the plants. At the moment this somewhat limits the use of the method in commercial practice.

Contribution from Mr. D. Tyson

We have found costs to work out at about £60 per acre but they could go up with an early crop. I would also like to confirm the fact that an efficient dilutor is necessary and phytotoxic effects may be felt if tomato plants are grown in soil badly infested with Root-knot Eelworm.

Q. Mr. D. G. Fenemore

Apart from the special consideration of Root-knot Eelworm, is metham-sodium recommended in Holland for use prior to growing cucumbers?

A. Dr. L. Flipse

Two reasons why we cannot recommend metham-sodium on cucumbers.
(a) It has been the experience for many years that other fumigants (D. D. and Nemagon) are superior. Root-knot eelworm in cucumber is a special problem, much more difficult than in tomatoes, I think because of much deeper rooting and much heavier infestation of Root-knot eelworm at a greater depth in the soil.
(b) Another reason is that cucumbers in glasshouses are grown in soil mixture of very high content of organic matter into which probably the chemical is too much absorbed. In fact we have seen several times failure from tests with metham-sodium on cucumber.

Q. Dr. L. Broadbent, Chairman

We have heard this afternoon something about the use of metham-sodium outside glasshouses on the Continent. Do nurserymen and foresters use it in this country on their seed beds?

A. Mr. D. Tyson

I can confirm that there is a recommendation on several product labels regarding this use. The crop must be a valuable one to justify the use of a high cost chemical. It is used in the Channel Islands at very low rates - 25/30 gallons/acre - for valuable tomato crops and on potato crops for partial control of potato root eelworm.

Q. Dr. L. Broadbent

Why does such good control of the nematodes on potatoes have such poor effect on yield? What is the effect of Heterodera rostochiensis on tuber formation? Does the number of tubers or does their size decrease? What is the effect of the nematodes on the plant?

A. Dr. J. E. Peachey

I think the problem of Heterodera rostochiensis is that we are dealing with a parasite which has a rather close relationship with the host. Very small infestations can do a great deal of damage. It is a question of the competition curve, and we come to a certain point with heavy infestations where reduction in population must be very extensive to show any effect on yield. Work in Scotland points to this conclusion. As regards tuber formation, the interesting thing about eelworm attack is that when you do get a crop failure, there are hardly any tubers at all. It is fair to say that under severe conditions, both tuber size and number decrease; under moderate conditions the tuber number decreases and with good control there is an improvement in both number and size but mainly in size.

Q. Dr. L. Broadbent, Chairman.

Does the nematode excrete any chemical which has an effect on tuber formation?

A. Dr. J. E. Peachey

Substantial reduction in root growth follows from heavy infestations and presumably the roots cannot support tuber formation.

Q. Dr. L Broadbent, Chairman.

In Mr. Tyson's paper, he refers to the control of Cladosporium fulvum by A.40 comparing it with liquid nabam. I agree that all these figures show that the level of leaf mould is about the same with the two. Is there any evidence that either of them caused any decrease in disease?

A. Mr. D. Tyson

As liquid nabam plus zinc sulphate is an approved control of Leaf Mould it was not felt necessary to do more than compare powder nabam, A.40, plus zinc sulphate with it.

However, considerable evidence is available to show that either liquid or powder nabam plus zinc sulphate does give a significant control of Leaf Mould when compared to untreated plots.

Q. Dr. Swarbrick

The pictures which Mr. Read showed of the damage to tomato plants caused by the vapour of sodium N methyl dithiocarbamate approaches that caused by certain growth regulating substances. This may help to explain two cases of plants sent to me during the summer. By the time I got them the leaves were nearly dead but there was epinasty and malformed tomatoes, although the information was that no growth regulators had been used.

My question is, is much of this material being used in practice, if so are growers aware of the damage that may be caused by even small amounts of the vapour of this substance?

A. Mr. W.H.Read

These serious phytotoxic effects were prevalent in the first year of its use but now, by conforming to instructions, these cases of acute damage are rare. A large quantity of metham-sodium is being used in glass-houses in this country.

Comment from Dr. Swarbrick

But the risk is there still?

A. Mr. W.H.Read

Yes, there is slight risk. We are trying to find out what is responsible.

Q. Mr. R.J.Collins

Could Dr. Peachey comment on recent reports on the use of sugar to control eelworm?

A. Dr. J.E.Peachey

The doses required and costs involved to put enough sugar in the soil would be quite impossible from the economic point of view, and certain preliminary experiments by Mr. Rao, suggest that the phytotoxic effect is great. The idea is a novel one but the dosages involved and problem of mixing would be fantastic.

THE ANALYSIS OF PESTICIDE RESIDUES IN FOODSTUFFS: A REVIEW OF RECENT DEVELOPMENTS IN ANALYTICAL METHODS

by H. Egan and E. Q. Laws
D.S.I.R., Laboratory of the Government Chemist

When pesticides are applied to growing crops, the active ingredient of the preparation used will either remain on the surface of the plant or be absorbed into the plant system, depending on the nature of the pesticide, the manner in which it is formulated and the nature of the plant. If a compound is taken into the plant circulation system and is subsequently translocated within the plant, it is said to be systemic. The main factor which determines whether a substance is systemic or not is whether it is itself water soluble or is easily converted to a water soluble form by oxidation.

In certain plants absorption of a non-systemic compound may take place in parts of the plant, without subsequent distribution throughout the plant. Systemic compounds may undergo enzymic oxidation and breakdown to other products within the plant, in some cases with the production of substances which are more toxic to mammals than the original pesticide. When pesticides are applied to living animals similar considerations apply but the results are not always the same as with plants owing to the greater complexity of the enzyme systems of the animal organism. Thus the same pesticide, gamma-BHC, is partially stored by rats but appears to be rapidly metabolised by rabbits (Jondorf et al. 1955). In general, substances which tend to persist are preferentially soluble in non-aqueous solvents and concentrate in the fatty tissues.

In any method of analysis, the above considerations must be taken into account. This paper does not set out to review methods of analysis as such: reviews of this kind are available elsewhere (Chilwell and Hartley, 1961; Westlake, 1961). The present purpose is to outline the problems which face the analyst examining foodstuffs for pesticide residues, to describe the various approaches which have been made to the problem and to say something of the present and future possibilities in the analytical field.

The essential problem presented to the analytical chemist in the detection and determination of pesticide residues in foodstuffs is that of specifically and accurately measuring a few micrograms of a complex, usually organic chemical in the presence of a much larger quantity of many other complex organic species. The general problem is presented in its most acute form to the public analyst (in the widest sense of the phrase) who, unlike his industrial colleague concerned with the company's field or other trials, may have no idea at all as to which, if any, of the possible residues may be present.

Virtually all methods for residue analysis are made up of three distinct stages: (a), extraction of the residue from the foodstuff into a suitable solvent; (b), the removal from this extract of co-extracted material which otherwise would interfere with the final method of determin-

ing the residue - the so-called 'clean-up' stage; and (c), the determination of the residue in the cleaned-up extract. The three stages usually follow the sequence above. Each stage presents its difficulties. Solvent extraction must be thorough but must nevertheless avoid the complication of intractable emulsion formation whereby although the residue is successfully separated from the food into the solvent, the solvent itself becomes inseparable from the sample. The choice of solvent may itself contribute to the clean-up process. The clean-up must not only remove interfering substances from the extract but it must not occasion losses of the extracted residue in the process. Specificity is a major difficulty in the final method of determination, even where the unknown residue is obtained as a simple solution: several complex pesticides may offer almost identical analytical characteristics and the fact that the unknown is in very dilute solution adds to the difficulty. Such difficulties are also illustrated by the fact that several technical pesticidal materials contain impurities of like composition, whilst others may give rise to metabolites which though of similar chemical constitution nevertheless have different toxicological properties. The isomers of hexachlorocyclohexane or of endosulfan, or the degradation of heptachlor to heptachlor epoxide and the oxidation products of phorate are examples.

In addition to the difficulties outlined above, the residue itself may be unstable (which may be all to the good so far as the consumer of the produce is concerned); the residue may be sensitive to change due to storage conditions or to circumstances arising from the analysis itself; and in all of these processes contributions to the final analytical result by the reagents themselves - the reagent blank - must be kept to a minimum, as must also losses of the residue during what may be a long series of laboratory operations.

Obviously the ideal approach to the problem of residue analysis would be to choose a highly sensitive and specific analytical method which will detect and measure a few micrograms of pesticide X in the presence of anything else. This is in fact the aim: more than this, the method should detect and measure pesticides X, Y, Z, ... in the presence of anything else. Here it can immediately be pointed out that in the search for such a method practically all the devices, old and new, known to the analytical chemist - and many of those known more particularly to his biologist, entomogist and other colleagues as well - have been investigated. The methods in the literature include the classical analytical techniques such as titrimetry or gravimetry; spectrophotometry in the ultra-violet, visible and infra-red regions; electrochemical methods including amperometric titrations, coulometry and polarography; column, paper and gas-liquid chromatography; and radio-chemical analysis. They also include bioassay methods and methods dependent on the selective action of enzyme preparations.

Methods can be roughly sub-divided into two kinds, specific and non-specific; in practice the division is not always a sharp one. Specific chemical methods often rely on a well-known property of one of the func-

tional groups of a molecule, such as a nitro group or a phenolic group, for the production of a measurable colour, and are in fact only specific in a relative sense. Again, the degree of specificity of such methods may depend as much on the extraction or clean-up processes as on the final method of analysis. The non-specific chemical methods also rely on preliminary extraction and clean-up stages but are dependent thereafter on the conversion of the residue to an inorganic form such as chloride, phosphate or sulphide, which is then measured by a suitable micro-method. It is particularly necessary in these methods to ensure that compounds of the element naturally present in the sample foodstuff do not interfere with the determination, a fairly straightforward matter for chlorine but less so for phosphorus or sulphur. It is also necessary to ensure that the reagents used are free from the element in question; this is a fairly easy matter for phosphorus but less easy for chlorine or sulphur.

When experimental samples, for example of a vegetable crop, are treated with known quantities of pesticide under controlled conditions, the results obtained by non-specific methods may provide very good evidence of the residue level present; but if the presence and nature of any residue is unknown, it is not possible to distinguish for example, which of the many organo-chlorine pesticides is present from a non-specific micro-chlorine determination. If the extraction and clean-up methods are properly chosen, the classical non-specific methods may be more precise (though perhaps less informative) than a specific chemical methods for synthetic pesticide residues. Successful design of the extraction and clean-up methods may go some way to introducing specificity into a general method; thus in the scheme devised by Laws and Webley (1961) for organo-phosphorus residues in vegetables the polar residues are first separated from the relatively non-polar residues by solvent partition and the non-polar compounds further divided into two groups according to their relative affinity in solution for an activated alumina column.

The critical stage in the analysis is frequently the clean-up. This usually depends on physical processes such as selective absorption from alumina, silicate or magnesia columns followed by elution of the residue with a solvent, or on partition of the residue and other interfering extractives between solvents as in the Jones and Riddick (1952) acetonitrile method. These solvent or column partition techniques require calibration for recovery, using known amounts of the pesticide in the presence of other constituents such as colours and waxes naturally present in the sample extract. There is an inherent difficulty in introducing a precisely known level of pesticide into a sample of plant or animal tissue in exactly the same form as it would occur as a result of field application: radiochemical methods have been used to monitor various alternative extraction methods (Klein, 1959).

Purely physical methods are also widely used for the final analytical stage, as where the residue itself possesses characteristic absorption properties in solution: the low ultra-violet region remains relatively unexplored in this connexion, due mainly to the difficulty of finding a suitable solvent transparent down to about 170 millimicrons. Infra-red

absorption curves are highly characteristic for individual compounds, but the sensitivity is much lower than the ultra-violet method.

The original aspiration of the residue analyst some ten or fifteen years ago was to devise an analytical system which resembled the old group analysis scheme, whereby an unknown residue could be isolated and by sequential tests its identity assigned to one or other of a number of groups of closely related substances and, ultimately by further eliminatory or confirmatory tests, assigned to a single pesticide. In the course of work to this end many useful general methods of chemical micro-analysis have been published but the more specific colorimetric methods, e.g. for DDT, (Report of DDT panel, 1960) parathion (Averell and Norris, 1948) BHC (Wood, 1960) or dieldrin (O'Donnell et al., 1955) are often preferred where the identity of the residue is not in question.

Some of the specific methods are tedious and require special training on the part of the analyst. In recent years a prospect has been offered that some of the newer physical techniques such as gas-liquid or paper chromatography and infra-red spectrography, can give qualitative and quantitative information for residue levels in a more direct way. Indeed, it is to some extent true that gas-liquid chromatography can dispense with the need for tedious clean-up methods (Goodwin et al., 1961). Investigations into the application of all of these methods are the subject of current work, both in industrial and government laboratories.

Gas-liquid chromatography is capable of very high sensitivity towards certain organo-chlorine compounds if an electron capture detection system is used: at the same time the response of much of the interfering co-extracted material from certain plant tissues is suppressed, so that again the clean-up problem is considerably simplified. Using a conventional argon detector, residues of a few micrograms can be separated, passed through a combustion furnace and the chloride produced measured micro-coulometrically, as in the equipment now commercially available in the United States which offers a complete screening test for several organo-chlorine residues at the 0.1 p.p.m. in less than an hour (Coulson et al., 1960).

Paper chromatography has proved useful for the separation and identification of many organo-chlorine residues, if care is taken to standardise the assembly of the chromatographic tank and the techniques of applying and measuring the spots, 0.5 micrograms of individual compounds can readily be detected and quantities of from 2.5 to 15 micrograms measured with reasonable accuracy (Evans, in press). Conventional extraction and clean-up methods are used, although it may be possible to adapt some systems so that part of the normal clean-up stage is done on the chromatographic paper (Menn et al., 1960). Both infra-red spectrometry and gas-liquid chromatography offer the prospect of a direct examination of simple extracts without extensive clean-up; they have the advantage of being rapid methods and both offer some degree of specificity. Infra-red examination can be highly specific but in order to use the method for micrograms quantities special instrumentation is needed; as with gas-liquid chromatography, the general accessibility of the method is bound

up with the increasing commercial availability of suitable instruments.

The present day tendency seems to be to take advantage of the modern rapid instrumental techniques by choosing a combination of these such that together they fulfil the two principal requirements of high sensitivity and high specificity. Thus, gas-liquid chromatography may provide an initial degree of specificity first by eliminating interferences due to background material and then by separating individual pesticides or groups of pesticides; from the peak height or area a quantitative indication is also given but this may be improved by the use of sub-micro chemical or electrochemical methods, or by the infra-red examination of the fractions using micro-cells and a beam condenser system. Alternatively an independent confirmatory identification may be obtained by arranging for the effluent to be run on a paper chromatogram followed by identification and measurement of the apots.

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ANALYSIS OF SYNTHETIC ORGANIC PESTICIDES IN WATER BY CHROMATOGRAPHY

by E. Hindin and G. H. Dunstan
Washington State University

The growing use of synthetic organic chemicals for the control of pests on croplands, forests and marshes poses a potential threat to the health of the populace.

In 1960 one sixth of the cultivated land in the U.S.A. was treated with synthetic organic insecticides and herbicides. Projecting the usage on a world-wide scale the area treated is enormous. Run off water draining these treated areas picks up minute quantities of pesticides.

DDT and aldrin have been found in several rivers in the United States, rivers which serve as raw water supplies for dozens of communities. So far the quantities of pesticide found have been far below human toxicity levels (Tarzwell, 1959). Studies by the Communicable Disease Center of the U.S. Public Health Service reveal that these chemicals may have a cumulative impact on human beings (Hollis and McCallum, 1959). Pesticides in water have been blamed for about 40% of the fish mortality in the United States in 1960, according to a preliminary report by the United States Public Health Service (1961).

Clearly there is a need for a suitable method for the separation, identification and estimation of pesticides in surface and ground water. This is no easy matter because of the solubility of the chlorinated organic and many of the new organic phosphorus and sulphur pesticides. The maximum concentration of most pesticides is one part or less in a billion parts of water. A means of removing the pesticides from water must be employed. Adsorption on activated carbon, ion exchange resins, and solvent extraction have been used with some success. The pesticides may be removed from the adsorbing medium by an appropriate solvent. Further concentration may be required to facilitate the handling of the solvent pesticide extract.

A screening programme was adapted by the Sanitary Engineering Section at Washington State University last year for the separation, identification and estimation of chlorinated and organophosphorus micro quantities. The screening programme uses two paper chromatographic methods and an enzymatic method as a presumptive test and gas chromatographic analysis as a confirmatory test.

The presumptive screening programme consists of three distinct methods: (a) a qualitative and semi-quantitative method developed by Mitchell (1958) of the U.S. Food and Drug Administration and modified by Zweig and Painter (1960) at the University of California for chlorinated pesticides, (b) a paper chromatographic method of Mitchell (1960) for the identification of organophosphate pesticides, and (c) the quantitative enzymatic method for organophosphate and carbamate pesticides by Cook (1954)

Paper chromatographic methods:

The paper chromatographic method for the analysis of chlorinated organic pesticides uses an immobile-mobile solvent. The immobile solvent is 5% mineral oil in ethyl ether while the mobile solvent is 75% acetone in water. A chromogenic reagent is used consisting of silver nitrate in 2-phenoxyethanol, water, and acetone. The pesticides are delineated by exposure to strong short-wave ultra violet light when they appear as dark brown spots against a white background. The Rf values indicate the specific compound or class of compounds. The quantity of pesticide may be determined by visual comparison with known quantities of the respective pesticide or by the concentration to spot intensity-area relationship. This method has a number of limitations: (a) a number of chlorinated pesticides have the same or approximate Rf values, (b) some pesticides appear as streaks rather than spots, and (c) a number of chlorinated organic pesticides do not respond. The first two limitations can be minimized, however, there is no solution for the last one.

Paper chromatography for the detection of organophosphate insecticides is, in principle, similar to that used for the separation and identification of the chlorinated pesticides. The immobile solvent is 50% aqueous solution of NN-dimethyl formamide. An activating reagent consisting of bromine must be used. The chromogenic reagent consists of fluorescein and NN-dimethyl formamide in ethyl alcohol. The spots containing the pesticides are delineated under short-wave ultra violet light. Under ultra violet light the pesticides appear as a quench area that is dark purple against a greenish yellow background. If the organophosphate contains isomers a primary and secondary spot may appear. This method has a serious limitation: the contrast between the background and quenched areas begins to dissipate after 3 minutes and thus the method is not quantitative.

Enzymatic method

In order to obtain information of quantitative significance another approach must be taken. The quantities of organophosphate and carbamate pesticides are determined by an enzymatic method. The degree of deactivation of the enzyme acetyl-cholinesterase is indicative of the quantity of pesticide. It has been found that pseudo-oxidation of phosphorothioate and dithioates increases by a hundred fold the sensitivity of this method.

Gas chromatographic method

To confirm findings of the preliminary examination, extracts containing pesticides are subjected to gas chromatographic analysis. The pesticides are separated on a 6 ft. column of chromosorb W coated with Dow 11 high vacuum silicone grease at 250° C. Helium is used as the elutant. The pesticides are detected and quantitatively measured using thermo-conductivity cells. The electronic impulses are translated to a potentio-metric recorder. The thermal-conductivity cell detector can detect 0.5 mg. of

most pesticides. A hydrogen flame detector can increase sensitivity so that 1 microgram quantities of the respective pesticide can be detected.

Virtually all organic compounds containing pesticidal properties can be detected by gas chromatography. The detention time, the time taken for the component to pass through the column, is indicative of the specific organic pesticide. The area of the peak is also of quantitative significance.

In testing more than 90 synthetic organic pesticides we have found no two with the same Rf value and detention time value. The detention time value is of extreme importance in the identification of a specific pesticide whose Rf value is the same as that of another pesticide.

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Q. Mr. G. Ordish

Could Dr. Egan tell us if many samples of retail produce are examined?

A. Dr. H. Egan

Where such samples are examined by local authorities, details may be found in the annual reports of the Public Analysts. I would refer Mr. Ordish to these reports.

Q. Dr. J. T. Martin

The methods to which Dr. Egan has referred have been of the greatest value in the analysis of bulk samples of crops, e.g. to ascertain whether they conform to prescribed limits of residues. We are, however, badly in need of methods for determining the distribution of deposits over sprayed targets in work on the assessment of formulations or spraying methods. Here rapid methods, permitting large numbers of tests and suitable for deposits on small areas of plant surfaces, are required but so far are available for few of the spray chemicals.

A. Dr. H. Egan

Dr. Martin and his colleagues have developed some very elegant methods, which are both simple and sensitive, for assessing surface deposits of certain pesticide formulations; but I agree with him that a general method of wider applicability is desirable. I think the more complicated methods, used earlier by Winteringham to follow the metabolism of insecticides by insects, could be used. These methods require radioactive pesticides specially prepared for experimental trials.