THE EFFECTS OF FUNGICIDES ON THE MICROBIOLOGY AND BIOCHEMISTRY OF SOILS

by

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1st May, 1974

To my wife Christine,my family,and my friends.

CONTENTS

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M. Wainwright

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SUMMARY

A study was made of the effects of a wide range of modern fungicides on the microbiology and biochemistry of soils.

The addition of fungicides to laboratory incubated soils led to marked changes in the soil microbial equilibrium. Initially fungal potential was decreased, but soon recovered to exceed that of the control at the end of 28 days. Bacterial numbers on the other hand increased dramatically following treatment. Similar changes were seen in field soils which had been treated with fungicides. The response of the cellulolytic fungal flora to field treatment with fungicides was also studied. Changes in the pattern of recolonization occurred, with species such as T. koningii and P. nigricans becoming dominant. Cellulolytic fungi were largely insensitive to the addition of fungicides to their growth medium.

Marked changes in the mineralization of nitrogen followed fungicide treatment. In the field all the fungicides used inhibited nitrification to a greater or lesser extent, while levels of the ammonium ion were increased. Similar changes were seen in laboratory incubated soils. Here treatment with high concentrations of fungicides led to nitrification-inhibition, while an increase in nitrate production often followed treatment with low concentrations. The amounts of ammonium-N in these soils increased dramatically following treatment.

Towards the end of the incubation period (28 days) low concentrations of fungicides led to increases in the total free amino-acid-N content in soil, while the converse was true of high concentrations. Addition of fungicides also led to qualitative changes in the free amino acid content of soils.

Increases were seen in certain metal ions including K, Na, Mn and Zn in fungicide treated soils.

A compound exhibiting auxin activity was extracted and characterised as 3-Indole-pyruvic acid. However, the exact origin of this compound is unknown. It seems likely that it was an extraction artefact since IPyA is highly unstable under the extraction conditions which were employed. The potential for soil auxin activity existed however, and was increased two-fold by the addition of the fungicide Captan to the soil.

Finally the possible effects of these changes on soil fertility are discussed, particularly in relation to the increased growth response phenomenon associated with the partial sterilization of soil.

GENERAL INTRODUCTION

Fungicides are used extensively in modern agriculture and horticulture in the control of plant pathogenic fungi. These chemicals are applied directly to the soil, or enter as run off from treated aerial systems, or from drifting sprays. Thus the soil receives an array of complex organic compounds many of which are active against micro-organisms other than the fungi. Since soil fertility is dependent to a large extent on the activities of micro-organisms it is essential that the effects of fungicides and other pesticides on the microbiology and biochemistry of soils be continually monitored.

The action of a fungicide in the soil depends on a number of complex chemical, biological and physical factors, which have been reviewed by Kreutzer (1963), Goring (1970) and Wolcott (1970). In particular the physical properties of a soil, including organic matter, clay content, pH, cation exchange capacity, adsorption, leaching and volatility, can influence the action of fungicides. Adsorption of fungicides onto clay minerals and organic matter is particularly important in relation to their availability to soil micro-organisms. Adsorption follows the formation of a variety of bonds, including covalent bonds, hydrogen bonds, and Van der Waal's forces.

The type of clay mineral in the micelle and the degree of micelle hydration is critical (Kreutzer, 1963). Similarly bonding is important in relation to organic matter, and increases in organic matter tend to lead to increased adsorption, and therefore decreased efficiency of the fungicide.

Another of the principle factors which determines the activity of a fungicide in soil is its degree of persistence. The persistence of a chemical in soil depends upon a number of factors which have been reviewed by Domsch (1964) and Munnecke (1967). The major factor appears to be the extent to which the chemical can be degraded by micro-organisms. Some workers consider that the soil microflora generally possess or can acquire the capacity to degrade any compound which is capable of being synthesised - a phenonomen described as "microbial infallibility". The implications of this concept have been discussed by Alexander (1968).

Other factors which affect the efficiency of fungicides in soils include, the type of formulation used, and such environmental variables as soil temperature and soil water content.

At present there is a great deal of controversy surrounding the use of pesticides, particularly the organo-chlorine insecticides such as D.D.T. (Gunn, 1972; Moore, 1972). Fungicides, with the exception of the organomercurials, have generally escaped such criticism, because they tend to be non-persistent and do not accumulate in food chains.

Most of the current emphasis in fungicidal research is being placed on the development of systemic compounds possessing a high degree of specificity. However, even these compounds may have a deleterious effect on the environment. Benomyl for example, has been found to reduce the earthworm populations of soils (Stringer and Wright, 1973).

It appears that fungicides will continue to be used in agriculture and horticulture in the foreseeable future, unless some major breakthrough in biological control takes place. Alexander (1968) summed up the current situation when he said, "Pesticides are hazardous chemicals. However, they are the best weapons for controlling pests that man has ever devised .... We have to live with pesticides, there are too many mouths to feed".

THE EFFECT OF FUNGICIDES ON THE MICROBIOLOGY OF SOILS

Introduction

The perfect fungicide is non-persistent, and selectively kills, or inhibits the growth of pathogenic fungi, without affecting non-target saprophytic micro-organisms (Corden and Young, 1965). However, since fungicides are designed to inhibit cellular processes common to most micro-organisms such specificity is rarely achieved.

The addition of a fungicide to soil results in a change in the microbial equilibrium. It favours those groups resistant to its action, or able to exploit the partial vacuum left by non-resistant forms. Since fungicides are formulated to eliminate pathogenic fungi it is reasonable to assume that all fungi will be more or less susceptible. However, the physiological activities of soil fungi are extremely variable, so that a fungicide which is capable of killing a mucoraceous species for example may have no effect on a member of the Deuteromycetes. Kreutzer (1965) reviewed the literature relating to the recolonization of treated soils. He considered the factors which determine the final degree of alteration of treated soils, which in turn determine the type and degree of re-infestation. These factors were :-

(1) The nature and quantity of biological and chemical residues remaining in the critical zone after treatment.

(2) The nature and quantity of available-substrates or specific energy sources remaining in, or introduced into the treated soil zone.

(3) The degree of competitive saprophytism of potential invaders, and from the resistant portions of the original microflora.

The following experiments were designed to determine what effect a wide range of fungicides would have on microbial populations in soils. In the first instance these involved monitoring the effects of the fungicides on actinomycete, bacterial and fungal populations in the field and in soil brought into the laboratory. These were followed by a study of the recolonization of treated field soils by cellulolytic fungi.

Captan was chosen for laboratory studies because it is a successful broad-range fungicide in wide use at the present time. Numbers of heterotrophic bacteria and fungi were determined over a 28 day period after treatment, while the initial effect of Captan on actinomycetes was determined after 2 days.

Evaluation of changes in the microbial population of soil is greatly influenced by the isolation technique used. In the present study the use of the dilution plate technique may give a distorted picture of the changes which take place in the microflora following treatment (Corden and Young, 1965). The technique favours fungi which sporulate heavily, and thus selects against sterile mycelia. In normal practice it also tends to isolate aerobic, heterotrophic and mesophilic species of bacteria. However both Jensen (1968), and Garrett (1963) have considered that the technique is capable of demonstrating the reaction of the soil microflora to different soil treatments.

Laboratory experiments were extended by the use of field trials at the University of Nottingham School of Agriculture, and at Rothamsted Experimental Station. Soil conditions in the field, where fungicides are normally applied, are far more variable than are those in soil sampleswhich are incubated in the laboratory. Field soils constitute an "open-system" in which micro-organisms can invade treated soils both vertically and laterally. The pattern of recolonization of such soil is likely to differ greatly from soils treated in the laboratory. Here the soils are incubated in sterile conditions, and thus begin with a "fixed" microbial population. In addition fungicides are intimately mixed with soil in the laboratory, while in the field the amount of active ingredient will vary from locally concentrated to absent. Because of these difficulties of obtaining good dispersal within the soil, changes seen in the micro-biology of treated laboratory soils are likely to be more pronounced than those observed in the field.

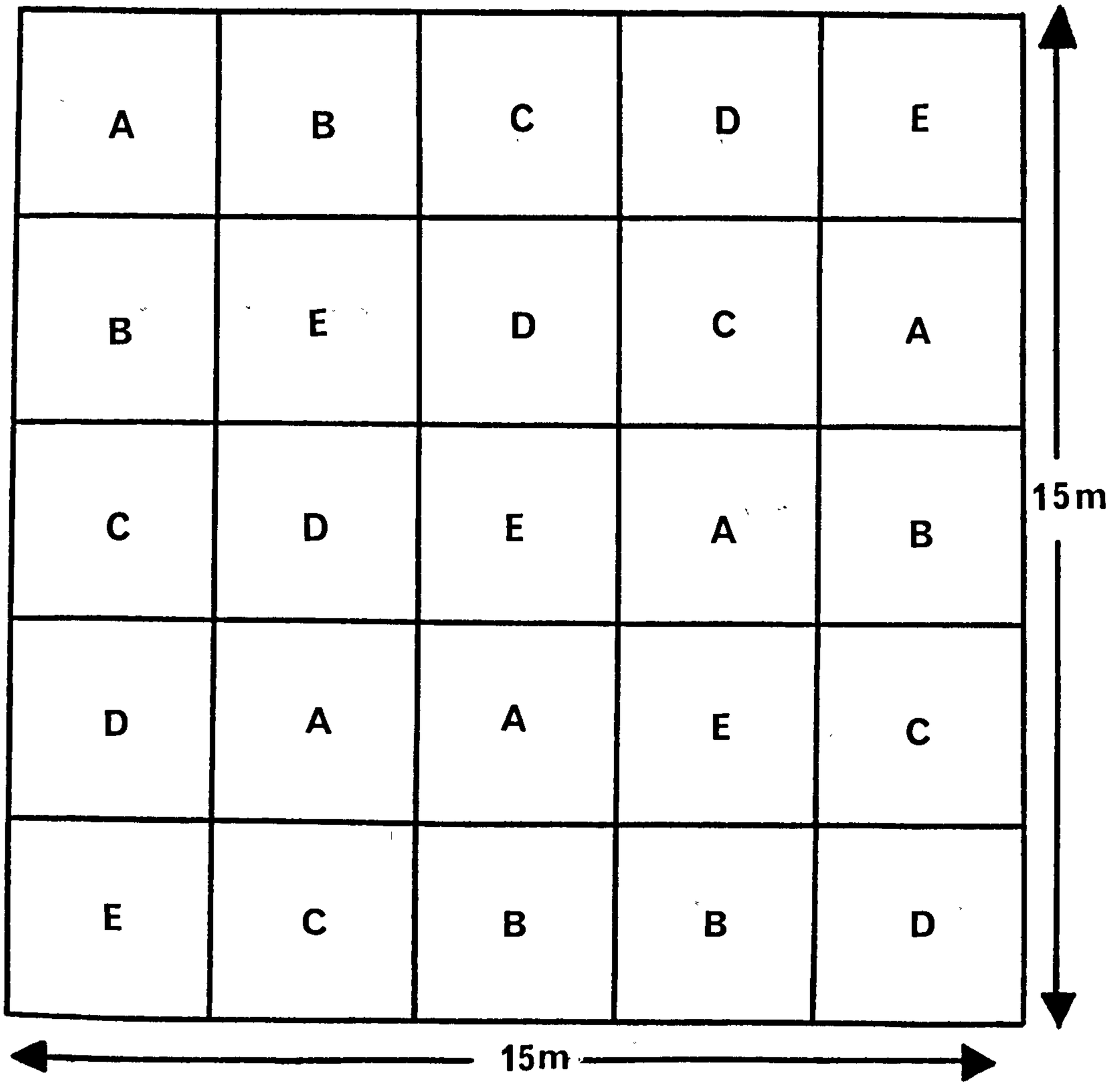
A large proportion of this section is devoted to the changes which take place in the cellulolytic fungi following the application of fungicides in the field. The pattern of recolonization of treated soils was determined over a 157 day period and the results show changes at the species level. Fungi are the major degraders of plant material in acid soils, (Alexander, 1961), and their elimination may have serious effects on the rate at which organic matter is broken down (Pugh and Williams, 1971; Williams, 1973).

Fungicides are now widely used in agriculture to control plant diseases caused by fungi. It is essential that their effects on the saprophytic microflora of soils be continually monitored. Mellanby

(1970) put it succinctly when he said "The effects of small doses of organomercury and other fungicides over long periods need to be observed. It is possible that effects on saprophytic soil fungi are more important than has been realized."

Fig. 1 Details of Latin Square at Sutton Bonington

Fig 1



Materials and Methods

(1) Incubation Experiments

Soil samples from Sutton Bonington were used in the incubation studies. The samples were incubated under conditions optimal for microbial activity, or for the process under study. Soil was freshly collected and sieved (<2 mm), fungicides were applied as solutions or suspensions and then mixed thoroughly. Soils were then brought to 66% of their water holding capacity and incubated in sterile Erlenmeyer flasks covered with "Parafilm" sealing tissue.

(2) Field Experiments

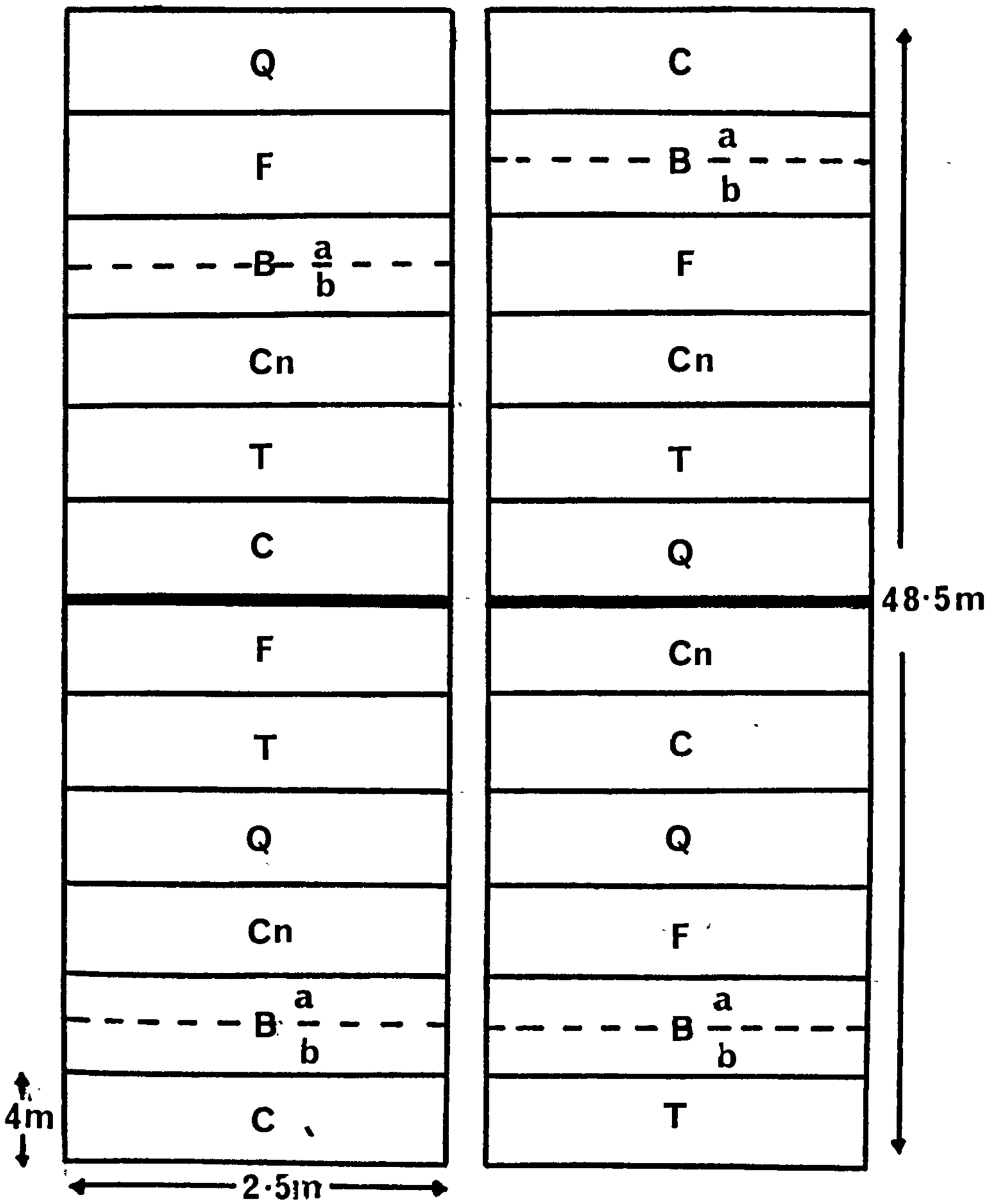
Incubation studies were supplemented by field trials. Experimental plots were laid out at Sutton Bonington and at Rothamsted Experimental Station. In both experimental plots, treatments were randomised.

(a) Sutton Bonington

A latin square was laid out at Sutton Bonington. The plot consisted of a square 15 x 15 m, divided into 25, 3 x 3 m sub-squares (Fig. 1). Fungicides were applied in solution or in suspension as the formulation, at the rates shown in Table I. Each treatment occurred once in each row and column (Fig. 1). The plots were sampled by placing a grid on each 3 m square. Numbers, taken from tables of random numbers, (Fisher and Yates, 1963) were then used to locate a point in the 3 m square. A soil core (10 cm deep x 3 cm) was then removed at this point with a soil-borer, and placed in a sterile polythene bag. Three random cores per 3 m square were taken, making a total of 15 samples per treatment.

Fig. 2 Details of randomised field plots at Rothamsted .

Fig 2



(b) Rothamsted

A split-plot design was used at Rothamsted (Fig. 2). Each "square" was 2.5 m x 4 m and each treatment was replicated four times. Fungicides were applied at the rates shown in Table 2. Sampling was achieved by distributing polythene bags randomly and then sampling where they fell. A soil core (10 cm deep x 3 cm) was removed at this point, and placed in sterile polythene bags. Four cores per square were taken, making a total of 16 samples per treatment. On arrival in the laboratory, samples were stored at $0^{o}C$ until required.

Laboratory Experiments

Sutton Bonington soil was sieved(< 2 mm) and weighed into 100 g. portions. Suspensions of Captan in sterile distilled water were then added and mixed thoroughly, so as to attain 66% of the water holding capacity and final concentrations of 5, 25, 50 and 100 µg active ingredient (AI) of Captan per g of soil. Samples of soil (250 g) were then transferred to sterile 250 ml Erlenmeyer flasks, which were covered with "Parafilm" sealing tissue and incubated at $25^{o}C$ for 28 days. Four replicates per concentration plus an equal number of controls were included. At 7 day intervals 1.0 g samples of soil were removed for the preparation of dilution plates. Twelve replicates of three serial dilutions were prepared using Czapek-Dox agar plus streptomycin (50 mg/1) for fungal enumeration; yeast-extract agar (Stevenson and Rouatt, 1953) for bacterial counts, and glycerol-arginine medium (Porter, Wilhelm and Tresner, 1960) for determination of the numbers of actinomycetes. Plates were incubated for 7 days for bacterial, and 10 days for fungal and actinomycete counts.

Table 1 Field Rates of Application of Fungicides (Sutton Bonington)

Fungicide	Rate of Application Kg./h.
Captan	52.6
Dicloran	1.65
Milcol	5 l./h.
Triarimol	2.0

Field Experiments

Numbers of bacteria and fungi were also determined following field treatment with fungicides. Four fungicides, Benomyl, Captan, Quintozene and Thiram, were applied to the Rothamsted field plots at the rates shown in Table 2. Twenty-eight days after application the numbers of both bacteria and fungi were determined as above. Twenty plates per treatment, plus controls were made from each of the four replicate samples.

In addition fungal numbers were determined over a 157 day period at Sutton Bonington following the application of fungicides at the rates shown in Table 1.

Field Experiments on the Effect of Fungicides on the Cellulolytic Microfungi of Soils

Water suspensions of Captan, Dicloran, Milcol and Triarimol were applied to the Sutton Bonington field plots at the rates shown in Table 1. Samples of soil were removed after 3, 15, 31, 58, 103 and 157 days, and transported to the laboratory in sterile polythene bags. Fifteen core-samples per treatment were taken and five soil plates per sample were prepared making a total of 75 plates per treatment. Cellulolytic fungi were isolated using Warcup's technique (1957) and cellulose agar (Eggins and Pugh, 1962). Cellulolytic species were identified after incubation at 25°C for 10 days. Species were recorded on a presence or absence basis on each plate, and the percentage frequency of isolation of each species was determined following fungicide treatment and compared with that of the control.

Table 2. Field Rates of Application of Fungicides (kg/ha)

(Rothamsted)

Fungicide	Application	Application
Benomyl (a)	-	20
Benomyl (b)	-	4
Captan	9	-
Dicloran	2	4
Formalin	500 l/ha	-
Quintozene	5.6	11.2
Thiram	6.7	13.4

Effect of Fungicides On The Growth of Selected Soil Fungi

The effect of dilutions of the fungicides used above on the growth of selected soil fungi was determined by dry weight of the mycelium after growth in a liquid medium plus fungicide.

The medium used in growth studies was modified Czapek's-Dox liquid medium pH 6.8 (Oxoid C.M.95). However, Mucoraceous forms failed to grow in this medium. The problem was overcome by the use of Dickinson and Boardman's (1970) medium. Aliquots of liquid medium (99 ml) were autoclaved at (15 p.s.i.) for 20 mins. in 250 ml conical flasks, stoppered with foam bungs. On cooling a 1 ml suspension of the fungicide in sterile distilled water was added aseptically, to give final concentrations of 5, 10, and 50μg (formulation) per ml of liquid medium. The flasks were then inoculated with a 5 mm disk removed from the margin of an asporogenous culture grown on solid medium. Care was taken to use colonies at the same growth stage. The flasks were incubated at 25°C, on a reciprocal shaker (100 strokes per minute). After 7 days incubation the medium was removed by suction filtration, and the mycelial pellets harvested onto tared filter papers which had been previously dried to constant weight at 100°C. After filtration the mycelium plus filter paper was dried at 100°C to constant weight, cooled in a desiccator, and then weighed. Four replicates per dilution were included plus an equal number of controls. The following fungi were used :-

Fusarium culmorum; Gliocladium roseum; Penicillium nigricans; Trichoderma koningii; and Zygorhynchus moelleri.

These Species were frequently isolated from the field soils and were of special interest because they were particularly resistant or susceptible to fungicide treatment.

Fig. 3. The effect of Captan on the heterotrophic bacteria of soils

Control

5 µg Captan per g of soil

25 µg Captan per g of soil

50 µg Captan per g of soil

100 µg Captan per g of soil

Fig 3

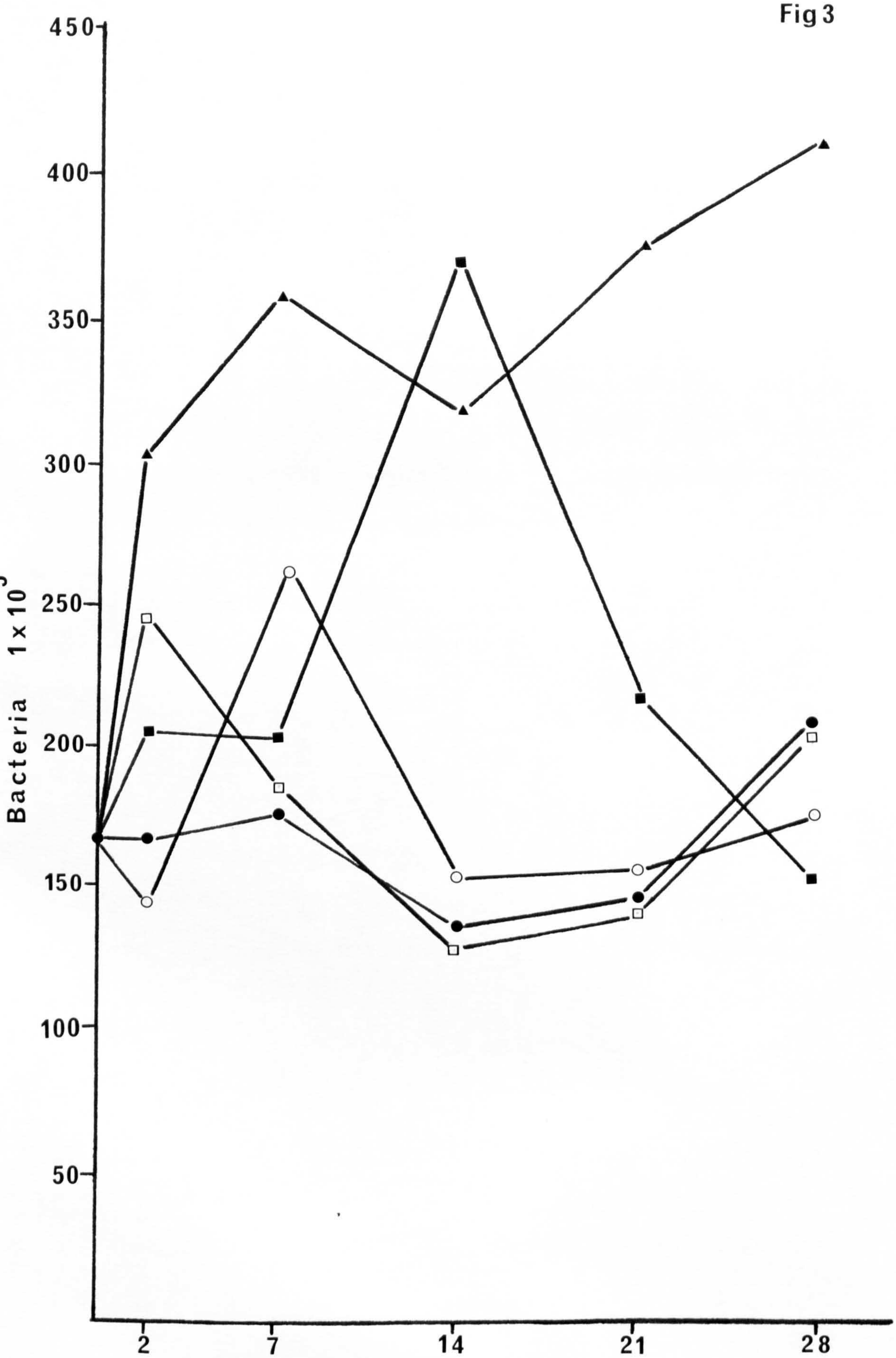
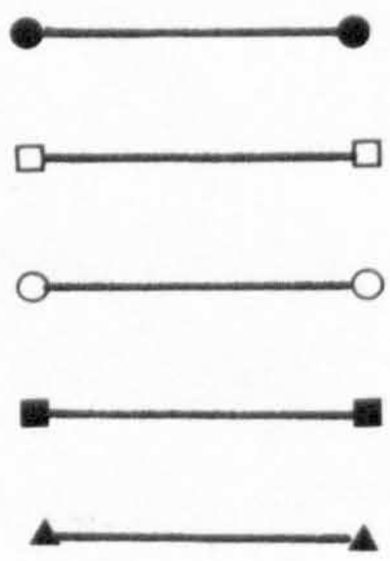

450
400
350
300
250
200
150
100
50
Bacteria 1x10^5
2
7
14
21
28
Time Days

Fig. 4(a) The effect of Captan on the numbers of actinomycetes in soil

Fig. 4(b) The effect of Captan on the numbers of fungi in soil.



Control

5 µg Captan per g. of soil

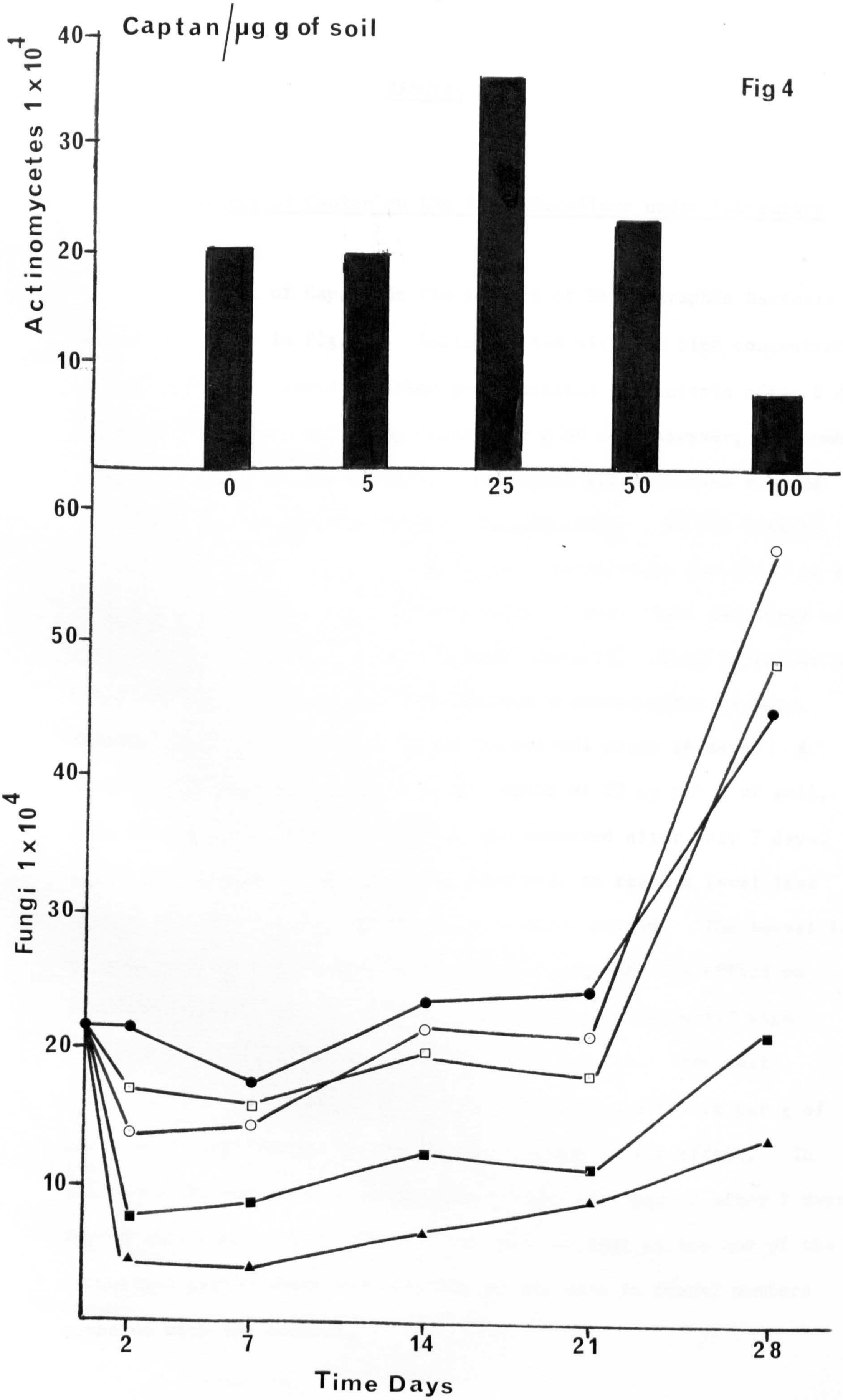
25 µg Captan per g. of soil

50 µg Captan per g. of soil

100 µg Captan per g. of soil

Captan/µg g of soil

Fig 4



RESULTS

1. The Effect of Captan on the Soil Microflora under Laboratory Conditions

The effect of Captan on the numbers of heterotrophic bacteria in soil is shown in Fig. 3. Soils treated with the high concentration of the fungicide showed a marked proliferation of bacteria after 2 days. Following treatment with 5 μg Captan per g of soil however, the numbers remained similar to the control. Different patterns then emerged depending on the concentration of fungicide used. At the highest concentration (100 μg per g), the numbers of bacteria continued to rise over the incubation period, so that after 28 days these contained over twice as many bacteria as the untreated controls. With Captan at 50 μg per g of soil a peak in bacterial numbers occurred after 14 days. Numbers then fell to a level below the control after 28 days. A similar peak followed application of Captan at 25 μg per g of soil. Here however, the peak was smaller, and occurred after only 7 days. Again the numbers of bacteria then declined, to reach a level less than the control at the end of the incubation period. The lowest level of application of Captan, 5 μg per g of soil had little effect on bacterial numbers beyond 2 days. Fungal numbers decreased with increasing concentration of the fungicide (Fig. 4). The most marked effect followed the application of Captan at 100 μg per g of soil, while application at the lowest rate had little effect. In all cases the decrease in fungal numbers was most marked after 2 days. Beyond this time numbers began to recover, so that at the end of the incubation period there was actually an increase in fungal numbers compared with the control,

Table 3 Microbial Numbers following Fungicide Treatment in the Field

Treatment	Fungi 1×10^4	Bacteria 1×10^6
Control	79	21.0
Benomyl (a)	103	20.6
Benomyl (b)	91	31.0
Captan	81	49.0
Quintozene	118	44.9
Thiram	94	44.1

Means of 20 replicate plates

Fig. 5 The effect of fungicides on the numbers of fungi in field soils

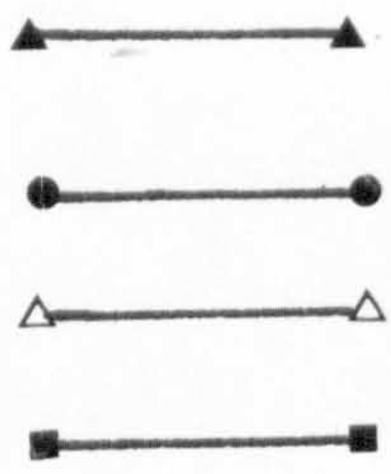
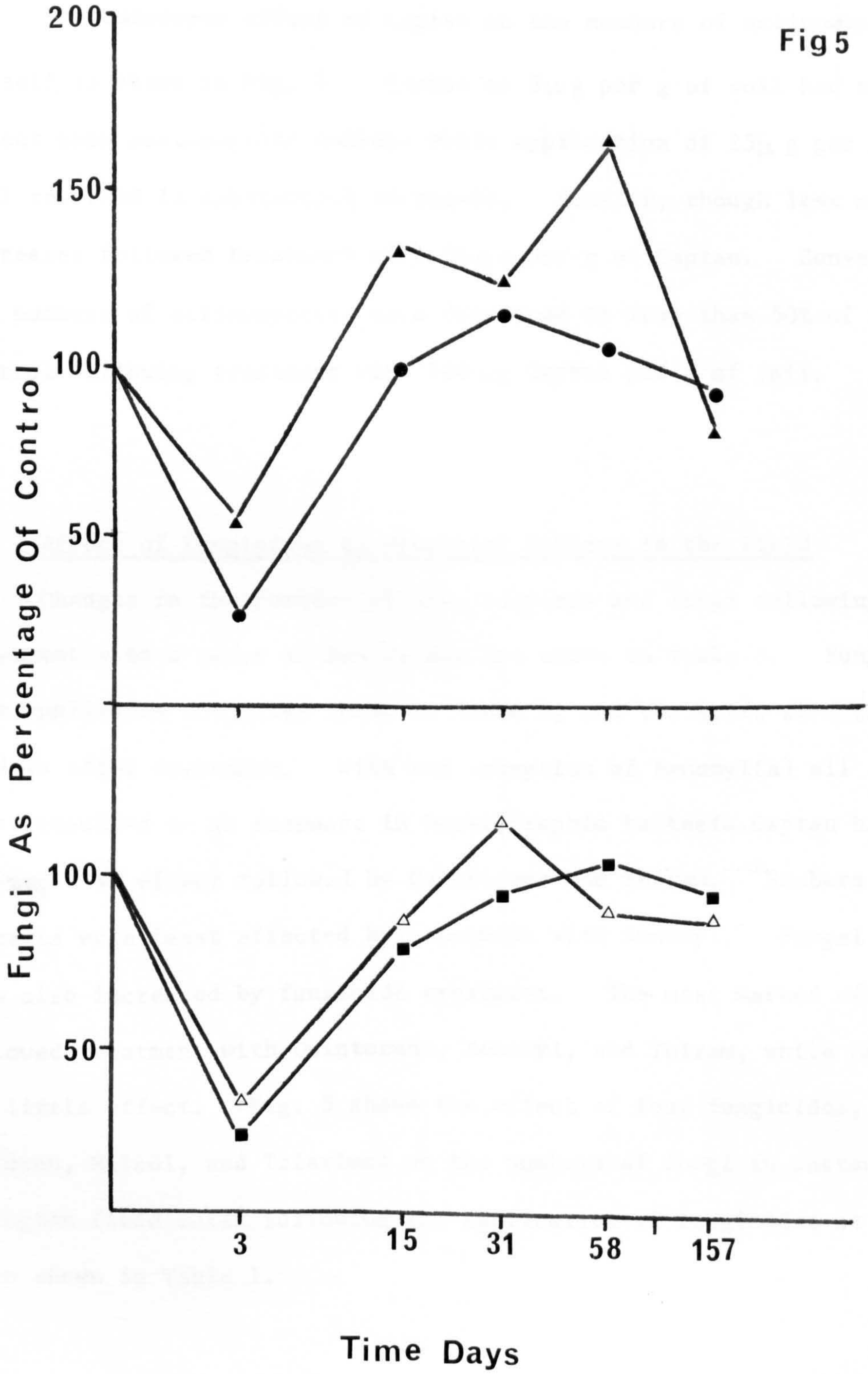


Fig 5


200
150
100
50
Fungi As Percentage Of Control
100
50
3
15
31
58
157
Time Days

The immediate effect of Captan on the numbers of actinomycetes in soil is shown in Fig. 4. Captan at 5μg per g of soil had no effect upon actinomycete numbers while application of 25μg per g of soil resulted in substantial increases. Similar, though less marked increases followed treatment with 50μg per g of Captan. Conversely the numbers of actinomycetes were decreased to less than 50% of the control following treatment with 100μg Captan per g of soil.

Effect of Fungicides on Microbial Numbers in the Field

Changes in the numbers of both bacteria and fungi following field treatment with a range of fungicides are shown in Table 3. Fungicides were applied at the rates shown in Table 2, and the soils were sampled 28 days after treatment. With the exception of Benomyl(a) all treatments resulted in an increase in heterotrophic bacteria.Captan had the greatest effect followed by Quintozene and Thiram. Numbers of bacteria were least affected by treatment with Benomyl. Fungal numbers were also increased by fungicide treatment. The most marked effect followed treatment with Quintozene, Benomyl, and Thiram, while Captan had little effect. Fig. 5 shows the effect of four fungicides, Captan, Dicloran, Milcol, and Triarimol on the numbers of fungi in Sutton Bonington field soils following the application of fungicides at the rates shown in Table 1.

In all cases, there was an initial decrease in numbers, with the greatest decrease following treatment with Dicloran where numbers were reduced to less than 25% of the control after 3 days. Fungal numbers recovered rapidly in all cases, so that numbers began to exceed the control towards the end of the isolation period. In the case of the Triarimol treated soil for example numbers reached a level 70% above the control after 58 days. After these initial increases, numbers decreased

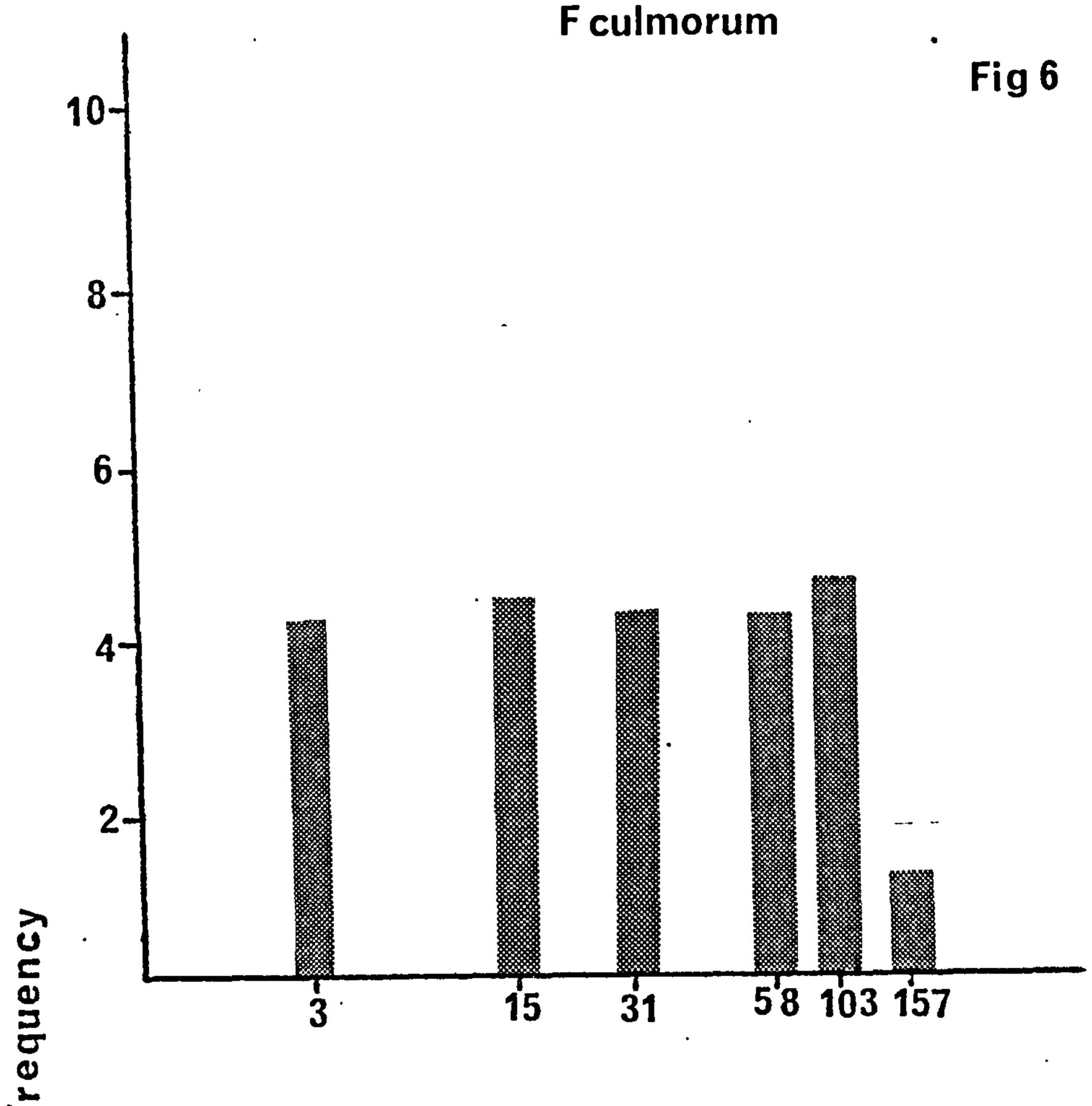
Fig. 6 The percentage frequency of isolation of F. culmorum following the treatment of field soils with fungicides.

(a) Control

(b) Captan

F culmorum

Fig 6



to a level below the control after 157 days.

Recolonization of Fungicide Treated Soils by Cellulolytic Fungi

Changes in the cellulolytic fungal flora of field soils (Sutton Bonington) following fungicide treatment are shown in Figs. 6 - 10. In addition their effect on the frequency of occurrence of Zygorhynchus moelleri, a non-cellulolytic species, is shown in Fig.11. Cellulose decomposing fungi were isolated from treated and control soils over a 157 day period. A large number of species was isolated (see Appendix) but only those species showing sufficiently high percentage frequencies to allow for meaningful analysis have been included. The effect of four fungicides, Captan, Dicloran, Milcol and Triarimol on the percentage frequency of the total isolates is shown in Figs. 6 - 11.

The frequency of Fusarium culmorum in both treated and untreated soils is low (Fig. 6). In control soils the frequency of isolation had a mean value of around 5%. Treatment with Captan had little initial effect on the frequency of F. culmorum, until after the 15 day sample when there was a decrease in frequency of isolation. Treatment with Dicloran, Milcol, and Triarimol, resulted in similar patterns. After application of Dicloran a peak in frequency was seen after 15 days after which, isolations fell and remained low after 58 days. Similarly following treatment with Milcol and Triarimol there was a peak, but here it occurred 31 days after treatment.

The frequency of Gliocladium roseum in the control soils varied between about 10% and 15% over the isolation period. Following the application of Captan there was a slight rise in frequency after 3 days, followed by a rise to over 20% after 15 days. The frequency then

to a level below the control after 157 days.

Recolonization of Fungicide Treated Soils by Cellulolytic Fungi

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decreased slightly, and then rose to a value above 25% after 157 days. Following the application of Dicloran there was an immediate rise in the frequency of isolation of G. roseum. This increase continued to the 31 day sample and was followed by a fall and then a minor peak after 103 days. A similar immediate increase in frequency occurred after both the Milcol and Triarimol treatments. In the latter case, the initial rise in frequency was followed by a marked decrease at the 103 day sample, followed by a return to increased frequency after 157 days.

Humicola grisea was the most frequently isolated fungus from both treated and control soils, accounting for around 20% of the total isolates (Fig. 8). Application of all four fungicides had similar effects. Except for the occasional slight increase, frequencies remained constant and similar to the control.

Penicillium nigricans was not isolated from untreated soils until the 157 day sample where it was isolated with low (5%) frequency (Fig. 9). Following fungicide treatment however, substantial increases in the frequency of this species occurred, in the main after 58 days. In Dicloran and Milcol treated soils however, there were increases after 31 days. After treatment with Captan, Milcol, and Triarimol, the frequency of isolations of P. nigricans reached a peak after 103 days while in Dicloran treated soils the peak occurred after 157 days (Fig. 9).

The percentage frequencies of isolation of Trichoderma koningii are shown in Fig. 10. In the untreated soils there was a mean frequency of around 10%. Following fungicide treatment however, this increased substantially. In soils treated with Captan, the frequency was doubled after 3 days. The increase was then maintained over the

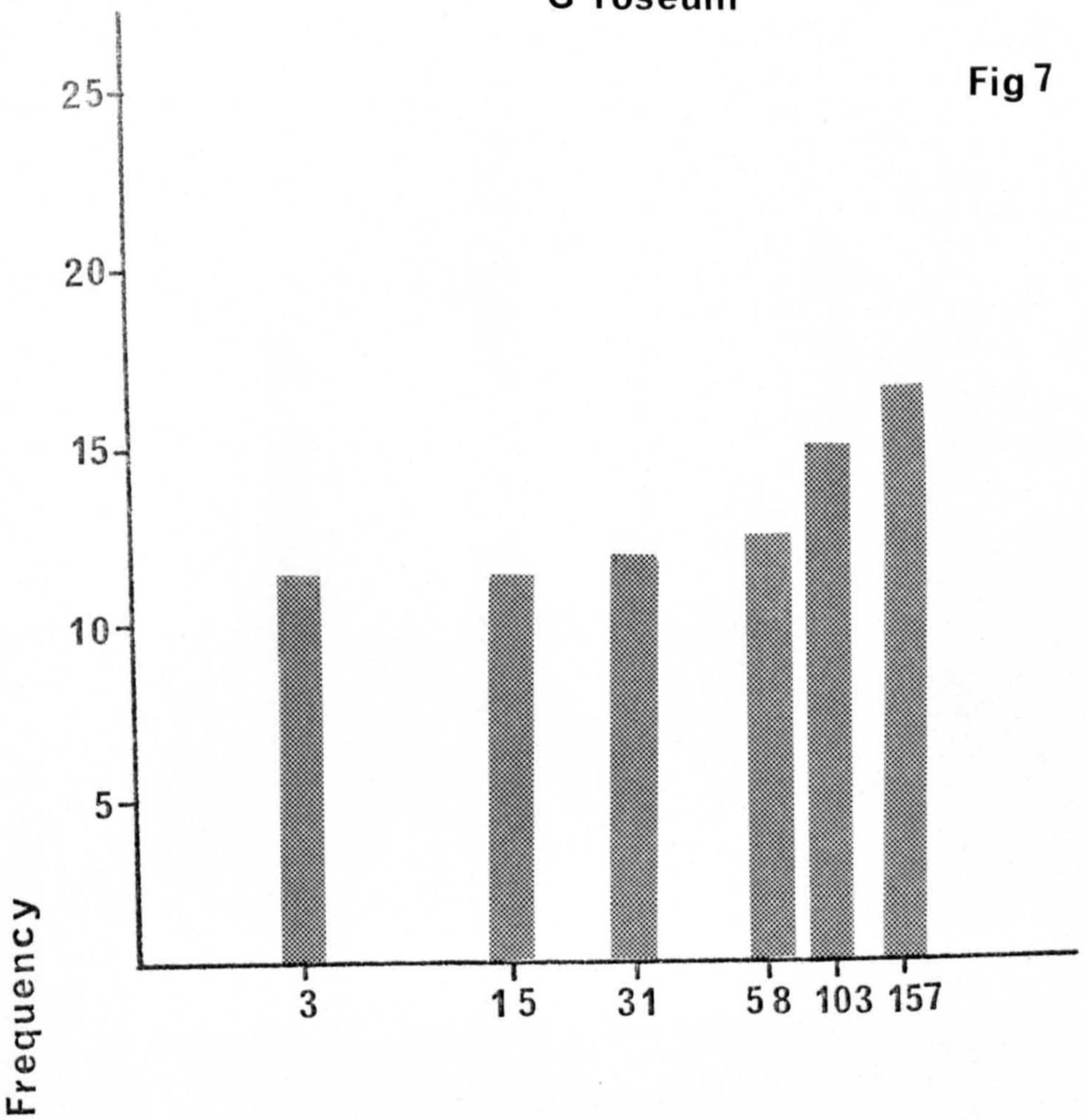
Fig. 7. The percentage frequency of isolation of G. roseum following the treatment of field soils with fungicides

(a) Control

(b) Captan

G roseum

Fig 7



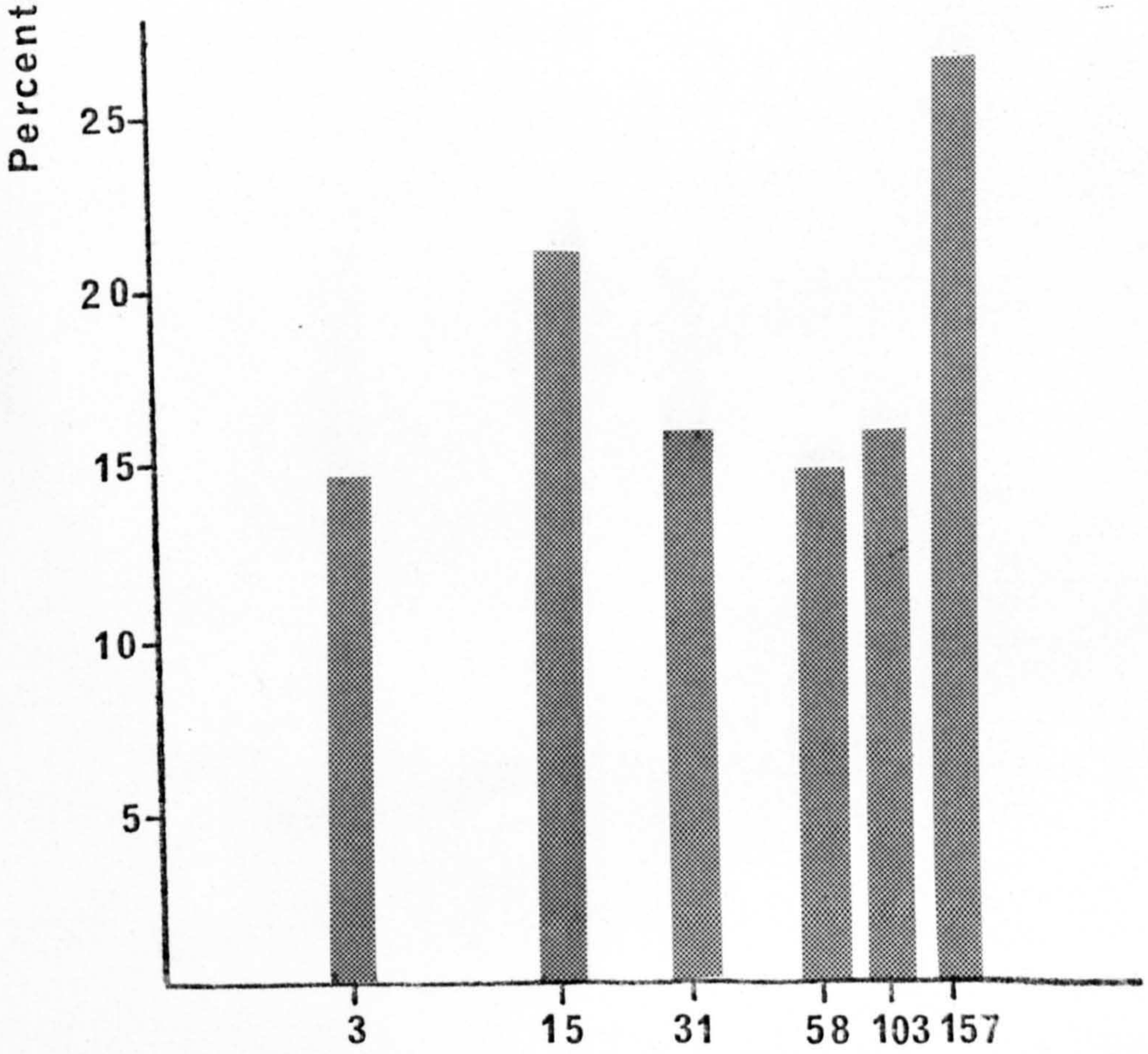


Fig. 7 The percentage frequency of isolation of G. roseum following the treatment of field soils with fungicides

(c) Dicloran

(d) Milcol

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Fig 7

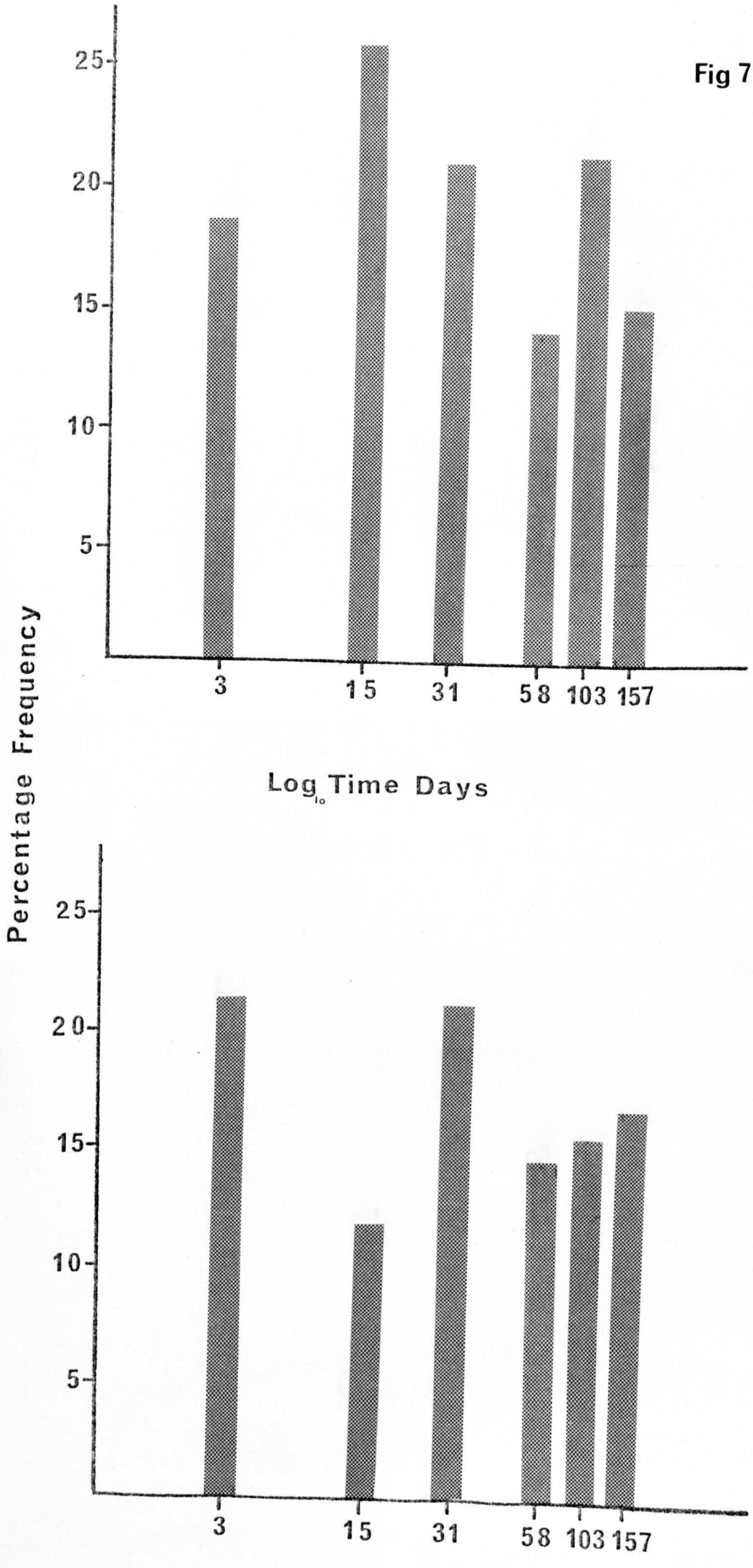
Percentage Frequency
25
20
15
10
5
3
15
31
58
103
157
Log10 Time Days
25
20
15
10
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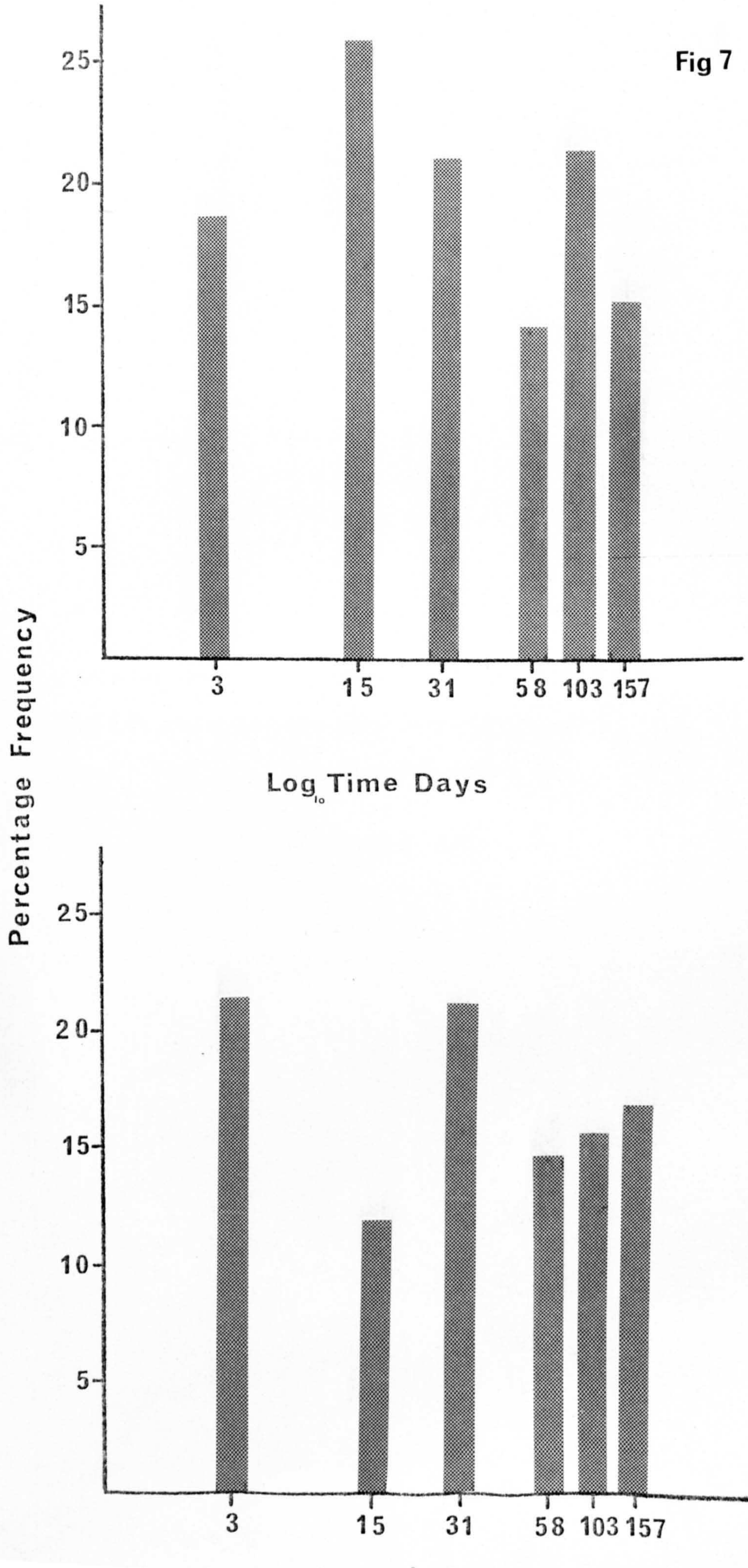
Fig. 7 The percentage frequency of isolation of G. roseum following the treatment of field soils with fungicides

(c) Dicloran

(d) Milcol

Fig 7


Percentage Frequency
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Log10 Time Days
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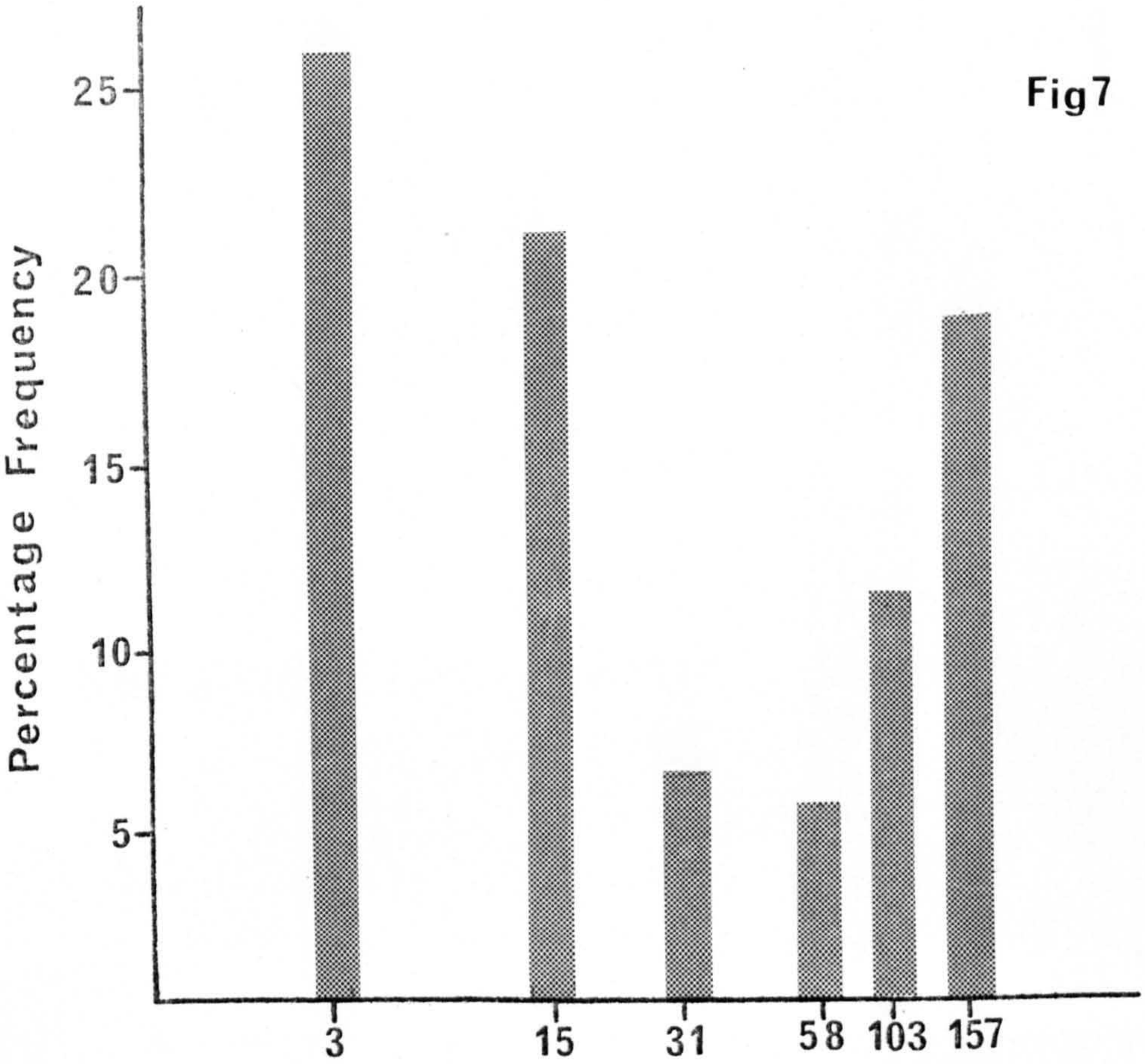
Fig. 7 The percentage frequency of isolation of G. roseum following the treatment of field soils with fungicides

(e) Triarimol

Fig7


Percentage Frequency
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Log Time Days
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Fig. 8 The percentage frequency of isolation of H. grisea following the treatment of field soils with fungicides

(a) Control

(b) Captan

H grisea

Fiq 8

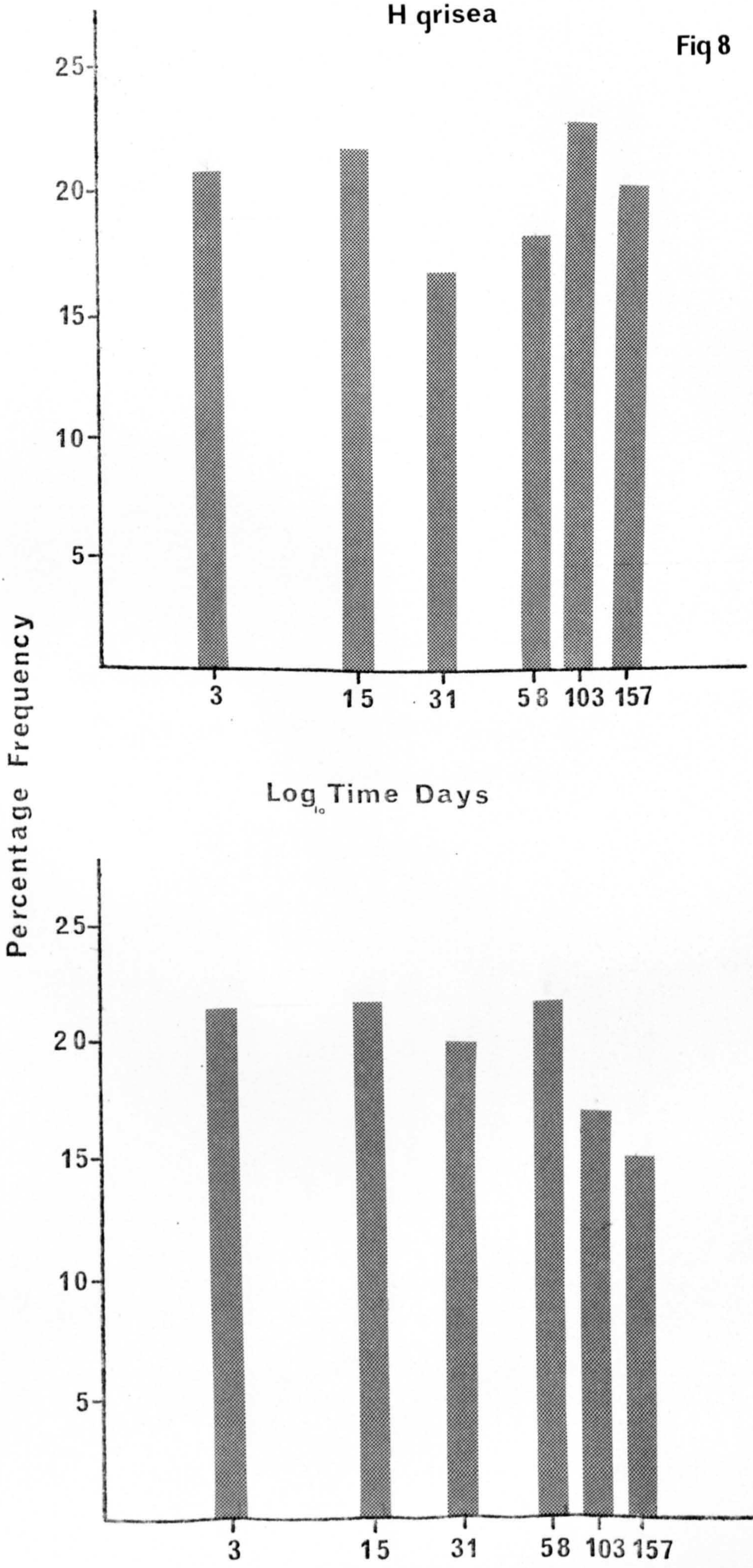
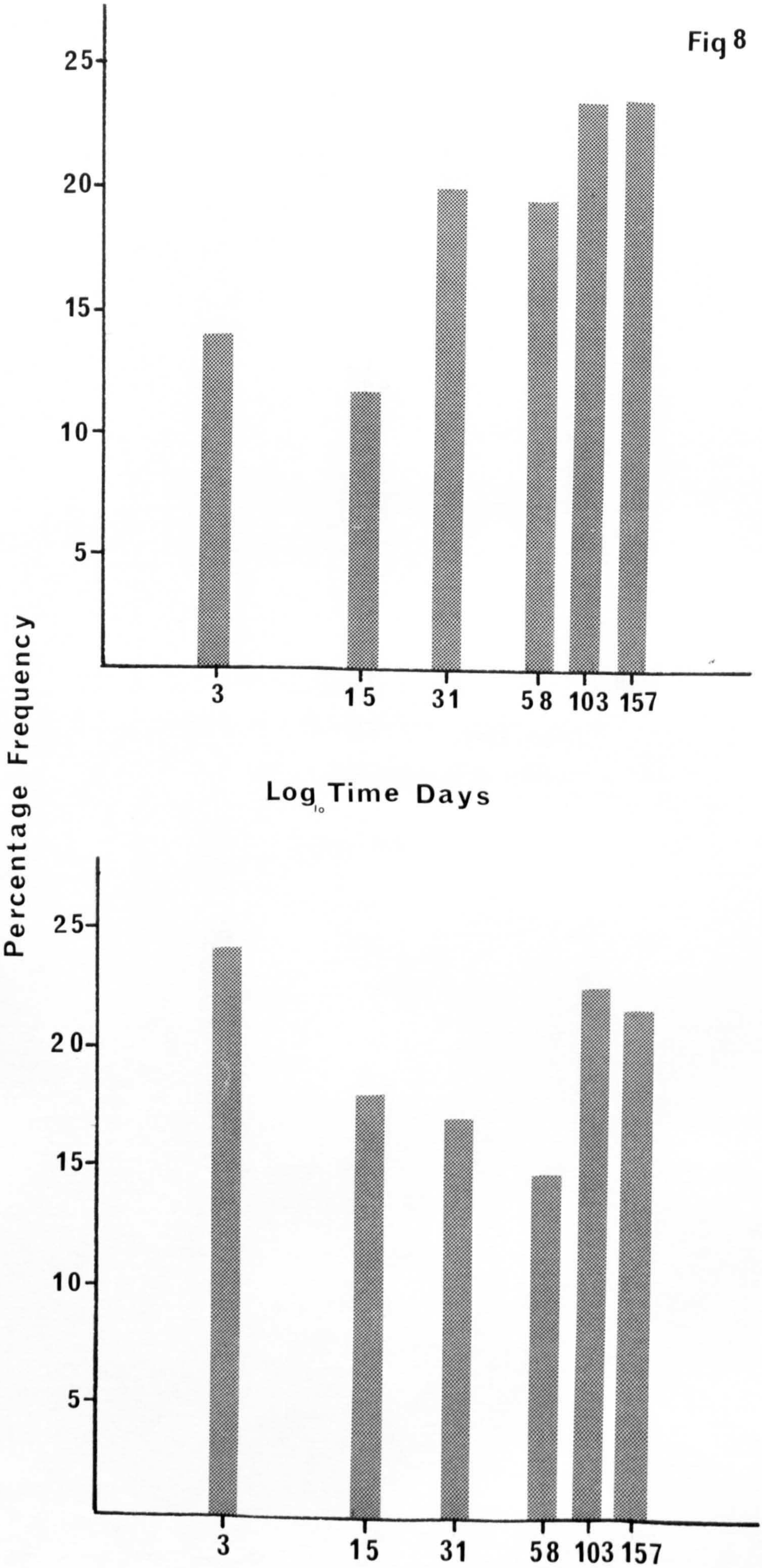


Fig. 8 The percentage frequency of isolation of H. grisea following the treatment of field soils with fungicides

(c) Dicloran

(d) Milcol

Fig 8


Frequency
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Log10 Time Days
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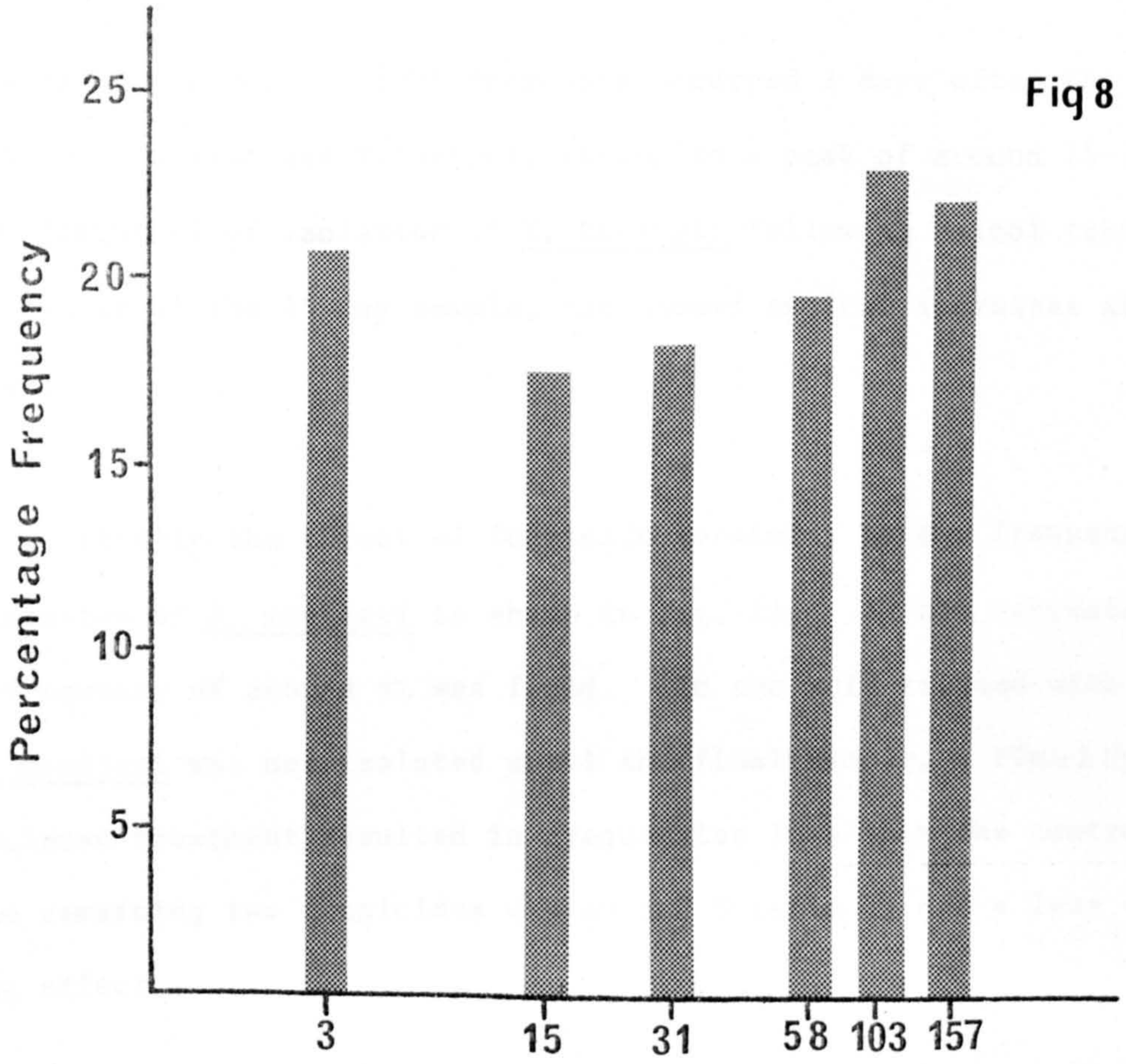
Fig. 8 The percentage frequency of isolation of H. grisea following the treatment of field soils with fungicides

(e) Triarimol

Fig 8


Percentage Frequency
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103
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Log10 Time Days

isolation period. Slight increases occurred 3 days after the application of Dicloran and Triarimol, rising to a peak of around 15-20%. The frequency of isolation of T. koningii following Milcol treatment decreased at the 15 day sample, but showed typical increases after 31 days.

Finally the effect of fungicide treatment on the frequency of isolation of Z. moelleri is shown in Fig. 11. In the untreated soils a frequency of around 5% was found. In the soil treated with Milcol, Z. moelleri was not isolated until the final sample. Finally Dicloran treatment resulted in frequencies less than the control, while the remaining two fungicides Captan and Triarimol, had a less devastating effect.

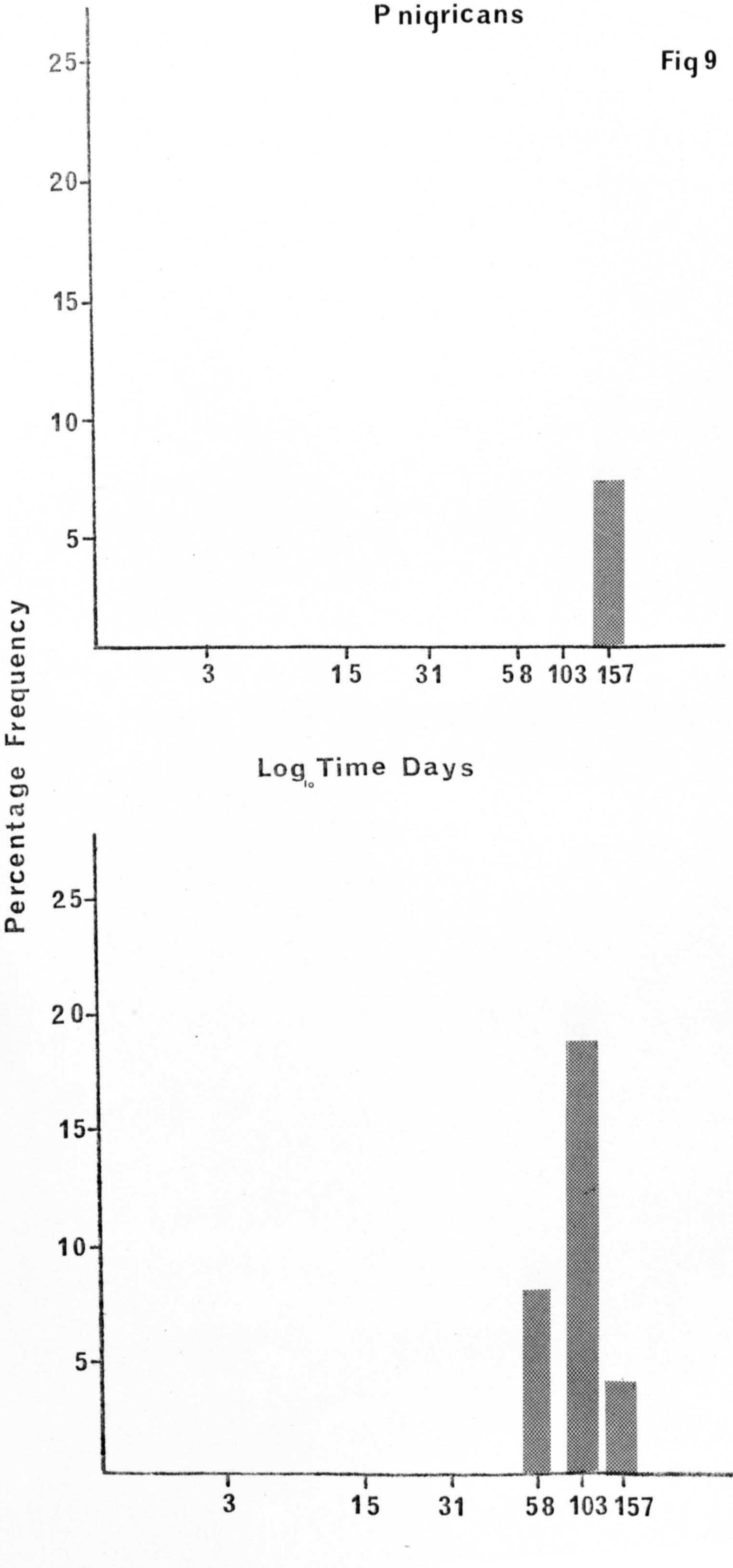
The effect of fungicides on the frequency of isolation of the fungal species can be summarised thus :-

1. F. culmorum and G. roseum. The frequency distribution of these fungi following treatment was typified by a peak in frequency higher than the control, in the middle of the isolation period.
2. P. nigricans. Showed an increased frequency towards the end of the incubation period, and was a major recolonizer of treated soils after 31 or 58 days.
3. T. koningii. The frequency of isolation of this fungus was generally markedly increased by fungicide treatment.
4. H. grisea. Was only marginally affected by treatment.
5. Z. moelleri. The only non-cellulolytic species included was markedly decreased in soils treated with Dicloran and Milcol.

Fig. 9 The percentage frequency of isolation of P. nigricans following the treatment of field soils with fungicides

(a) Control

(b) Captan


P nigricans
Fig 9
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Frequency
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Log10 Time Days
Percentage
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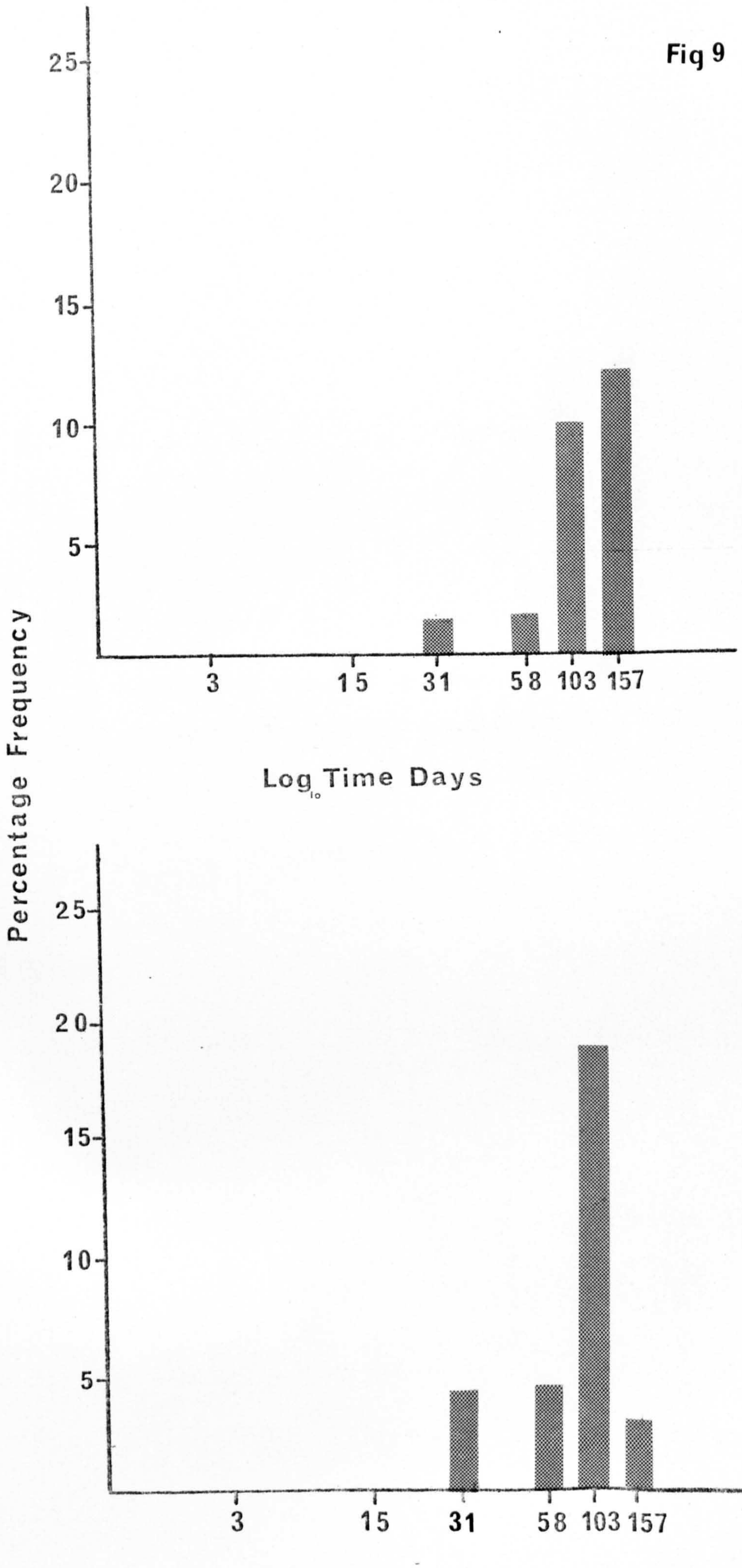
Fig. 9 The percentage frequency of isolation of P. nigricans following the treatment of field soils with fungicides

(c) Dicloran

(d) Milcol

Fig 9


Frequency
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Log10 Time Days
Percentage
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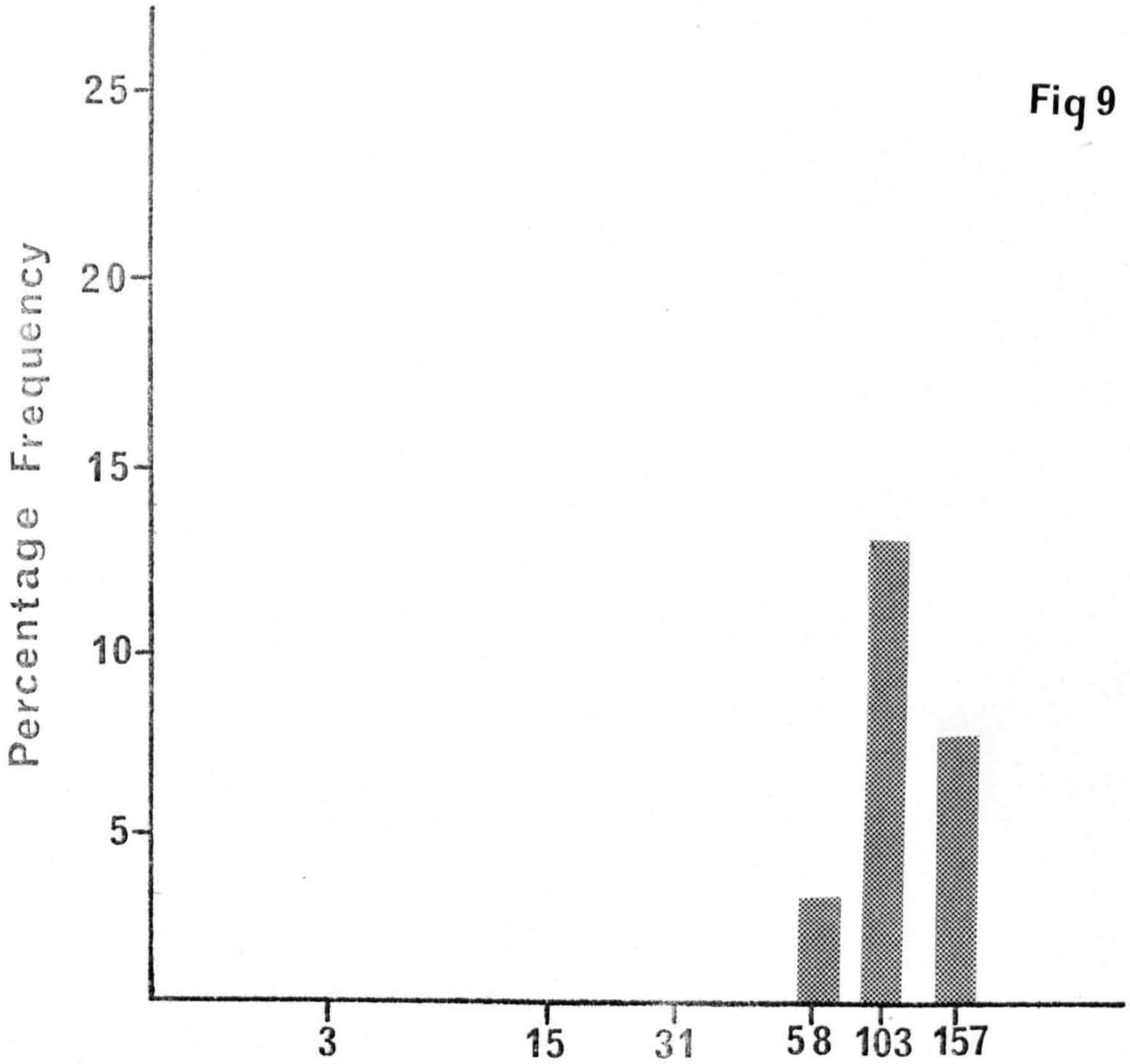
Fig. 9 The percentage frequency of isolation of P. nigricans following the treatment of field soils with fungicides

(e) Triarimol

Fig 9


Percentage Frequency
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103
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Log Time Days
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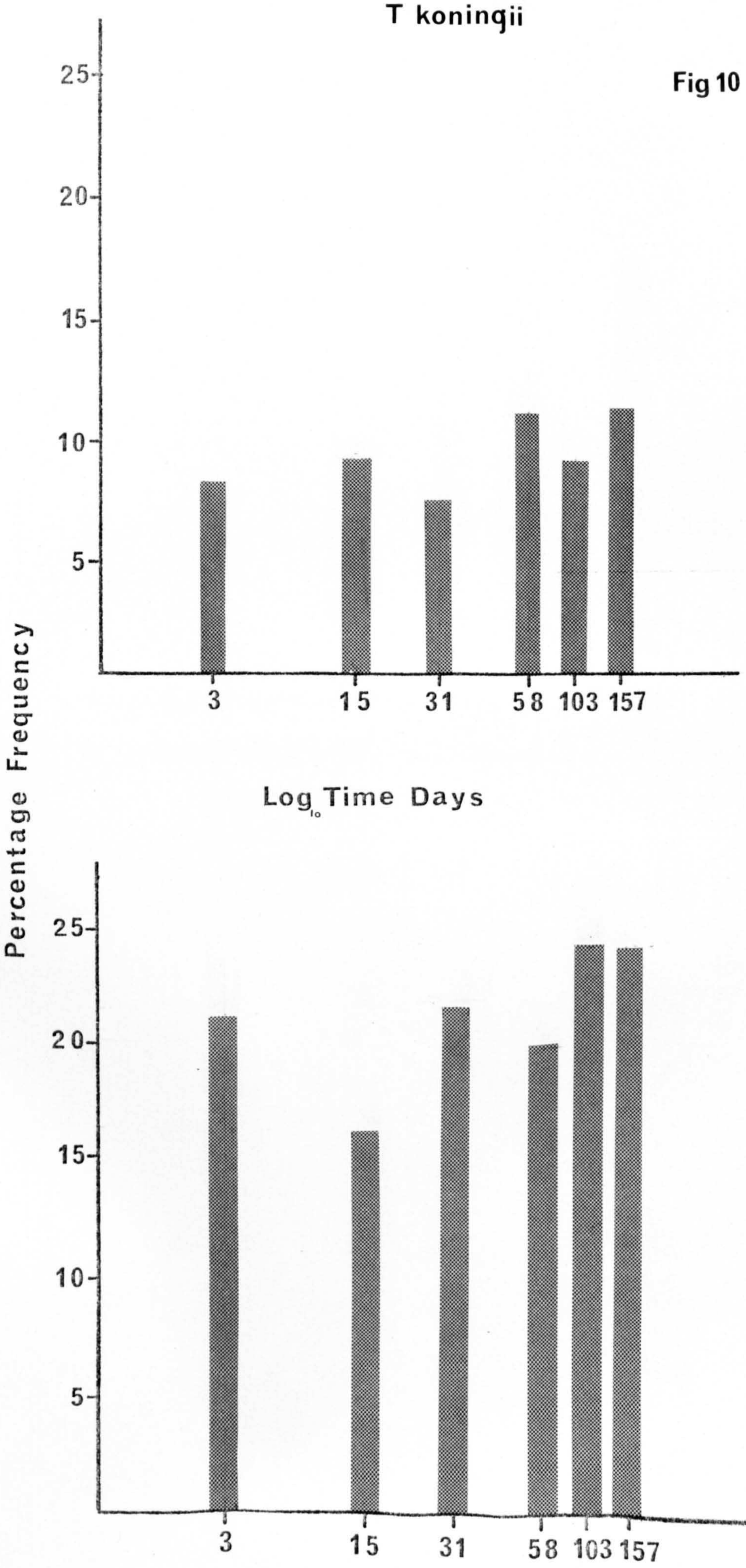
Fig. 10 The percentage frequency of isolation of T. koningii following the treatment of field soils with fungicides

(a) Control

(b) Captan


T koningii
Fig 10
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Frequency
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Log10 Time Days
Percentage
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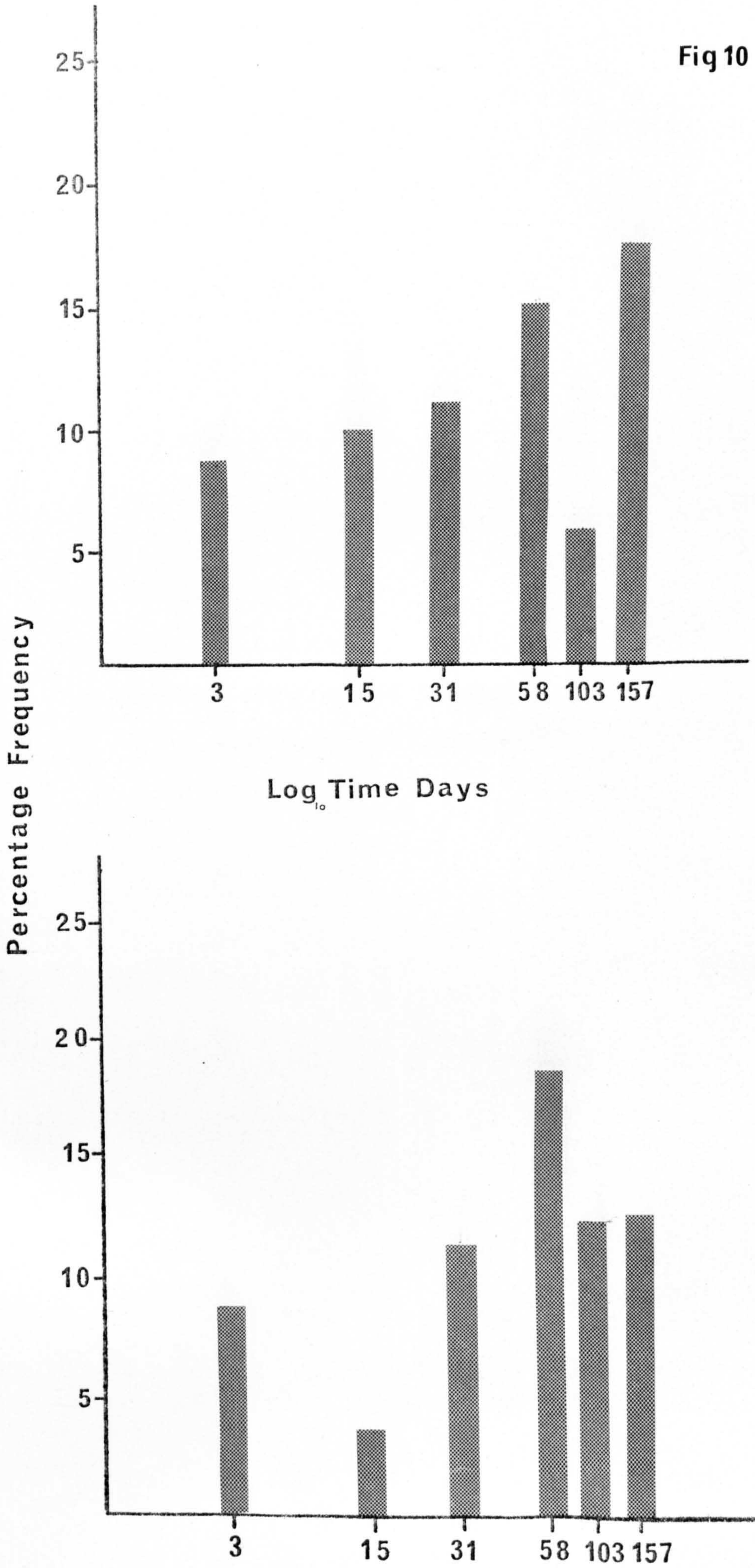
Fig. 10 The percentage frequency of isolation of T. koningii following the treatment of field soils with fungicides

(c) Dicloran

(d) Milcol

Fig 10


Frequency
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Log10 Time Days
Percentage
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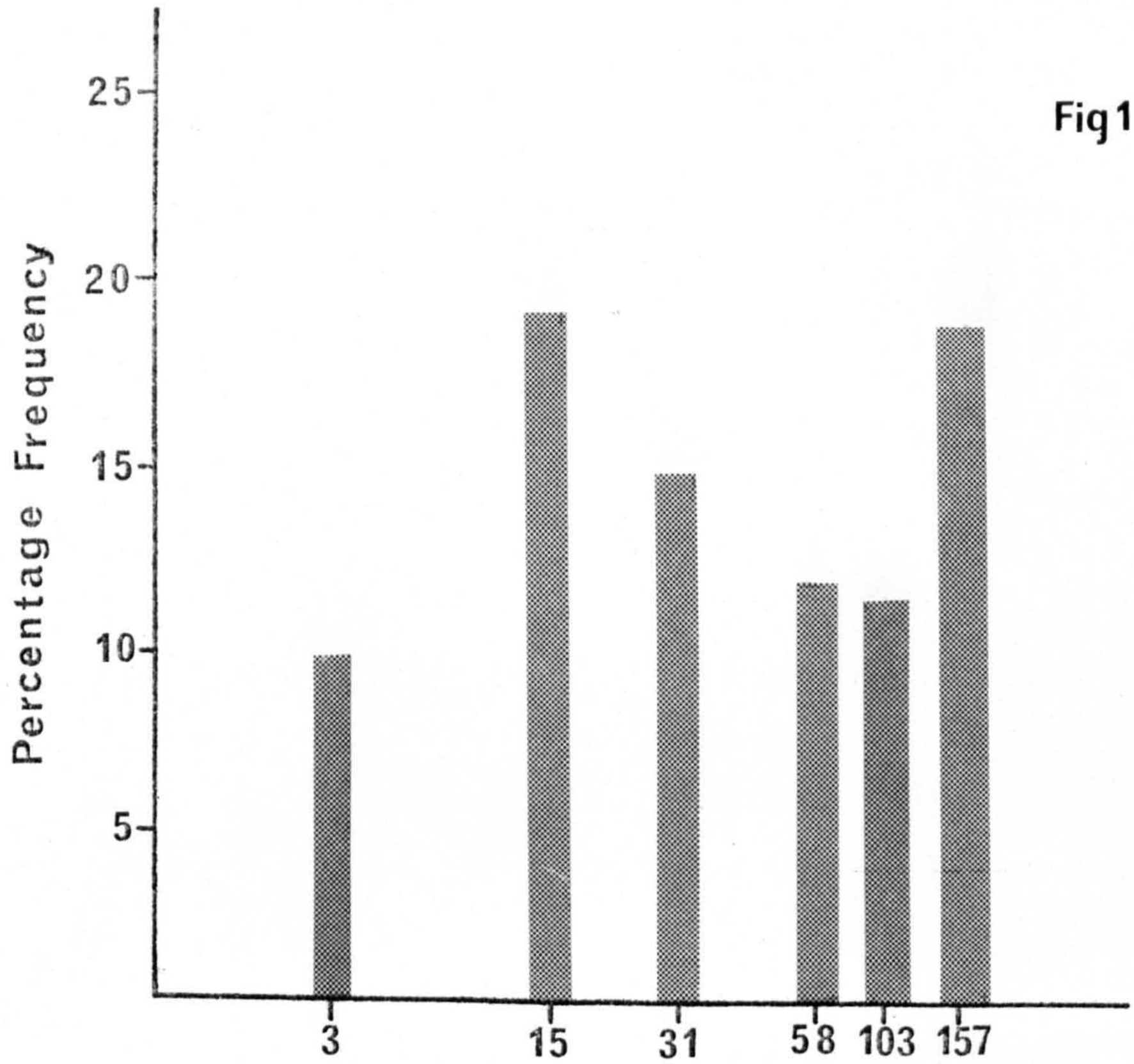
Fig. 10 The percentage frequency of isolation of *T. koningii* following the treatment of field soils with fungicides

(e) Triarimol

Fig 10


Percentage Frequency
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Log Time Days
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Fig. 11 The percentage frequency of isolation of Z. moelleri following the treatment of field soils with fungicides

(a) Control

(b) Captan

Z moelleri

Fig 11

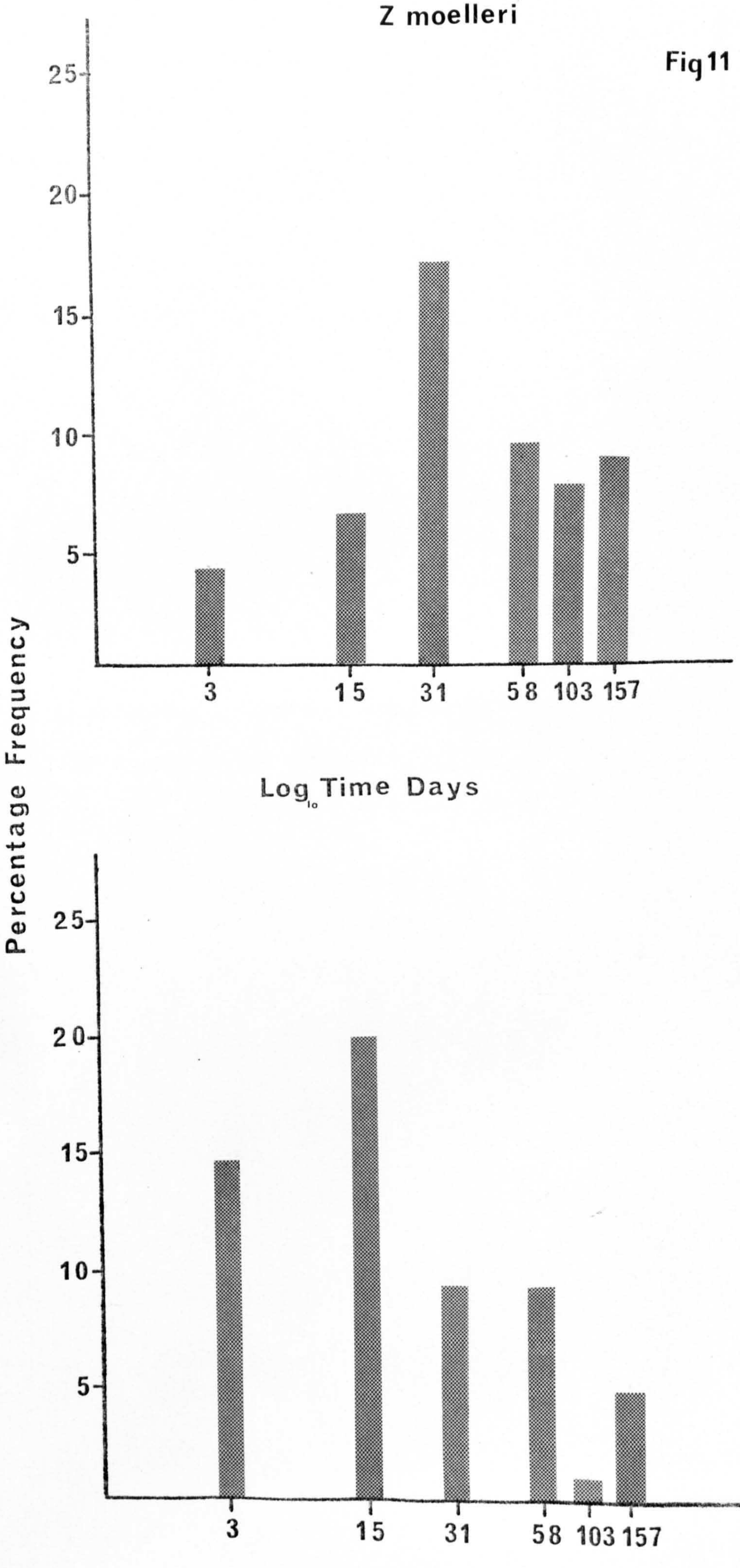
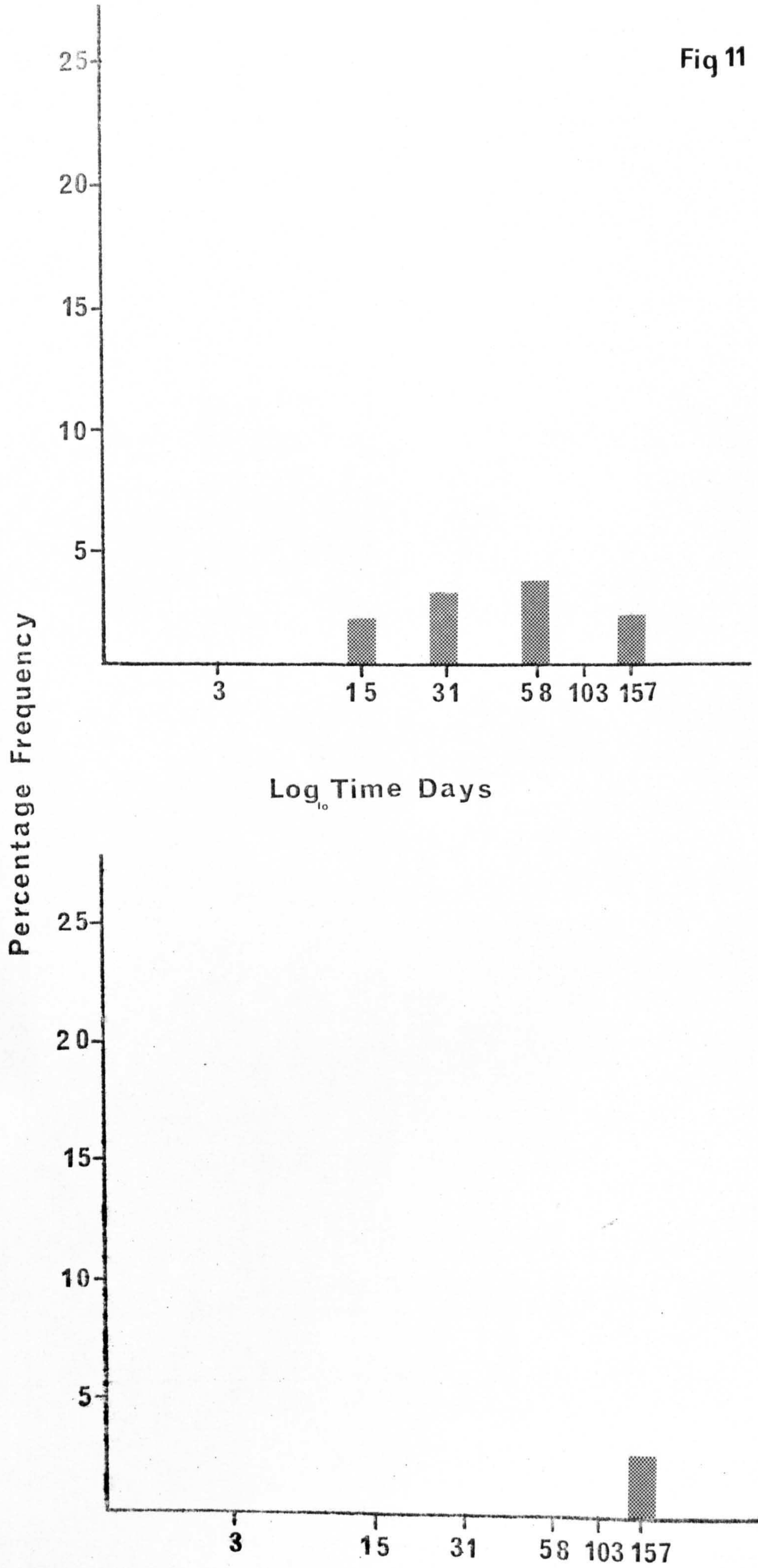


Fig. 11 The percentage frequency of isolation of *Z. moelleri* following the treatment of field soils with fungicides

(c) Dicloran

(d) Milcol

Fig 11


Frequency
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103
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Log10 Time Days
Percentage
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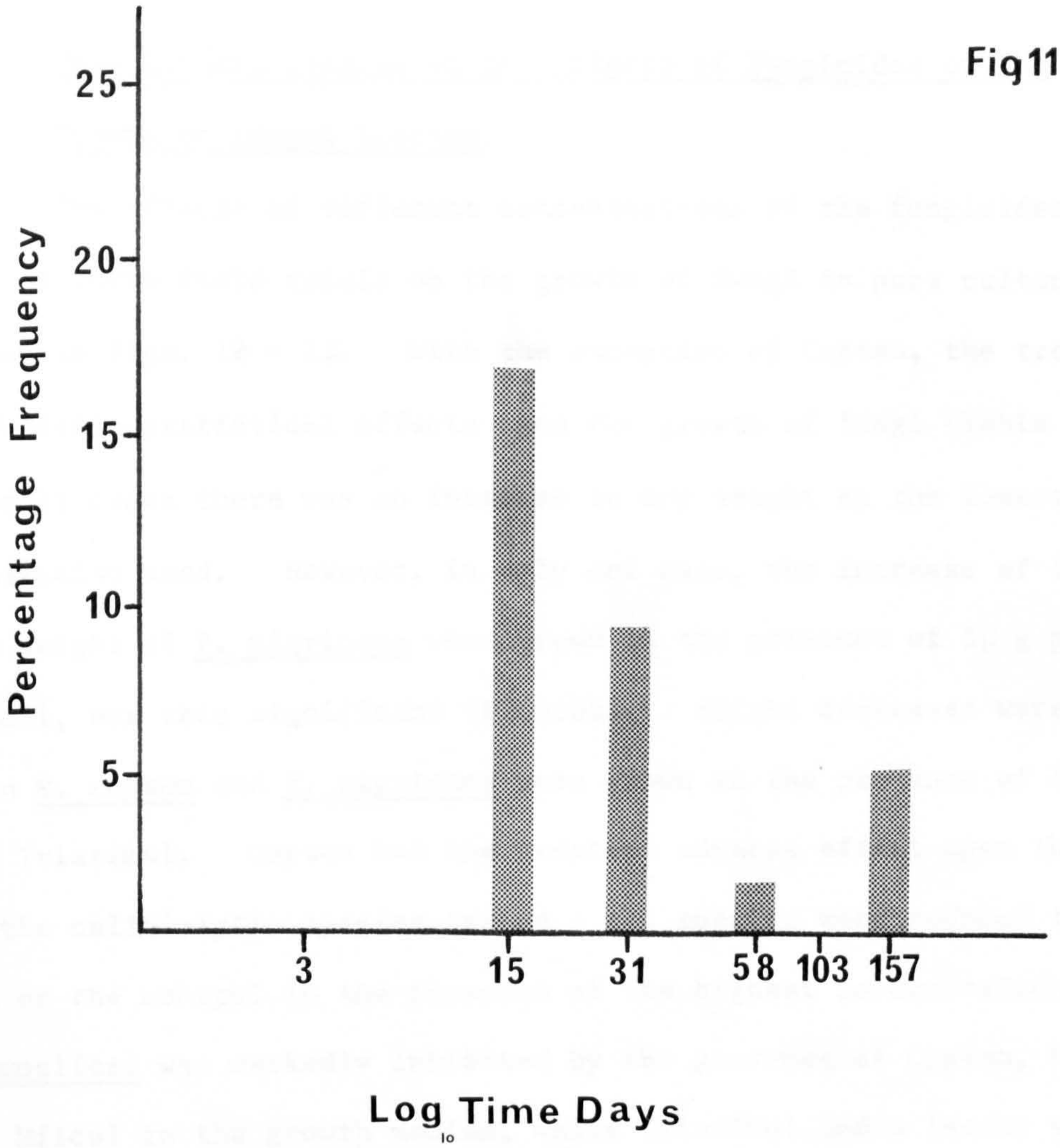
Fig. 11 The percentage frequency of isolation of Z. moelleri following the treatment of field soils with fungicides

(e) Triarimol

Fig 11


Percentage Frequency
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31
58
103
157
Log Time Days
10

Pure Culture Studies on the Effects of Fungicides on the Growth of Fungal Species.

The effects of different concentrations of the fungicides used in the above field trials on the growth of fungi in pure culture are shown in Figs. 1a - 1z. With the exception of Captan, the treatments had little statistical effects upon the growth of fungi (Table 4). In most cases there was an increase in dry weight at the lowest concentration used. However, in only one case, the increase of 50% dry weight of P. nigricans when grown in the presence of 5μ g per ml Milcol, was this significant ($P > 0.05$). Slight decreases were seen when G. roseum and P. nigricans were grown in the presence of Dicloran and Triarimol. Captan had the greatest adverse effect upon the growth of the cellulolytic species tested - all species were reduced to below 25% of the control in the presence of the highest concentrations. Z. moelleri was markedly inhibited by the presence of Captan, Dicloran and Milcol in the growth medium, while Triarimol had a lesser effect.

Fig. 12 Effect of fungicides on the growth of F. culmorum

Mycelial dry wt. as a percentage of the control

(a) Captan

(b) Dicloran

(c) Milcol

(d) Triarimol

Fig 12

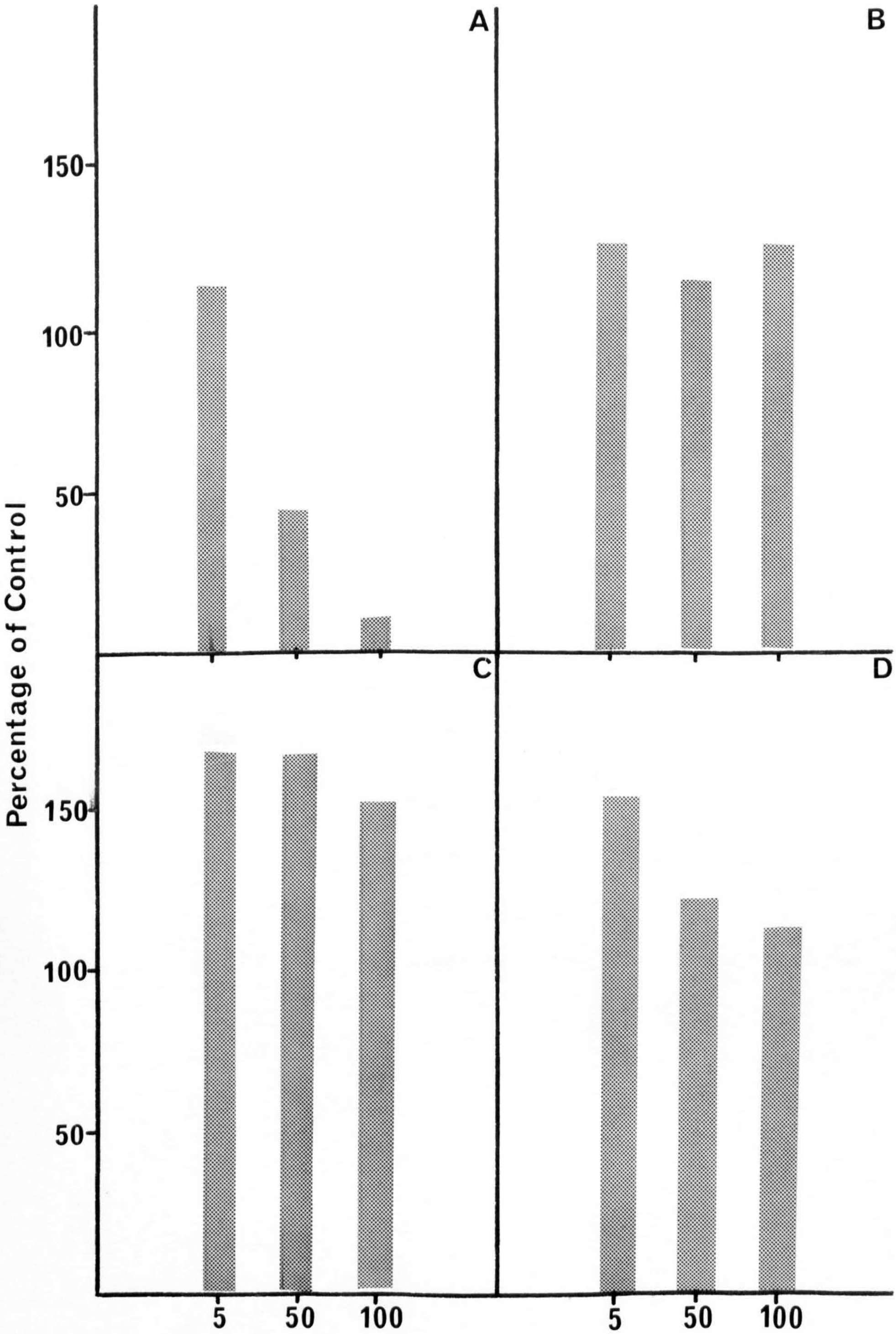

A
B
C
D
Percentage of Control
150
100
50
150
100
50
5
50
100
5
50
100
Fungicide µg/ml

Fig. 13 Effect of fungicides on the growth of

G. roseum

Mycelial dry wt. as a percentage of

the control

(a) Captan

(b) Dicloran

(c) Milcol

(d) Triarimol

Fiq 13

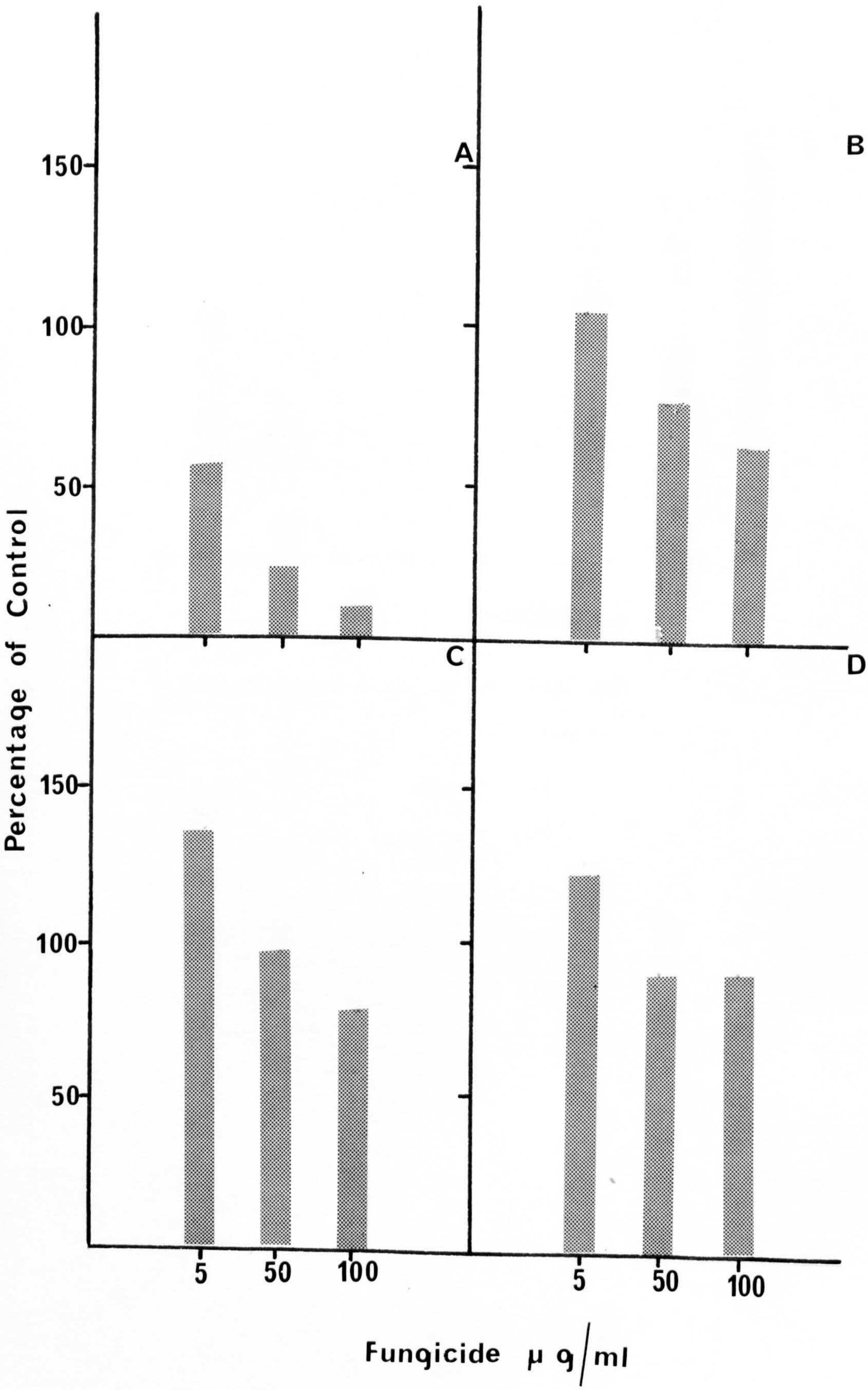

A
B
C
D
150
100
50
Percentage of Control
150
100
50
5
50
100
5
50
100
Fungicide μg/ml

Fig. 14 Effect of fungicides on the growth of *P. nigricans*

Mycelial dry wt. as a percentage of control

(a) Captan

(b) Dicloran

(c) Milcol

(d) Triarimol

Fig 14

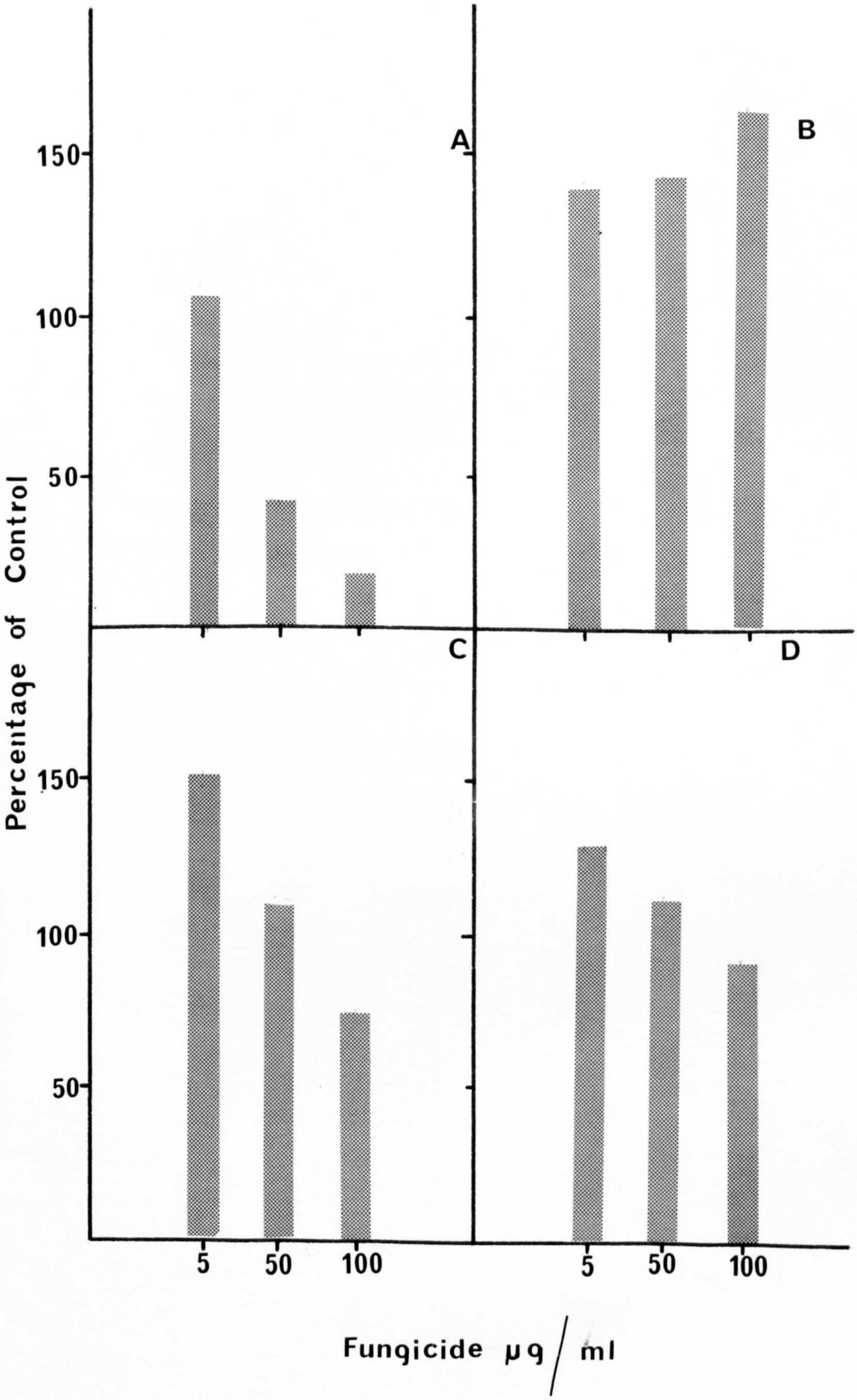

A
B
C
D
150
100
50
Percentage of Control
150
100
50
5
50
100
5
50
100
Fungicide µg / ml

Fig. 15 Effect of fungicides on the growth of

T. koningii

Mycelial dry wt. as a percentage of control

(a) Captan

(b) Dicloran

(c) Milcol

(d) Triarimol

Fig 15

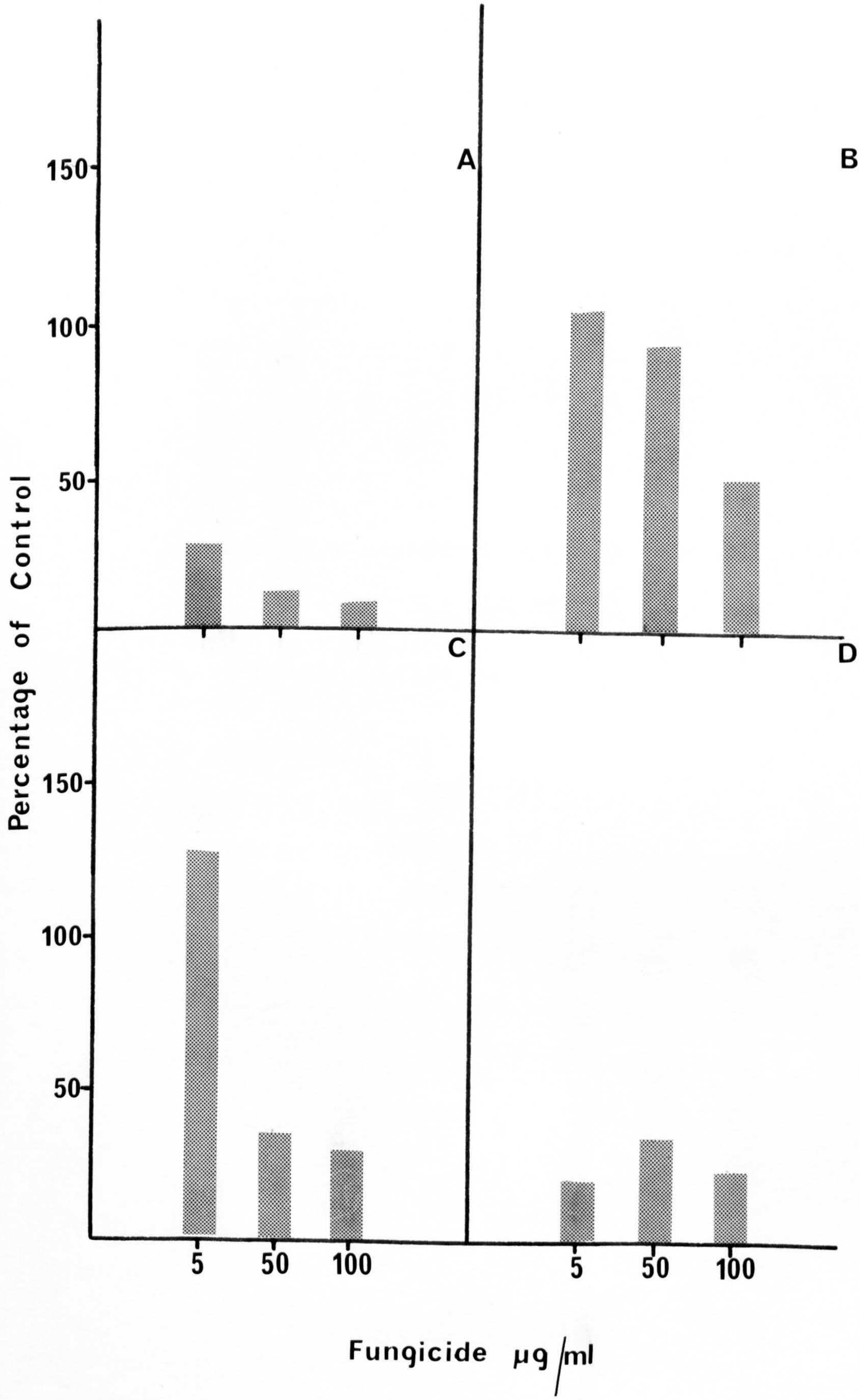

A
B
C
D
150
100
50
Percentage of Control
150
100
50
5
50
100
5
50
100
Fungicide µg/ml

Fig. 16 Effect of fungicides on the growth of Z. moelleri

Mycelial dry wt. as a percentage of control

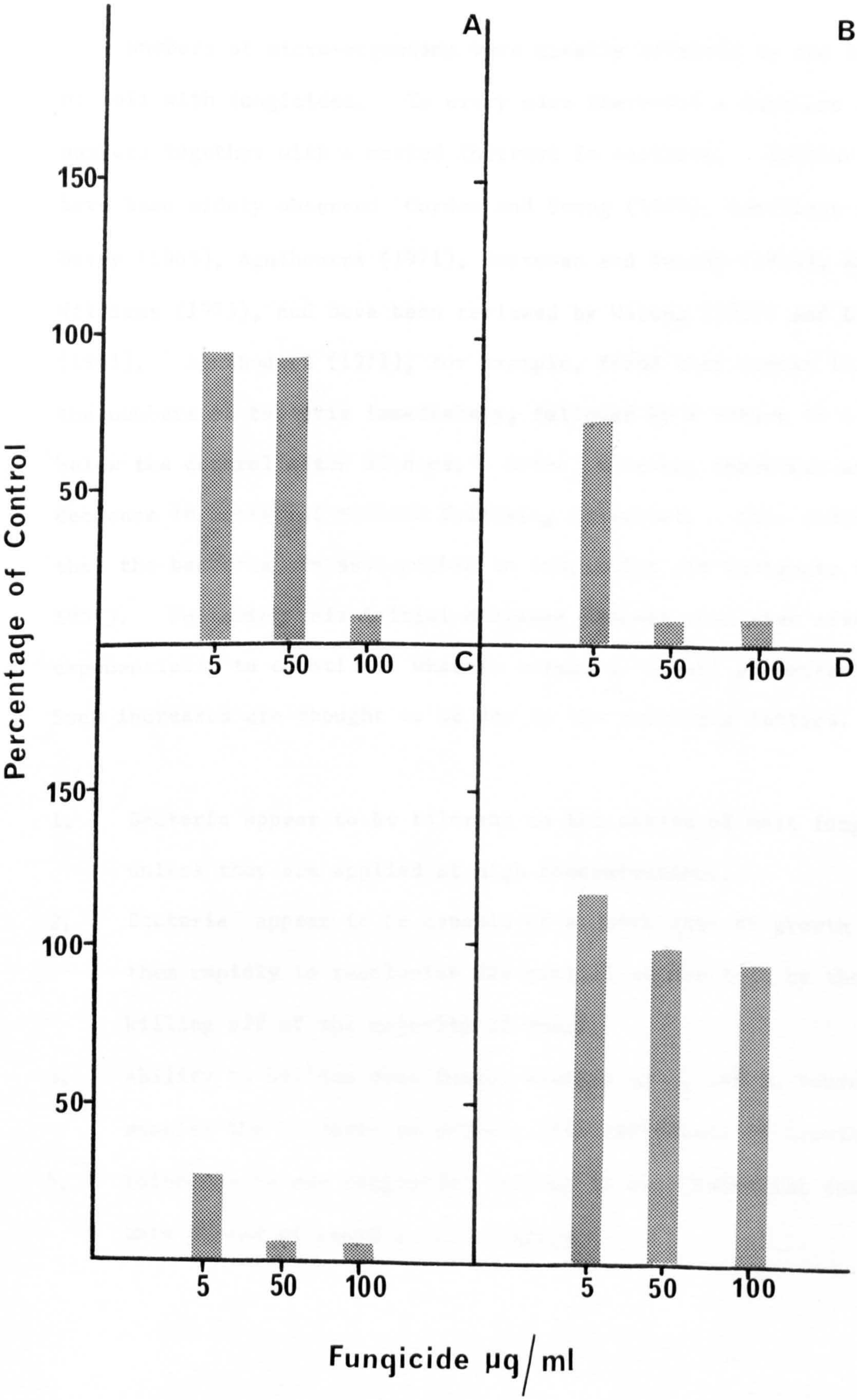
(a) Captan

(b) Dicloran

(c) Milcol

(d) Triarimol

Fig 16


A
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C
D
150
100
50
Percentage of Control
5
50
100
Fungicide µg/ml

DISCUSSION

Numbers of micro-organisms were greatly affected by the treatment of soil with fungicides. In every case there was a decrease in fungal numbers together with a marked increase in bacteria. Similar effects have been widely observed Corden and Young (1965), Danielson and Davey (1969), Agnihoutri (1971), Houseman and Tweedy (1973), and Williams (1973), and have been reviewed by Warcup (1957) and Bollen (1961). Agnihoutri (1971), for example, found that Captan increased the numbers of bacteria immediately, followed by a return to a level below the control after 35 days. Often, however, there was an initial decrease in bacterial numbers following treatment. This indicates that the bacteria are susceptible to fungicides and fumigants (Warcup, 1957). Following this initial decrease numbers then rise often exponentially to constitute what is termed a "flush" of bacteria. Such increases are thought to be due to the following factors.

1. Bacteria appear to be tolerant to the action of most fungicides, unless they are applied at high concentrations.
2. Bacteria appear to be capable of a quick rate of growth enabling them rapidly to recolonize the partial vacuum left by the killing off of the majority of fungi.
3. Ability to utilize dead fungal biomass as C. and N. sources enables the bacteria to achieve this rapid rate of growth.
5. Tolerance to any fungicidal residue is also essential during this period of rapid recolonization.

After the flush, bacterial numbers decline rapidly. As fungal numbers have yet to recover, this decline is more likely to be due to exhaustion of nutrients, rather than to a return of fungal competition. An interesting feature shown in Fig. 3 is the way in which the peaks in bacterial numbers were dependent upon concentration of Captan. Similar findings were reported by Agnihoutri (1971). The highest concentration of fungicide did not produce a peak until after 28 days, while there is a direct relationship between the position of the peak and concentration of Captan for the lower concentrations. This can be explained by assuming that the highest concentration of fungicide, by killing off fungi and other susceptible organisms, provided a large amount of biomass available for use by bacteria. As the concentration of fungicide decreased the amount of biomass made available decreased, so that the flush in bacteria reached a low peak and then declined rapidly. Again this suggests that nutrient availability is the principal factor determining the extent of the bacterial flush.

Little work is available on the species or group composition which makes up this bacterial flush. Williams (1973) found that most of the bacterial colonies found in soils treated with the organo-mercurial fungicide Verdasan were Gram-negative, non-motile bacilli, measuring 4.5 - 5.5 x 1.5 μ. Ridge and Theodoru (1973) found that fluorescent pseudomonads were the dominant type in fumigated soils. This group contains bacteria which are generally considered to be poor competitors, but which are capable of a rapid growth rate in the presence of organic compounds.

An interesting theory was suggested by Dransfield (1956) to account for this increase in bacterial numbers. He found an inverse relationship

between numbers of bacteria in soils treated with T.C.N.B., and protozoa. Protozoa are known to be predatory on soil bacteria and their decline in treated soils may help to explain increases in bacterial numbers.

Fungal numbers, in contrast to bacterial numbers, remained low throughout the early stages of the incubation period, and in soils treated with low concentrations of fungicides eventually exceeded the control. The population at this point however, was not the same as the control population consisting mainly of species of Penicillium and Trichoderma. Again, the inherent defect of the dilution technique of favouring sporulating species may have given a result which is biassed against sterile mycelia and those which only sporulate lightly. Decreases in fungal numbers were also seen in Sutton Bonington field soils. However, numbers of fungi in both sets of field treatments rapidly rose to exceed the control at the end of the isolation period.

Unlike the evidence relating to changes in bacterial and fungal numbers in treated soils, that relating to numbers of actinomycetes is inconclusive. Richardson (1954) found that the dithiocarbamate fungicides had no effect upon actinomycetes. Similarly Wensley (1953) found that actinomycetes were more resistant than either bacteria or fungi to fumigation with ethylene dibromide or methyl bromide. However, Corden and Young (1965) found that Mylone, Nabam and Metasol initially reduced numbers, while Agnihoutri (1971), found that the actinomycete population decreased in the first week after Captan treatment, followed by a marked increase. In the current work it was found that concentrations of Captan approaching 100 µg per g of soil were required to depress actinomycete numbers, while concentrations of 25µ g Captan per g of soil resulted in substantial increases. Clearly the nature and concentration of the fungicide used markedly affects the numbers of actinomycetes which follow its application.

Kreutzer (1965) has reviewed the data relating to the microbial recolonization of treated soils. He listed the following groups in order of decreasing resistance to fungicide or fumigant treatment :-

(1) Actinomycetes

(2) Heterotrophic bacteria

(3) Autotrophic bacteria

(4) Fungi

Amongst the fungi he listed Pythiaceous species as most susceptible followed by fungi of intermediate susceptibility such as Rhizoctonia, Myrothecium, Phoma, Cladosporium, and Paecilomyces. The most resistant of all fungi appeared to be species of Penicillium, Aspergillus and Trichoderma.

The cellulose decomposing fungi as a group have been largely neglected in studies relating to the recolonization of treated soils. Pugh and Williams (1971) and Williams (1973) found that cellulose decomposing fungi were more sensitive to treatment with the mercurial fungicide Verdasan than were non-cellulose decomposers. They found a general reduction in Chaetomium and Fusarium species, Gliomastix murorum var. felinum, Myrothecium striatisporum, Paecilomyces and Penicillium species; while Trichocladium asperum and Trichoderma hamatum were isolated more frequently. However, the fungal recolonization of these soils was complicated by secondary changes, including pH water content, and aeration, which resulted from fungicide treatment. The experiments involving the use of field plots at Sutton Bonington avoided these complications, and demonstrated the direct effect of these fungicides on the frequency of isolation of the cellulolytic species. In addition the effect of treatment on Z. moelleri has been included. Although this fungus is not a cellulose degrader, it is often isolated

on cellulose agar. No doubt its ability to grow as a secondary sugar fungus, utilizing the sugars released by true cellulose-decomposers, together with a fast growth rate, enabled it to do this.

Generally the results of the trials indicated that cellulolytic species were resistant to fungicide treatment. This resistance was further emphasised by lack of inhibition when grown in pure culture in the presence of fungicides.

The frequency of isolation of T. koningii was markedly increased by all treatments. Species of Trichoderma are major recolonizers of soils treated with either fungicides or fumigants. (Martin, 1950; Warcup, 1951; Mollison, 1953; Evans, 1955; Saksena, 1960; Moubasher, 1963; Corden and Young, 1965; Moubasher and Mazen, 1971; Agnihoutri, 1971; Williams, 1973; Wainwright and Pugh, 1974.) This list is by no means exhaustive, but amply demonstrates the importance of this genus as a major recolonizer of treated soils.

A number of workers have studied the effect of fungicides or fumigants on this fungus, in order to determine why it is consistently dominant in treated soils. Evans (1955) and Saksena (1960) looked at the effect of carbon disulphide fumigation on T. viride. These studies were prior to the key of Rifai (1969), so that T. viride quoted by early workers is not necessarily synonomous with T. viride of the Rifai key. Saksena (1960) found that the degree of tolerance towards carbon disulphide shown by T. viride was by no means exceptional and that this alone failed to account for its dominance. He considered that the dominance of Trichoderma in fumigated soils was due to a moderate degree of fumigant tolerance and an intrinsically rapid growth

rate. Some other fungi, particularly members of the Phycomycetes have more rapid growth rates, but are much less tolerant than Trichoderma to fumigants. In contrast fungi which are tolerant of chemical treatment are often slow growers. Saksena (1960) also suggested that the high concentrations of ammonium-N in treated soils may be utilized by Trichoderma enabling it to grow rapidly. A similar view was expressed by Wainwright and Pugh (1974).

Mughugho (1968) has analysed the dominant Trichoderma species in fumigated soils using the Rifai (1969) key. The analysis revealed that several species groups, namely T. hamatum, T. harzianum, T.koningii and T. viride were consistently present.

Much of the interest in the increase of Trichoderma in treated (particularly fumigated) soils was aroused by the hypothesis of Bliss (1951), who suggested that the killing of Armillaria mellea in infected roots by carbon disulphide was not due to direct fungicidal action, but to the activity of T. viride. The experiments of Garrett (1957), and Saksena (1960) suggested that this hypothesis was partially correct.

Mughogho (1968) also found that Penicillium nigricans was a dominant recolonizer of fumigated soils, as did Martin, Baines and Erwin (1957). In alkaline soils P. nigricans tended to replace Trichoderma as the dominant recolonizer. Lilly (1961), for example, found it to be the dominant species in fumigated soils of pH 7.0 - 7.5.

In this work P. nigricans was isolated with negligible frequency in the control soils, but became a dominant recolonizer following fungicide treatment.

The frequency of H. grisea appeared to be only marginally affected by fungicide treatment, except for the occasional slight increase.

Fusarium culmorum and Gliocladium roseum were dominant in treated soils 15 - 31 days after application of fungicide but declined towards the end of the isolation period. Species of Gliocladium were also found to be dominant in soils treated with a variety of fumigants (Martin, Baines and Erwin, 1957).

The only fungus represented in the results which showed a marked decrease in frequency following treatment was Z. moelleri. This agrees with the general finding that Phycomycetes are very susceptible to fungicide treatment.

The growth of these species in culture medium to which fungicide was added was determined in an attempt to the patterns seen in the field. Only Captan had a markedly deleterious effect on the growth of cellulolytic species, while Captan, Milcol, and Dicloran adversely affected the growth of Z. moelleri; the non-cellulolytic species included for comparison. There were increases in dry weight, most of which were not statistically significant. Certain treatments however, (for example the effect of Milcol on the growth of P. nigricans) did result in a significant increase in dry weight. Similar increases were found by Nutman and Roberts (1962), and Williams (1973), who found that addition of the mercurial fungicide Verdasan increased the dry weight of P. crustosum, C. pannorum; and F. moniliforme by as much as 20%. Similarly Nutman and Roberts (1962) found that at low concentrations a wide range of fungicides including organo-mercurials, Captan, and dithiocarbamates stimulated both conidial germination and growth of

Colletotrichum coffeanum. They found that widely dissimilar compounds had similar stimulatory effects. It is doubtful whether the concentrations of fungicides were sufficient to act as added substrate, particularly when the increases occurred at the lowest fungicide concentration, and in nutrient rich conditions. A more likely explanation was that the fungicides inhibited a feedback mechanism, or stimulated the production of a storage material like lipid, which in turn increased the dry weight. Clearly further physiological work is required to elucidate this problem.

Fungicides have a marked effect on the microbial equilibrium of soils, resulting in large increases in bacterial numbers, and a decrease in fungal numbers. Cellulose decomposing fungi on the whole did not appear to be adversely affected by the treatments. Even so after only one application at normal rates, changes in the pattern of fungal colonization occurred. Many of these changes are difficult to interpret, and even more difficult to relate to pure culture studies. The latter in fact bear little relation to the natural environment of soil micro-organisms. It is now generally considered that micro-organisms exist in soil in a state of semi-starvation, flaring up only intermittently with the appearance of new substrates. Pure cultures on the other hand provide optimum nutrient conditions, and direct contact between the fungus and the fungicide.

Williams (1973) has shown that continuous treatment of soils with an organo-mercurial fungicide can have a deleterious effect on the structure and microbiology of soils. It is possible that continued treatment with the fungicides used here may have similar though less marked effects. However, there is little evidence in the literature to suggest that this is the case, mainly because these fungicides exhibit low persistence in soils.

There can be no doubt that disturbances in the microbial equilibrium has a profound effect upon the biochemistry of treated soils. Again however, the problem arises of how to assess the effects of these changes on soil fertility.

THE EFFECT OF FUNGICIDES ON NITROGEN MINERALIZATION IN SOILS

Introduction

Nitrogen, a major building block of the protein molecule, is essential for the growth of plants, animals and micro-organisms. Viets (1965), for example, found that plants contain more nitrogen than any other element with the exception of hydrogen. It is not surprising then that the growth of agricultural plants is limited more often by a deficiency of nitrogen than by any other element (Black, 1968). There is an extensive literature on soil nitrogen including monographs by Alexander (1961), Bartholomew and Clark (1965), Bremner, (1965) Campbell and Lees (1967), and Black (1968).

Nitrogen undergoes a number of transformations in soil involving organic, inorganic and volatile compounds. These transformations result from the activities of micro-organisms, particularly the bacteria (Alexander, 1961); and are generally viewed of in terms of a nitrogen cycle. Campbell and Lees (1967) however, state that there is "neither in the soil nor anywhere else, a nitrogen cycle;" but for the sake of simplicity a cycle in which the form of the element is constantly altered by the activities of micro-organisms can be assumed. This cycle can be summarized thus:-

1. A small part of the large quantities of N_2 in the atmosphere is converted to the organic form by bacteria which are either free-living, or associated with plants. This is termed NON-SYMBIOTIC and SYMBIOTIC NITROGEN FIXATION, respectively.

2. This nitrogen is then utilized by plants, animals, and micro-organisms to form proteins. In this state the element is largely UNAVAILABLE.

3. On death of the organism this protein-nitrogen is freed by micro-organisms and converted to amino acids and then to the ammonium ion. A process which is termed AMMONIFICATION.

4. The liberated ammonium ions are then converted by bacteria via the nitrite ion to nitrate, in the process of NITRIFICATION.

5. In its ionic state nitrogen may be taken up by plants or micro-organisms, may be leached, or alternatively may be lost to the atmosphere as a result of microbial convertion of nitrate to gaseous nitrogen, termed DENITRIFICATION.

The formation of ionic, or mineral nitrogen, from organic nitrogen is termed NITROGEN MINERALIZATION, while the reverse process by which mineral nitrogen is converted to the organic form is termed NITROGEN IMMOBILIZATION.

MOBILIZATION

The following section is devoted to the effects of fungicides on the mineralization of nitrogen a process which can be summarized:-

$$\text{ORGANIC N} \xrightarrow{(1)} \text{AMINO ACIDS} \xrightarrow{(2)} NH^{+}_{4} \xrightarrow{(3)} NO^{-}_{2} \longrightarrow NO^{-}_{3}$$

The conversion of organic nitrogen to ammonium (1) is mediated by non-specific micro-organisms, while transformation (2) and (3) involve mainly specialized autotrophic bacteria.

(1) AMMONIFICATION

The conversion of organic nitrogen to ammonium (1) is termed ammonification. Research on the subject has been reviewed by Harmsen and van Schreven (1955), Alexander (1961), Harmsen and Kolenbrader (1965), and van Schreven (1965). Ammonification involves non-specific micro-organisms including actinomycetes, bacteria and fungi. These micro-organisms utilizing protease enzymes, break down plant, animal, and microbial proteins which find their way into the soil. Proteases in the soil are under intensive study at the moment. They are intimately associated with organic matter, and appear to be the product of plant debris or of microbial activity (Kadd, 1972). Proteases released by fungi generally function best at pH 4-8, while bacterial proteases have optima around pH 7-8. The free enzymes produced by these micro-organisms hydrolyze the peptide bonds of proteins and peptides and liberate free amino acids, and free carboxyl groups. The amino acids thus liberated serve as both carbon and nitrogen sources for heterotrophic micro-organisms and higher plants. Amino acids however, are generally broken down prior to utilization. Such degradation involves removal of the carboxyl group, by DECARBOXYLATION, thus:-

(a) Deamination by direct removal of ammonia

$$R\ CH_2\ CH\ NH_2\ COOH \longrightarrow RCH = CH\ COOH + \underline{NH_3}$$

(b) Oxidative deamination

$$RCH\ NH_2\ COOH + \tfrac{1}{2}\ O_2 \rightarrow RC\ O\ COOH + \underline{NH_3}$$

(c) Reductive deamination

$$R\ CH\ NH_2\ COOH + 2H \longrightarrow R\ CH_2\ COOH + \underline{NH_3}$$

(d) Decarboxylation

$$R\ CH\ NH_2\ COOH \longrightarrow RCH_2\ NY_2 + \underline{CO_2}$$

(2) SOIL AMINO ACIDS

Amino acids are present in the soil in either the free or the bound form. Between 20 and 50% of the total nitrogen in soils is in the form of α amino acids (Bremner, 1967). The amount and type of amino acid present is generally determined following hydrolysis under reflux with 6.5 NHCl for from 12-24 hours. Chromatographic analysis indicated that soils do not differ greatly in amino acid composition, and that the factors affecting their distribution include climate, cultivation and fertilization (Bremner, 1967). It is generally assumed that the bound amino acids in soils are mainly in the form of proteins or polypeptides. Much of the combined amino acids are presumably in the form of mucopeptide or techoic acids, which are the major constituents of bacterial cell walls.

Free amino acids are present in the soil in very small amounts rarely exceeding 2 µg per g of soil. While some free soil amino acids appear stable, most are extremely labile (Greenwood and Lees, 1960). Greenwood and Lees (1956), reported that most amino acids when added to the soil disappeared within 2-9 days, a pattern confirmed by Schmidt,Putman and Paul (1960), and by Van Driel (1961). The amount of free amino acid extracted from soils is largely dependent upon the extraction technique employed. Numerous extracting agents have been used to characterise the free amino acid content of soil. Early workers used water, but obtained poor recovery of added amino acids. Recently more sophisticated extracting agents have been employed. The most extensive work on free soil amino acids, (Paul and Schmidt, 1961) involved the use of barium hydroxide or ammonium acetate; for which good recovery of both added and residual amino acids was reported. Similarly Sowden and Ivarson (1966) reported good recovery using carbon tetrachloride in con-

junction with a complexing agent.

Free amino acids exist in either the basic acidic, or neutral form. The form in which they exist determines the ease with which they are extracted. Gilbert and Altman (1966) used 20% ethanol as an extracting agent, and obtained good recovery of the acidic and neutral fractions, but poor recovery of the basic ones. The latter are usually unavailable in agricultural soils, while the acidic and neutral amino acids are free in soil solution. In the present study 20% ethanol was used as an extracting agent because of its ability to extract the fractions which are potentially the most important in plant and microbial nutrition.

Free amino acids are produced by the lysis of dead micro-organisms, or by direct excretion from soil micro-organisms. They can be taken up directly by both plants and micro-organisms and appear to be a potentially important nutrient source.

(3) NITRIFICATION

Nitrification is the microbial formation of nitrate from ammonium, via nitrite. The reactions involved are mediated in the main by specific, autotrophic bacteria. Recent evidence however, indicates that the fungi are capable of the limited ability to nitrify. Schmidt (1954), Hora and Iyengar (1960), Odu and Adeoya (1970), and Hatcher and Schmidt (1971). Thus a distinction between AUTOTROPHIC and HETEROTROPHIC NITRIFICATION can be made.

Alexander (1961) considers that there still exists considerable uncertainty as to the status of many of the organisms capable of catalysing ammonium or nitrite oxidation. However, two groups of

bacteria appear to be involved in the process of nitrification. The first obtains energy by the oxidation of ammonium ions to nitrite, while the second oxidizes nitrite ions to nitrate. Species of the genus Nitrosomonas are generally considered to be the most important group involved in the first oxidation; while species of Nitrobacter are important in the second. (Meiklejohn, 1953; 1954). Most workers in the past have regarded the nitrifying bacteria as obligate autotrophs, gaining energy from the oxidative reactions involved in nitrification. Carbon for cell synthesis is thought to be derived from CO_2, carbonates, and bicarbonates, using the energy derived from oxidative reactions. Until recently it was thought that these bacteria were incapable of utilizing organic nutrients. Recently however, Smith and Hoare (1968) found that Nitrobacter agilis could grow on acetate without nitrite or CO_2. This suggested that the bacterium can act as a faculative autotroph. The ability to switch from CO_2 and inorganic N to acetate could confer advantages when growing in the environment, if the former were rate limiting, i.e. nitrifying bacteria can now be considered to be mixotrophs. The whole concept of obligate autotrophy is now under scrutiny, and at least one author considered it to be "a demise of a concept" (Rittenberg, 1972).

The biochemistry of the nitrifying micro-organisms has been reviewed by Lees (1955), Alexander (1961), and Wallace and Nicholas (1969). The biochemical mechanisms whereby the chemoautotrophic bacteria convert ammonium to nitrate was for a long time obscure, but over the past decade the following outline has appeared.

(1) The primary oxidation by Nitrosomonas converts ammonium to nitrite.

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H_2O + 2H^+ \quad F - 84 \text{ K cal.}$$

A process known to occur in at least two steps.

$$NH_4^+ + 0.5O_2 \longrightarrow NH_2OH + H^+ \quad F - 0.7 \text{ K cal.}$$

$$NH_2OH + O_2 \longrightarrow NO_2^- + H_2O + H^+ \quad F - 8.33 \text{ K cal.}$$

(2) The second oxidation involves Nitrobacter and is the oxidation of nitrite ion to nitrate

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^- \quad F - 17.8 \text{ K cal.}$$

Nitrification proceeds most rapidly in near-neutral to alkaline soils. Both Nitrosomonas and Nitrobacter are strict aerobes and so nitrification is inhibited by waterlogging. Nitrification is also a temperature dependant process, which functions best at an optimum temperature of 28-30°C.

In the present study the optimal conditons, in terms of water content, temperature, and pH of the soil used were determined, prior to fungicide treatment, in order to demonstrate how dependant the process of nitrification is on environmental variables. Nitrification can occur in a number of different ecosystems, including forest and grassland soils (Chase, Corke, and Robinson, 1968). However inhibition of nitrification often occurs in grassland soils, presumably due to the presence of an inhibitor (Neal, 1969), which is excreted by grass roots. More recent evidence however suggests that this inhibition is due mainly to the build up of ammonium in these soils. (Odu and Akerele, 1973).

The work presented in this section is a study of the effects of fungicides on nitrogen mineralization. Qualitative and quantitative changes in the free amino acid content of soil have been determined, together with the changes in nitrification and ammonification which follow the application of fungicides.

Materials and Methods

Effect of Fungicides on the Free Amino Acid Content of Soils

The effect of three fungicides, Benomyl, Thiram, and Verdasan, on the acidic and neutral free amino acids was determined. Fungicides were added to fresh sieved Sutton Bonington soil (<2 mm) on the basis of their active ingredient to give final concentrations of 250 and 50 µg of Benomyl and Thiram per g of soil, and 50 and 10 µg Verdasan per g of soil. Duplicate samples (50 g) were weighed into sterile Erlenmeyer flasks which were sealed with "Parafilm" and incubated at 25°C ±1 for 28 days.

The technique of Gilbert and Altman (1966) was used with the following modifications for the extraction of free amino acids. Samples of soil (25 g) were extracted by shaking with 50 ml of 20% V/V ethanol for 20 h at 25°C. The suspensions were filtered by suction and the filtrate was pre-cooled in a methcol/dry-ice slurry then lyophilized to dryness. Finally the residue was taken up in 10 ml of 70% V/V ethanol and stored at 10°C prior to de-salting.

After a period of at least 48 h storage to dissolve the residue, the solutions of free amino-acids in 70% V/V ethanol were de-salted using Dowex 50 W - X8. Cation exchange resin buffered to pH 2 with acetate/HCl buffer. Ammonium hydroxide-washed resin (4 g) was added to 10 mls of solution plus 0.5 ml of buffer. After 20 h the resin was filtered off and eluted with 2 x 10 ml portions of 2N NH_4 Oh; which were evaporated to dryness at 30°C. The residue was then taken in 0.5 ml of propan-2-ol ready for chromatography (Altman, 1969).

The amino acids were detected using two dimensional paper chromatography. Each solution (60 ml) was spotted onto Whatman No. 1 chromatography paper and run at 25°C. The solvents used were firstly butan-1-ol/glacial acetic acid water (2 : 1 : 1); and secondly 2 methyl propan-2-ol/butan-2-one/water solvent (4 : 3 : 3). The individual amino acids were then detected by spraying with 0.05% ninhydrin in butan-1-ol and heating for 2 mins. at 100°C. Identification was based upon spots developed from known standards. A yellow spot, located close to that of the proline standard was found in all extracts. However tests with isatin (0.4% V/V in butan-1-ol) failed to give the blue coloration typical of proline, (Pasieka and Morgan, 1956).

Amino acids were determined quantitatively by cutting out the chromatogram spots and eluting with 4 ml of 1% ninhydrin in water-saturated butan-1-ol buffered by the addition of 1 ml of acetate /HCl buffer (pH.7). The colour was extracted from the paper by immersing the tubes in a water bath at 55°C for 5 mins, followed by heating in a water bath to 80°C for 1 - 2 mins. Final extraction was achieved by the addition of 1 ml of 1% V/V acetone. The intensity of the colour was then determined colorimetrically at 550 nm, using a Unicam sp.600 Spectrophotometer. Development of the chromatograms was strictly standarised, and the results are expressed as a percentage of the control.

Determination of the Optimum Conditions for Nitrification

(1) Effect of pH on Nitrification

Sutton Bonington soil was sieved (<2 mm) and weighed into 100 g.portions. One set of four replicates received calcium

hydroxide (lime), while the other was left unamended. The soils were brought to 66% of their water holding capacity and incubated at 25°C in Erlenmeyer flasks sealed with "Parafilm". At 7 day intervals samples were removed. The nitrate content and pH of the samples was then determined.

(2) Effect of Water Content on Nitrification

Soils were incubated at the following percentages of their water holding capacity (30 ml per 100 g of soil), for 7 days at 25°C. At the end of this period the respecrive amounts of nitrate-N were determined.

(3) Effect of Temperature on Nitrification

Soils were incubated for 7 days at the following temperatures

0, 5, 10, 20, 25, 30, 35, 40, 45, 50, ° C

(4) Effect of Fungicides on Ammonification and Nitrification in soils

The effect of three fungicides, Captan, Thiram and Verdasan on ammonification and nitrification were next determined. Fungicides were added to Sutton Bonington soils as commercial preparations on the basis of their active ingredients. Fresh soil collected immediately before each experimental run was sieved (< 2 mm) and air-dried overnight. Excessive air drying was avoided, since this tends to lead to the increased mineralization of both carbon and nitrogen (Birch, 1959).

To 200 g of air dry soil was added 1 ml of a $(NH_4)_2\ 50_4$ solution contining 200 μg of ammonium-N. Fungicides in solution or in suspension were added at varying concentrations. The soils were then brought to 66% of their water holding capacity with sterile

de-ionized water, to give final concentrations of 1, 5, 10, 15, 25 and 50 μg of Verdasan per g of soil; and 10, 25, 50, 100 and 250 μg of Captan and Thiram per g of soil. Controls with and without an ammonium sulphate amendment were also included. Samples of the soils (50 g) were then weighed into sterile Erlenmeyer flasks. Sufficient $Ca(OH)_2$ (about 0.2 g) was thoroughly mixed with the soils, in order to neutralize the acid produced by nitrification. The flasks were sealed with "Parafilm" to maintain the initial water content throughout the experimental period, and were incubated at 28 ± 1^{o} C Four replicates per treatment were included.

The respective amounts of ammonium and nitrate-N were determined at 7 day intervals for 28 days.

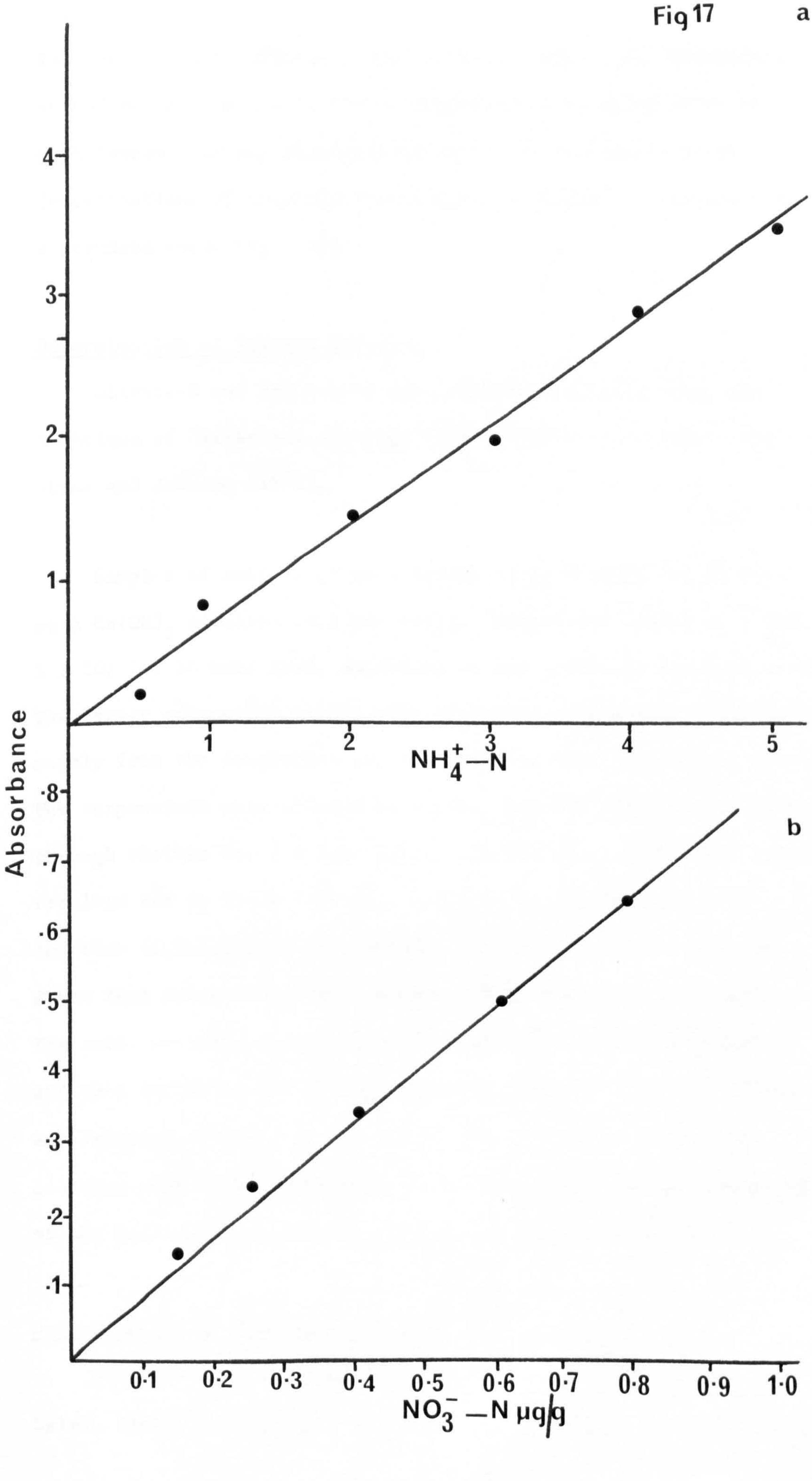
Determination of Ammonium Nitrogen.

Ammonium-N was determined by the indophenol blue method. Samples of soil (2 g) were extracted by shaking with 20 ml of 1.5N KCl for 1 h. The suspensions were filtered through Whatman No. 1 filter paper and then 1 ml of 6% E.D.T.A. was added to 2 ml of filtrate to chelate any metal ions (Tetlow and Wilson, 1964). Then 7 ml of de-ionized water, 5 ml of phenolate and 3 ml of sodium hypochlorite were added from fast-flowing burrettes. The reagents were freshly made up at the concentrations suggested by Parkinson, Gray and Williams (1971). The resulting solutions were thoroughly mixed and incubated at 25^{o}C in a water bath for 20 mins.

Tetlow and Wilson (1964) have shown that the reaction is photosensitive, so the analysis was performed in diffuse daylight and incubated in a darkened water bath. After incubation the solutions were made up to 50 ml with de-ionized water, mixed again, and the

Fig. 17 Ammonium and Nitrate-Nitrogen standard curves

Fig 17 a


4
3
2
1
1
2
3
4
5
NH_4^+—N
Absorbance
·8
·7
·6
·5
·4
·3
·2
·1
b
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
NO_3^-—N µg/g

intensity of the indophenol blue-ammonium complex was determined at 630 nm in a Unicam Sp.600 spectrophotometer, using cells of path-length 1 cm and phenolate-hypochlorite as reagent blank. Concentrations of ammonium-N were then determined by reference to a standard curve (Fig. 17).

Determination of Nitrate Nitrogen

Nitrate-N was determined spectrophotometrically using the technique of Clarke and Jennings (1965) with modifications after Simms and Jackson (1971).

Samples of soil (5 g) were extracted by shaking for 15 mins with $Ca(OH)_2$ solution (0.2 per cent). Extraction ratios of 1 :5; 1 : 10; 1 : 20 were used, depending on the predicted level of nitrate. The larger extraction ratios were preferred to minimize interferences mainly from the fungicides and from nitrite (West and Lyles, 1960). The suspensions were allowed to settle, and the supernatants filtered through Whatman No. 1 filter paper. In all cases clear test solutions resulted and to these 7 ml of a 0.01% (w/w) chromotropic acid solution (C.T.A.) were then added to 3 ml of test solution in Pyrex test tubes (Simms and Jackson, 1971) and mixed thoroughly. The tubes were immediately cooled below 40°C in running tap-water and then incubated for 20 min in a water bath held at 40°C (Clarke and Jennings, 1965). At the end of the incubation period the contents were mixed throughly, cooled below 40°C and the intensity of the yellow C.T.A.-nitrate complex was determined at 430 nm.

Determination of Nitrite Nitrogen

Nitrite-N can interfere with the C.T.A. technique (West and Lyles, 1960). Throughout the course of the experiments semi-

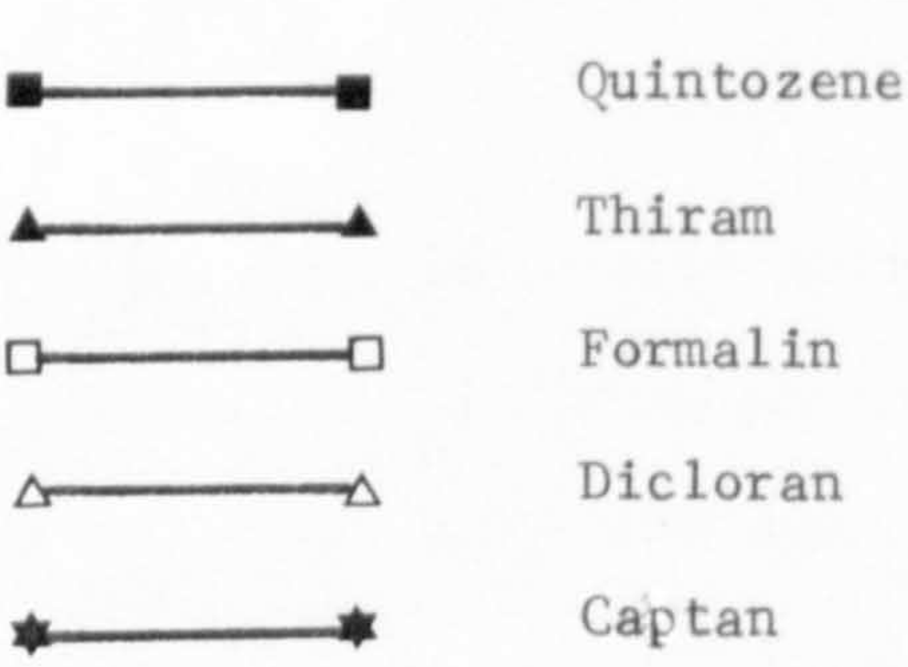
quantitative random checks for nitrite-N were performed using modified Gries Ilovsay's reagent (Prince, 1945). At no point did nitrite concentrations capable of interfering with the C.T.A. method accumulate.

The Effect of Fungicides on Ammonification and Nitrification in the Field

The following fungicides, Captan, Dicloran, Formalin, Thiram and Quintozene were applied to Rothamsted soils at the rates shown in Table 2. Soil samples were transported to the laboratory at intervals of 28 days over a period of 12 weeks. On arrival the samples were incubated at 25°C for 10 days in sterile Erlenmeyer flasks sealed with "Parafilm". Samples of soil (5 g) were extracted with 50 ml of 0.2% W/V solution of $Ca(OH)_2$ by shaking for 15 mins. The amount of nitrate-N was then determined using C.T.A.

In addition the respective amounts of ammonium and nitrate-N were determined 28 days after application of the following fungicides Benomyl, Captan, Quintozene, and Thiram at twice the field rate (Fig. 18).

Fig. 18 The effect of fungicides on nitrification in the field



Fiq 18

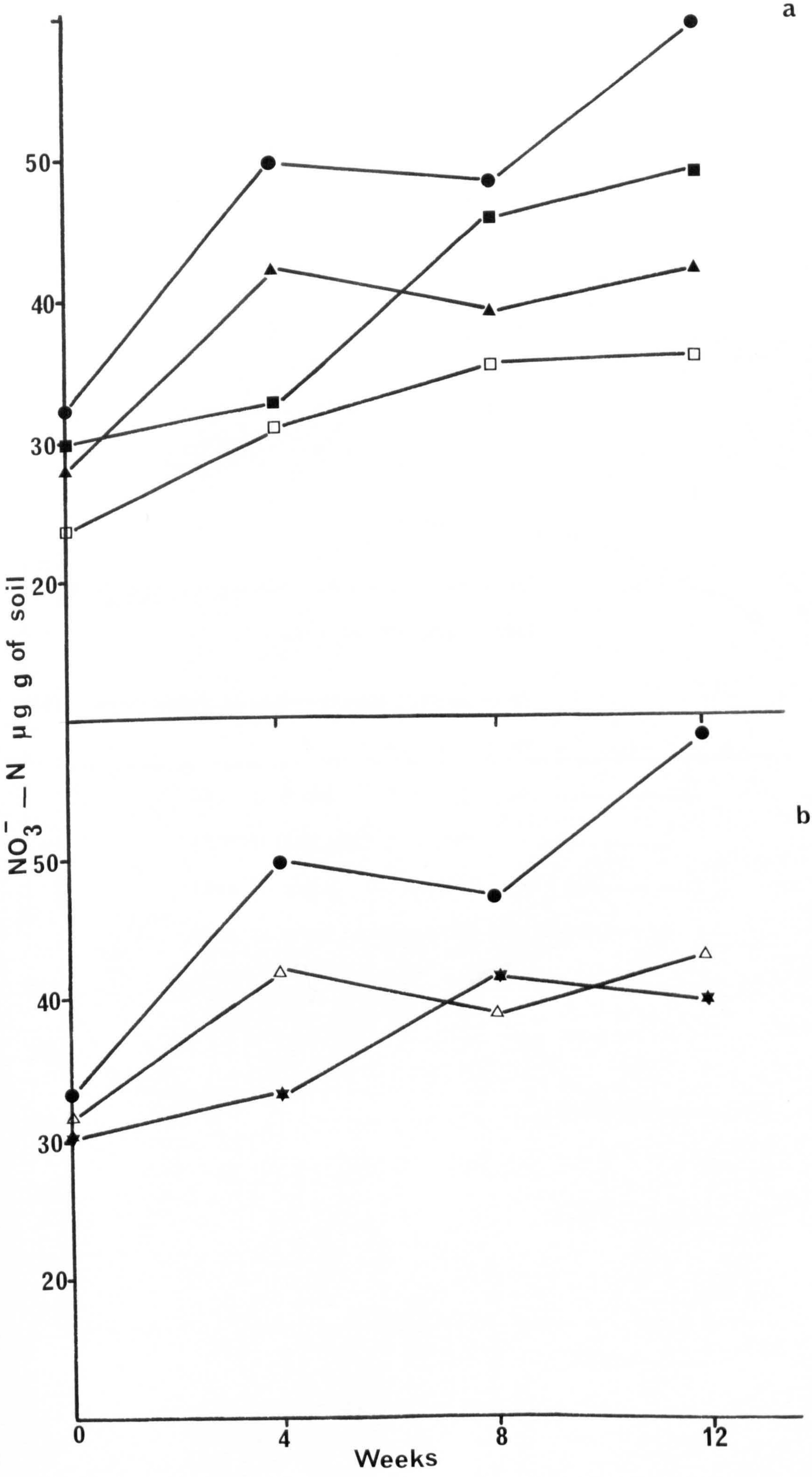


Fig. 19 The effect of fungicides on the free amino acid-N content of soil

Amino acid-N expressed as a percentage of the control

(a) Benomyl 50 μg/g of soil

Benomyl 250 μg/g of soil

(b) Thiram 50 μg/g of soil

Thiram 250 μg/g of soil

Fig 19

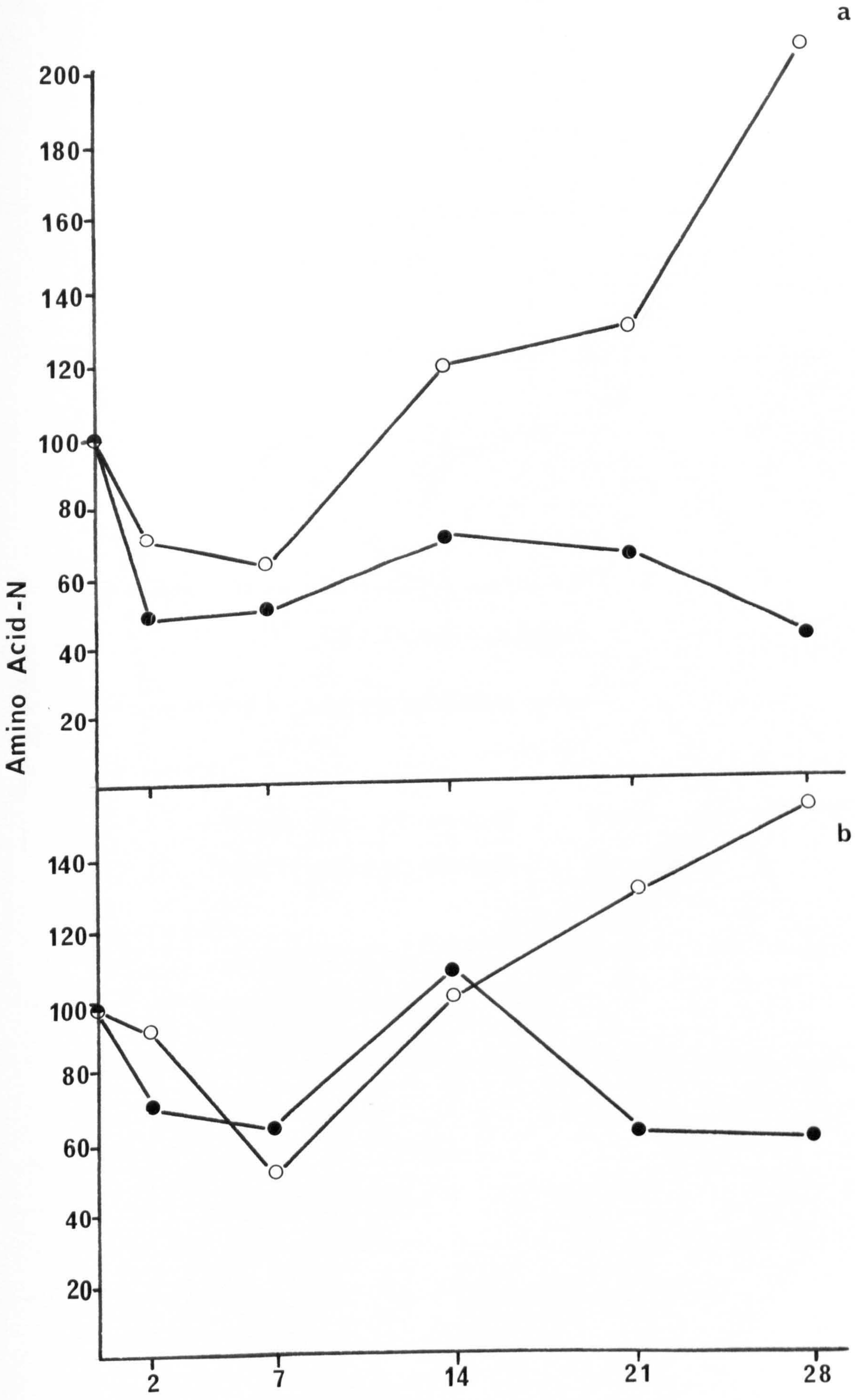

a
200
180
160
140
120
100
80
60
40
20
Amino Acid-N
b
140
120
100
80
60
40
20
2
7
14
21
28
Time Days

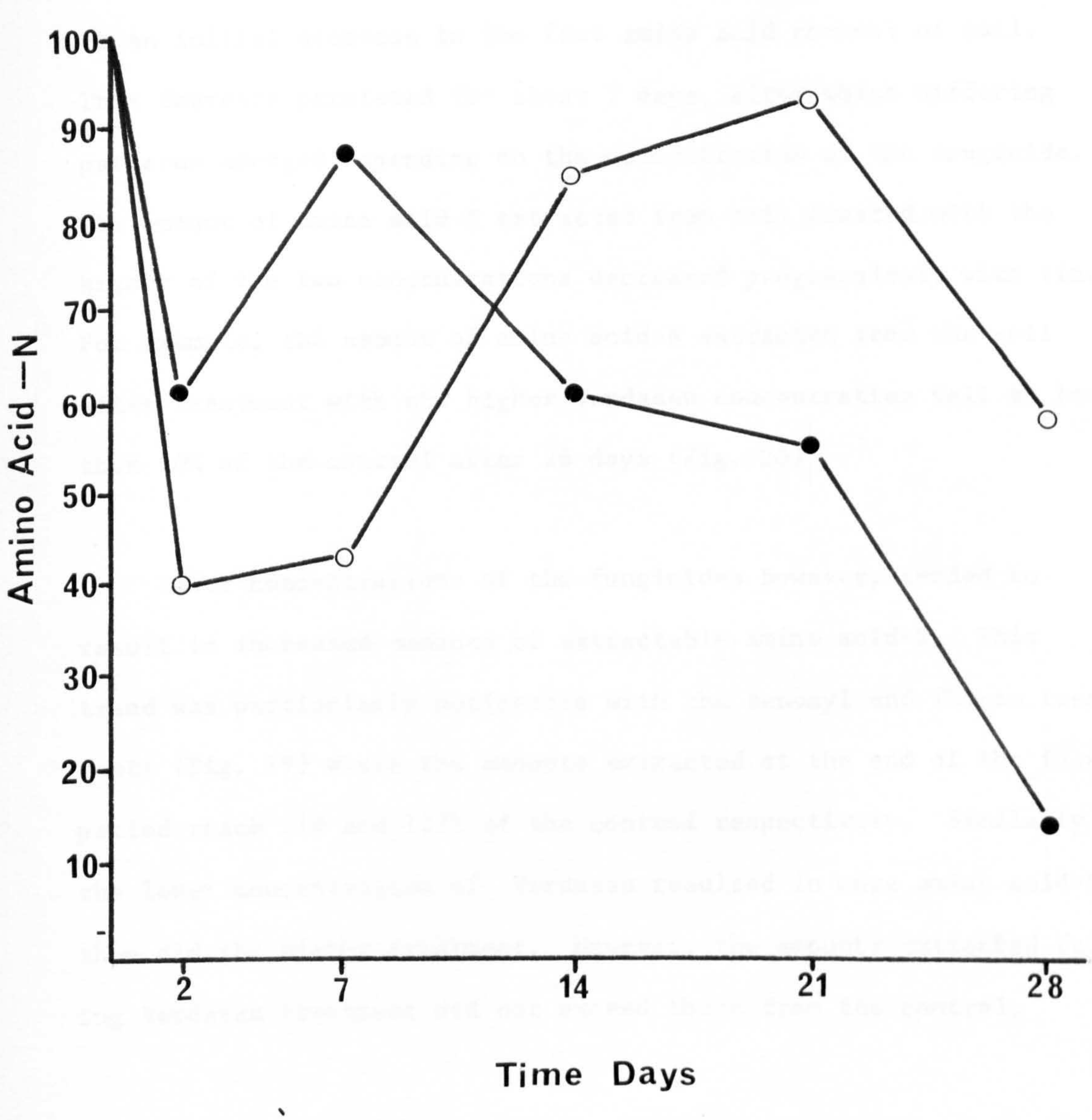
Fig. 20 The effect of fungicides on the free amino acid-N content of soil

Amino acid-N expressed as a percentage of the control

○——○ Verdasan 10 μg/g of soil

●——● Verdasan 50 μg/g of soil

Fig 20



RESULTS

(1) Effect of Fungicides on the Free Amino Acid Content of Soil

Quantitative effects of the fungicides on the free amino acid content of soil are shown in Figs. 19,20. All treatments resulted in an initial decrease in the free amino acid content of soil. This decrease persisted for about 7 days, after which differing patterns emerged depending on the concentration of the fungicide. The amount of amino acid-N extracted from soil treated with the higher of the two concentrations decreased progressively with time. For example, the amount of amino acid-N extracted from the soil after treatment with the higher Verdasan concentration fell to less than 20% of the control after 28 days (Fig. 20)

Lower concentrations of the fungicides however, tended to result in increased amounts of extractable amino acid-N. This trend was particularly noticeable with the Benomyl and Thiram treatments (Fig. 19) where the amounts extracted at the end of the incubation period reach 218 and 127% of the control respectively. Similarly the lower concentration of Verdasan resulted in more amino acid-N than did the higher treatment. However, the amounts extracted following Verdasan treatment did not exceed those from the control.

A total of 9 different amino acids were extracted and typified (Tables 5 - 7). Seven of these were consistently extracted from the untreated soils. The most frequently extracted amino acids were aspartic acid, glutamic acid, leucine, and tryptophan. Serine, phenylalanine, threonine, tyrosine and glycine were extracted less frequently.

Table 4 The Effect of Fungicides (applied at twice the normal rates) on Levels of NH_4^+ and NO_3^- in the field.

Treatment	NH_4^+ -N µg/g soil	NO_3^- -N µg/g soil
Control	3.6	26.1
Benomyl (a)	4.0	25.4
Benomyl (b)	6.0	19.2
Captan	6.5	21.3
Quintozene	6.8	20.5
Thiram	5.0	16.5

Means of 8 replicates

Table 5 Qualitative effects of Benomyl on the free amino acid content of soil

Treatment	Days After Treatment	Aspartic Acid	Glutamic Acid	Glycine	Leucine	Phenyl-Alanine	Serine	Threonine	Tryp-tophan	Tyrosine
Control	2	+	+		+		+	+	+	
	7	+	+		+	+	+		+	
	14	+	+		+	+	+		+	
	21	+	+		+				+	
	28	+	+		+				+	
Benomyl 50 µg/g	2	+	+	+	+	+	+		+	
	7	+		+	+			+	+	
	14	+	+	+	+	+		+	+	
	21	+	+	+	+	+		+	+	
	28	+	+	+		+	+	+		
Benomyl 250µg/g	2	+			+			+	+	
	7	+	+				+			
	14	+	+	+	+	+		+	+	
	21	+	+	+			+		+	
	28	+	+	+	+	+		+		

Table 6 Qualitative effects of Thiram on the free amino acid content of soil

Treatment	Days After Treatment	Aspartic Acid	Glutamic Acid	Glycine	Leucine	Phenyl-Alanine	Serine	Threonine	Tryp-tophan	Tyrosine
Control	2	+	+		+		+	+	+	
	7	+	+		+	+	+		+	
	14	+	+		+	+	+		+	
	21	+	+		+				+	
	28	+	+		+				+	
Thiram 50 µg/g	2	+			+	+	+	+	+	
	7	+	+		+	+		+	+	
	14	+	+			+	+	+	+	
	21	+	+		+	+		+	+	
	28	+	+			+	+	+	+	
Thiram 250 µg/g	2	+			+	+			+	
	7	+			+			+	+	
	14	+	+		+				+	
	21				+				+	
	28				+				+	

Table 7 Qualitative effects of Verdasan on the free amino acid content of soil

Treatment	Days After Treatment	Aspartic Acid	Glutamic Acid	Glycine	Leucine	Phenyl-Alanine	Serine	Threonine	Tryp-tophan	Tyrosine
Control	2	+	+		+		+	+	+	
	7	+	+		+	+	+		+	
	14	+	+		+	+	+		+	
	21	+	+		+				+	
	28	+	+		+				+	
Verdasan 10 µg/g	2	+		+	+		+	+		+
	7	+	+	+	+			+	+	+
	14	+	+	+	+			+	+	
	21	+	+		+		+	+	+	
	28	+	+		+	+	+	+		
Verdasan 50 µg/g	2	+	+	+	+		+	+		
	7				+		+	+		
	14	+	+		+	+	+	+	+	
	21	+	+	+	+	+	+	+		
	28		+							

Following the addition of the fungicides aspartic acid was extracted with only minor differences from the control. All other amino acids showed greater or lesser degrees of variation from the control. Glutamic acid, leucine, and phenylalanine, were less common with the Thiram treatments, and tryptophan was less frequently extracted with Verdasan at 50 μg per g of soil. Two of the treatments brought about a marked change in the amino acid fraction of the soils. Twenty-eight days after adding 50 μg of Verdasan only glutamic acid could be extracted. Similarly after 28 days incubation with 250 μg Thiram only glycine and tryptophan were found.

In contrast however, other free amino acids increased in frequency in soils following fungicide treatment. Glycine, not found in the controls, appeared in all three treatments at both concentrations. Threonine which was found only in control soils after 2 days was frequent in all treated soils except those receiving 250 μg Thiram per g of soil.

Results of the Determination of Optimal Conditions for Nitrification in Soil

The optimum conditions for nitrification were determined prior to application of fungicides and are shown in Figs. 21 - 23.

The effect of pH on nitrification is demonstrated in Fig. 21. The soil to which lime was added had a mean pH of 9, while the unlimed soil had an average value of 7. Comparison of the corresponding rates of nitrification show that the limed soil produced nitrate at a faster rate than did the neutral unlimed soil. The amount of nitrate formed in the limed soil after 14 days was over twice that in the unlimed soils.

Fig. 21 The effect of pH on nitrification

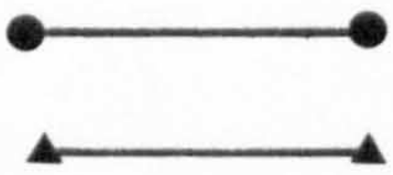


Fig 21

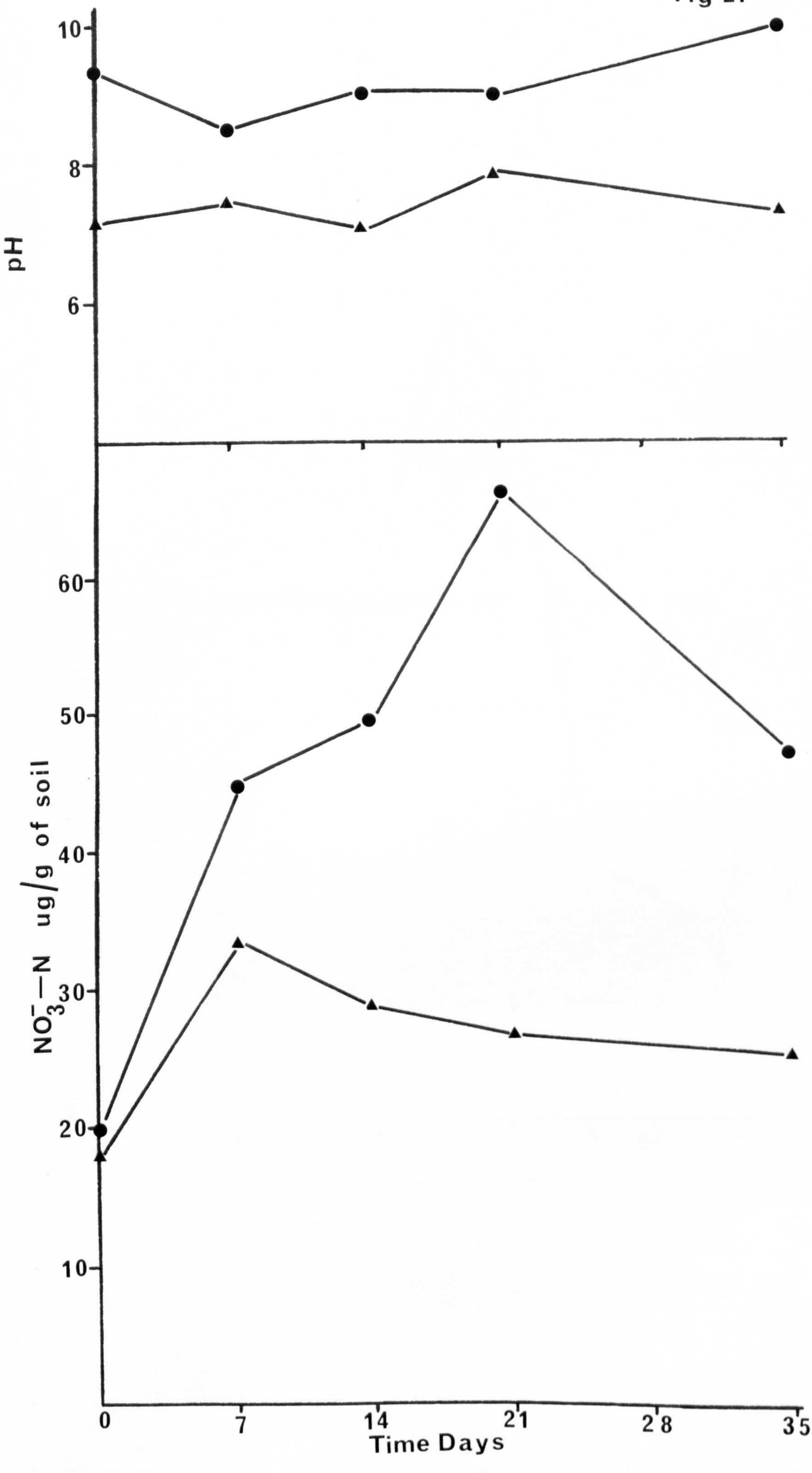


Fig. 22 The effect of temperature on nitrification

Fig 22

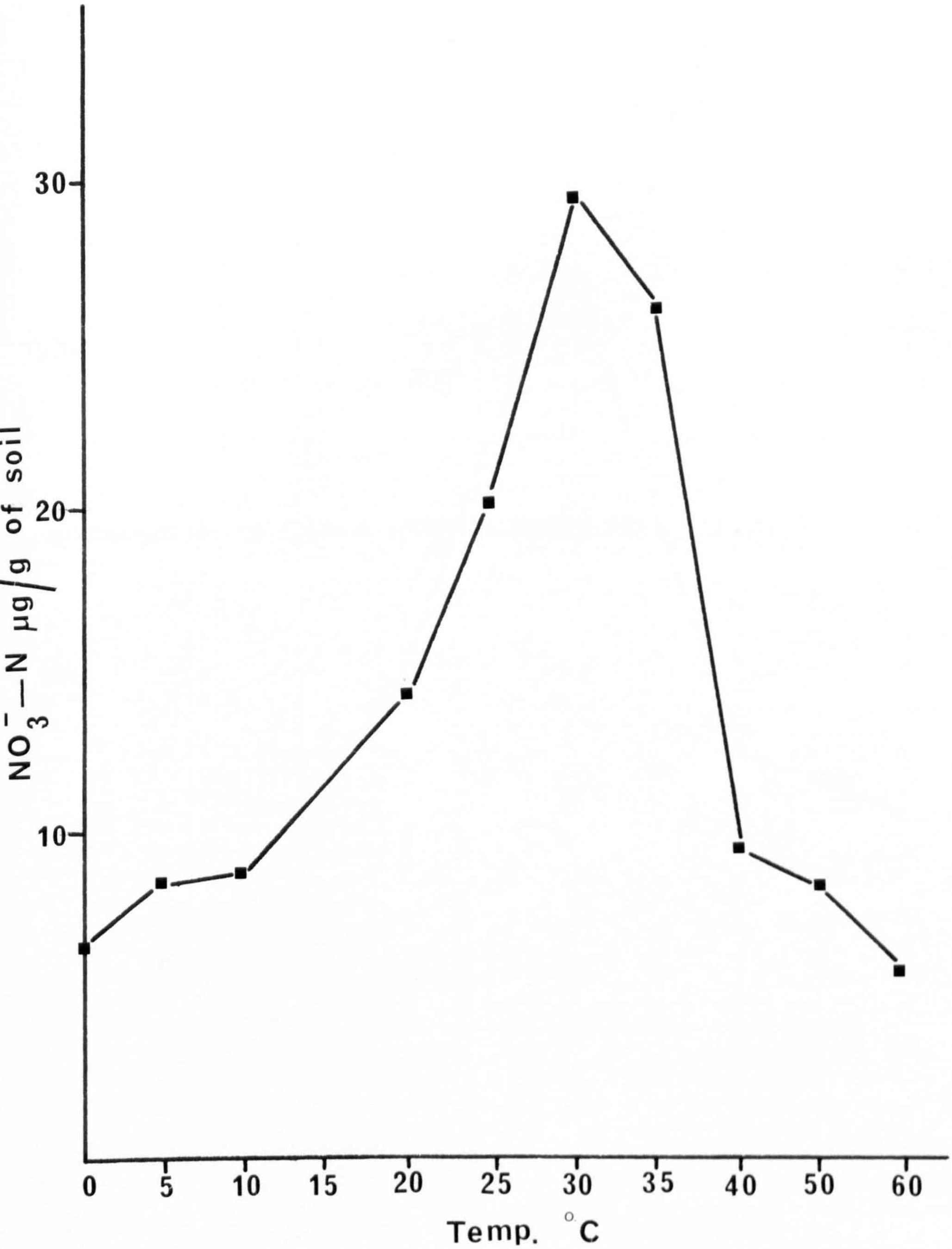
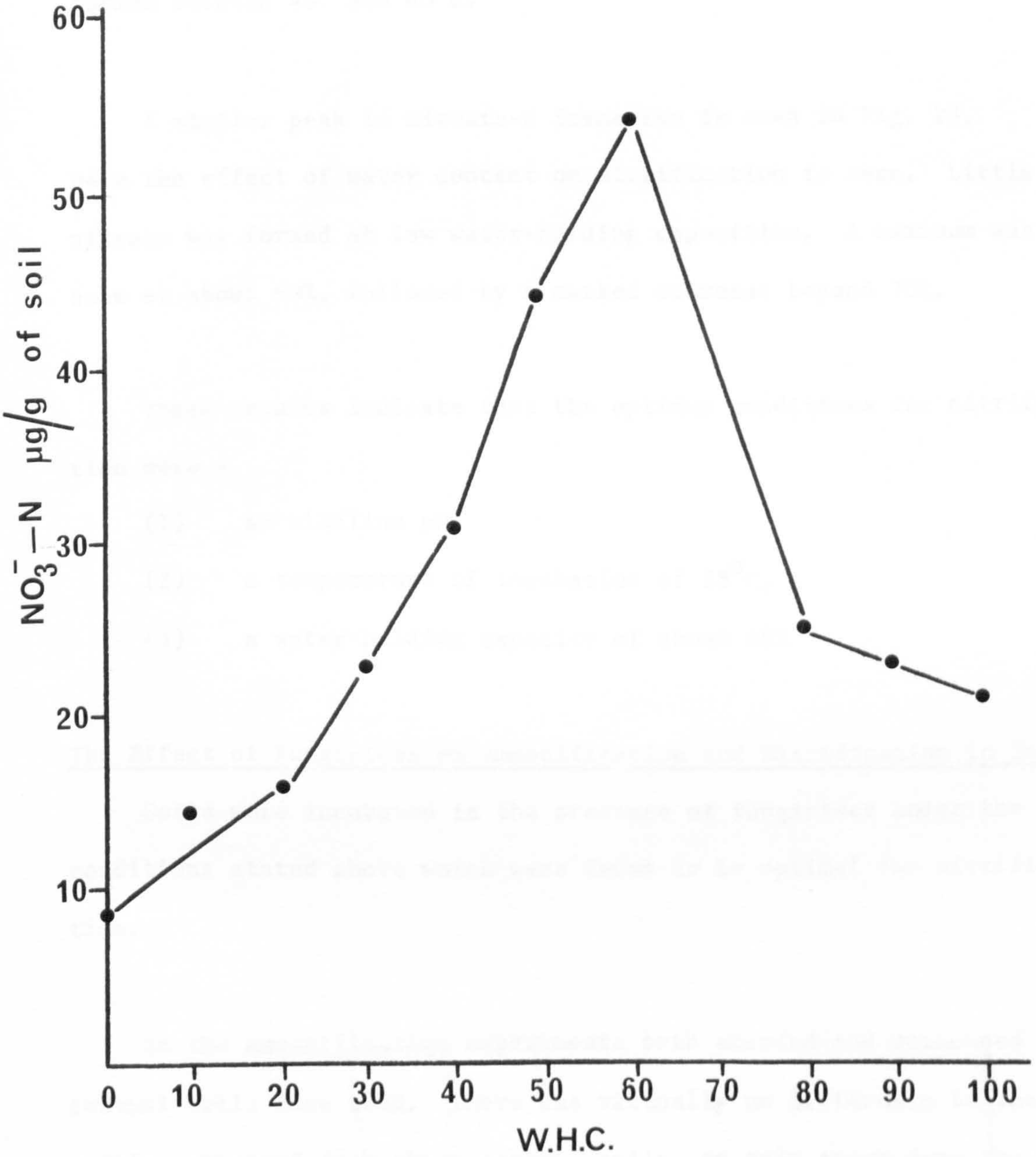


Fig. 23 The effect of water content on nitrification

Fig 23


$NO_3^- - N$ µg/g of soil
60
50
40
30
20
10
0
10
20
30
40
50
60
70
80
90
100
W.H.C.

The effect of temperature is shown in Fig. 22. The amount of nitrate formed at low temperatures over the incubation period was almost negligible. At 20°C there was a marked increase in nitrate which reached a maximum at 28°C. Beyond 35°C however, the amount of nitrate-N formed decreased markedly, so that very little was formed between 40° and 60°C.

A similar peak in nitrate-N formation is seen in Fig. 23. Here the effect of water content on nitrification is seen. Little nitrate was formed at low water-holding capacities. A maximum was seen at about 60%, followed by a marked decrease beyond 70%.

These results indicate that the optimum conditions for nitrification were -

(1) an alkaline pH,

(2) a temperature of incubation of 28°C,

(3) a water-holding capacity of about 66%

The Effect of Fungicides on Ammonification and Nitrification in Soils

Soils were incubated in the presence of fungicides under the conditions stated above which were found to be optimal for nitrification.

In the ammonification experiments both amended and unamended control soils were used. There was virtually no difference in the results obtained from these control soils, so only those from the amended soils have been plotted in Figs. 24 - 26. The amount of ammonium-N formed in the control over the incubation period were very small. Addition of concentrated solutions of fungicides to soil however, resulted in a marked effect. At low concentrations all three fungicides did not greatly affect ammonification. At

Fig. 24 The effect of Thiram on Ammonification

(a) Control +$(N_4)_2SO_4$

Thiram 50 μg/g of soil

Thiram 25 μg/g of soil

Thiram 10 μg/g of soil

(b) Control +$(NH_4)_2SO_4$

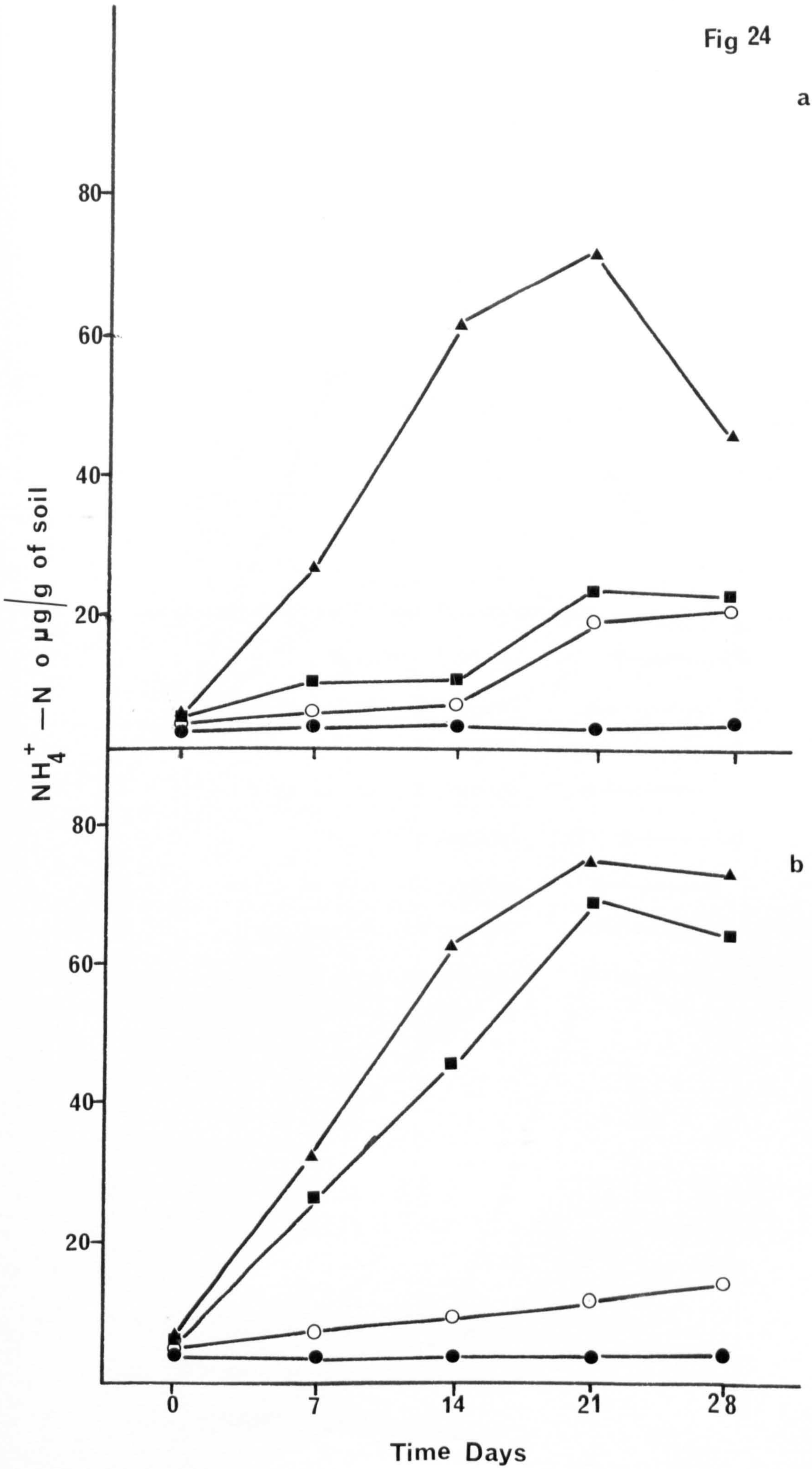
Thiram 250 μg/g of soil

Thiram 100 μg/g of soil

Thiram 5 μg/g of soil

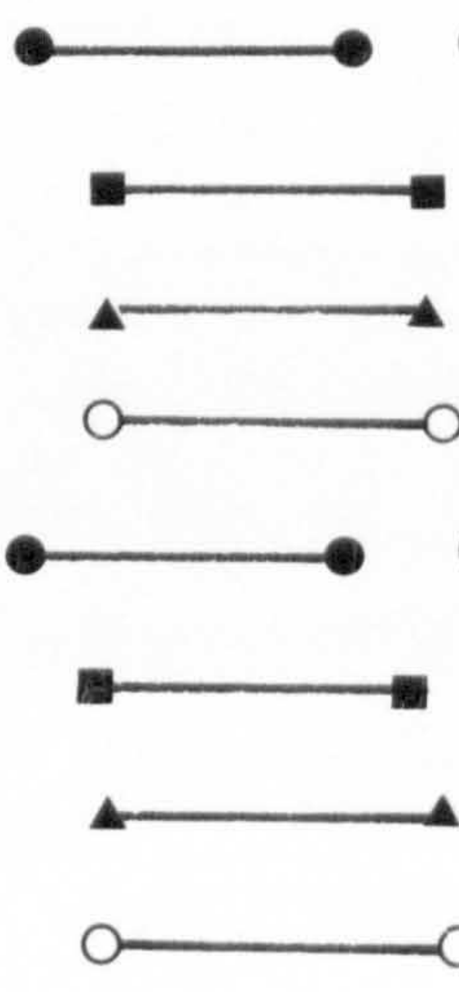
Fig 24

a



b

Fig. 25 The effect of Captan on Ammonification



(a) Control +$(NH_4)_2SO_4$

Captan 50 μg/g of soil

Captan 25 μg/g of soil

Captan 5 μg/g of soil

(b) Control +$(NH_4)_2SO_4$

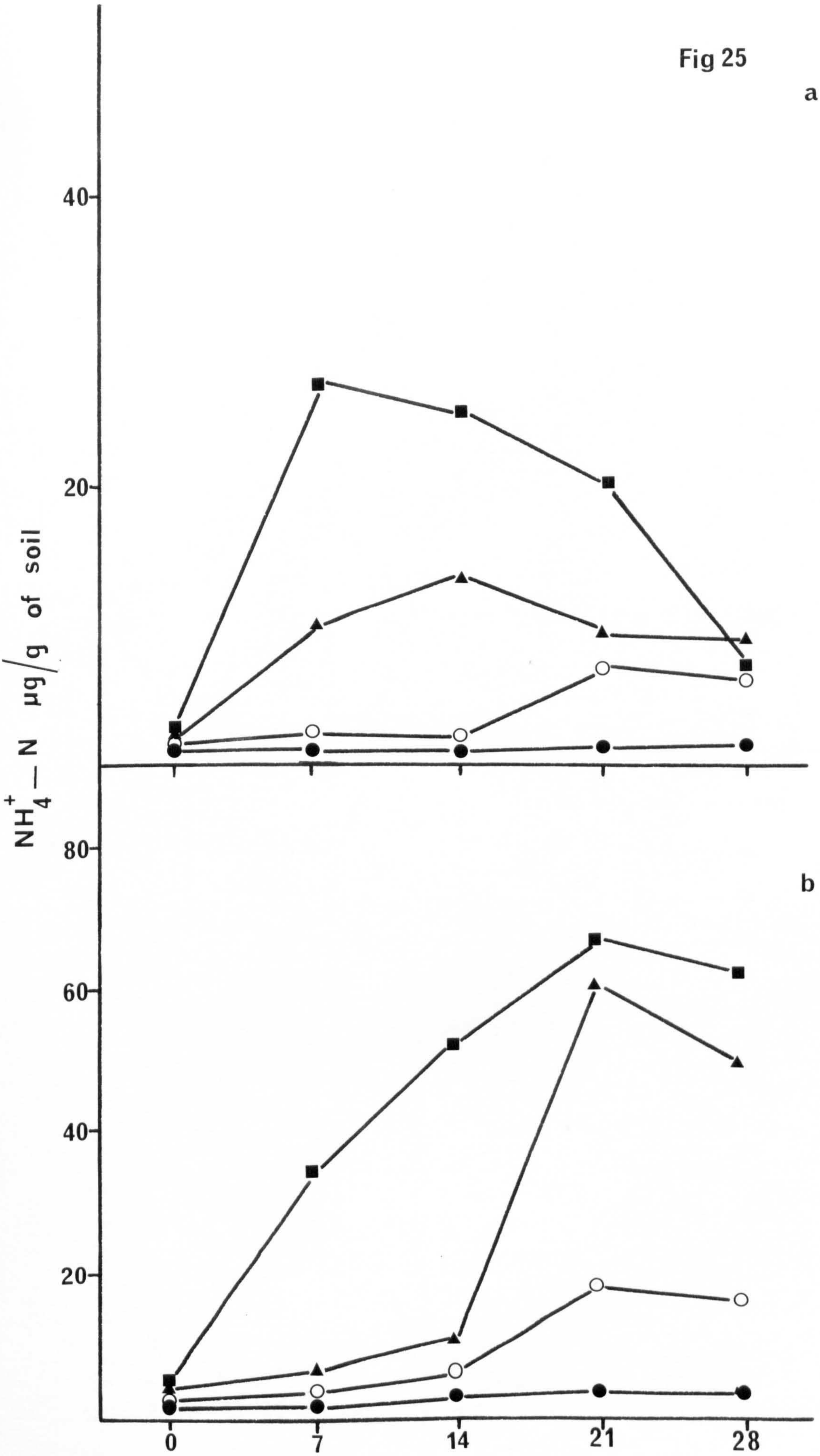
Captan 250 μg/g of soil

Captan 100 μg/g of soil

Captan 10 μg/g of soil

Fig 25

a



b

Fig. 26 The effect of Verdasan on Ammonification

(a) Control +$(NH_4)_2SO_4$

Versasan 25 μg/g of soil

Verdasan 10 μg/g of soil

Verdasan 5 μg/g of soil

(b) Control +$(NH_4)_2SO_4$

Verdasan 50 μg/g of soil

Verdasan 15 μg/g of soil

Verdasan 1 μg/g of soil

Fig 26

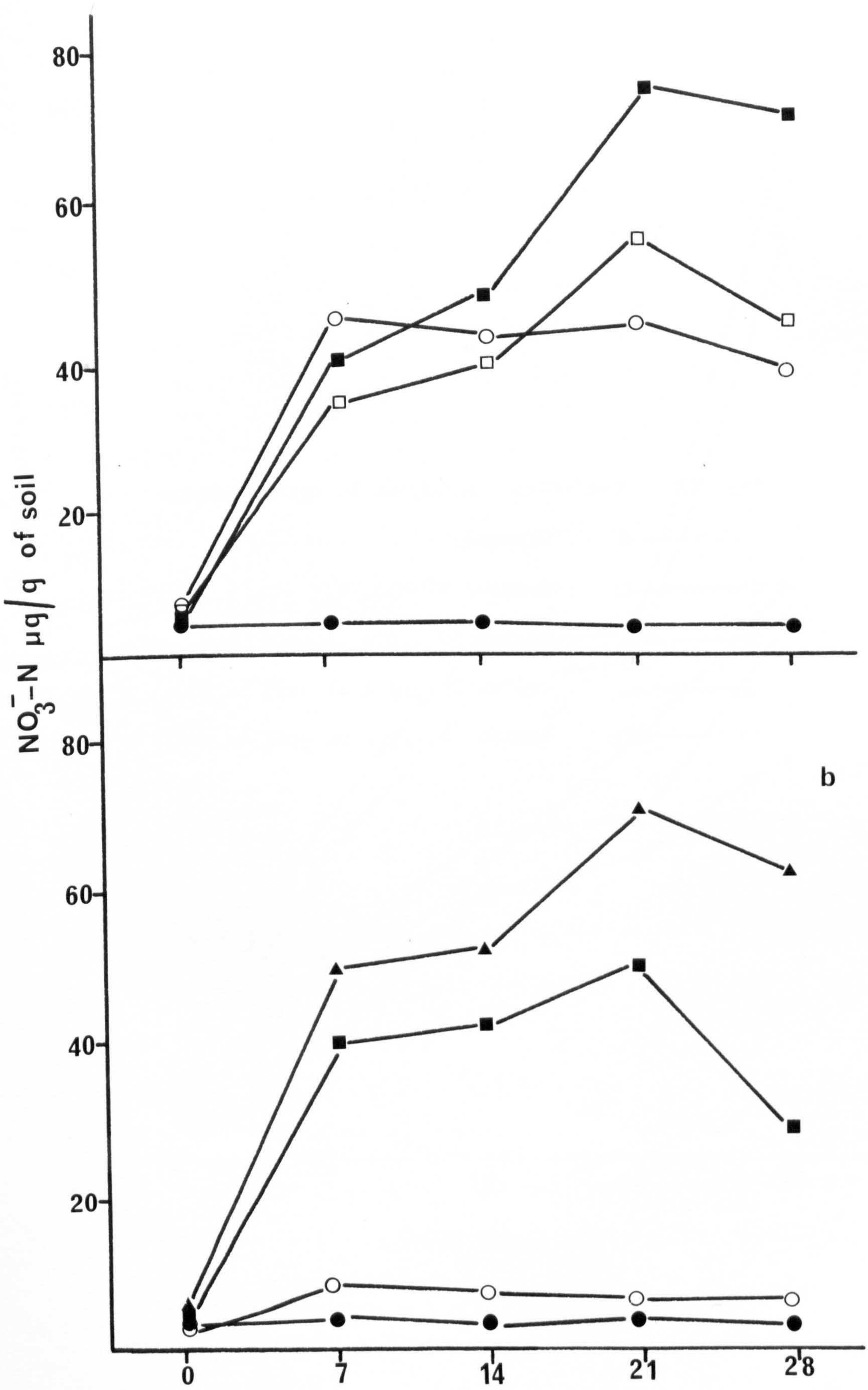


Fig. 27 The effect of Captan on Nitrification

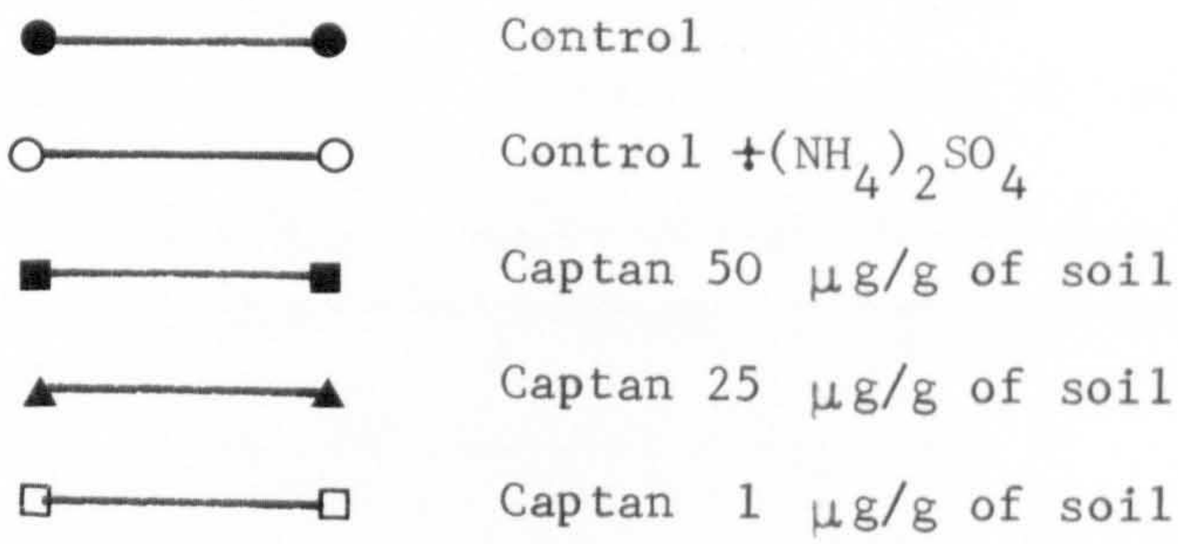
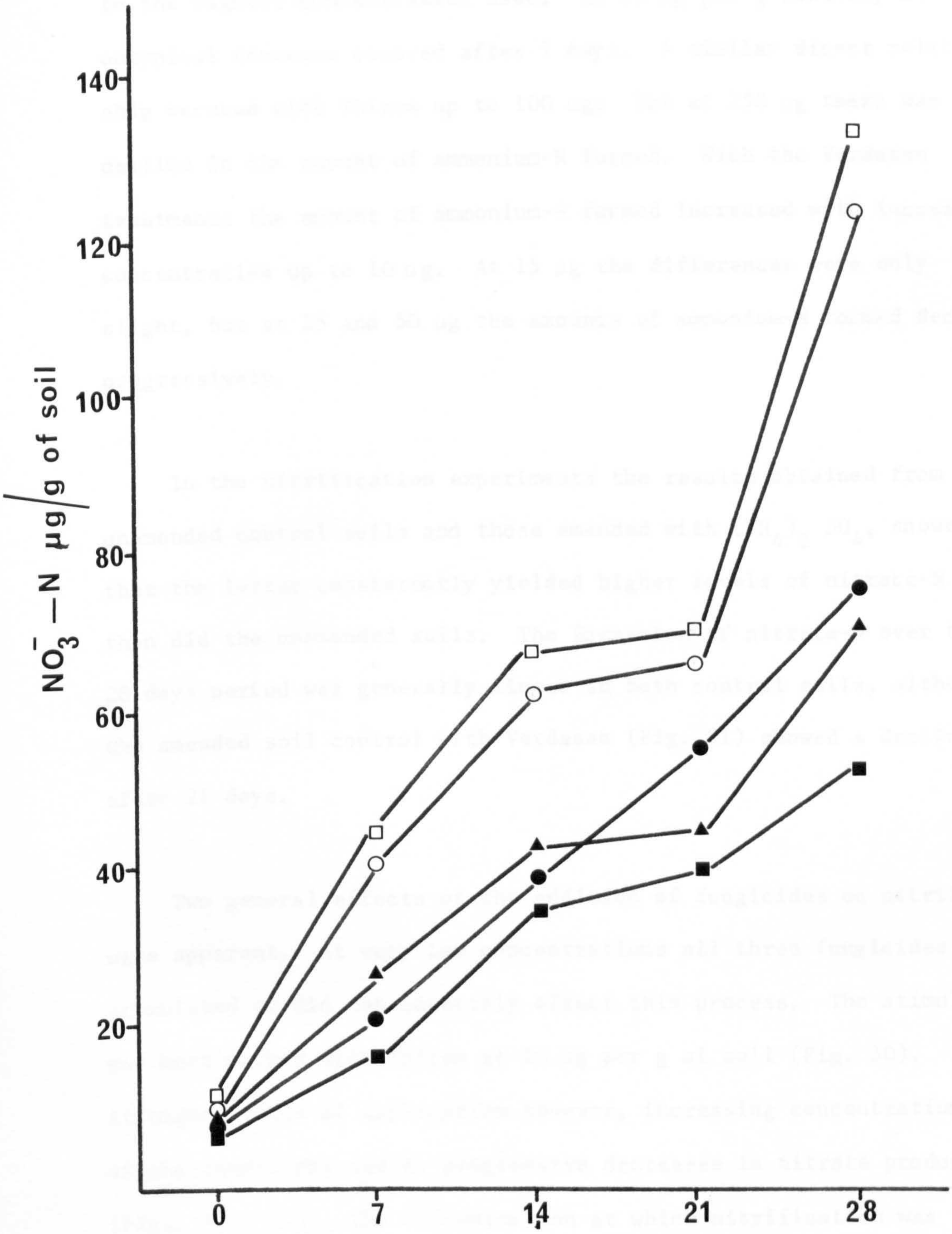


Fig 27



increasing concentrations however, different patterns emerged. Captan brought about a progressive rise in the amount of ammonium-N to the highest concentration used. At 50 µg per g however, an untypical decrease occured after 7 days. A similar direct relationship occured with Thiram up to 100 µg; but at 250 µg there was a decline in the amount of ammonium-N formed. With the Verdasan treatments the amount of ammonium-N formed increased with increasing concentration up to 10 µg. At 15 µg the differences were only slight, but at 25 and 50 µg the amounts of ammonium-N formed decreased progressively.

In the nitrification experiments the results obtained from the unamended control soils and those amended with $(NH_4)_2\ SO_4$, showed that the latter consistently yielded higher levels of nitrate-N than did the unamended soils. The formation of nitrate-N over the 28 days period was generally linear in both control soils, although the amended soil control with Verdasan (Fig. 31) showed a decline after 21 days.

Two general effects of the addition of fungicides on nitrification were apparent. At very low concentrations all three fungicides stimulated or did not adversely affect this process. The stimulation was most marked with Thiram at 10 µg per g of soil (Fig. 30). At higher rates of application however, increasing concentration of the fungicides led to progressive decreases in nitrate production (Figs. 28 - 31). The concentration at which nitrification was inhibited differed for the three fungicides: thus it occured with Verdasan at 10 µg; with Thiram at 100 µg and with Captan at more than 250 µg fungicide per g of soil.

Fig. 28 The effect of Captan on Nitrification

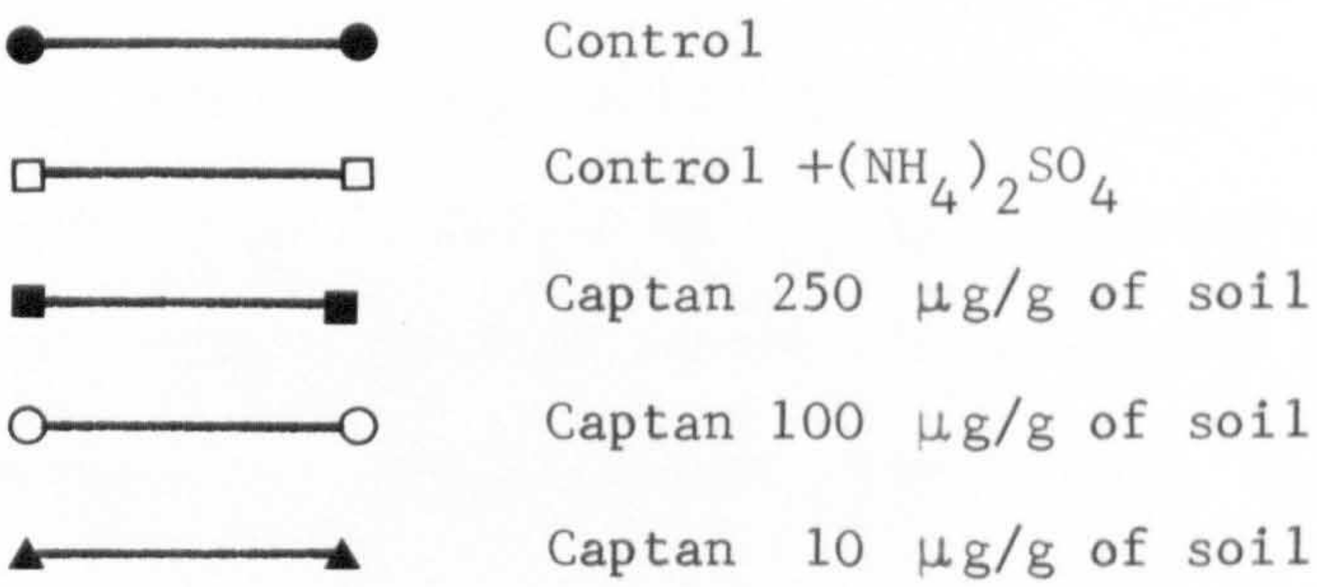


Fig 28

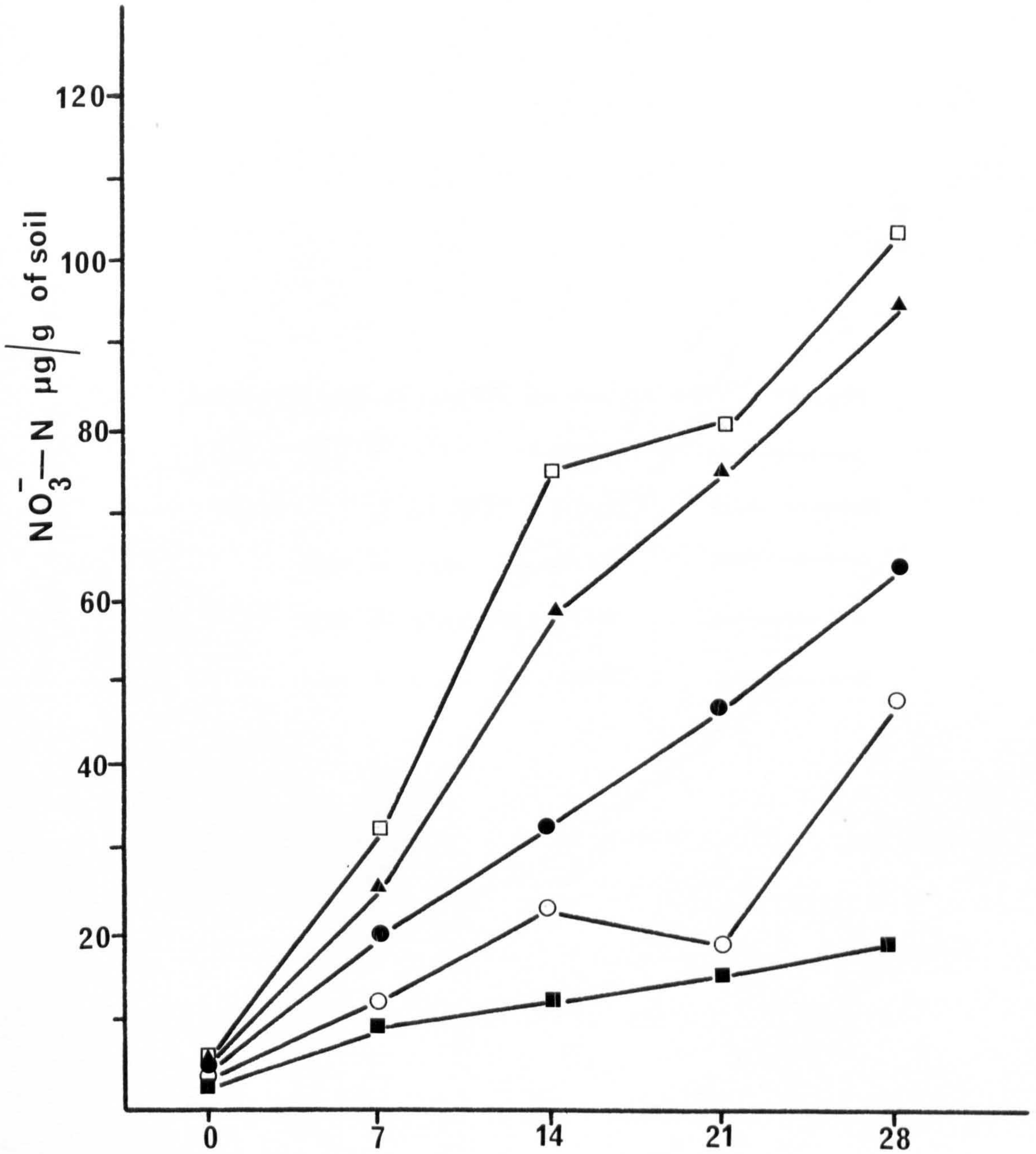
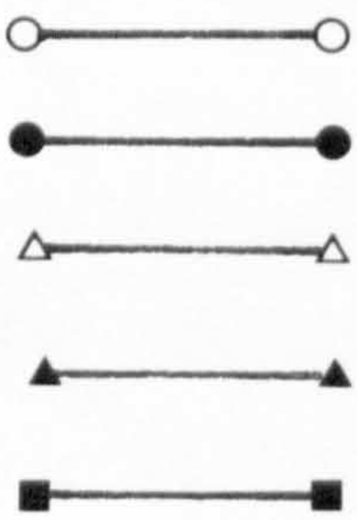


Fig. 29 The effect of Thiram on Nitrification



Control

Control $+(NH_4)_2SO_4$

Thiram 50 μg/g of soil

Thiram 25 μg/g of soil

Thiram 5 μg/g of soil

Fig 29

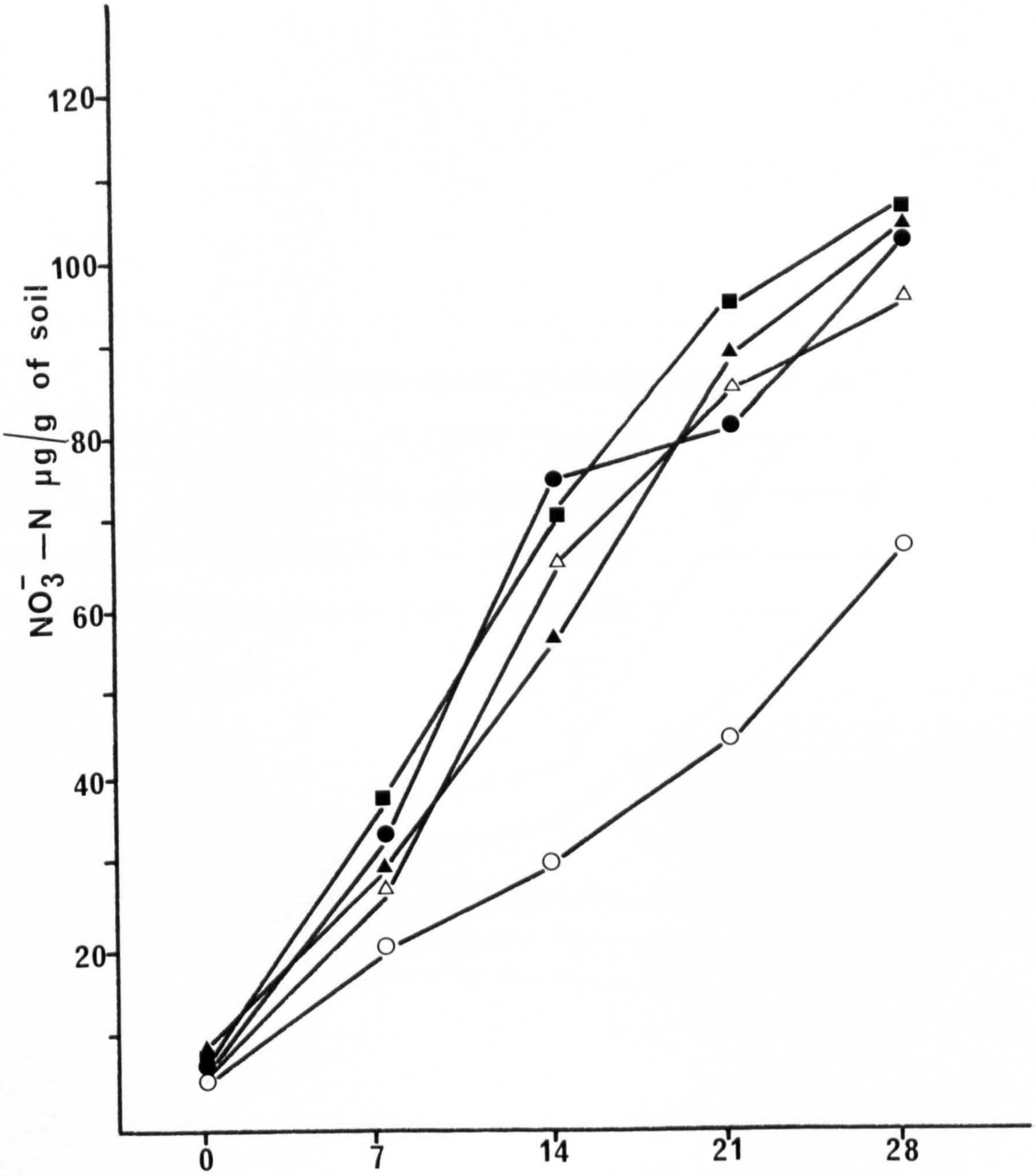
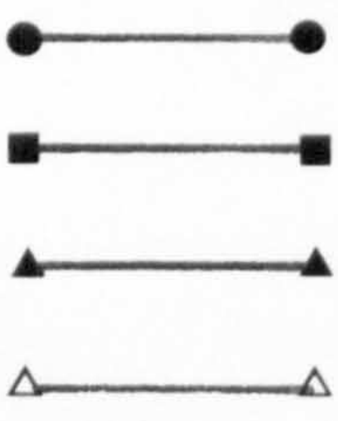


Fig. 30 The effect of Thiram on Nitrification



Control + $(NH_4)_2SO_4$

Thiram 250 μg/g of soil

Thiram 100 μg/g of soil

Thiram 10 μg/g of soil

Fig 30

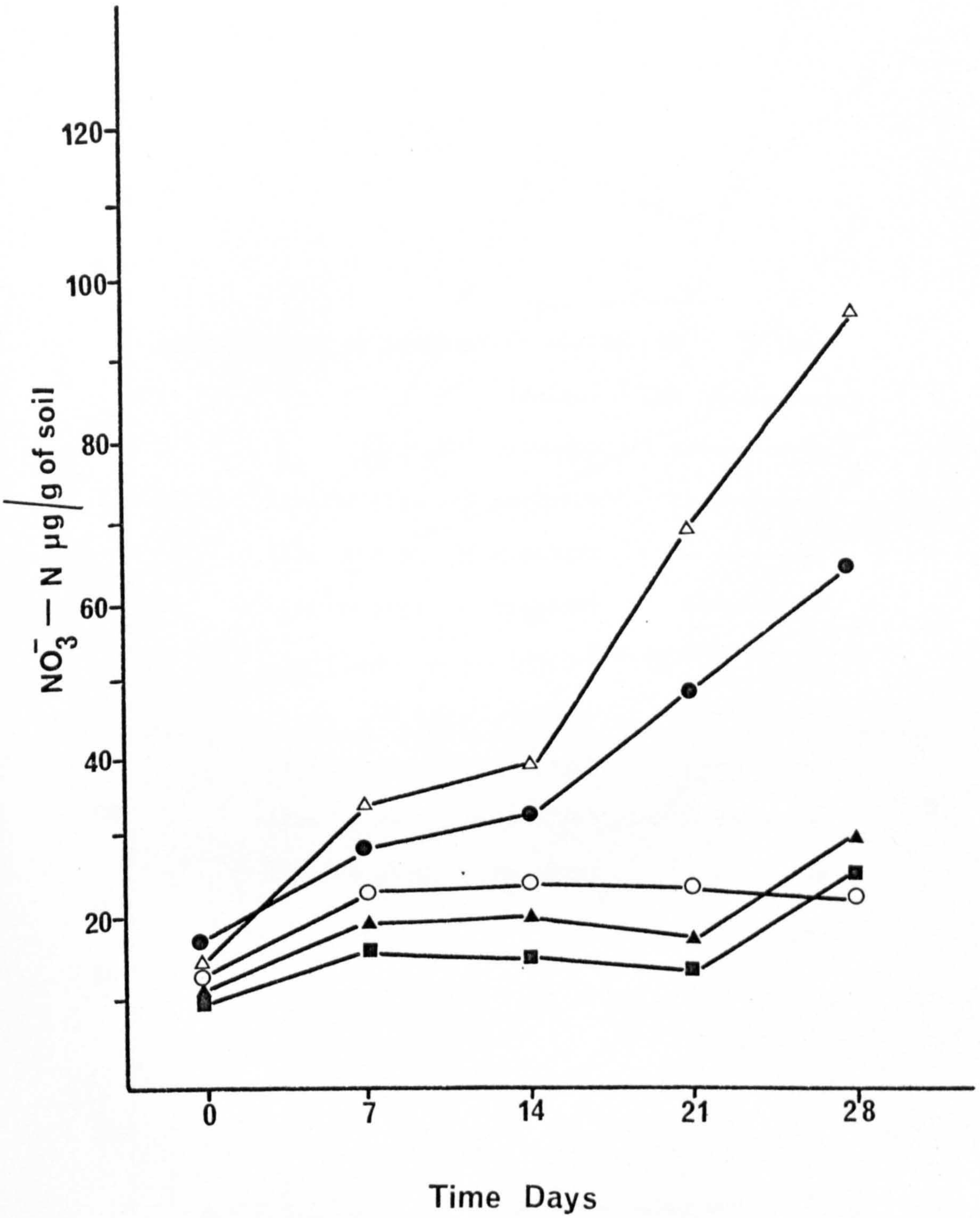
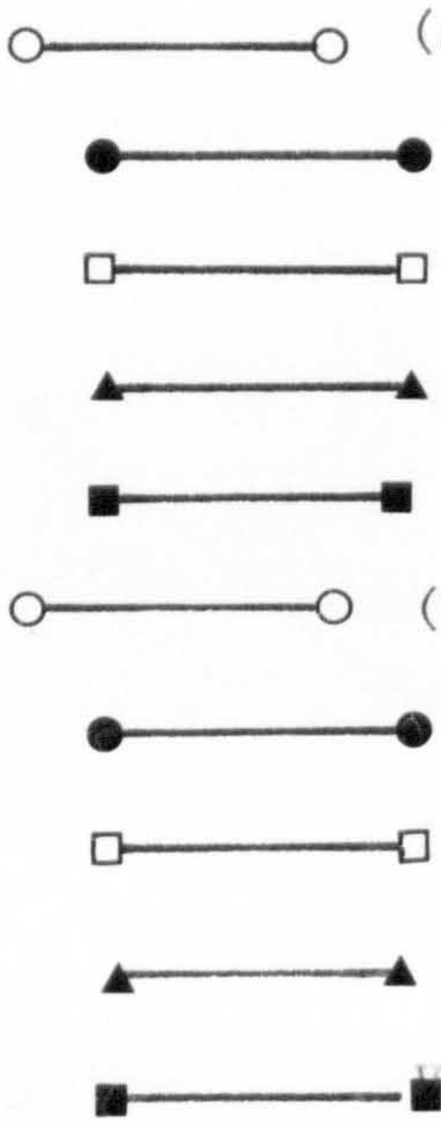


Fig. 31 The effect of Verdasan on Nitrification



(a) Control

Control +$(NH_4)_2SO_4$

Verdasan 15 μg/g of soil

Verdasan 10 μg/g of soil

Verdasan 1 μg/g of soil

(b) Control

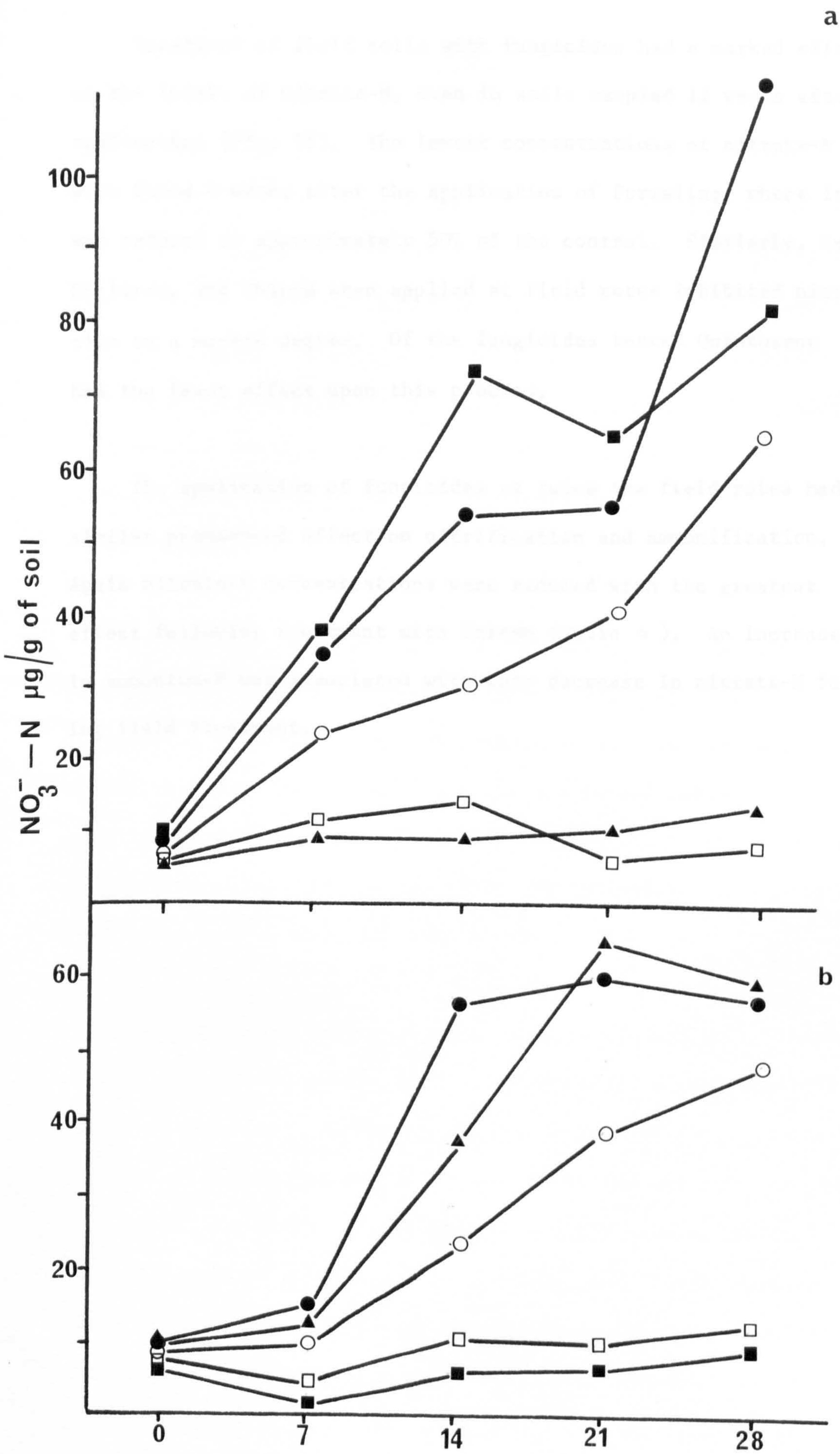
Control +$(NH_4)_2SO_4$

Verdasan 50 μg/g of soil

Verdasan 25 μg/g of soil

Verdasan 5 μg/g of soil

Fig 31



Treatment of field soils with fungicides had a marked effect on the levels of nitrate-N, even in soils sampled 12 weeks after application (Fig. 18). The lowest concentrations of nitrate-N were found 4 weeks after the application of Formalin; where it was reduced to approximately 50% of the control. Similarly, Captan, Dicloran, and Thiram when applied at field rates inhibited nitrification to a marked degree. Of the fungicides tested Quintozene had the least effect upon this process.

The application of fungicides at twice the field rates had a similar pronounced effect on nitrification and ammonification. Again nitrate-N concentrations were reduced with the greatest effect following treatment with Thiram (Table 4). An increase in ammonium-N was associated with this decrease in nitrate-N following field treatment.

DISCUSSION

It is not surprising, in view of the central importance of nitrogen to soil fertility, that the effects of fungicides and other pesticides upon the mineralization of the element have received intensive study. Emphasis has been placed upon nitrification-retardation and changes in the level of the ammonium ion. With the exception of the work of Altman (1969) there are few results available on the effects of fungicides on the early stages of nitrogen mineralization.

Fungicide application had a marked effect upon the mineralization of nitrogen both in the field and in the laboratory. Treatment resulted in a change of the predominant form of the element from nitrate to ammonium. In addition the nature and amounts of free amino acids were altered. While the changes which take place in the ionic form of nitrogen are simple to interpret, changes in the free amino acid content are more complex.

The free amino acid content of soil is dynamic and probably results from the excretory or lytic products of micro-organisms and of plant roots. Following the application of fungicides a portion of the soil microbial biomass is killed and provides substrates for the flush of resistant or quickly growing forms. Work presented in Section I indicates that this flush consists mainly of heterotrophic bacteria. Initially following treatment large amounts of proteinaceous material will be made available to these bacteria and other surviving micro-organisms. Free amino acids will be released into the soil solution following decomposition

of this proteinaceous material. Some of these, mainly the basic fraction, will be fixed while large amounts of acidic and neutral amino acids will be available for deamination to release ammonia (Alexander, 1961).

The amount of free amino acid extracted from the soil following the application of fungicides will be dependent on both the size and nature of the flush following the initial kill off. Many micro-organisms are known to produce amino acids as excretory products. Balicka and Kosinkienick (1963) claimed that the majority of soil micro-organisms are able to excrete amino acids in the presence of N and C. They noted that ammonium-N is the best source of nitrogen in this synthesis. Following treatment, large amounts of carbon, and nitrogen in the form of ammonium are made available (Wainwright and Pugh, 1973).

Quantitative results showed three trends :

(1) following treatment the amount of free amino acid-N decreased - a finding contrary to the assumption that large amounts of amino acids are freed by the addition of fungicides. However, Van Driel (1961) reported that the amount of mineralization of added amino acid-N was dependent on concentration and that mineralization increased with increasing concentration. Any large amounts of amino acids released into the soil due to the lysis of micro-organisms following fungicide treatment would thus be quickly mineralized.

(2) low concentrations of fungicides tended to result in increased levels of excreted amino acid-N.

(3) The converse was true of high concentrations.

These findings indicate that large concentrations of fungicides when added to the soil favour amino acid mineralization. This would agree with the findings presented below, that large amounts of ammonium-N accumulated in soils treated with high concentrations of fungicide, while the converse was found following low rates of application.

The amino acids extracted in this work are typical of those reported to occur free in soils (Paul and Schmidt, 1960; Gilbert and Altman, 1966). Glycine was not found, and threonine was uncommon in the control soils, although they have been reported to occur in other soils (Sowden and Ivarson, 1966). Following the addition of fungicides however, these two amino acids were commonly extracted. Their appearance in treated soils calls for comment. Possible reasons include:-

(1) that they were produced by lysis of dead micro-organisms rather than by excretion of the active soil microflora,

(2) that they may have been excreted during the microbial flush when a limited flora was growing actively on the increased substrate,

(3) that they are resistant to degradation, so that once produced they remain in the soil.

Greenwood and Lees (1956) found that lysine, threonine, and methionine were particularly resistant to degradation whereas tryptophan, aspartic acid, and glutamic acid appeared more labile.

Halvorsan (1972) has reported that single amino acids can be utilised as both carbon and nitrogen sources in bacterial nutrition. Amino acids which resulted in good growth of a range of soil bacteria included aspartic and glutamic acids and tryptophan. However, he also found that glycine and threonine were not utilised to any great extent. This reinforces the view that these amino acids are resistant to degradation.

Nitrification Assessment of Methods

The development of spectrophotometric techniques to measure the amounts of ammonium and nitrate-N in soils enabled large scale evaluation of the effects of fungicides on ammonification and nitrification. In the past the basic methods used to determine these ions by colorimetry have been the phenoldisulphonic acid method for determination of nitrate (Eastoe and Pollard, 1950) and the Nessler method for ammonium determination (Yuen and Pollard, 1952). However, both of these techniques have been found to be inadequate, mainly because of their sensitivity to trace amounts of metal ions. Other techniques used in the determination of the forms of nitrogen have suffered from similar patterns. The brucine hydrochloride method of Fisher, Ibert and Beckman (1958) for example is very sensitive to temperature and light.

In 1965 Clarke and Jennings successfully modified a technique used by West and Lyles (1960) to measure nitrate-N preseent in water. The technique involved the use of Chromotropic acid (C.T.A.) (4, 5, - dihydroxy-2 7 napthalenedisulfonic acid disodium salt.) The method was further improved by Simms and Jackson (1971). The C.T.A. method is rapid, highly sensitive and practically free from

interferences. However the presence of large amounts of chloride and nitrite ions can interfere with colour formation. In some soils the presence of sugars or carbohydrates can cause difficulties. For example, McNeilly and Howard (1973) found that the technique was unsuitable for use in woodland soils because of the development of a pink-purple colour, which masked the colour reaction. For the purposes of the present study however, it proved ideal.

Nitrate-N is present in the soil in solution and therefore its extraction presents no difficulty. Calcium hydroxide was used as an extracting agent to help clarify the soil extracts. A linear relationship between colour intensity and nitrate-N concentration was found (Fig. 17).

Unlike nitrate-N, the ammonium form is fixed in soils and cannot be removed by the use of water as an extracting agent (Nommik, 1957). The extracting agent most commonly used is potassium chloride. In these studies normal solutions were used.

The colorimetric technique used for the determination of ammonium-N in these studies is based on that used to determine urease activity by McGarity and Myers (1967); as detailed by Parkinson, Gray and Williams (1971). A complete discussion of the reaction conditions for the method is given by Bolleter, Bushman and Tidwell (1961), and by Tetlow and Wilson (1964). A similar technique used by the Russian workers Shkonde and Koroleva (1964) employs phenol/hypobromite in preference to phenol/hypochlorite. The former however is a highly poisonous vapour a fact which has discouraged its use.

The reaction between ammonium ions and phenolate/hypochlorite is complex and is susceptible to variations in light and temperature. However, with adequate controls the technique proved ideal.

One problem which arose from the use of these techniques was that two separate soil extracts, one using calcium hydroxide, and the other potassium chloride, were required. However, McNeilly and Howard (1973) used KCl with the C.T.A. method. The use of KCl extracts for both determinations should make future work less laborious.

The optimum conditions for nitrification were determined prior to the addition of fungicides and the results are in broad agreement with those found by other workers.

The major ecological influence on nitrification is pH. Typically the rate falls off below pH 6.0 and becomes negligible below 5.0, although nitrate-N may be present in soils of pH 4.0 and below (Alexander, 1961). There is clear evidence that the autotrophic ammonium-oxidising organisms is greater in neutral and alkaline soils than in acid ones (Morrill, 1959).

Nitrification is markedly affected by temperature. Numerous investigations have shown that below 5°C and above 40°C the rate is very slow. Nevertheless, Broadbent, Tyler and Hill (1958) found that although greatly reduced nitrification was still measurable at 3°C Frederick (1956) reported that a temperature of between 27-37°C is the most favourable for the activities of the nitrifying bacteria, while Meiklejohn (1954) considered the temperature optimum for the process to be between 30 and 36°C.

The nitrifying bacteria are faculative aerobes and anything which limits aeration (e.g. water-logging) adversely affects nitrification. In both dry and wet soils there is a tendency for the accumulation of ammonium-N. The nitrifying population appears to be sensitive to drying, as suggested by the occurence of a lag phase in ammonium oxidation when soils are moistened and incubated. (Grunes and Viets, 1966). Thus at low soil water contents nitrification is inhibited by a lack of water, while under water-logged conditions aeration is the limiting factor. The optimum water content tends to vary with soil type, but is generally between 50% and 60% of the water holding capacity (Alexander, 1961).

The three fungicides used in this work were associated with a decrease in nitrification and an increase in ammonification at the higher rates of application. At lower concentrations, nitrification and ammonification were not adversely affected, and nitrification was stimulated by Thiram at 10 µg per g of soil. A similar increase in nitrate production was shown by Jacques, Robinson and Chase (1959) after adding low concentrations (12 ppm) of dithiocarbamates to soil. They regarded this as being caused by some indirect effect on the microbial population.

The lowest rates of application of the three fungicides resulted in most nitrification and least ammonification. In both processes Verdasan was effective at lower concentrations than Thiram, while Captan required highest concentration to give similar results: thus inhibition of nitrification and maximum ammonification were obtained with 10 µg Verdasan, 100 µg Thiram and 250 µg per g of soil Captan.

The various components of the microbial population of soil are differentially susceptible to the action of fungicides. The nitirfiers are a very specialised group restricted to a few genera of bacteria. Kreutzer (1963) believed that this specialisation resulted in the nitrifiers being susceptible to fungicides and other partial sterilants. The ammonifiers however, include a wide spectrum of organisms, including bacteria, fungi, and to a lesser extent the actinomycetes. In neutral to alkaline soils the bacteria tend to predominate. Alexander (1961) noted that liberation of ammonium-N from organic matter is an action associated with many physiologically dissimilar micro-organisms, and that nitrogen is mineralized even under the most extreme conditions. The amount of C and N is increased by partial sterilants (Jenkinson, 1966), due mainly to changes in the microflora. Following the application of fungicides to the soil, the numbers of heterotrophic bacteria are at first reduced and then increased dramatically. The increase is probably responsible for the upsurge in ammonification.

The increased nitrate production at low application rates of the fungicides may have resulted from the killing of a portion of the soil microflora (mainly the fungi). However, these low concentrations did not affect the nitrifying and ammonifying bacteria which were able to utilize the newly available substrates.

The amount of nitrite-N remained low throughout the period of incubation of the soils. This agrees with the suggestion (Jacques, Robinson and Chase, 1959) that the ammonium oxidising organisms are susceptible, while nitrite-oxidising bacteria are not.

Marked effects upon both processes were also seen in field soils.

There is an extensive literature dealing with the effects of fungicides and fumigants on nitrification and ammonification, including :- Thiegs (1955); Jacques, Robinson and Chase (1959); Gasser and Peachey (1964); Good and Carter (1965); Dubey (1970); Prasad, Rajale and Lakhdive (1971); Prasad and Rajale (1972); Bundy and Bremner (1973).

The addition of fungicides to soils both in the field and in the laboratory had a marked effect upon the mineralization of nitrogen. Such changes will have a direct effect upon soil fertility. In the past the importance of the nitrate form of the element has been emphasised. Recently however, it has been found desirable to change the equilibrium of soil nitrogen in favour of ammonium-N. A more detailed account of these changes in relation to soil fertility follows in the general discussion.

THE EFFECT OF FUNGICIDES ON THE EXCHANGEABLE METAL IONS OF SOILS

Introduction

Micro-organisms, especially bacteria and fungi, play a major role in influencing the availability of plant nutrients in soils (Alexander, 1961; Erlich, 1971). In general micro-organisms are active in interconverting various of the oxidation states of the element, or by the production of acids which solublize the mineral from the parent rock. Differential effects of fungicides upon the microbial population may thus alter the concentration of various metal ions in soil. Such changes may be beneficial to plant growth, or may result in a build up of the ion to toxic levels.

In the present study the effects of fungicide treatment on the level of exchangeable sodium, potassium, copper, manganese, and zinc were determined both in the field and in the laboratory. A short review of the properties, and the extent to which micro-organisms influence the availability of these cations follows -

Potassium

Potassium is one of the major macronutrients needed by higher plants, and is present in these in quantities larger than any mineral nutrient with the exception of nitrogen (Black, 1968). The content of potassium in mineral soils is larger than that of nitrogen or phosphorous, and the supply of this element is rarely limiting to plant growth. In most soils however, the great bulk of potassium is non-

exchangeable, with as little as 0.4% of the total potassium content of soil in the exchangeable or water soluble form (Black, 1968). Anderson, Keyes and Cromer (1942) found that the exchangeable potassium content of normal soils ranged from 66-341 μg/g of soil. Ammonium salts are generally used to displace potassium in soil, despite the criticism of Merwin and Peech (1951) who found that sodium acetate and 0.2 N-acetic acid extracted more potassium than did ammonium acetate.

Potassium is solubilized through the microbial liberation of organic and inorganic acids which react with potassium containing minerals. However, the element is also immobilized by micro-organisms, so that the amount present in the soil at any one time is an equilibrium concentration between microbial mobilization and immobilization (Alexander, 1961).

Certain bacteria including Bacillus spp., and fungi including Asperigillus niger are capable of releasing potassium from native aluminosilicate minerals. The production of the following mineral acids, carbonic, nitric, sulphuric and certain organic acids are important in the mobilization of this element (Alexander, 1961).

Sodium

Sodium is an important plant nutrient mainly because of its relationship with potassium. Plants tend to vary in their sodium requirement, but nevertheless together with potassium it is the principle monovalent cation found in plants. The relationship between sodium and potassium in plant nutrition has been reviewed by Black (1968).

The remaining metal ions which have been studied in relation to fungicide application are the micro-nutrients copper, manganese and zinc. The relationship between these cations and microbial activity has been reviewed by Erlich (1971). He considered that the following factors affect the availability of these nutrients :-

(i) Micro-organisms may contribute directly to weathering of the parent rocks by the production of acids.

(ii) Micro-organisms may precipitate or solubilize minor elements by catalysis involving enzymatic oxidation or reduction reactions in which the minor element acts as an electron acceptor or donor.

(iii) Micro-organisms may liberate minor elements by metabolic intervention, that is by the production of an end product which reacts with minor elements in non-biological redox reactions, salt formation, acid-base reactions or complexing agents.

Copper

Copper is essential to all cellular forms of life. Higher plants suffer when copper is deficient, exhibiting impaired carotene and chlorophyll synthesis. Copper is found in a number of enzymes including phenol- and cytochrome oxidases.

In normal soils copper concentrations vary between 0.1 and 1000 μg per g of soil. The solubility of the ion is very dependent upon, pH, Eh, and carbonate and sulphide concentrations. In addition certain forms of organic matter complex Cu^{2+} and increase its stability in solution.

Bacteria, such as Thiobacillus thioxidens are able to solubilize Copper compounds from carbonates, silicates, and copper oxides, mainly by the production of sulphuric acid formed in the oxidation of sulphur. In addition bacteria, such as B. subtillus and B. firmis can produce complexing agents which can separate the ion from the mineral by binding it more firmly (Erlich, 1971).

Zinc

Like copper, zinc is required by all cellular forms of life. A deficiency in higher plants results in reduced production of hormones such as indoleacetic acid.

In soil the concentration of the ion may vary from 10 µg to 1000 µg per g and like copper, its mobility is affected by pH and adsorption and binding in organic matter. Again micro-organisms appear to influence the production of this ion by the formation of complexing agents. Desulfovibrio desulfuricans for example can form ZnS from $2ZnCo_3$- $3Z_n(OH)_2$, from $ZnCo_3$ and from Zn^+ adsorbed on clays. Similarly the ferrobacillus-thiobacillus group of bacteria can oxidise ZnS to $ZnSO_4$ aerobically under acid conditions (Erlich, 1971; Bryner and Jameson, 1958).

Manganese

The concentration of manganese in the soil appears to fluctuate more than most elements. It exists as a bivalent ion, and as the higher oxides of different valencies, and degrees of reactivity. The bivalent reduced state is the one most readily available to plants, while the higher valency forms tend to be largely unavailable.

These various forms of the element are in equilibrium with one another, the direction of which depends on redox potential and moisture status of the soil.

The oxidation state in which manganese is present in soils depends to a large extent on the activities of micro-organisms. Manganese occurs mainly in the Mn^{2+} state, which is soluble in soil solution and is available to both plants and micro-organisms; and in the tetra-valent Mn^{4+} state which is generally present as MnO_2 and is insoluble and unavailable. The overall reactions involved in these changes in oxidation states are :-

$$(1)\quad Mn^{2+} + 2OH^- + \tfrac{1}{2}O_2 \longrightarrow MnO_2 + H_2O$$

$$(2)\quad MnO_2 + 4H^+ + 2e^- \longrightarrow Mn^{2+} + 2H_2O$$

Oxidation of Mn^{2+} yields Mn^{4+} while reduction of the tetra-valent form yields Mn^{2+}. The form of the element which predominates is largely dependent on soil pH. As the soil pH rises above 8.0 manganese tends to oxidize, while soil acidity below pH 5.5 favour the reduced state. Because the oxidised state is largely unavailable to plants manganese deficiency symptoms are usually seen in such alkaline soils (Christensen, Toth and Bear, 1950), but deficiences have also been found in near neutral soils (Grasimanis and Leeper, 1966).

The oxidation of manganese in neutral and acidic soils is largely biological, with a pH optimum of between 6 and 7.5 (Leeper and Swaby, 1940). The reduction reactions on the other hand tend to be non-biological. Anything which checks the microbial oxidation of Mn^{2+} causes the exchangeable and available form to predominate.

Organisms which have been shown to be involved in Mn oxidation include Aerobacter, Bacillus, Corynebacterium and Pseudomonas, Spp; and the fungal genera Alternaria, Cladosporium, Curvularia, Helminthosporium and Cephalosporium (Ivarson and Heringa, 1972). There is little conclusive evidence to suggest that micro-organisms obtain energy from the oxidation of manganese, i.e. they are not chemoautotrophic micro-organisms. However, there are indications that such chemoautotrophy may exist (Alexander, 1961).

It appears that the oxidation of manganese involves the microbial production of the following organic acids, citrate, malate, tartarate and lactate. The micro-organisms which produce these compounds appear to immobilize only a fraction of the total available soil manganese, so that unlike potassium, there is little competition between plants and micro-organisms for this element.

Little work is available on the effects of modern fungicides on soil chemical properties, although there is an extensive literature dealing with the effects of steam sterilization and fumigation on the nutrient status of soils (Warcup, 1957).

MATERIALS AND METHODS

(1) Laboratory Experiments

Fungicides were applied as solutions or suspensions to Sutton Bonington soil. Samples were then incubated in Erlenmeyer flasks sealed with "Parafilm", at 25°C for 21 days. At intervals samples were removed for determination of cations present.

(2) Field Experiments

Fungicides were applied to the Rothamsted field plots at the rates shown in Table 2. Twenty eight days after treatment samples were transported to the laboratory and stored overnight at 0°C. Cation analysis was then performed within 3 days.

Determination of Cations

Cations were extracted with a molar solution of ammonium acetate, as suggested by Hesse (1971). 10 g of soil (<2 mm) were leached by shaking for 2 h with 100 ml of M ammonium acetate (pH7). The suspension was then filtered through Whatman No. 1 filter paper and the filtrate was analysed quantitatively for the following elements : Na., K., Cu., Zn. and Mn.

Determination of these cations was carried out using a Unicam SP.90 atomic absorption spectrophotometer. Stock solutions containing 1,000 µg/ml of the cation were prepared by dissolving the following salts in double distilled, de-ionized water : Potassium - 1.90 g. KCl per 1.: Sodium - 2.54 g. NaCl per 1.; Copper - 3.92 g $CuSO_4$ $5H_2O$ per 1.; Zinc - 4.39 g $ZnSO_4$ $7H_2O$ per 1.; Manganese - 4.05 g $MnSO_4$ $4H_2O$ per 1. From the stock solutions, appropriate dilutions were made so that the highest concentration gave at least 80-90% absorption or emission. At least three lesser dilutions were then used

to prepare a standard curve. Na and K were determined by emission - the rest by absorption spectrophotometry.

Table 8 Laboratory Studies on the Effect of Fungicides on Exchangeable Trace Elements

Treatment Fungicide µg/g active ingredient	Time(Days) 2	7	14	21
Cu. mg/100 g of soil				
Control	30	22	10	10
Benomyl 50	20	25	17	22*
Benomyl 250	20	27	12	20
Captan 50	20	32	17	17
Captan 250	20	30	17	17
Thiram 50	20	17	17	22*
Thiram 250	12*	30	17	25*
Verdasan 10	30	27	22*	15
Verdasan 50	25	60*	37*	15
Mn. mg/100 g of soil				
Control	32	20	25	25
Benomyl 50	25	37*	55*	47*
Benomyl 250	32	52*	45*	50*
Captan 50	32	52*	45*	50*
Captan 250	40	60*	60*	60*
Thiram 50	40	37*	57*	60*
Thiram 250	50*	40*	50*	50*
Verdasan 10	32	35*	55*	50*
Verdasan 50	32	37*	52*	47*
Zn. mg/100 g of soil				
Control	62	65	45	72
Benomyl 50	42*	52	56	55
Benomyl 250	41*	80	60	59
Captan 50	55	56	45	41
Captan 250	57	80	49	46
Thiram 50	44*	60	47	64
Thiram 250	47	72	45	63
Verdasan 10	62	67	50	45
Verdasan 50	45	55	37	57

Means of 4 replicates *Significantly different from control at P>0.05

Table 9 Laboratory Studies on the Effect of Fungidices on Na and K

Treatment: Fungicide µg/g active ingredient	Time(Days) 2	7	14	21
Na. µg/g of soil				
Control	262	116	137	134
Benomyl 50	231	123	162*	150
Benomyl 250	237	145	146	182
Captan 50	262	127	144	146
Captan 250	237	189	142	121
Thiram 50	237	127	162*	185*
Thiram 250	244	135	151	236*
Verdasan 10	406*	137	162*	247*
Verdasan 50	712*	662*	656*	989*
K. µg/g of soil				
Control	175	220	137	139
Benomyl 50	170	236*	139	155
Benomyl 250	165	246*	146	161*
Captan 50	187*	237*	144	154*
Captan 250	182	254*	143	159*
Thiram 50	179	232*	162	151
Thiram 250	179	236*	152	177
Verdasan 10	182*	246*	152*	145
Verdasan 250	190	250*	156*	155

Means of 4 replicates

*Significantly different from control at $p > 0.05$.

Table 10. Changes in Soil Exchangeable Cations Following Application of Fungicides in the Field

	Cu (Mg/100g)	Mn (Mg/100g)	Zn (Mg/100g)	Na (ug/g)	K (ug/g)
Control	40	55	30	184	250
Benomyl (a)	16*	66	31	230	325
Benomyl (b)	24	120	41*	236	293
Captan	41	56	27	231	308
Thiram	35	150	59*	243*	400*
Quintozene	32	272*	61*	278*	240

Means of 8 replicates.

*Significantly different from control at $p > 0.05$.

RESULTS

The application of fungicides to field soils generally increased the amounts of exchangeable Mn, Na and Zn (Table 10). In Thiram treated soils there were statistically significant increases in Na, K and Zn. The only other significant changes followed the application of Benomyl. At the higher rate Cu was significantly decreased while at the lower rate Zn was increased.

Laboratory studies on the effects of fungicides on exchangeable cations are shown in Tables 8, 9. Throughout the incubation period there was a general increase in the amount of exchangeable K following fungicide treatment, while amounts of exchangeable Na were not generally significantly different from the control, except following the application of Verdasan. Small increases in Na were seen in Benomyl and Thiram treated soils.

The effects of the fungicides on the concentration of trace elements in the laboratory incubated soils are shown in Table 8. Significant increases in the concentration of Mn followed the addition of fungicides. Generally with the exception of the two day sample, fungicide application also increased the amounts of Cu, while the only changes in the concentration of Zn were decreases after the application of Thiram at 50 μg/g of soil and at both concentrations of fungicides.

DISCUSSION

Changes in the exchangeable cation status of soils following the application of fungicides have rarely been reported. However, similar though more pronounced effects have been shown following fumigation and steam sterilization. Aldrich and Martin (1952) found increases in Ca, Mg, Mn and K following fumigation with D-D and chloropicrin. Similarly Smith (1963) reported increases in Fe, Mn and P in fumigated soils, and Skipper and Westerman (1973) have recently detailed numerous changes following propylene oxide and sodium azide treatment. Steam sterilization in particular has a marked effect on the availability of Mn in soils. Often the amounts of available Mn are increased to toxic levels (Boyd, 1971; Sonneveld and Voogt, 1973).

In this study significant increases in exchangeable K and Mn occured, with smaller increases in Cu, Zn and Na. Most of these changes have been observed in partially sterilized soils, particularly following fumigation (Warcup, 1957). The following factors are important in determining the degree of change in the nutrient status of soils following fungicide treatment.

(1) Activities of Micro-organisms

Many of the biochemical changes which follow fungicide treatment can be attributed to the marked flush of heterotrophic bacteria. Micro-organisms, particularly bacteria, are important in influencing the availability of soil cations, either directly or by the formation of exogenous products. The concentration of a metal ion depends, to a large extent, on the balance between microbial mineralization and

immobilization. Bacteria in particular, can mobilize metal ions from the native mineral by the production of organic acids. Large quantities of these acids, in particular 2-Keto-gluconic acid, are likely to be produced by the increased bacterial population in treated soils (Webley and Duff, 1965; Barber, 1969). The increases in K seen in fungicide treated soil may result from such mineralization. Balancing this release of cations into soil solution is the microbial utilization of metal ions, i.e. immobilization.

Casida and Santolo (1961) found an increase in the growth of bacteria in the presence of manganese and ion, similarly Weinberg (1964) found that Mn is required for the growth of Bacillus spp., and Heim and Lechevalier (1956) found that strains of Streptomyces increased their dry weight in the presence of iron and zinc.

Very small amounts of cations will also be released following lysis from micro-organisms which are killed by the fungicides. However this is unlikely to contribute greatly to the cation content of the treated soil.

Differential effects of fungicides on the microbial population may also influence the level of cations. For example, if the manganese oxidising bacteria are more susceptible to fungicides than are the Mn reducers, this will result in an increase in the available form of the element.

(2) The Presence of Organic Matter.

Allison (1951) found that water soluble organic matter was increased in soils following fumigation with ethylene oxide solution. Similar increases in organic matter will occur in soils treated with fungicides. It appears that enzymes liberated from such organic

matter continue to degrade compounds, forming, amongst other things, soluble complexing agents, which interfere with cation fixation.

Organic matter has the greatest effect on the availability of manganese. Heintze (1957), for example, found that the higher oxides of Mn did not commonly occur in organic soils. Thus fungicide treated soils containing large amounts of water-soluble organic matter would favour the reduced and exchangeable form of the element. The effect of organic matter on Mn transformations must take at least three forms.

(i) The production of complexing agents to effectively reduce the activity of the free ion solution.

(ii) A decrease in the oxidation potential of soil, either directly or indirectly through increased microbial activity.

(iii) A stimulation of microbial activity which results in incorporation of Mn in biological tissue.

(3) Hydrogen - Ion Concentration (pH)

The pH of soil increases markedly following partial sterilization, particularly fumigation (Allison, 1951; Skipper and Westerman, 1973). Similar increases in pH occurred when soils were treated with fungicides. In some cases this increase is probably due to the presence of basic substances in the formulation, thus, for example, Verdasan contains basic sodium carbonate, and has an initial high pH. However, even in Verdasan treated soils the pH continued to rise over the incubation period. Such pH increases are probably due to the formation of basic NH_4^+ ions in soil solution. Changes in pH are important because, in addition to moderating the absorption and precipitation of micro-nutrients in soil, pH also alters plant uptake of elements.

Fungicides can, by altering the pH, microbiology and water soluble organic content of soils, markedly influence the nutrient status of soils. In addition, most fungicides contain metal ions within their formulation which directly increases the soil cation content. Increases in cations are consistently found in partially sterilized soils, whether the agent of sterilization is steam, fumigant, or fungicides. The degree of alteration in the nutrient relates directly to the "strength" of the partial sterilant. Thus, of the three, fungicides have the least effect upon the soil nutrient status. However, such changes may have a marked influence on the growth of both plants and micro-organisms.

THE EFFECT OF FUNGICIDES ON THE AUXIN CONTENT OF SOILS

Introduction

Soils are known to contain compounds which exhibit biological activity (Parker-Rhodes, 1940; Stewart and Anderson, 1942; Hamence, 1946; Whitehead, 1963 and Sheldrake, 1971). This activity is thought to be due to the auxin 3-indoleacetic acid (IAA). As yet however, no soil auxin has been fully chemically characterized (Stevenson, 1967). The auxin content of the soil depends on the soil type, and the extraction method employed. Values of apparent IAA content rante from 0.062 - 45 $\mathring{\mu}$g. per kg of soil, with soils rich in organic matter containing the largest amounts (Hamence, 1946). Auxins appear to be non-uniformily distributed in soils, and are assumed to originate mainly as the result of microbial activity. Micro-organisms can synthesise IAA and other indoles *in vitro* and appear potentially capable of producing large quantities in the soil (Roberts and Roberts, 1939).

The study of auxin biosynthesis *in vitro* is relatively straightforward; in the environment however, it is complicated by the fact that both micro-organisms and plants synthesise indoles from the same precursor L-tryptophan.

The microbial degradation of tryptophan has been reviewed by Phelps and Sequeira (1968). The basic pathways which are involved are shown in Fig. 37. Thimann (1935) showed that L-tryptophan was required by *Rhizopus suinus* in the synthesis of IAA, and that 3 indolepyruvic acid (IPyA) was a possible intermediate. Subsequently a number of

workers have showed that tryptophan is the major precursor in the microbial production of auxin (Kaper and Veldstra, 1958; Larsen, Harbo, Klungsoyn and Aasheim, 1962; and Buckley and Pugh, 1971).

Recent work by Gordon and Paleg (1961) suggests that auxins may be formed from tryptophan by the action of phenols, and phenol oxidases, without the direct intervention of plants or micro-organisms.

Definite statements on the role of soil auxins must await further studies to determine their exact chemical nature, and elucidate their effects on plant growth. However auxins are known to have a direct effect upon root growth (Sheldrake, 1973) and this, coupled with their importance as plant growth regulators, suggests that they may play an important role in soil fertility.

The purpose of this work was to characterize soil auxins and determine what effect the addition of the fungicide Captan would have upon their nature and concentration.

MATERIALS AND METHODS

(1) Incubation

Fresh Sutton Bonington soil was sieved (<2 mm) and brought to 66% of its water holding capacity with sterile distilled water. Soils treated with Captan received 50 μg/g active ingredient per g of soil. Soils (300 g) were then incubated in 1 litre polythene bottles sealed with "Parafilm" for 7 days at 25°C. prior to extraction. Ten replicates of treated soils and controls were included.

(2) Extraction

Extraction of auxin was based upon the method of Hamence (1946). The soils (300 g) were extracted with 1% W/V $Ca(OH)_2$ (250 ml) by shaking for 2 h in the dark at 25°C. The soil slurry was then filtered under pressure into a Buchner flask darkened with aluminium foil and cooled in a salt-ice freezing mixture.

A portion of the filtrate (200 ml) was then adjusted to pH 2.5 with 2.5 NHCl and extracted with 2 x 100 ml of diethyl ether. The ether was washed with water and the acidic fraction removed by partitioning with 5% Na HCO_3. This fraction was then adjusted to pH 3.4 and extracted with a final volume of ether which was concentrated in vacuo at 37.5°C and completely reduced with nitrogen. The residue was then taken up in 1 ml of ether for chromatography.

(3) Chromatography

All chromatography was performed at 25°C in the dark.

In the first instance 100 μg of the extracts were spotted onto Whatman No. 1 Chromatography paper and run in propan-1-ol/NH_3/ water solvent (10 : 1 : 1). A number of indole compounds were run alongside the extract as standards.

The chromatograms were then sprayed with Erlich's reagent (1% p-dimethyl-amino-benzaldehyde in N.HCl). The extracts produced a pattern of spots which was similar to that produced by the 3-indole pyruvic acid standard.

In order to demonstrate that only one compound was present the extracts were next run two dimensionally in acetic acid/butan-1-ol/ water solvent (16 : 25 : 5) and then at right angles in 20% KCl. The chromatograms were examined under u/v light and then sprayed with the following locating reagents (1) Erlich's reagent, (2) Salkowski's reagent, (3) ammoniacal silver-nitrate reagent (4) nitric-nitrite reagent.

(4) Paper Electrophoresis.

The extracts were spotted onto Whatman No. 1 Chromatography paper and run for 4 h at low voltage (7.35 volts per cm), in a Shandon paper electrophoresis unit. The following buffer solution was used - glacial acetic acid + 25 ml pyridine diluted to 625 ml with water (pH 6.2).

(5) Ultra-violet Spectroscopy

Final characterisation of the unknown was achieved by extracting the spot located with u/v light with 50% v/v ethanol/water and determining its u/v spectrum using a Unicam Sp. 800 Spectrophotometer. For

comparison the u/v spectra of the following indoles, 3-indoleacetic acid (IAA), 3-indolealdehyde (IA), 3-indoleacetaldehyde (IAC), 3-indolepyruvic acid (IPyA), 3-indolecarboxylic acid (ICA); were determined.

(6) Quantitative Determination

Depulicate samples of the ten replicates were chromatogrammed. The fluorescent spots were located under u/v light and cut out. The two duplicate spots were then combined and eluted with 50% V/V ethanol/water. The amount of fluorescence was then determined at 312.5 nm, using a Unicam Sp. 600 ultraviolet spectrophotometer. The amount of unknown in the fungicide treated soils was then calculated as a percentage of the control.

(7) Determination of Certain Properties of IPyA

The breakdown of IPyA under specific conditions was determined :-

(a) The Effect of pH on the on the Stability of IPyA.

A 0.001M solution of IPyA in 50% V/V ethanol/water was prepared. Citrate-phosphate buffer (1 ml) was added to 4 ml of solution to produce solutions of IPyA at the following pH. 2,4, 6 and 8. The u/v spectra of the solutions were determined immediately.

(b) The degradation of IPyA with Time at pH 8.0.

The breakdown of IPyA at pH 8.0 was determined after 0, 10, 15, 25 and 40 mins.

(8) Determination of Biological Activity Using the Wheat Straight Coleoptile Test.

Wheat seeds were germinated on pads of damp tissue paper in the dark at 25°C. When the coleoptile reached 10 cms they were removed and floated in distilled water in the dark (25°C).

Sections of coleoptile (4 mm) were then cut using a standardised cutter, and were floated in distilled water for a short period. The chromatogram spots were located on the chromatography paper under u/v light and cut out. Two spots were then cut into small pieces and eluted in glass vials containing citrate-phosphate buffer (pH 5.0) + 2% sucrose (1 ml). Controls were also included. These consisted of areas of solvent impregnated paper, similarly eluted. The coleoptile lengths were added to the elutants and shaken (1 stroke/min) in the dark at 25°C for 20 h. At the end of this period the coleoptile sections were removed and changes in their length determined.

Table 11 Amounts of Auxin Characterised from Control and Captan Treated Soils

Control	Captan 50 ug/g of Soil
Soil Sample	Auxin Expressed as Percentage of Control
1	212.3
2	221.0
3	223.0
4	121.1
5	124.0
6	30.6
7	211.4
8	211.4
9	216.3
10	211.4
Mean	178.0

RESULTS

(1) Chromatography

The pattern of spots produced when the unknown substances were run in propan-1-ol/NH_3/water solvent is shown in Fig. 32. Five definite spots were produced, together with two faint ones. The pattern was almost identical to that produced by the IPyA Standard. None of the other indoles tested produced a similar breakdown pattern.

When the unknown extracts were run two-dimensionally only one spot resulted. This spot fluoresced white/blue and was located in an area equivalent to the IPyA standard.

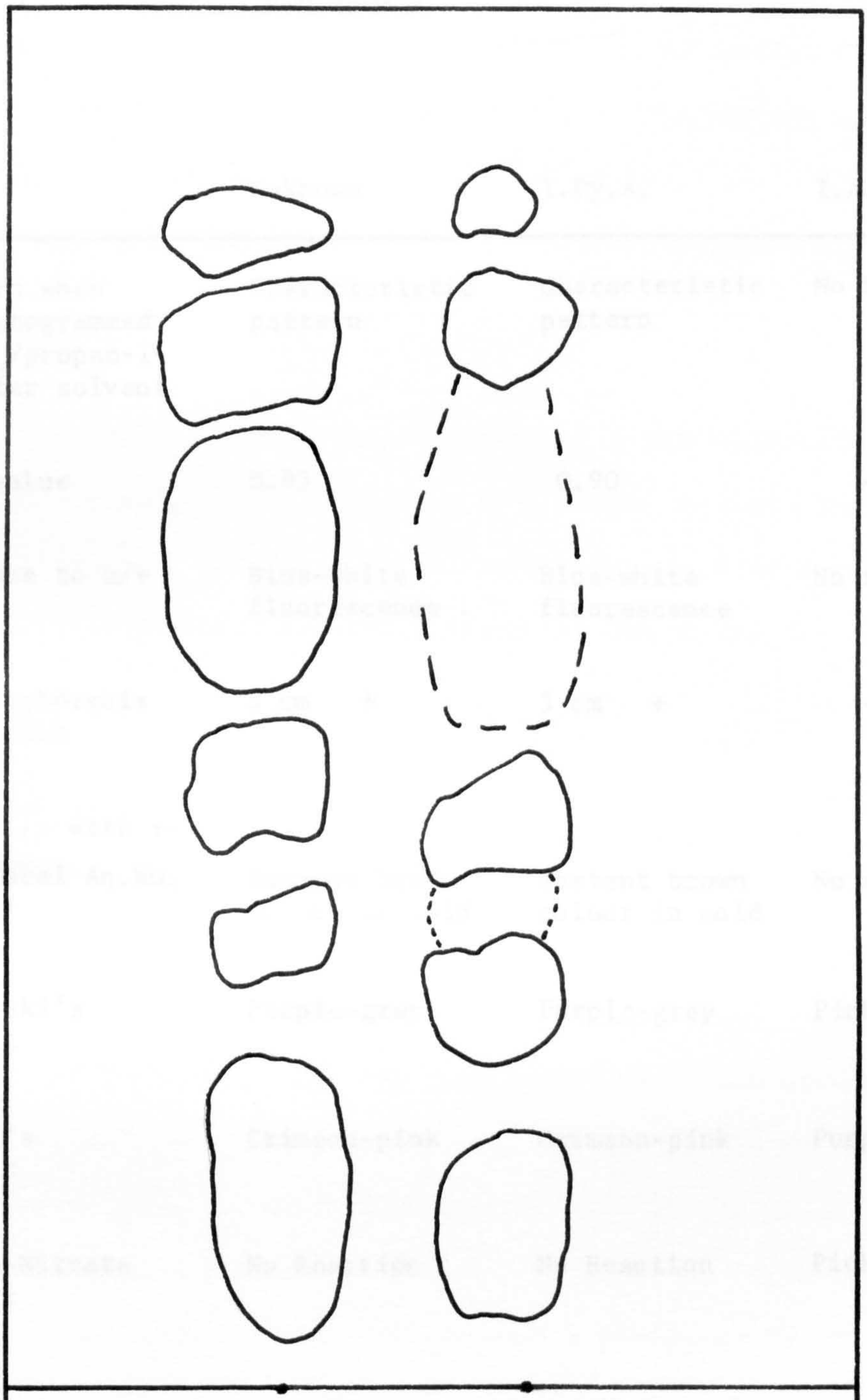
The unknowns and standards were run one dimensionally in butanol/acetic acid/water solvent, and the Chromatograms were developed with various locating reagents which are specific for indoles (Table 12). The results show that both the unknown and IPyA standard had similar r.f. values and gave identical reactions with the locating reagents.

Ultra violet light was a particularly useful tool in the characterisation of the unknown - of the following indoles, IAA, IAC, IPyA, IAN, ICA; only IPyA and IAC fluoresced. The latter fluoresced bright white, while both the IPyA standard and the unknown fluoresced blue/white.

Paper electrophoresis was used to further characterise the unknown. Both the unknown and IPyA standard were located by u/v 3 cms towards the anode following electrophoresis.

Fig. 32 The breakdown pattern of the unknown and IPyA in propan-1-ol/NH_3/Water (10 : 1 : 1) solvent

Fig 32



I. $P_{y.}$ A

Unknown

Table 12 Comparison of the Properties of the Unknown I.Py.A. and I.A.A.

Test	Unknown	I.Py.A.	I.A.A.
Pattern when chromatogrammed in NH_3/propan-1-ol/water solvent	Characteristic pattern	Characteristic pattern	No breakdown
R.f. value	0.93	0.90	-
Response to u/v light	Blue-white fluorescence	Blue-white fluorescence	No fluorescence
Electrophoresis R.f. value	3 cm +	3 cm +	-
Reactions with :-			
Ammoniacal Aq.NO_3 Reagent	Instant brown colour in cold	Instant brown colour in cold	No reaction
Salkowski's Reagent	Purple-grey	Purple-grey	Pink
Erlich's Reagent	Crimson-pink	Crimson-pink	Purple
Nitric-Nitrate Reagent	No Reaction	No Reaction	Pink

(2) Ultra-violet Spectroscopy

The unknown was finally characterized using u/v spectroscopy. The u/v spectra of a number of indoles were also determined for reference purposes. The results are shown in Fig. 33. The unknown produced a characteristic spectrum with a broad peak at 312.5 nm. The u/v spectrum of IPyA was almost identical to that of the unknown, with a peak at 312 nm.

The other indoles, with the exception of 3-indolealdehyde had markedly different spectra, consiting of a number of peaks in the 275 nm region (Figs. 36, 37(a)). 3-indolealdehyde exhibited a peak at 300 nm, but also produced two other peaks, at 245 nm and 262 nm (Fig. 36(b)).

(3) Investigation of the Breakdown Properties of IPyA.

The results indicated that the unknown was IPyA. The breakdown of pure IPyA was next determined at various pH. IPyA appeared very susceptible to changes in pH. At a pH of between 2 and 7, the typical broad curve of IPyA was given, the position of this peak altered however. At pH 2, for example, the peak was in the 330 nm region. At pH 8.0 minor peaks in the 290 nm region began to appear, indicating that the pure IPyA was breaking down into a number of components. The breakdown at pH 8.0 was determined over a period of 40 min. (Fig. 35). The breakdown began immediately and continued, so that after 40 mins two distinct minor peaks resulted.

(4) Determination of Biological Activity.

The elutants of the unknown spots exhibited definite biological activity. There were increases in wheat coleoptile length of between 18 and 94%.

Fig. 33 The ultra-violet absorption spectra of the unknown and IPyA.

Fig 33

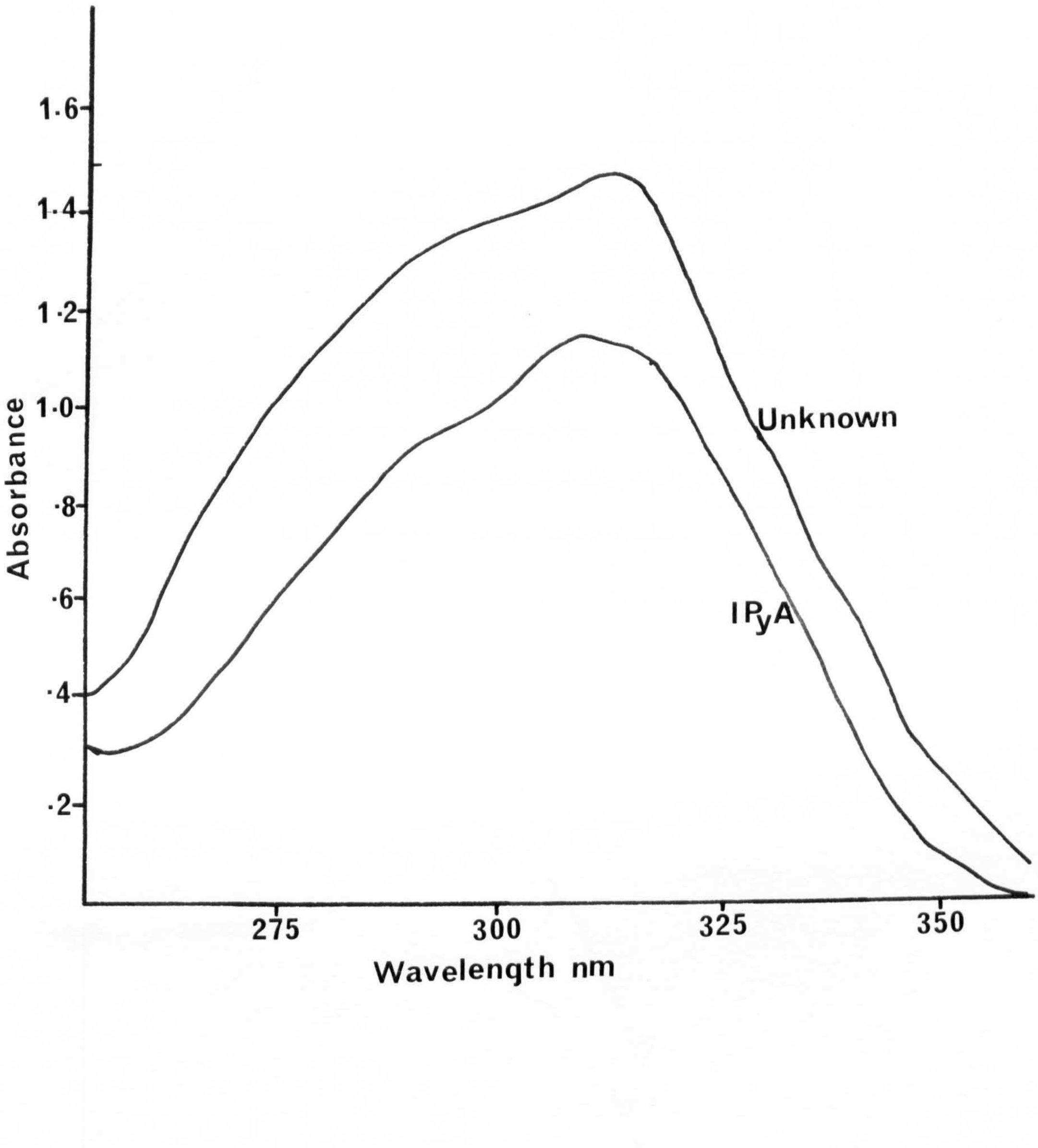
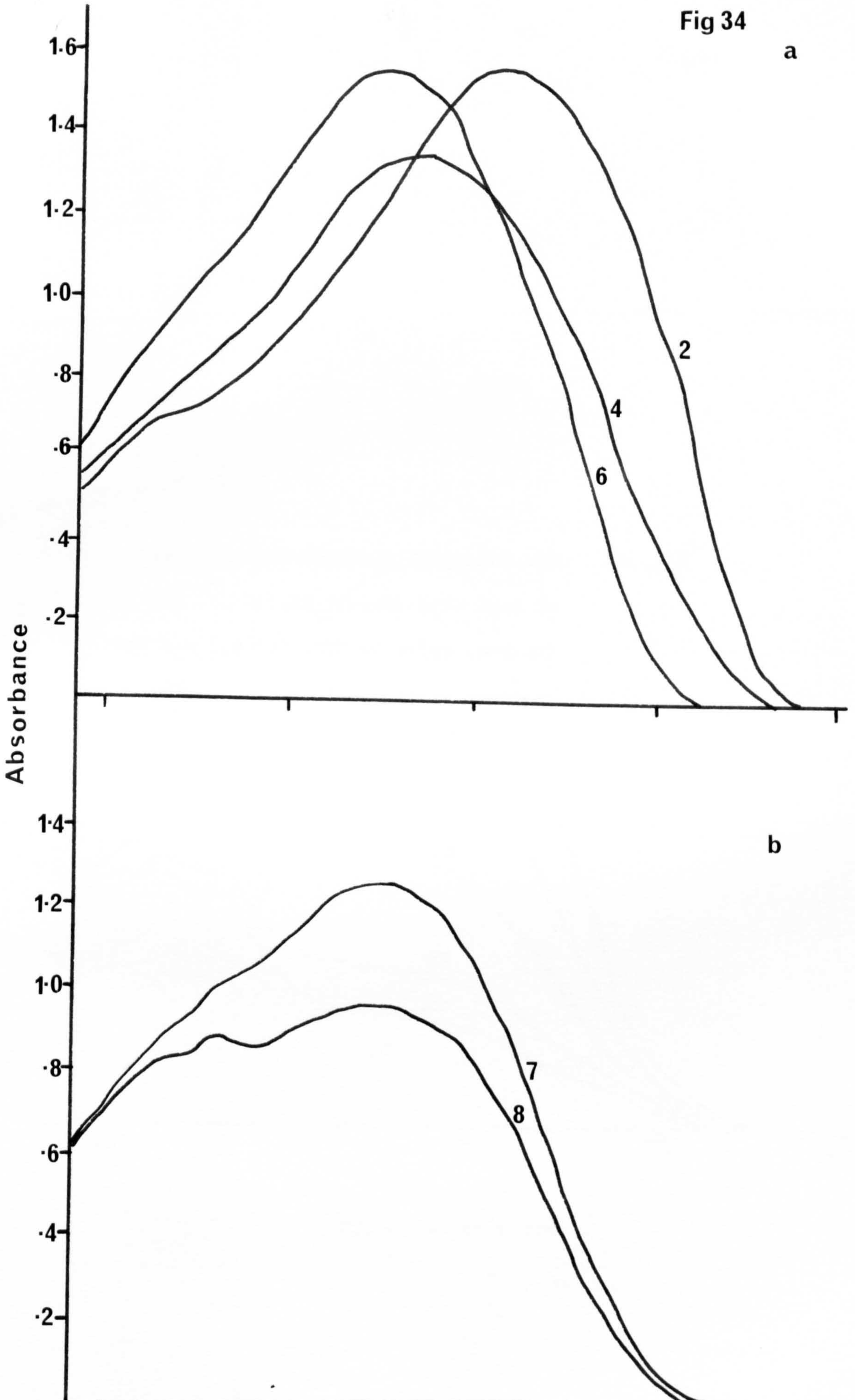

1·6
1·4
1·2
1·0
·8
·6
·4
·2
Absorbance
Unknown
IPyA
275
300
325
350
Wavelength nm

Fig. 34 The effect of pH on the ultra-violet absorption spectrum of IPyA.

Fig 34

a


1·6
1·4
1·2
1·0
·8
·6
·4
·2
Absorbance
2
4
6
1·4
1·2
1·0
·8
·6
·4
·2
7
8
275
300
325
350
400
Wavelength nm

b

Fig. 35 The change in spectral characteristics of IPyA with time at pH 8.0 (numbers refer to time (mins.) and the spectrum at pH 4.0 is included for comparison).

Fig 35

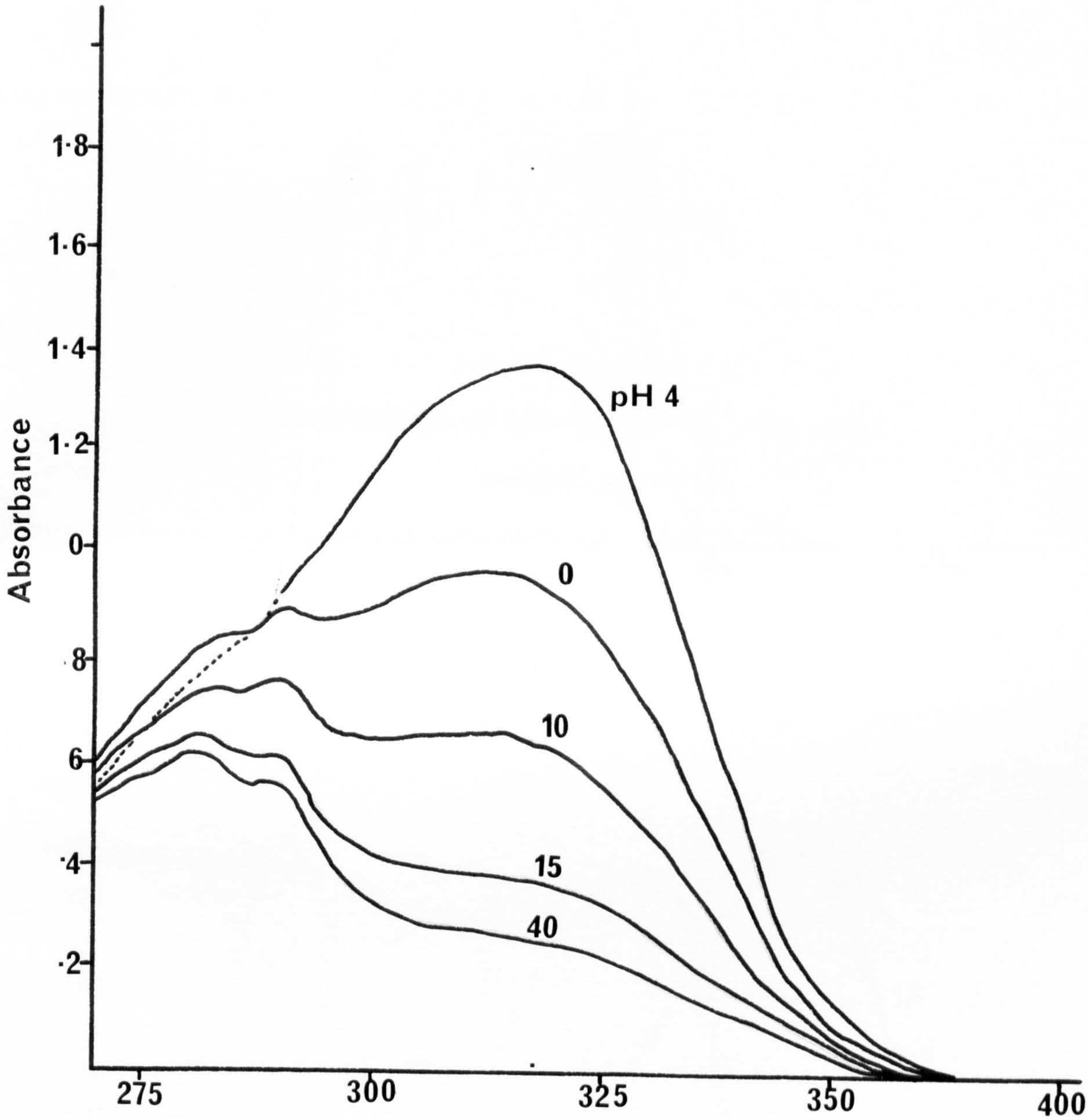

Absorbance
1·8
1·6
1·4
1·2
0
·8
6
·4
·2
pH 4
0
10
15
40
275
300
325
350
400
Wavelength nm

Fig. 36 The ultra-violet absorption spectra of certain indoles

(a) 3 - indoleacetic acid

3 - indoleacetonitrile

(b) 3 - indolealdehyde

3 - indolecarboxylic acid

Fig 36

a

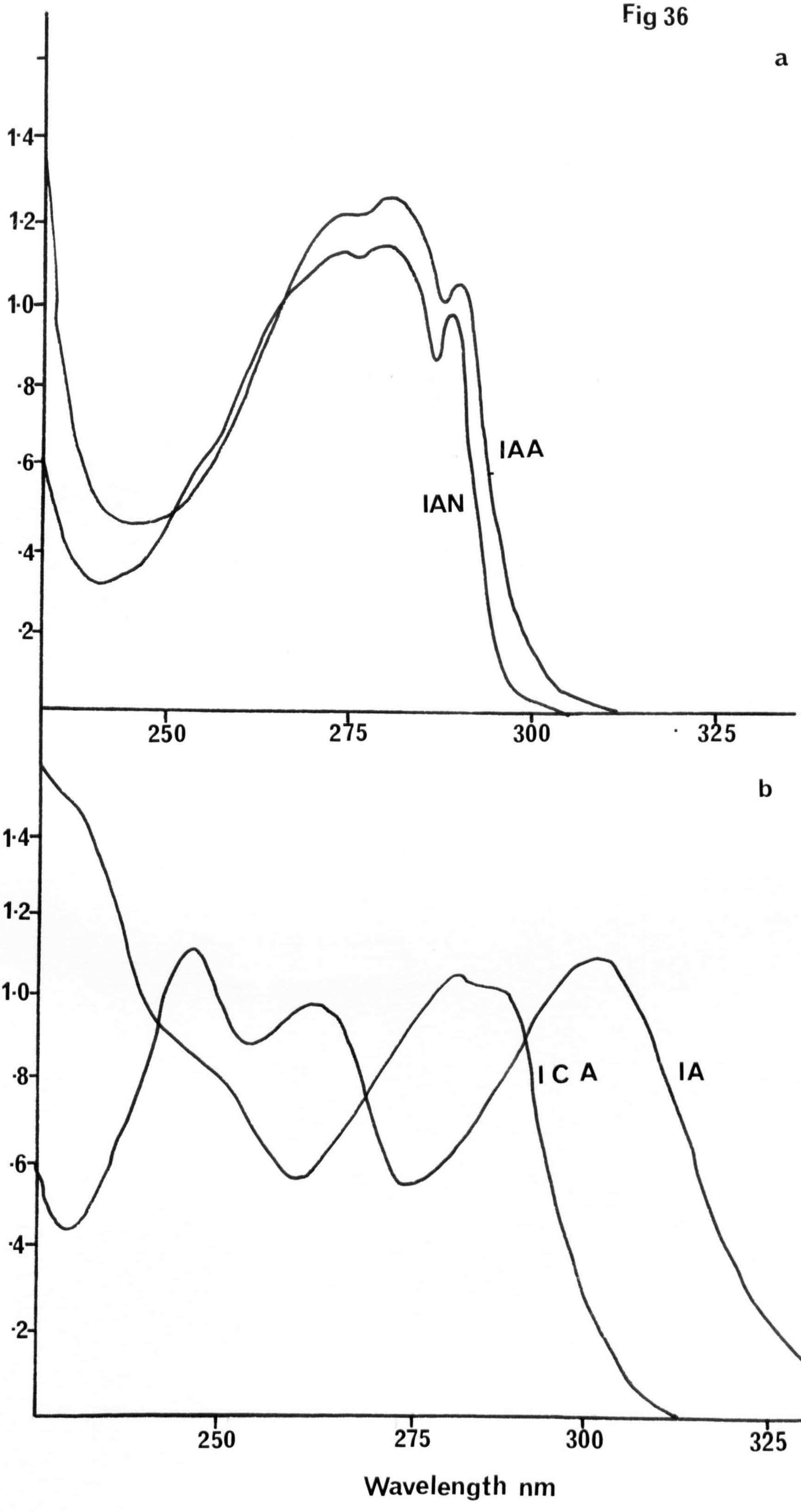

Absorbance
1·4
1·2
1·0
·8
·6
·4
·2
IAN
IAA
250
275
300
325
b
I C A
IA
1·4
1·2
1·0
·8
·6
·4
·2
250
275
300
325
Wavelength nm

Fig. 37 (a) The ultra-violet absorption spectra of certain indoles

Tryptophan

3 - Indoleacetaldehyde

(b) The effect of the addition of $CA(OH)_2$ followed by acidification to pH 2.5 on certain indoles.

Trytophan

3 - Indolepyruvic acid

3 - Indoleacetic acid

Fig 37

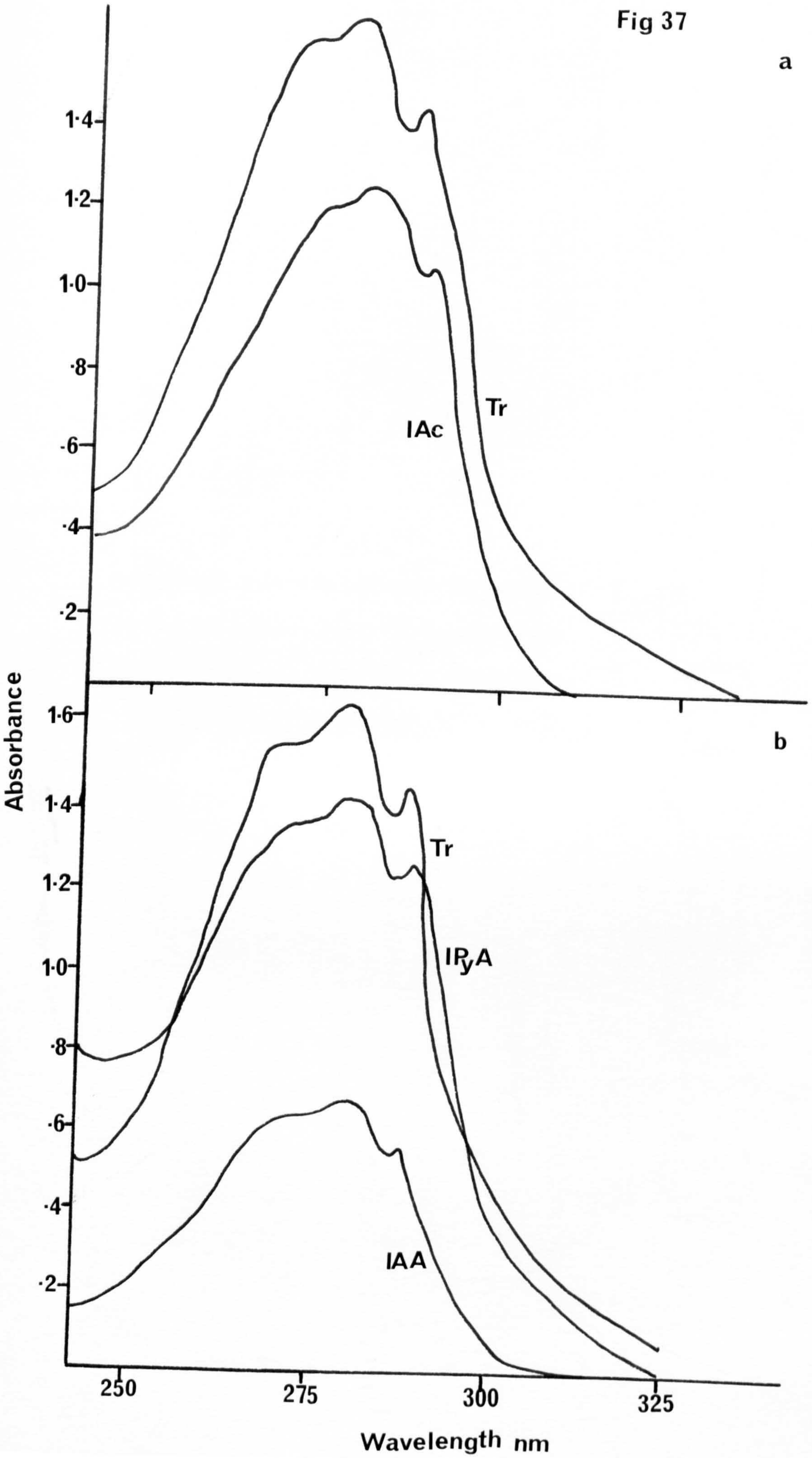
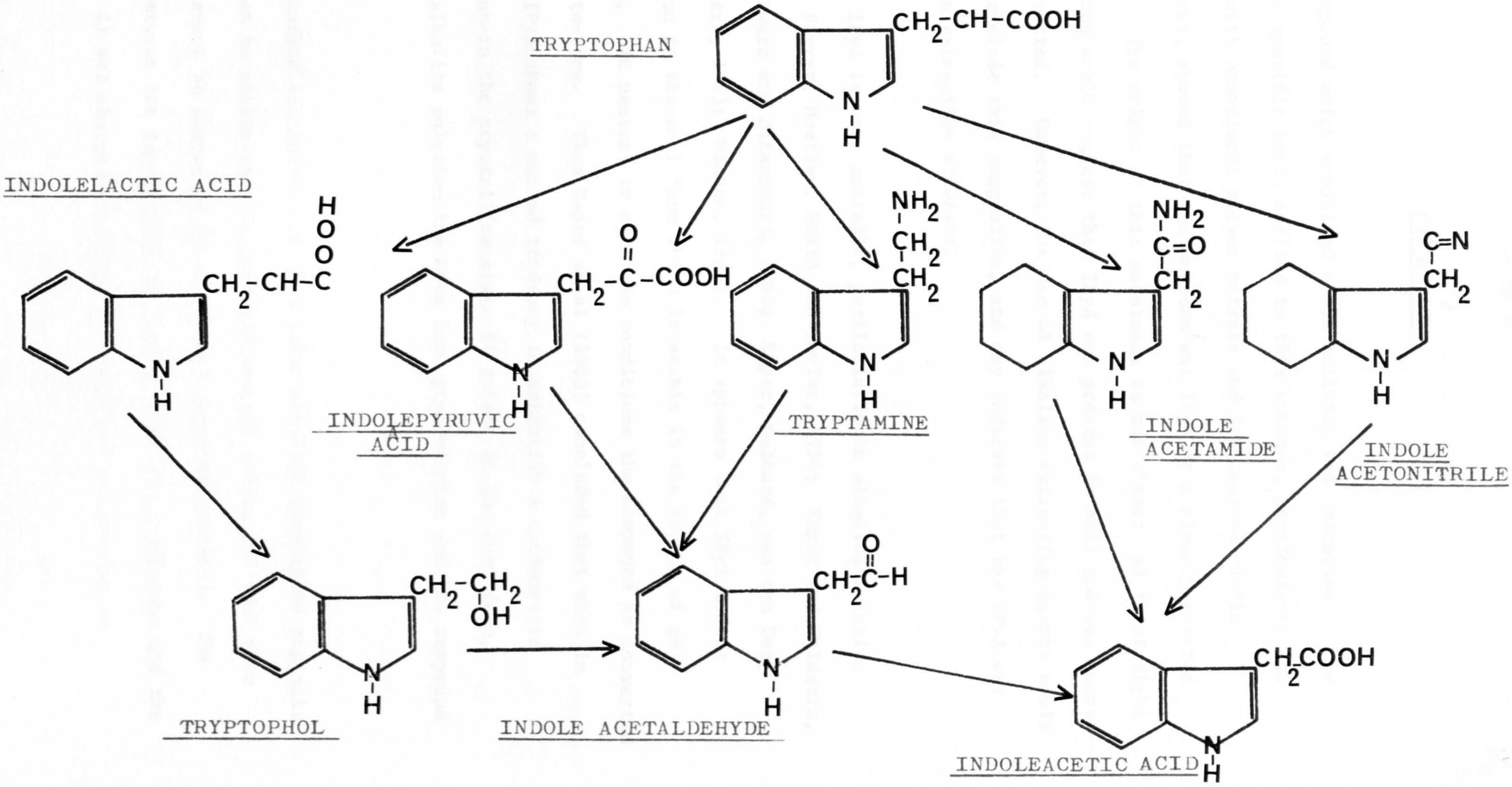

a
1·4
1·2
1·0
·8
·6
·4
·2
IAc
Tr
b
Absorbance
1·6
1·4
1·2
1·0
·8
·6
·4
·2
Tr
IPyA
IAA
250
275
300
325
Wavelength nm

Fig. 38 Summary of possible pathways in the bio-synthesis of 3 - indoleacetic acid from tryptophan
(Phelps and Sequira, 1968)


TRYPTOPHAN
INDOLELACTIC ACID
INDOLEPYRUVIC ACID
TRYPTAMINE
INDOLE ACETAMIDE
INDOLE ACETONITRILE
TRYPTOPHOL
INDOLE ACETALDEHYDE
INDOLEACETIC ACID

DISCUSSION

A compound which exhibited auxin activity was extracted. The results of specific tests applied to this unknown, particularly its reaction with ammoniacal silver nitrate and its characteristic u/v spectrum, showed that the unknown was IPyA or a closely related compound. The origin of this substance is not clear: at first sight the evidence would suggest that IPyA was present in soil and was successfully extracted. However, the use of alkaline extracting agents would probably exclude this suggestion, and may indicate that the IPyA was in fact an extraction artefact.

Pure IPyA is very unstable, particularly in alkaline solution (Bentley, Farrar, Housley, Smith and Taylor, 1956; Kaper and Veldstra, 1958; Schwarz and Bitancourt, 1960; Kaper, Gebhard, van den Berg and Veldstra, 1963; Winter, 1964). It appears that IPyA exists in solution in the enol form which is stable in the region of pH 2.9 - 6.0. In neutral to alkaline conditions the compound is converted to the keto-form. Thus Kaper et al (1963) concluded that when in solution IPyA shows a marked tendency to establish a tautometric equilibrium-in the crystalline state it exists in the enol form, while in alkaline solution the keto form predominates and the compound degrades.

The marked instability of IPyA under alkaline conditions suggests that it can be stable only in acid and neutral soils, and that when present cannot be extracted by the use of alkaline solvents. The anomaly between the instability of IPyA in alkaline conditions and the fact that it was characterised can be explained by assuming :-

(1) That it was present in the soil and was successfully extracted. This would suggest that IPyA can form protective bonds with for example organic matter which prevent it from breaking down in alkaline solvent.

(2) That IPyA was not present in the soil, but a stable compound possessing similar properties was.

(3) IPyA was not present in the soil, but a precursor (for example tryptophan) was. During the exrtaction precursor was converted to IPyA

(4) A compound which was not a precursor of IPyA (for example IAA) was present in the soils and was converted to IPyA during extraction.

The last two hypotheses were tested by dissolving pure IPyA, IAA and tryptophan in 1% v/v $Ca(OH)_2$ followed by acidification to pH 2.5 - i.e. the compounds were exposed to the same conditions used to extract the soil. After 2 h incubation at 25°C, the u/v spectra of the individual compounds were determined to see if they were stable under these conditions. The results show (Fig. 38) that tryptophan and IAA were stable under these conditions but that IPyA was converted to a compound exhibiting a markedly different spectrum in the 275 nm region. This suggests that (1) IPyA could not have been extracted in a stable form by the extracting agent used, (2) tryptophan and IAA, which were stable under these conditions, were unlikely to have been the origin of the IPyA which was characterised.

The evidence at this point suggests that IPyA was not extracted from the soil, but was an extraction artefact, derived from an as yet unknown precursor. However, the possiblity that the unknown which was characterised was a closely allied stable compound cannot be ruled out.

The major works on soil auxins have involved the use of alkaline extracting agents (Hamence, 1946; and Stewart and Anderson, 1942). However, other extracting agents have been used. Parker-Rhodes (1940) used water extracts, while more recently Sheldrake (1971) used ether extracts. Stewart and Anderson (1942) after studying the efficiency of a number of extracting agents concluded that both water and ether were incapable of extracting auxin from mineral soils. Sheldrake (1971) used ether to remove auxin from organic woodland soils and found that ether extracted only one fifth of the total soil auxin. The use of alkaline extracting agents by early workers suggests that their work may have been based on extraction artefacts, and that the presence of auxins in mineral soils has not yet been conclusively demonstrated. Clearly further work on the auxin content of soil is required.

The results presented here suggest that over twice the amount of the auxin-potential was found in the extracts from Captan treated soils compared with the controls. This increase in soil auxin-potential was probably due to the marked increase in heterotrophic bacteria found in such treated soils. A wide variety of micro-organisms have the ability to synthesise auxins including, fungi (Gruen, 1965; Valadon and Lodge, 1970; and Buckley and Pugh, 1971); bacteria (Kaper and Veldstra, 1958; Larsen et al, 1962; Sequiera and Williams, 1964); and algae (Mowat, 1963).

The presence of auxins in these soils has not been demonstrated conclusively, although the results show that the potential for auxin production was present. The use of alkaline extracting agents for soil auxins may result in extraction artefacts, and should be carefully examined in the light of the present evidence. Fungicide treatment appears to increase at least the potential for soil auxin activity.

GENERAL DISCUSSION

The fungicides used in this study range from a broad-spectrum biocide, formalin, to Benomyl, a modern selective, systemic fungicide. These compounds generally had a marked effect on the microbiology and biochemistry of soils. The changes which were seen following treatment can be summarised :-

(1) At most rates of application changes in the microbial equilibrium of the soils occurred. Fungal numbers were reduced initially, but were often in excess of the control value at the end of the incubation period. Cellulolytic fungi however, appeared to be largely insensitive to the action of fungicides. Bacterial numbers increased dramatically following treatment, to reach a peak which was dependent upon the fungicide concentration applied.

(2) High concentrations of fungicides led to a decrease in nitrate production, and a marked increase in the levels of the ammonium ion. Very low concentrations of fungicides however, tended to favour nitrification.

(3) Towards the end of the incubation period low concentrations of fungicides led to increases in the total free-amino acid-N concentration in soil, while the converse was true of high concentrations. Addition of fungicides also led to qualitative changes in the free amino acid content of soils.

(4) Increases were also seen in certain metals including K, Na, Mn and Zn, in fungicide treated soils.

(5) The auxin-potential of the soil was doubled following treatment with Captan.

Soil is a very complex environment. The activity of micro-organisms living in this environment is dependent upon a whole range of biological, chemical and physical factors. In a climax soil substrates for microbial colonization are limiting. Potential energy for growth is therefore low and organic entropy approaches maximum. Kreutzer (1963) states that under these conditions the ruling condition is one of quiescence.

When a fungicide is added to a soil it has an effect on the soil microbial population. This may be a blanket effect, or may be specific to one group of micro-organisms. Following the killing of a portion of the soil microbial biomass, substrates are released into the soil environment. Potential energy for the growth of heterotrophic organisms is raised and entropy decreases (Kreutzer, 1963).

At the climax of these changes the soil consists of an almost pure culture of heterotrophic bacteria, together with a number of resistant fungi and actinomycetes, which are able to exist in this highly competitive environment. There is an abundance of nitrogen in the form of ammonium-N and free amino acids. Certain metal ions are released into the soil, following mineralization, while others are immobilized by the flush of heterotrophic bacteria. This proliferating bacterial population is active in producing metabolic bi-products. Changes also occur in the physical properties of soil, particularly in pH.

These changes determine the nature of the microbial recolonization of treated soils and in turn have a direct bearing on soil fertility:-

(1) The Effects of Fungicide Application On Plant Pathogenic Fungi.

In addition to the direct killing of the target organisms, three direct effects of fungicides on plant pathogenic fungi have been recognised.

(i) Disease Trading - This is the situation where a dominant pathogen is controlled by the fungicide, but a minor parasite is elevated to major importance. For example, Huber, Watson and Steiner (1965) showed that Telone provided excellent control of Verticillium wilt of potatoes, but increased the severity of diseases caused by Rhizoctonia.

(ii) Boomerang Effect - This occurs when a dominant pathogen is controlled initially by the fungicide treatment, but later returns with increased virulence (Gibson, 1956; Kreutzer, 1960).

(iii)Indirect Control of Pathogens - The classical case of this phenomenon is the biological control of Armillarea mellea on citrus roots by Trichoderma spp, following soil fumigation (Garrett, 1957). An added facet of this phenomenon has been provided by Ohr and Munnecke (1974), who have suggested that sublethal concentrations of a fumigant (methyl bromide) may reduce the ability of A. mellea to produce antibiotics inhibiting fungi such as Trichoderma.

Most of these phenomona result from changes in the microbial equilibrium of treated soils. During the recolonization of soil, following the formation of a biological vacuum certain species may appear which would not have previously been dominant. Similarly a pathogenic species may be initially controlled by the fungicides, but may reappear with greater virulence due to the lack of competition from other fungi.

(2) Increased Growth Response

An increase in higher plant growth, above that resulting from elimination of pathogenic fungi, often follows fumigation or fungicide treatment. Such increases have been extensively reported. Benzian (1965) for example, showed that the sterilization of forest nursery soils with formalin led to increases in weight and vigour of conifer seedlings.

Similar increases in yield have been reported by Widdowson and Penny (1956), Salt (1967), Ebbels (1969), Cooke and Hull (1972), and Jenkinson, Nowakowski and Mitchell (1972). The phenomonen of increased growth response following treatment was reviewed by Altman (1964).

Increased growth of plants following the partial sterilization of soils is generally thought to result from the increased mobilization and availability of nitrogen. However, opposition to this view has been expressed (Wilhelm, 1965). A number of authors have suggested that other, more subtle factors are involved (Aldrich and Martin, 1952; Smith, 1963; Altman, 1970; and Rawlinson and Colhoun, 1970), for example, considered that increases in plant growth resulted in part from the increased availability of certain cations following fumigation. Wilhelm (1965) and Rawlinson and Colhoun (1970) found that the mercurial fungicide Cerasan protected the mesocotyl of oat seedlings from contamination and invasion by certain soil fungi which are normally regarded as saprophytes. One factor which has not been considered is the possibility of an increase in soil amino acids or auxin, or other growth regulators. The effects of auxin on root growth is well known (Sheldrake, 1973), and the evidence suggests that they could be responsible in part for this increased growth response.

This work indicates that there are a number of changes in soil population which may account for this phenomonen, and it is probable that no single factor is responsible.

The addition of fungicides to soil led to increases in ammonium-N and corresponding decreases in nitrate-N. In the past nitrate has been considered to be the most important form of the element. However, it has been recognised that ammonium-N can play a dominant role in plant nutrition (Black, 1968). Thus the inhibition of nitrification following fungicide treatment may be beneficial to soil fertility. Ammonium-N is fixed by cation exchange in soils, while nitrate-N is free in solution. Thus ammonium-N is less likely to be leached from the soil and in this form, this essential element is conserved. Recent work indicated that nitrification inhibitors can have a beneficial effect on soil fertility. Probably the most extensive research on any single inhibitor has been on 2-chloro-6-(trichloromethyl) pyridine (Goring, 1962). This compound has been found to be extremely specific for the autotrophic ammonium-oxidising micro-organisms.

Field experiments with cotton, sweetcorn, and sugar beets under irrigation (Swezey and Turner, 1962) showed that both the yield of the crop and the nitrogen content were increased where 2-chloro-6-(trichloromethyl) pyridine was applied with ammonium nitrogen.

In the short term, changes in the microbiology and biochemistry of soils which result from fungicide treatment appear to be marked. There is very little evidence to suggest however, that in the long term the use of modern fungicides presents a great threat to the soil environment. Indeed the evidence presented in this thesis indicates

that the converse is true, and that the changes which ensue from fungicide treatment are likely to be beneficial to soil fertility - at least in the short term. Nevertheless, constant testing and vigilence are needed in the future to avoid any major problems which could result from the continual use of fungicides and other pesticides in the soil.

A P P E N D I C E S

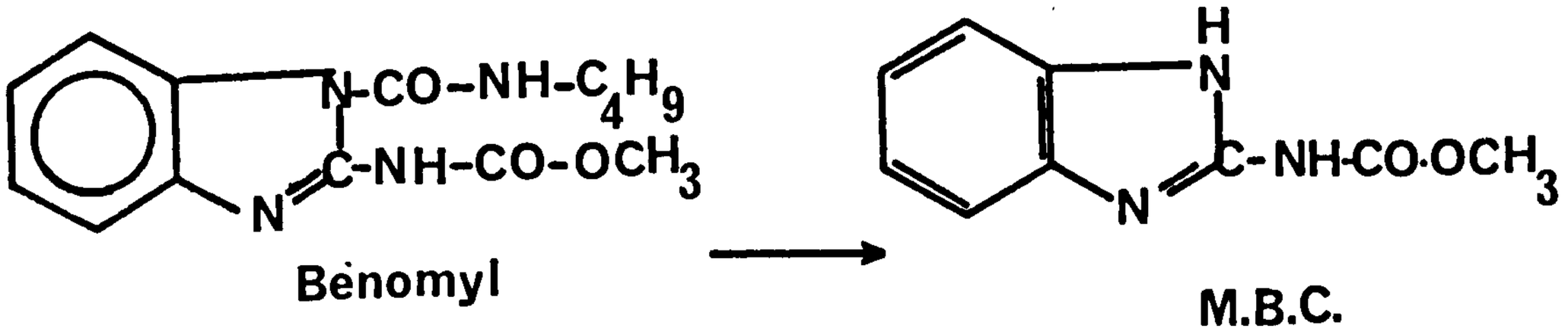
APPENDIX A. DETAILS OF THE FUNGICIDES USED

(1) Benomyl

Active ingredient Methy (1-butylcarbamoyl) - benzimadazol 2-yl carbamate 50% W/W.

In addition to its eradicant and proctectant fungicidal properties and action as a mite ovicide, benomyl possesses considerable systemic fungicidal properties (Delp and Lapping, 1968).

It is effective against Verticillium albo-atrum, powdery mildews and black spot of roses, but not against Helminthosporium spp. (Greenaway, 1972), Phytophthora infestans, or Pythium Spp. Bollen and Fuchs (1970) found that Benomyl had a striking selectivity of action within the Deuteromycetes. While certain species were sensitive members of the Porosporae and Annellosporae were not. Similarly members of the Oomycetes were found to be insensitive. Benomyl resistance has been shown in Botrytis cinerea (Bollen and Scholten, 1971) and in Penicillium brevicompactum and P. corymbiferum (Bollen, 1971).



In aqueous solution Benomyl is rapidly hydrolyzed to methyl benzimidazol-2-yl Carbamate (M.B.C.).

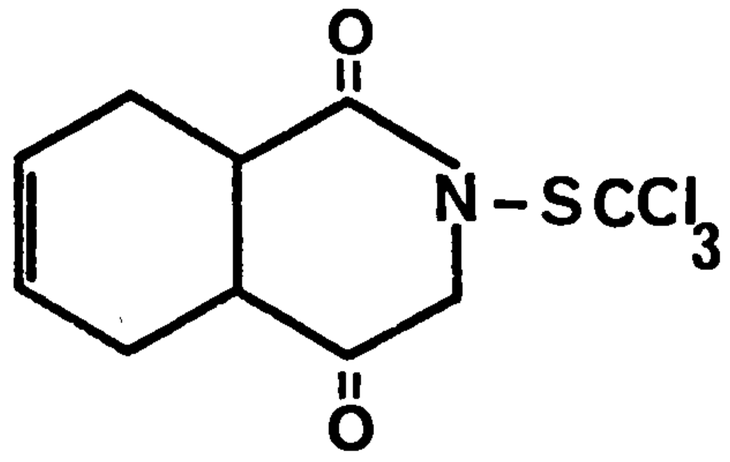
The systemic fungicides including Benomyl have been recently reviewed (Marsh, 1972).

(2) Captan

Active ingredient N-trichloromethylthio-4-cyclohexene-1, 2 di-carboximide 50% W/W.

Captan is used to control diseases of fruit and vegetable diseases, and as a seed treatment, or drench against damping off. Its fungitoxic properties were discovered by Daines (1953).

Captan has a broad spectrum of action, but is ineffective against rusts and powdery and downy mildews. Fungi differ in their sensitivity to the fungicide. McCallen et al (1954), for example found the following order of sensitivity of spore germination : Monilinia > Altenaria> Neurospora > Aspergillus. The structural formula of Captan is :-



The activity of Captan appears to be associated with the destruction of thiol groups in the sensitive fungi. Lukens (1969) has reviewed the role of Captan as a fungicide.

In soil Captan hydrolyses to non-toxic products, and has an half life of 2-4 days (Burchfield, 1959).

(3) Dicloran

Active ingredient 2, 6 Dichloro-4-nitroaniline 4% W/W.

Dicloran is used primarily to control grey mould of lettuce caused by Botrytis cinerea. Other uses include the control of stem rot of tomatoes and "fire" in tulips. The fungicide was discovered during routine testing of aniline derivatives as herbicides. The marked action of the nitro-anilines on the germination of B. cinerea was observed. Weber and Ogawa (1963) concluded that Dicloran interferes with protein synthesis, while Sharples (1961) found that at concentrations of 1 ppm it increased nucleic acid levels in Botrytis.

Recent work however, indicates that a considerable degree of resistance is shown by species of Botrytis and of Rhizopus to Dicloran and the other nitroanilines (Ogawa and Moore, 1968; Esurouso and Wood, 1971).

(4) Formalin

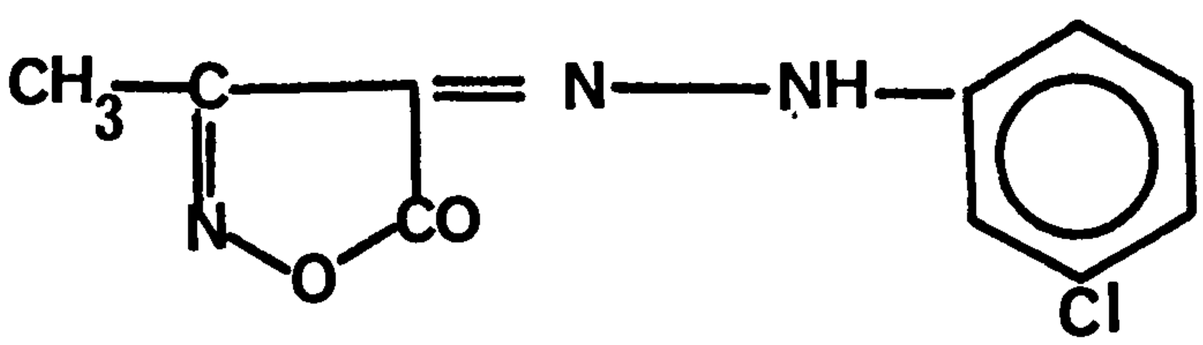
Active ingredient Formaldehyde 37.5-40.5% W/V.

Formaldehyde is a colourless pungent gas which is very soluble in water. The solution of formalin may also contain up to 14% methyl alcohol to prevent polymerization to paraformaldehyde. Formaldehyde, HCHO, has long been used as a partial sterilant. Its first recorded use was by Loew in 1888, (Martin, 1973) as a disinfectant against fungi. Since then it has been used as a soil disinfectant against Ustilago hordei on barley, and against Septoria apii on celery. Formaldehyde was reviewed by Byrde (1969).

(5) Milcol

Active ingredient - drazoxolon, C4-C2 chlorophenyl-hydrazone)-3 methy-5-isoxatzolone.

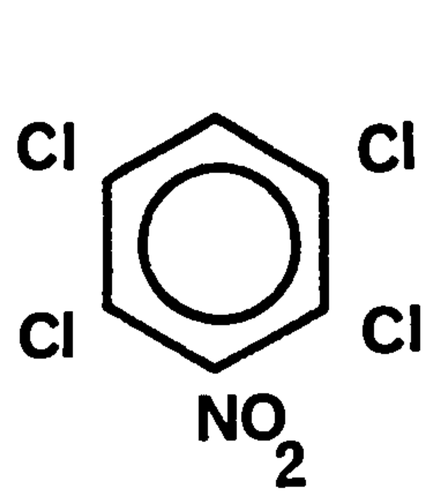
The structural formula is :-



Milcol is effective against foliage diseases, and is used as a seed treatment against seedling blights. It is particularly effective against mildews.

(6) Quintozene

Active ingredient - pentanitrochlorabenzene. Structural formula :-



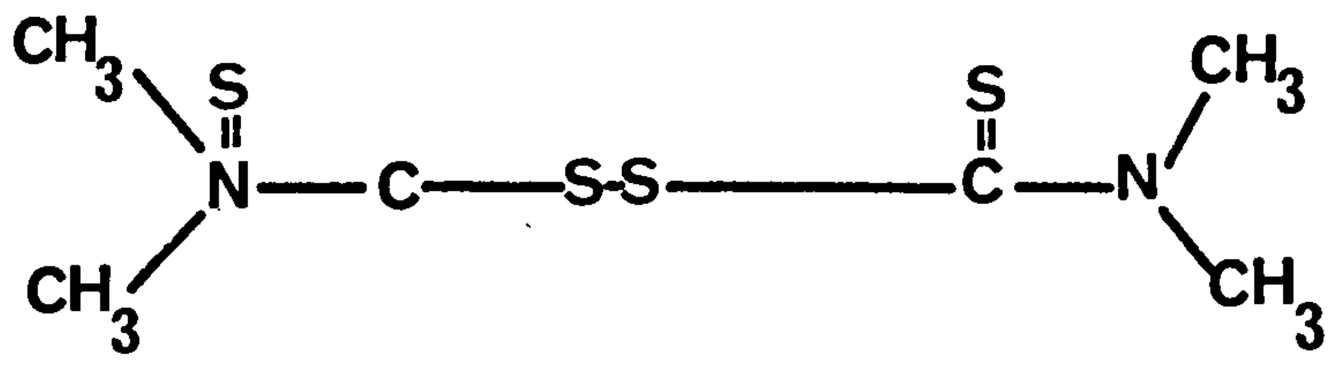
Quintozene is used extensively in the soil to control plant diseases caused by Rhizoctonia solani, and by species of Botrytis and of Sclerotinia.

Quintozene has been found capable of reducing fungal growth rates and sporulation, but not spore germination (Priest and Wood, 1961). The chlorinated nitrobenzenes are capable of affecting plant growth and have systemic activity against Fusarium wilt of tomato (Grossman, 1958).

The properties of Quintozene were reviewed by Corden (1969) and its effects on soil microflora were discussed by Dransfield (1956).

(7) Thiram

Active ingredient - tetramethylthiuram disulphide. Structural formula :-



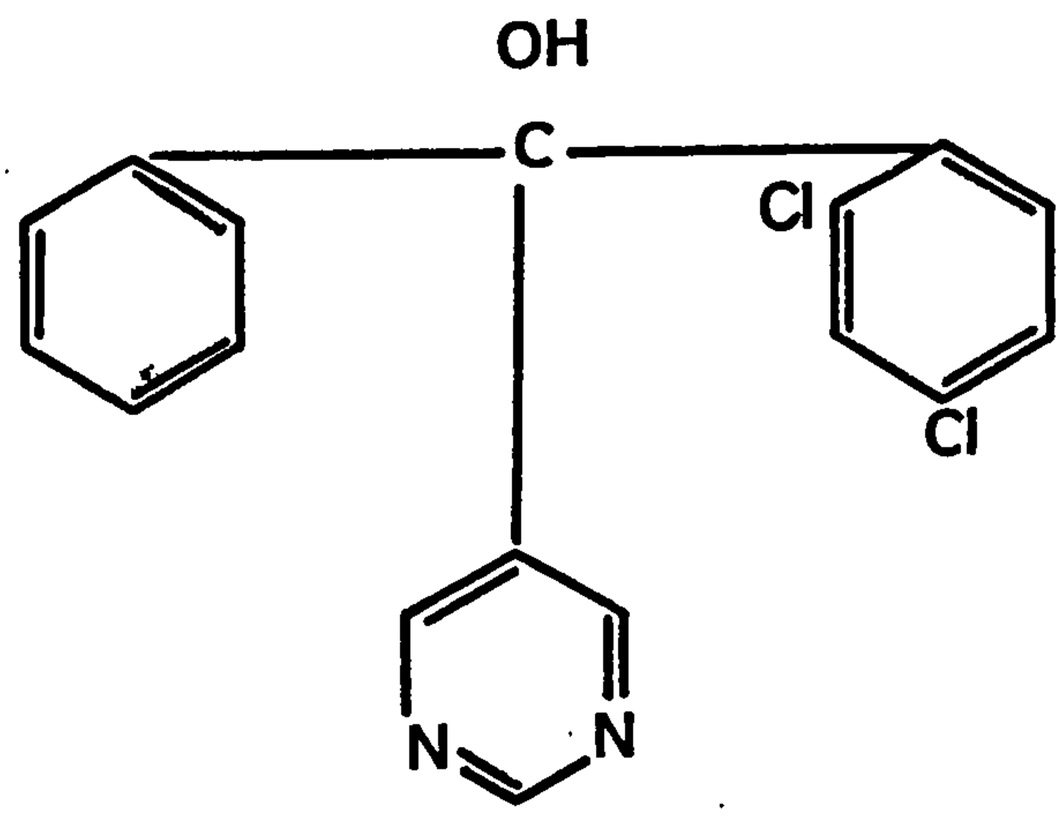
Thiram belongs to the dithiocarbamate group of fungicides, which includes Ferbam, Nabam and Ziram. Tetramethyl thiuram disulphide was already used in rubber processing before its fungicidal properties were recognised. The compound resembles a polysulphide in possessing a sulphur atom in excess of a monosulphide. It is a derivative of dithiocarbamic acid which has both insecticidal and fungicidal properties. Thiram is used to control flax seedling blight (caused by Colletotrichum lini), as a seed dressing, and in the control of damping off in vegetables.

(8) Triarimol

Active ingredient - (2, 4 - Dichloropenyl)- - phenyl-5-pyrimidinemethanol 4% W/W.

Triarimol is a promising systemic fungicide which can control numerous diseases of fruit and vegetables including apple scab (Venturia inaequalis) and blackcurrent leaf spot (Pseudopezzia ribes). It is also effective against powdery mildews, and against wheat and barley rusts.

The structure formula is :-



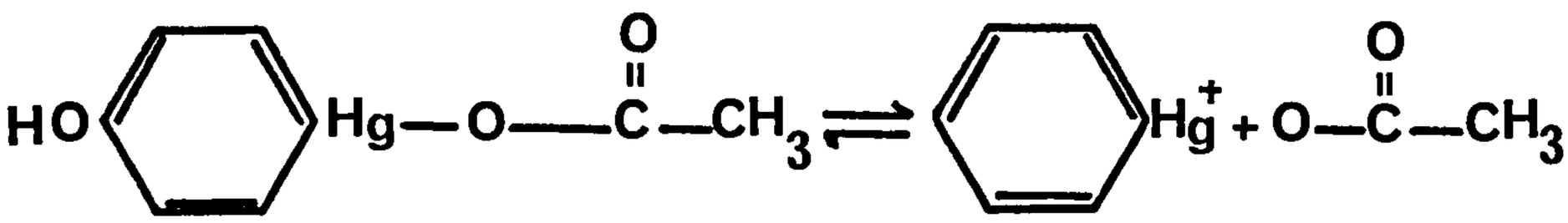
Triarimol is ineffective against species of Pythium, Rhizoctonia, and Botrytis. Houseworth, Brunton and Tweedy (1971) found that the fungicide did not effect respiratory pathways during the initial stages of spore germination, but appeared to arrest germ-tube elongation.

The makers of Triarimol, Eli Lilly, state (technical report 12.70) that this fungicide has a low acute oral LD_{50} and that feeding studies produce no adverse effects to rats at 400 ppm. However, Triarimol has now been removed from the United States market on suspicion of being a carcinogen. The continued use of this fungicide in this country and elsewhere is under review.

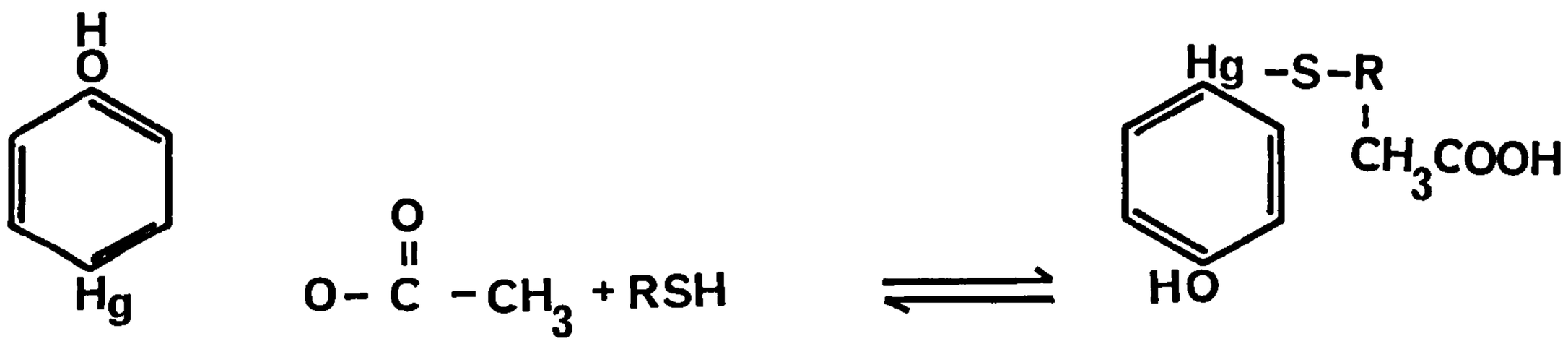
(9) Verdasan

Active ingredient phenyl mercuryacetate. 2.5% W/W.

Verdasan is an organomercurial used as a seed dressing, and in turf management. Phenyl mercury acetate has the following configuration -



The biological activity shown by this compound is due to the formation of ionic bonds between the phenylmercury acetate cation and certain anions present in living tissue, or by the formation of mercaptide with living tissue thus :-



The properties and action of Verdasan and other organo-mercurials` have been reviewed by Martin (1973).

THE FUNGICIDES USED

A wide range of fungicides was used in this study ranging from a convential partial sterilant, formalin, to a modern systemic fungicide Benomyl. These fungicides are in common use at the present time, and together make up the bulk of those used in agriculture, forestry and horticulture.

The list includes representatives of various fungicide groups -

Benomyl	Modern Systemic
Captan	Heterocyclic Nitrogen Compound
Dicloran	Nitroaniline
Formalin	Partial Sterilant

Milcol	Modern Mildewicide
Quintozene	Nitrobenzene
Thiram	Dithiocarbamate
Triarimol	Modern Systemic

Details of these fungicides are given in the Appendix (AI).

PHYSICO-CHEMICAL PROPERTIES OF THE SOILS

The following physical and chemical parameters were determined for both Sutton Bonington and Rothamsted soils, prior to determining the effects of fungicides on their microbiology and biochemistry :

(1) Loss of weight on ignition

(2) Total Organic Carbon (Walkley and Black's method)

(3) Total Nitrogen (Kjeldahl method)

(4) Water holding capacity

(5) Soil pH

Details of the methods used are given in Appendix B.

APPENDIX B.

METHODS USED TO DETERMINE THE PHYSICAL AND CHEMICAL PROPERTIES OF SOILS

(1) Loss of weight on ignition

2 g of fresh soil were oven dried and ignited in weighed silica crucibles. The samples were charred at 25°C for 2 h before ignition at 500°C in a muffle furnace for 20 minutes . After cooling in a desiccator the samples were re-weighed and the percentage loss on ignition was calculated.

(2) Determination of total Organic Carbon

Total organic carbon was determined using Walkley and Black's rapid titration method, outlined by Piper (1947). The procedure involved wet digestion of the sample with chromic and sulphuric acids. The chromic acid not reduced by the organic matter was then titrated with standard ferrous sulphate solution. A 0.25 g sample of 2 mm sieved air-dry soil was transferred to a 500 ml wide mouth conical flask. N potassium dichromate solution (10 ml) was added followed by 20 ml of concentrated sulphuric acid. The mixture was shaken and left for 30 minutes after which it was diluted with 200 ml of distilled water. Phosphoric acid (10 ml of 85%) was then added using a safety pipette followed by diphenylamine indicator (1 ml). Fresh N. ferrous sulphate solution stored under hydrogen was then run in from an automatic burette. The end point was reached when the purple colour "flashed" green. A further 1 ml of dichromate solution was then added and ferrous sulphate solution was

added dropwise to establish an accurate end-point. The percentage carbon was calculated from the formula :-

$$\% \quad C. \quad = \quad \frac{V_1 \quad - \quad V_2}{W} \quad x \quad 0.003 \times 100$$

Where V_1 = vol. of N. potassium dichomate (ml)

V_2 = vol. of N. ferrous sulphate (ml)

W = weight of sample in g.

A blank titration using potassium dichromate was also carried out. The method gives about 76% recovery, so the figures were corrected by multiplying by 1.32 (Walkley and Black, 1934).

(2) Determination of total nitrogen

The technique used was the macro-Kjeldahl method outlined by Jackson (1958), and modified by Williams (1973).

5 g of air-dry soil (< 2mm) was wrapped in a 12.5 cm Whatman No. 1 filter paper and dropped into a clean 500 ml Kjeldahl flask, containing 20 g of sodium sulphate + catalyst mixture. The latter was prepared by thoroughly mixing 20 g of $CuSO_4$ $5H_2O$ (ground, and dried at 110°C overnight) with 3 g of HgO, and 1 g of selenium powder. Concentrated H_2SO_4 (35 ml) was then poured carefully into the Kjeldahl flask and the contents mixed thoroughly. Digestion was carried out on a Kjeldahl rack at first at low heat until frothing ceased, and then more strongly until the sample was completely charred. The mixture was boiled until the organic matter was destroyed, followed by heating for 4 - 5 hours until the mixture was completely cleared.

Deionized water (300 ml) was then added and the contents mixed thoroughly. Finally the supernatant was poured into a clean Kjeldahl flask prior to distillation.

A few small pieces of porcelain (to prevent bumping) were added to the ammonium sulphate solution followed by several pieces of granulated zinc. 4% boric acid 25 ml was pipetted into a 250 ml conical flask and four drops of bromocresolgreen - methylred indicator solution were added. The receiver tube of the still was adjusted so that its end was just below the surface of the boric acid, and water was allowed to flow through the condenser. About 125 ml of NaOH (40%) containing $Na_2 S_2 O_3$ were carefully run into the Kjeldahl flask. The flask was then quickly attached to the still using a splashead and the contents were mixed thoroughly by swirling. The solution was gently heated until about 150 ml of the distillate were collected at a rate of 10 ml per minute. Finally the boric acid was titrated with standard HCl when the blue colour just disappeared at the end point. A blank digestion using the same reagents, but without soil was also carried out.

The percentage N content was calculated from the formula :-

$$\% \ N = (T - B) \times N \times \frac{1.4}{5}$$

Where T = sample titration in ml

B = blank titration in ml

N = normality of HCl

S = sample weight in g

(3) Determination of soil pH

Soil pH was determined using a 2 g sample of soil and a soil : water ratio of 1 : 2. The pH of the soil : water suspension was then determined using a Pye glass electrode pH meter (Pye/Unicam model 292). Periodic calibration of the pH meter was made using phosphate buffers of pH 4 and pH 7.

(4) Determination of water holding capacity

Water holding capacity trays were used to determine this parameter. A filter paper was cut to fit the bottom of the tray and then wetted. Small amounts of sieved ($<$2 mm) air dry soil was then placed into the tray and tapped down to achieve a loose, even distribution of soil. When the tray was full it was placed in a petri dish so that the perforated base was in contact with the water overnight. After weighing the tray + soil was dried to constant weight in an oven at 105^{o}C. On cooling in a desiccator the tray + soil was weighed and the percentage loss in weight was determined.

B1 Soil Characteristics

Characteristic	Rothamsted	Sutton Bonington
pH (Mean)	5.3	6.7
pH (Range)	5.0-6.8	6.3-7.3
Organic C (%)	3.1	2.9
Total N (%)	0.27	0.22

B2 The Effect of Fungicides on Soil pH.

Days Incubation	2	7	14	21
Treatment µg/g soil				
Control	6.3	6.4	6.5	6.5
Verdasan 10	6.7*	7.2*	7.1*	6.7
Verdasan 50	7.8*	8.2*	7.6*	8.1*
Captan 50	6.5	6.5	6.8*	6.5
Captan 250	6.3	6.6	6.8*	6.6
Thiram 50	6.2	6.5	6.8*	6.5
Thiram 250	6.0*	6.6	6.9*	6.6
Benomyl 50	6.2	6.4	6.8*	6.5
Benomyl 250	6.2	6.6	6.7	6.4

*Significant Difference from Control $P > 0.05$

C1 List of Species Isolated from Sutton Bonington Soils

Species isolated on cellulose agar.

Absidia glauca Hagen
Arthrobotrys oligospora Fres.
Aspergillus fumigatus Fres.
Botryotrichum piluliferum Sacc. and March.
Chaetomium angustum Chivers.
C. globosum Kunze ex Fries.
C. mollicellum Ames
Doratomyces sp.
Fusarium culmorum (W.G.Sm) Sacc.
F. solani (Mart.) Appel. et Wollenw.
Gliocladium roseum Banier.
Gliomastix murorum (Corda) Hughes
Humicola grisea Traaen.
Mortierella sp.
Monillia brunnea Gilman and Abbot.
M. geophila Oud.
Mucor hiemalis Wehmer
M. plumbeus Bon.
Penicillium brevicompactum Dierckx
P. citrinum Thom.
P. expansum Link.
P. nigricans
P. rubrum.
Stemphyllium botryosum Wallr.
Trichoderma album Preuss.
T. koningii Oud.
Trichocladium asperum Harz.
Trichurus sp.
Verticillium sp.
V. Lateritium Berk.
Zygorhynchus moelleri Vuill.

C2 The Effect of Fungicides on Growth of Selected Fungi (mycelial dry wt. gms.)

Fungicide µg/ml	CAPTAN 5	50	100	DICLORAN 5	50	100	MILCOL 5	50	100	TRIARIMOL 5	50	100
F. culmorum	114.0	46.5	9.21	127.0	120.0	120.5	168.5	169.4	151.2	155.0	157.5	74.4
G. roseum	66.6	24.8 ****	10.7 ****	79.1	64.7	57.7 *	135.0	99.0	77.7	126.0	92.6	91.6
T. koningii	28.7	11.6	7.6	118.4	94.3	50.0	132.5	35.6	25.0	23.1	29.5	33.4
P. nigricans	110.4	41.4 *	17.0 ****	73.0	95.3	118.7	151.2 *	111.2	73.9	125.1	112.4	92.5
Z. moelleri	98.7	95.8	9.1	74.7	8.4 ****	9.5 ****	28.0 ****	6.3 ****	3.6 ****	121.5	102.5	99.9

**** Significant difference P > 0.05

* Significant difference P > 0.1

C3 The Effect of Captan On Numbers Of Heterotrophic Bacteria in Soils (1×10^5)

Captan Conc. µg/g	Days of Incubation 0	2	7	14	21	28
Control	157.5	160.0	165.0	137.6	145.1	207.0
C5	157.5	245.1	182.0	134.0	142.1	202.0
C25	157.5	142.5	257.4	155.0	156.0	170.0
C50	157.5	207.5	205.0	365.5	210.0	150.2
C100	157.5	305.0	355.6	312.6	360.2	397.5

The Effect of Captan On Numbers of Fungi in Soils (1×10^4)

Captan Conc. µg/g Soil	Days of Incubation 0	2	7	14	21	28
Control	24.0	25.0	18.0	24.5	26.0	43.0
C5	24.0	17.5	16.0	18.5	17.5	47.5
C25	24.0	14.5	15.4	19.5	18.0	57.5
C50	24.0	8.5	9.0	10.5	9.75	15.0
C100	24.0	3.5	3.0	4.5	7.0	9.5

C4 The Effect of Captan on Numbers of Actinomycetes in Soil

Conc. Captan µg/g Soil	Nos. Actinomycetes 2 days After Treatment
Control	20.0
C5	19.0
C25	35.5
C50	22.5
C100	6.5

C5 Effect of Fungicides on Fungal Numbers in Field Soils

(expressed as % of Control)

Fungicide	Time (Days)				
	3	15	31	58	157
Triarimol	51	145	120	165	60
Milcol	20	85	108	102	90
Captan	40	83	112	80	78
Dicloran	20	68	86	97	88

C6 The Effect of Fungicides on Free Amino Acid-N Content of Soils

(Expressed as % of Control)

Fungicide (µg/g soil)	Time (Days) 2	7	14	21	28
Benomyl 50	72.5	61.5	119.5	121.0	201.5
Benomyl 250	49.4	50.1	62.5	59.7	38.9
Thiram 50	68.5	62.0	105.8	58.5	57.5
Thiram 250	98.0	50.0	100.0	124.8	144.7
Verdasan 10	40.2	42.0	83.5	90.5	58.0
Verdasan 50	61.9	88.0	60.0	54.6	13.5

C7 The Effect of pH on Nitrification

pH	Time in Days 0	7	14	21	35
Limed Soil	9.3	8.4	9.0	8.9	9.8
Unlimed Soil	7.1	7.3	6.9	7.5	7.0
Nitrate-N µg/g Soil					
Limed Soil	20.0	44.2	49.3	68.7	47.0
Unlimed Soil	18.3	32.3	29.1	27.3	26.8

C8 The Effect of Temperature On Nitrification

Temp. $0^{o}C$	NO_3^-N µg/g Soil
0	6.5
5	8.2
10	8.1
15	14.0
25	20.1
30	29.4
35	26.6
40	9.55
50	8.00
60	5.95

C9 The Effect Of Water Content On Nitrification

% W.H.C.	NO_3^-N µg/g soil
0	8.9
10	13.8
20	15.0
30	22.0
40	29.1
50	40.2
60	50.1
80	24.8
90	22.8
100	20.0

C10 The Effect of Fungicides on Nitrification in the Field

($\mu g\ NO_3^-$ per g soil)

Fungicide	Time (Weeks) 0	4	8	12
Control	32.5	49.3	48.1	58.5
Quintozene	30.0	31.5	43.0	45.2
Thiram	28.5	40.5	36.5	38.0
Formalin	23.2	28.1	31.5	30.5
Dicloran	33.0	39.5	37.0	39.0
Captan	30.5	32.4	39.0	36.5

C11 The Effect of Fungicides on Ammonification

(NH_4^+ -N µg/g soil)

Fungicide (µg/g soil)	Time (Days) 0	7	14	21	28
Control	3.0	3.5	3.6	3.7	4.0
Captan 250	5.0	35.1	47.5	65.6	61.5
100	5.1	7.0	10.4	59.9	44.7
50	3.0	27.1	25.0	20.0	7.0
25	2.5	10.0	13.6	10.2	9.5
10	2.5	2.6	2.5	6.9	6.0
5	3.9	4.1	6.7	19.0	16.4
Thiram 250	7.0	32.8	63.5	69.2	72.4
100	5.0	25.6	45.9	68.9	64.5
50	5.0	26.1	64.2	72.2	27.1
25	4.0	9.8	9.7	23.2	23.0
10	3.0	3.8	7.1	18.9	19.5
5	4.0	8.0	9.5	11.6	14.0
Verdasan 50	5.4	44.9	50.8	64.6	61.7
25	6.5	25.5	48.1	73.9	71.0
15	4.0	40.0	42.1	50.1	28.9
10	5.1	45.6	42.5	43.5	37.8
5	5.0	33.9	40.0	55.9	38.0
1	3.0	7.0	6.0	5.9	5.8

Means Four Replicates

C12 The Effect of Fungicides on Nitrification

(NO_3^- -N µg/g soil)

Treatment µg/g soil		Time (Days) 0	7	14	21	28
Control		5.3	21.5	32.3	46.4	48.2
C + N		6.1	32.1	76.2	81.2	102.3
Captan 250		3.0	4.6	11.6	16.5	17.3
	100	5.1	12.1	23.2	19.9	47.1
	50	10.1	16.8	34.4	39.9	52.1
Control		7.0	21.2	38.5	54.2	75.3
C + N		12.0	40.2	61.3	64.1	120.1
Captan	25	10.0	26.2	41.9	44.2	70.3
	10	5.0	25.5	58.1	75.5	93.1
	5	13.1	44.2	66.8	70.1	132.3
Control		12.2	23.6	24.1	24.2	23.6
C + N		16.4	28.7	29.2	49.5	65.3
Thiram 250		9.1	17.8	18.2	17.8	16.5
	100	10.0	205.	21.1	18.4	30.8
	50	5.0	26.3	64.2	85.5	96.6
Control		5.1	20.3	24.9	44.3	63.9
C + N		4.6	32.6	75.1	80.4	102.6
Thiram	25	4.9	25.4	57.6	89.7	107.5
	10	10.0	36.8	40.3	68.2	96.4
	5	4.9	37.2	71.5	95.7	108.2
Control		6.Q	10.0	23.4	38.6	46.4
C + N		10.0	16.1	55.1	60.0	56.5
Verdasan	50	6.4	2.9	6.4	6.3	10.6
	25	8.o	4.9	10.9	10.3	11.9
	15	6.9	11.9	14.2	6.1	8.2
Control		7.0	23.2	30.1	40.0	65.2
C + N		10.0	28.9	54.2	55.6	111.9
Verdasan	10	5.0	8.2	8.9	10.9	13.2
	5	10.9	11.9	36.9	63.5	58.9
	1	10.0	16.1	55.1	60.0	56.5

C13 Composition of the Locating Agends Used in the Determination of Soil Auxin

(1) Ammoniacal-Silver Nitrate Reagent

Silver Nitrate	11 g
Ammonium hydroxide	33 ml
Distilled Water	67 ml

(2) Erlich's Reagent

4-dimethylaminobenzaldehyde	4 gms
Ethanol (100%)	380 ml
Hydrochloric Acid (SGR. 1.18)	80 ml

(3) Nitric-Nitrite Reagent

Potassium nitrite	1 gm
Nitric Acid (Conc)	20 ml
Ethanol (95%)	80 ml

(4) Salkowski's Reagent

Perchloric Acid (5%)	100 ml
Ferric Chloride (0.05M.)	2 ml

REFERENCES

AGNIHOUTRI, V.P. (1971). Persistence of Captan and its effects on microflora, respiration and nitrification of a forest nursery soil. Can. J. Microbiol. 17, 377-383.

ALDRICH, D.G. and MARTIN, J.P. (1952). Effect of fumigation on some chemical properties of soils. Soil Sci. 73, 149-

ALEXANDER, M. (1961). Introduction to Soil Microbiology. John Wiley, New York.

ALEXANDER, M. (1968). Degradation of Pesticides by soil bacteria. Soil Bacteria ed. Gray, T.R.G. and Williams, S.T., Liverpool Univ. Press.

ALLISON, L.E. (1951). Vapour-phase sterilization of soil with ethylene oxide. Soil Sci. 72, 341-352.

ALTMAN, J. (1964). The effect of chlorinated C_3 hydrocarbons on growth and amino acid production of indigenous soil bacteria. Phytopathology 54, 886.

ALTMAN, J. (1969). Effect of chlorinated C_3 hydrocarbons on amino acid production by indeginous soil bacteria. Phytopathology 59, 762-766.

ALTMAN, J. (1970). Increased and decreased plant growth responses resulting from soil fumigation. Ecol. Soil Borne Dis. Univ. California Press.

ANDERSON, M.S., KEYES, M.G. and CROMER, G.W. (1942). Soluble material of soils in relation to their classification and general fertility. U.S. Dept. Agr. Tech. Bul. 813.

BALICKA, N. and KOSINKIEWICZ, B. (1963). Soil Organisms ed. Doeksen J. and Vander Drift J. North Holland, Amsterdam.

BARBER, D.A. (1969). The influence of the microflora on the accumulation of ions by plants. Ecol. Aspects of Min. Nut. of Plants. Brit. Ecol. Soc. Sym. 9, 191-199.

BARTHOLOMEW, W.V. and CLARK, F.E. (1965). Soil Nitrogen Agronomy 10. Am. Soc. Ag. Madison.

BENTLEY, J.P., FARRAR, K.R., SMITH, G.F., and TAYLOR, W.C. (1956). Some chemical and physiological properties of 3-Indolepyruvic acid. Biochem. J. 64, 44-49.

BENZIAN, B. (1965). Experiments on nutrition problems in forest nursery soils. Bulletin Forestry Commission 37, 1-251. H.M.S.O. London.

BIRCH, H.F. (1959). Further observations on humus decomposition and nitrification. Pl. Soil. 11, 262-286.

BLACK, C.A. (1968). Soil Plant Relationships. John Wiley, New York.

BLISS, E.D. (1951). The destruction of Armillaria mellea in citrus soils. Phytopathology 41, 665-683.

BOLLEN, G.J. (1971). Resistance of benomyl and some chemically related compounds in strains of Penicillium species. Neth. J. Pl. Path. 77, 187.

BOLLEN, G.J. and FUCHS, A. (1970). On the specificity of the in vitro and in vivo antifungal activity of benomyl. Neth. J. Pl. Path. 76, 299-312.

BOLLEN, G.J. and SCHOLTEN, G. (1971). Acquired resistance to benomyl and some other systemic fungicides in strains of B. cinerea in cyclamen. Neth. J. Pl. Path. 77, 83-90.

BOLLEN, W.B. (1961). Interactions between pesticides and soil micro-organisms. Ann. Rev. Microbiol. 15, 69-92.

BOLLETER, W.T., BUSHMAN, C.J. and TIDWELL, P.W. (1961). Spectro-photometric determination of ammonia as indophenol. An. Chem. 33, 592-594.

BOYD, H.W. (1971). Manganese toxicity to peanuts in autoclaved soil. Pl. Soil 34, 133-144.

BREMNER, J.M. (1965). Inorganic forms of nitrogen. Agromony 9, 1179-1237.

BREMNER, J.M. (1967). Nitrogenous compounds. Soil Biochemistry V.1. McLaren, A.D. and Peterson, G.H. Marcel Dekker, N. York.

BROADBENT, F.E., TYLER, K.E. and HILL, G.N. (1958). Low temperature effects on nitrification in four Californian soils. Soil Sci. 87, 123-128.

BRYNER, L.C. and JAMESON, A.K. (1958). Micro-organisms in leaching sulphide minerals. Appl. Microbiol. 6, 281-287.

BUCKLEY, N.G. and PUGH, G.J.F. (1971). Auxin production by phylloplane fungi. Nature 231, 332.

BUNDY, L.G. and BREMNER, J.M. (1973). Inhibition of nitrification in soils. Soils Sci. Soc. Amer. Proc. 37, 396-398.

BURCHFIELD, H.P. (1959). Comparitive stabilities of Pyrene 1-flouro-2, 4 dintrobenzene, dichlone, and Captan in a silt loam soil. Contrib. Boyce Thompson Inst. 20, 205-215.

BYRDE, R.J.W. (1969). Non aromatic organics. Fungicides Vol. 2 ed. Torgeson, D.C. Academic Press, New York.

CAMPBELL, N.E.R. and LEES, H. (1967). Soil Biochemistry ed. McLaren, A.D. and Peterson, G.H. Marcel Dekker, New York.

CASIDA, L.E. and SANTORO, T. (1961). Growth response of some micro-organisms to manganese. Soil Sci. 92, 287-297.

CHASE, F.E., CORKE, C.T. and ROBINSON, J.B. (1968). Nitrifying bacteria in soil. Ecology of Soil Bacteria ed. Gray T.R.G. and Williams, S.T. Liverpool Univ. Press.

CHRISTENSEN, P.D., TOTH, S.J. and BEAR, F.E. (1950). The status of soil manganese as influenced by moisture, organic matter and pH. Soil. Sci. Soc. Amer. Proc. 14, 279-282.

CLARKE, A.L. and JENNINGS, A.C. (1965). Spectrophotometric estimation of nitrate in soil using chromotropic acid. Agric. Fed. Chem. 13, 174-176.

COOKE, D.A. and HULL, R. (1972). The effect of soil fumigation with D-D on the yields of sugar beet and other crops. Ann. Appl. Biol. 71, 59-67.

CORDEN, M.E. (1969). Aromatic compounds. Fungicides Vol. 2, 477-529. ed. Torgeson, D.C. Academic Press, New York.

CORDEN, M.E. and YOUNG, R.A. (1965). Changes in the soil microflora following fungicide treatments. Soil Sci. 99, 272-277.

DAINES, R.H. (1953). Captan - the new discovery in fruit fungicides. Am. Fruit. Grower, 73, 16.

DANIELSON, R.M. and DAVEY, C.B. (1969). Microbial recolonization of a fumigated nursery soil. Forest Sci. 15, 368-380.

DELP, C.J. and LAPPING, H.L. (1968). Performance attributes of a new fungicide and mite ovicide candidate. Pl. Dis. Rept. 52, 95.

DICKINSON, C.H. and BOARDMAN, F. (1970). Physiological studies of some fungi isolated from peat. Trans. Br. mycol. Soc. 55, 293-305.

DOMSCH, K.H. (1964). Soil fungicides. Ann. Rev. Phytopathol. 2, 293-320.

DRANSFIELD, M. (1956). The effects of tetrachloronitrobenzene on the soil microflora. Ph.D. Thesis, Univ. Nottingham.

DUBEY, H.D. (1970). A nitrogen deficiency disease of sugar cane probably caused by repeated pesticide application. Phytopathology 60, 485-487.

EASTOE, J.E. and POLLARD, A.G. (1950). A modified phenoldisulphonic acid method for determining nitrate in soil extracts. J. Sci. Fd. Agric. 1, 266-269.

EBBELS, D.L. (1969). Effects of soil fumigation on disease incidence, growth and yield of spring wheat. Ann. appl. Biol. 63, 81-903.

EGGINS, H.O.W. and PUGH, G.J.F. (1962). Isolation of cellulose decomposing fungi from soil. Nature 193, 94-95.

ERLICH, H.L. (1971). Biogeochemistry of the minor elements in soil. Soil Biochemistry vol. 2, ed. McLaren, A.D. and Skujins, J. Marcel Dekker, N. York.

ESURUOSO, O.F. and WOOD, R.K.S. (1971). The resistance of spores of resistant strains of B. cinerea to quintozene tecnozene and dicloran. Ann. appl. Biol. 68, 271, 279.

EVANS, E. (1955). Survival and recolonization by fungi in soil treated with formalin, and carbon disulphide. Trans. Brit. mycol Soc. 38, 335-346.

FISHER, F.L., IBERT, E.R. and BECKMAN, H.F. (1958). Inorganic nitrate, nitrite, or nitrate-nitrite. Rapid colorimetric determination of microgram quantities in aqueous solution. Anal. Chem. 30, 1972-74.

FISHER, R.A. and YATES, F. (1963). Statistical tables for biological agricultural and medical research. Oliver and Boyd, London.

FREDERICK, L.R. (1956). The formation of nitrate from ammonium nitrogen in soils : 1 Effect of temperature. Soil Sci. Soc. Am. Proc. 20, 496-500.

GARRETT, S.D. (1957). Effect of a soil microflora selected by carbon disulphide on the survival of Armillaria mellea in woody host tissue. Can. J. Microbiol. 3, 135-149.

GARRETT, S.D. (1963). Soil Fungi and Soil Fertility. Pergamon Press, London

GASSER, J.K.R. and PEACHEY, J.E. (1964). A note on the effects of some soil sterilants on the mineralization and nitrification of soil nitrogen. J. Sci. Fd. Agric. 15, 142-146.

GIBSON, I.A.S. (1956). An anamolous effect of soil treatment with ethyl mercury phosphate on the incidence of damping-off in pine seedlings. Phytopathology, 46, 181-182.

GILBERT, R.G. and ALTMAN, J. (1966). Ethanol extraction of free amino acids from soil. Pl. Soil 24, 229-238.

GOOD, J.M. and CARTER, R.L. (1965). Nitrification lag following soil fumigation. Phytopathology 55, 1147-1150.

GORDON, S.A. and PALEG, L.G. (1961). Formation of auxin from tryptophan through action of polyphenols. Plant Physiol. 36, 838-845.

GORDING, C.A.I. (1962) Control of nitrification by 2, chloro-6-(trichloromethyl) pyridine. Soil Sci. 93, 211-218.

GORING, C.A.I. (1970). Nutrient cycling implications of pesticides. Pesticides in the soil: Ecol. Deg. Mov. Symposium, Michigan State Univ.

GRASMANIS, V.O. and LEEPER, G.W. (1966). Toxic manganese in near neutral soils. Pl. Soil 15, 41-48.

GREENAWAY, W. (1972). Growth of helminthosporium on media containing the fungicide benomyl and the assay of benomyl using B. cinerea. Ann. appl. Biol. 73, 319-324.

GREENWOOD, D.J. and LEES, H. (1956). Studies on the decomposition of amino acids in soils. 1. Pl. Soil 7, 253-268.

GREENWOOD, D.J. and LEES, H. (1960). Studies on the decomposition of amino acids in soils. 2. Pl. Soil 12, 175-194.

GROSSMAN, F. (1958). Untersuchungen über die innertherapeutische Wirkung organischer fungizide 2 Chlornitrobenzole. Z Pflanzenkrunkh. Pflanzerschutz 65, 594-599; Chem. Abstr. 54, 6011 (1960).

GRUEN, H.E. (1965). The production of I.A.A. by Phycomyces blakesleeanus. Mycologia 57, 683-695.

GRUNES, D.L. and VIETS, F.G. (1966). Effect of soil moisture on ammonification and nitrification in two northern plain soils. Soil Sci. Soc. Amer. Proc. 30, 363-366.

GUNN, (1972) Dilemmas in conservation for applied biologists. Ann. appl. Biol. 72, 105-127.

HALVORSON, H. (1972). Utilization of single L-amino acids as sole source of carbon and nitrogen by bacteria. Can. J. Microbiol. 18, 1647-1650.

HAMENCE, H.J. (1946). The detection and determination of auxins in organic manures. Analyst. 71, 111-116.

HARMSEN, G.W. and KOLENBRADER, D.A. (1965). Soil inorganic nitrogen. Agronomy 10, 43-92.

HARMSEN, G.W. and VAN SCHREVEN, D.A. (1955). Mineralization of organic nitrogen in soil. Adv. Agron. 7, 299-398.

HATCHER, H.J. and SCHMIDT, E.L. (1971). Nitrification of aspartate by A. flavus. Appl. Microbiol. 21, 181-186.

HEINTZE, S.W. (1957). Studies on soil manganese. J.Soil Sci. 8, 287-300.

HEIM, A.H. and LECHEVALIER, H. (1956). Effect of iron, zinc, manganese and calcium on the growth of various strains of Streptomyces. Mycologia 48, 628-636.

HESSE, P.R. (1971). A textbook of soil chemical analysis. J. Murray, London.

HORA, T.S. and IYENGAR (1960). Nitrification by soil fungi. Arch. fur. Mikrobiologie 35, 252-257.

HOUSEMAN, L.D. and TWEEDY, B.G. (1973). Effect of atrazine in combination with Captan or Thiram upon fungal and bacterial populations in the soil. Pl. Soil 38, 493-501.

HOUSEWORTH, L.D., BRUNTON E.W., and TWEEDY, B.G. (1971). The effect of triarimol on oxidation of glucose and acetate to CO_2 and on spore germination of A. niger and C. cucumerinum. Phytopathology 61, 896 (Abst).

HUBER, D.M., WATSON, R.D., and STEINER, G.W. (1965). Crop residues, nitrogen and plant diseases. Soil Sci. 100, 302-308.

IVARSON, K.C. and HERINGA, P.K. (1972). Oxidation of manganese by micro-organisms in manganese deposits in Newfoundland soils. Can. J. Soil Sci. 52, 401-416.

JACKSON, M.L. (1958). Soil Chemical Analysis. Constable, London.

JACQUES, R.P., ROBINSON, J.B. and CHASE, F.E. (1959). Effects of thiourea, ethyl urethane, and some dithiocarbonate fungicides on nitrification in fox sandy loam. Can. J. Soil Sci. 39, 235-243.

JENKINSON, D.S. (1966). Studies on the decomposition of plant material in soil. 2. Partial sterilization of soil and the soil biomass. J. Soil Sci. 17, 280-302.

JENKINSON, D.S., NOWAKOWSKI, T.Z. and MITCHELL,J.D.D. (1972). Growth and uptake of nitrogen by wheat and ryegrass in fumigated and irradiated soils. Pl. Soil 36, 149-158.

JENSON, V. (1968). The plate count technique Soil Bacteria. ed. Gray T.R.G and Williams S.T. Liverpool Univ. Press.

KAPER, J.M., GEBHARD, O., BERG VAN DEN. and VELDSTRA, H. (1963) Studies on indolepyruvic acid. ". Ultra violet spectrophotometry. Arch. Biochem. Biophys. 103, 475-487.

KAPER, J.M. and VELDSTRA, H. (1958). On the metabolism of tryptophan by Agrobacterium tumefaciens. Biochem. Biophys. Acta. 30, 401-420.

KREUTZER, W.A. (1963). Selective toxicity of chemicals to soil micro-organisms. *Ann. Rev. Phytopathol. 1*, 101-126.

KREUTZER, W.A. (1965). The reinfestation of treated soil. Ecol. Soil Borne Plant Pathogens. 495-507, ed. Baker, K.F. and Snyder, W.C. Univ. California Press.

LADD, J.N. (1972) Properties of proteolytic enzymes extracted from soil. Soil Biol. Biochem. 4, 227-237.

LARSEN, P., HARBO, A., KLUNGSOYN, S. and AASHEIM, T. (1962). On the biogenesis of some indole compounds by Acetobacter xylinum. Physiol. Plant 15, 552-565.

LEES, H. (1955). The nitrifying bacteria. Biochemistry of autotrophic bacteria, 44-58.

LEEPER, G.W. and SWABY, R.J. (1940). The oxidation of manganous compounds by micro-organisms in the soil. Soil Sci. 49, 163-168.

LILLY, K. (1961). Ecological Studies on Soil Fungi. Ph.D. Thesis, Univ. Saugar, India.

LUKENS, R.J. (1969). Heterocyclic nitrogen compounds. Fungicides vol. 2 ed. Torgeson, D.C. Academic Press, New York.

MARSH, R.W. (1972). Systemic Fungicides, Longman, England.

MARTIN, J.P. (1950). Effects of fumigation and other soil treatments in the greenhouseon the fungus population of old citrus soil. Soil Sci. 69, 107-122.

MARTIN, H. (1973). The Scientific Principles of Crop Protection. Edward Arnold, London.

MARTIN, J.P., BAINES, T.P. and ERVIN, J.O. (1957). Influence of soil fumigation for citrus replants on the fungus population of the soil. Soil Sci. Soc. Amer. Proc. 21, 163-166.

MCCALLEN, S.E.A., MILLER, L.P. and WEED, R.M. (1954). Comparative effects of fungicides on oxygen uptake and germination of spores. Contrib. Boyce Thompson Inst. 98, 39-68.

MCGARITY, J.W. and MYERS, M.G. (1967). A survey of urease activity in soils in Northern New South Wales. Pl. Soil 27, 217-238.

MEIKLEJOHN, J. (1953). Microbial aspects of soil nitrification. E. Afric. Ag. J. 19, 54-6.

MEIKLEJOHN, J. (1954). Some aspects of the physiology of the nitirifying bacteria. Autotrophic micro-organisms 68-83. Cambridge Univ. Press.

MELLENBY, K. (1970). Pesticides and Pollution. Collins, London.

MERWIN, H.D. and PEECH, M. (1951). Exchangeability of soil potassium in the soil, silt and clay fractions as influenced by the nature of the complementary exchangeable cation. Soil Sci. Soc. Amer. Proc. 15, 125-128.

MCNEILLY, B.A. and HOWARD, P.J. (1973). Limitations on the use of chromotropic acid for determining nitrate in woodland soil. Soil Biol. Biochem. 5, 689-693.

MOLLISON, J.E. (1953). Effect of partial sterilization and acidification of soil on the fungal population. Trans. Br. mycol. Soc. 36, 215-228.

MOORE, N.W. (1972). Toxic chemicals and wildlife. Monk's Wood exp. Stn. Report for 1969-71 p 24. Nature Conservancy.

MORRIL, L.G. (1959). An explanation of the nitrification patterns observed when soils are perfused with ammonium sulphate. Diss. Abstr. 20, 30005.

MOUBASHER, A.H. (1963). Selectivity effects of fumigation with carbon disulphide on the soil fungus flora. Trans. Br. mycol. Soc. 46, 338-344.

MOUBASHER, A.H. and MAZEN, M.B. (1971). Selective action of three fumigants on Egyptian soil fungi. Trans. Br. mycol. Soc. 57, 447-454.

MOWAT, J.A(1963). Auxins and gibberellins in marine algae. Proc. 4th Int. Seaweed Symp. 352-359.

MUGHOGHO, L.K. (1968). The fungus flora of fumigated soils. Trans. Br. mycol. Soc. 51, 441-459.

MUNNECKE, D.E. (1967). Fungicides in the soil environment. Fungicides v. 1. ed. Torgeson, D.C. Academic Press, New York.

NEAL, J.L. (1969). Inhibition of nitrifying bacteria by grass and forb root extracts. Can. J. Microbiol. 15, 633-635.

NOMMICK, H. (1957). Fixation and defixation of ammonium in soils. Acta. Agric. Scand. 7, 395-436.

NUTMAN, F.J. and ROBERTS, F.M. (1962). Stimulation of two pathogenic fungi by high dilutions of fungicides. Trans. Br. mycol. Soc. 45, 449-456.

ODU, C.T.I. and ADEOYE, K.B. (1970). Heterotrophic nitrification in soils. - a preliminary investigation. Soil Biol. Biochem. 2, 41-45.

ODU, C.T.I. and AKERELE, R.B. (1973). Effects of soil, grass and legume root extracts on hetetertrophic bacteria, nitrogen mineralization and nitrification in soils. Soil Biol. Biochem. 5, 861-867.

OGAWA, J.M. and MOORE, C.J. (1968). The occurrence and behaviour of variants of Rhizopus stolonifer tolerant to 2, 6 dichloro-4-nitroanaline. Phytopathology. 58, 997-1003.

OHR, H.D. and MUNNECKE, D.E.(1974). Effects of methyl bromide on antibiotic production by A. mellea. Trans. Br. mycol. Soc. 62, 65-72.

PARKER-RHODES, A.F. (1940). Preliminary experiments on the estimation of traces of heteroauxin in soils. J. agric. Sci. 30, 654-671.

PARKINSON, D., GRAY, T.R.G. and WILLIAMS, S.T. (1971). Methods for Studying the Ecology of Soil Micro-organisms. I.B.P. Handbook No. 19, Blackwell, Oxford, England.

PASIEKA, .A.E. and MORGAN, J.F. (1956). Specific determination of proline in biological materials. Proc. Soc. Exp. Biol. Med. 93, 54-57.

PAUL, E.A. and SCHMIDT, E.L. (1960). Extraction of free amino acids from soil. Soil Sci. Soc. Amer. Proc. 24, 195-198.

PAUL, E.A. and SCHMIDT, E.L. (1961). Formation of free amino acids in rhizosphere and non-rhizosphere soils. Soil Sic. Soc. Amer. Proc. 25, 359-362.

PHELPS, R.H. and SEQUIRA, L. (1968). Auxin biosynthesis in a host-parasite complex. Biochem. and Physiol. Plant Growth Sub. 197-212. Proc. 6th Int. Conf. Plant Growth Sub. Ottawa.

PIPER, C.S. (1947). Soil and Plant Analysis. Univ. Adelaide Press.

PORTER, J.N., WILHELM, J.J., and TRESNER, H.D. (1960). Method for the preferential isolation of actinomycetes from soil. Appl. microbiol. 8, 174-178.

PRASAD, R. and RAJALE, G.B. (1972). The influence of nitrification inhibitors and slow release nitrogen materials on transformations of fertilizer nitrogen in soils of fluctuating water content. Soil Biol. Biochem. 4, 451-457.

PRASAD, R., RAJALE, G.B. and LAKHDIVE, B.A. (1971). Nitrification retarders and slow release nitrigen fertilisers. Adv. in Agron. 23, 337-383.

PRIEST, D. and WOOD, R.K.S. (1961). Strains of Botrytis allii resistant to chlorinated nitrobenzenes. Ann. appl. Biol. 49, 445-460.

PRINCE, A.L. (1945). Determinations of total nitrogen ammonia, nitrates and nitrites in soils. Soil Sic. 59, 47-52.

PUGH, G.J.F. and WILLIAMS, J.I. (1971). Effects of an organo-mercury fungicide on saprophytic fungi and on litter decomposition. Trans. Br. mycol. Soc. 57, 164-166.

RAWLINSON, C.J. and COLHOUN, J.,(1970). Chemical treatment of cereal seed in relation to plant vigour and control of soil fungi. Ann. appl. Biol. 65, 459-472.

RICHARDSON, L.T. (1954). The persistence of Thiram in Soil and its relationship to the microbial balance and damping-off control. Can. J. Bot. 32, 335-346.

RIDGE, E.H. and THEODOROU, C. (1973). The effect of soil fumigation on microbial recolonization and mycorrhizal infection. Soil Biol. Biochem. 4, 295-305.

RIFAI, M.A. (1969). A revision of the genus Trichoderma. Mycol. paper 116, 9-56.

RITTENBERG, S.C. (1972). The obligate autotroph - the demise of a concept. Antonie van leewenhoek, 38, 457-478.

ROBERTS, J.L. AND ROBERTS, E. (1939). Auxin production by soil micro-organisms. Soil Sci. 48, 135-139.

SAKSENA, S.B. (1960). Effects of carbon disulphide fumigation on T. viride and other soil fungi. Trans. Br. mycol. Soc. 43, 111-116.

SALT, G.A. (1967). Formalin fumigation of Spring Wheat. Rep. Rothamsted Exp. Stn. 1966, 124.

SCHMIDT, E.L. (1954). Nitrate formation by a soil fungus. Science 119, 187-189.

SCHMIDT, E.L., PUTMAN, H.D. and PAUL, E.A. (1960). Behaviour of free amino acids in soil. Soil Sci. Soc. Amer. Proc. 24, 107-109.

SCHREVEN, D.A. van (1965). Quelques aspects microbiologiques du metabolisms de l'azote dans le sol. Ann. Inst. Pasteur, 109, Suppl. 3, 19-49.

SCHWARZ, K. and BITANCOURT, A.A. (1960). Further evidence for tautomerism in chromatograms of Indole-3-Pyruvic acid. Biochem. J. 75, 182-187.

SEQUEIRA, L.and WILLIAMS, P.H. (1964). Synthesis of I.A.A. by Psuedomonas solanacearum. Phytopathology 52, 439-448

SHELDRAKE, A.R. (1971). The occurrence of and significance of auxin in the substrate of bryophytes. New Phytol. 70, 519-526.

SHELDRAKE, A.R. (1973). The production of hormones in higher plants. Biol. Rev. 408, 509-559.

SHARPLES, R.O. (1961). The fungitoxic effects of dichloran on Botrytis cinerea. Proc. 1st Br. Insectic. Fungic. Conf. 2, 327-336.

SHKONDE, E.I. and KOROLEVA, V.V. (1964). Determination of ammonium nitrogen in soil extracts using indophenol die. Soviet Soil Sic. 6, 609-613.

SIMS, J.R. and JACKSON, G.D. (1971). Rapid analysis of soil nitrate with chromotrophic acid. Soil Sci. Soc. Am. Proc. 35, 603-606.

SKIPPER, H.D. and WESTERMAN (1973). Comparative effects of propylene oxide, sodium azide, and autoclaving on selected soil populations. Soil Biol. Biochem. 5, 409-414.

SMITH, D.H. (1963). Effects of fumigants on the soil status and plant uptake of certain elements. Soil Sci. Am. Proc. 27, 538-541.

SMITH, A.J. and HOARE, D.S. (1968). Acetate assimilation by N. agilis in relation to its obligate autotrophy. J. Bact. 95, 844-855.

SONNEVOLD, C. and VOOGT, S. (1973). The effect of soil sterilization with steam-air mixtures on the development of some glasshouse crops. Pl. Soil 38, 415-423.

SOWDEN, F.J. and IVARSON, K.C. (1966). The free amino acids of soil. Can. J. Soil. Sci. 46, 110-114.

STEVENSON, F.J. (1967). Organic Acids in Soil. Soil Biochemistry V. 1, 119-146. Marcel Dekker, New York.

STEVENSON, I.L. and ROUATT, J.W. (1953). Qualitative studies of soil micro-organisms. Can. J. Botany 31, 438-447.

STEWART, W.S. and ANDERSON, M.S. (1942). Auxins in some American soils. Bot. Gaz. 103, 570-575.

STRINGER, A. and WRIGHT, W.A. (1973). The effects of benomyl and some related compounds on L. terrestris and other earthworms. Pestic. Sci. 4, 165-170.

SWEZEY, A.W. and TURNER, G.O. (1962). Crop experiments on the effect of 2-chloro-6-(trichloramethyl) pyrimidine for the control of nitrification of ammonium and urea fertilisers. Agron. J. 54, 532-535.

TETLOW, J.A. and WILSON, A.I. (1964). An absorptiometric method for determining ammonia in boiler feed water. Analyst. 89, 453-465.

THIEGS, B.J. (1955). Effects of soil fumigation on nitrification. Down to Earth 11, 14-15.

THIMANN, K.V. (1935). On the plant growth hormone produced by Rhizopus suinus. J. Biol. Chem. 109, 279-291.

VALADON, L.R.G. and LODGE, E. (1970). Auxins and other compounds of Cladosporium herbarum. Trans. Br. mycol. Soc. 55, 9-15.

VAN DRIEL, W. (1961). Studies on the conversion of amino acids in soil. Acta. bot. neerl. 10, 209-247.

VIETS, F.G. (1965). The plant's need for nitrogen. Agronomy 10, 503-549.

WAINWRIGHT, M. and PUGH, G.J.F. (1973). The effect of three fungicides on nitrification and ammonification in soil. Soil Biol. Biochem. 5, 577-584.

WAINWRIGHT, M. and PUGH, G.J.F. (1974). The effect of fungicides on certain chemical and microbial properties os soils. Soil Biol. Biochem. 6. (In Press)

WALLACE, W. and NICHOLAS, D.J.D. (1969). The biochemistry of nitrifying micro-organisms. Biol. Rev. 44, 359-391.

WALKLEY, A. and BLACK, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29-38.

WARCUP, J.H. (1951). Effect of partial sterilization by steam or formalin on the fungus flora of an old forest nursery soil. Trans. Br. mycol. Soc. 34, 519-532.

WARCUP, J.H. (1957). Chemical and biological aspects of soil sterilization. Soils Fert. 20, 1-5.

WEBER, D.J. and OGAWA, J.M. (1963). Mode of action of Z, 6-chloro-4-nitroaniline in R. arrihizms (Abstr) Phytopathology 53, 893.

WEBLEY, D.M. and DUFF, R.B. (1965). The incidence in soils and other habitats of micro-organisms producing 2-Ketogluconic acid. Pl. and Soil 307-313.

WEINBERG, E.D. (1964). Manganese requirement for sporulation and secondary biosynthetic processes in Bacillus. Appl. Microbiol. 12, 436-441.

WENSLEY, R.N. (1953). Microbiological Studies of the action of some selected soil fumigants. Can. J. Bot. 31, 277-308.

WEST, P.W. and LYLES, G.L. (1960). A new method for the determination of nitrate Anal. Chim. Acta. 23, 227-232.

WHITEHEAD (1963). Some aspects of the influence of organic matter on soil fertility. Soils Fert. 26, 217-223.

WIDDOWSON, F.V. and PENNY, A. (1965). Effects of formalin on the yields of spring wheat . Rep. Rothamsted. Exp. Stn. 1965, 65.

WILHELM, S. (1965). Pythium ultinum and the soil fumigation growth response. Phytopathology 55, 1016-1020.

WILLIAMS, J.I. (1973). The effect of an organo-mercury fungicide on saprophytic soil fungi. Ph.D. Thesis, Univ. Nottingham.

WINTER, A. (1964). Evidence for the occurrence of I. Py.A. in vivo. Arch. Biochem. Biophys. 106, 131-137.

WOLCOTT, A.R. (1970). Retention of pesticides by organic materials in soils. Pesticides in the Soil Ecology, Degradation and Movement. Symp. Michigan State Univ.

YEUN, S.H. and POLLARD, A.G. (1952). The determination of nitrogen in agricultural materials by the Nessler reagent. J. Sci. Fd. Agric. 3, 441-447.