STUDIES IN THE CONTROL OF COMMON SCAB OF POTATO TUBERS

A thesis presented

by

PAUL FERDINAND ROGERS, B.Sc., A.R.C.S.

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of the University of London.

Department of Botany and Plant Technology,
Imperial College of Science and Technology,
Prince Consort Road,
London, S.W.7.

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ABSTRACT

A method was developed for testing the effect of soluble compounds on the growth of Streptomyces scabies. Manganese was tested in the form of a soluble salt, a glass frit and a number of organic complexes. As a soluble salt it was less toxic than had been suggested by other workers but it was considerably more toxic in the form of chelate complexes.

Three field trials were undertaken to test the effect of chelated manganese on common scab, but no control of the disease was obtained.

Twenty metals were tested for their effect on S.scabies in liquid culture. Six were sufficiently toxic to S.scabies to be of possible use in scab control, but of these, cadmium, arsenic, antimony, mercury and silver were either too dangerous or not economical for use as soil fungicides. Copper had some potential and liquid culture tests and pot trials showed that its use in the form of a glass frit might overcome problems of phytotoxicity.

A number of other chelated metals were tested in liquid culture but their effect on S.scabies was not related to their effect when applied as soluble salts, nor was it related to their chelate stability constants.

It has been suggested that organic amendment of soil controls scab by the reduction of insoluble soil manganese to soluble forms which are toxic to S.scabies. This was investigated by means of pot and field trials, a method being devised to assess numbers of manganese-reducing microorganisms in soil.

Dried grass meal at 2 tons/acre gave significant control of scab ($P,0.1$) but increases in soluble manganese were not sufficient to account for this. Soil microorganism populations increased after adding grass meal but had fallen again by the time tuber initiation and scab infection had commenced. Results suggested that manganese reduction could affect scab provided there were sufficiently high levels of easily reducible manganese in the soil.

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INTRODUCTION

Common scab disease of potatoes is an economically important disease. Although the development of acceptable resistant varieties is a possible answer to the problem, there is no prospect of such varieties becoming widely used in the near future.

The main problems in working with this disease are the difficulty of identifying the pathogen by standard methods, its slow growth in pure culture and the disagreements in the published results on many aspects of the disease, particularly those involving pot and field trials.

Although there has been extensive work with many chemicals for their possible use as fungicides against scab, as yet, none is widely acceptable in this role, mainly because the dosage required for control is seldom economically feasible.

The work on minor elements is promising, primarily because these elements may be present in the soil naturally even if they are not in a form which will have any effect on the scab organism.

Past work on fungicides and on minor elements has suffered the disadvantage that it has been necessary to assess their effect by means of pot and field trials. The

present work was initially directed towards developing a quantitative method of assessing possible fungicides and applying it to study the effect of a range of minor elements on S.scabies with a view to undertaking pot and field trials later on.

The effectiveness of some soil amendments in controlling scab implicates the soil microflora in control. There is circumstantial evidence suggesting a link between this and the availability of minor elements in the soil which are toxic to the scab organism. This aspect of the problem occupies the second part of the experimental section.

REVIEW OF THE LITERATURE

The Potato Crop

The world production of potatoes has remained almost constant over the last 15 years at an annual figure of about 250 million long tons. The total acreage is about 60 million with an average yield of 4.3 tons/acre. Of this total, about one third is produced in Europe excluding the USSR (FAO, 1963).

In Great Britain there is little industrial use for potatoes and the main demand is for human consumption which runs at about 4¼ million tons per year. Some 700,000 acres are planted giving a national yield of 6 million tons at 8 - 9 tons per acre. This satisfies national demand except in low yield years when maincrop potatoes are imported. Demand for earlies exceeds supply and on average 250,000 tons are imported each year (PMB, 1964).

Half of the national yield goes for home consumption and a further 18 per cent. for caterers. There is about 15 per cent. wastage and a similar percentage is used as seed, the remainder being used for stock feed (Hessayon and Fenemore, 1961).

The main potato growing area of Britain is the Fens. Less important are west Lancashire, north west Lincolnshire, the west Midlands and the East Riding of Yorkshire. Parts

of the home counties, particularly in Essex, and also in the Tyne/Tees area have risen in importance, mainly because of their proximity to markets. In Scotland, Perthshire is the most important area, with potato growing concentrated in the east coast counties from Morayshire southwards.

The value of the potato crop to the grower exceeds £100 million and is probably double that to the retailer. The size of the crop is notoriously variable, the average yield in 1959 being 8.6 tons/acre as against 6.9 tons/acre the previous year.

The main task of the Potato Marketing Board as constituted in 1955 is to ensure a balance between supply and demand. All growers of an acre or more of potatoes are required to be registered producers, this covering 93 per cent. of the total potato acreage. Each grower pays a levy of £1 per acre to the PMB.

The Board undertakes its task in a number of ways:

a) Advertising directed at the consumer.

b) Control of acreage, designed to prevent overproduction; a grower who exceeds his quota must pay an excess acreage contribution of £10 per acre.

c) Control of riddle size; only a bottom riddle size is normally employed and this controls the quantity of potatoes being marketed by eliminating small tubers, but

occasionally an upper limit is imposed to remove large or mis-shapen tubers.

d) Surplus ware may be purchased when there is a glut, and the surplus used for stock feeding.

In Britain, nearly half of the total potato crop is made up of the variety Majestic. The remainder consists largely of King Edward, Redskin and Kerr's Pink, and Arran Pilot.

The Disease

Common scab of potatoes, caused by Streptomyces scabies (Thaxt.) Waksman and Henrici, is distinguished by being the only plant disease of economic importance caused by an actinomycete. Although the potato is the main host, it has been reported on a number of other root crops such as carrots, radish, mangold, swede, turnips and leeks (Jones, 1953; Palm, 1934). In addition to causing tuber scab it will cause a stem necrosis on potatoes (Hooker and Sass, 1948; Hooker et al., 1950) and will cause root necrosis when seedlings of soybean, wheat, pea, radish and beet are inoculated (Hooker, 1949).

The pathogen causes surface scabs which do not affect the size of the tuber very much but do blemish the tuber surface. The economic significance of the disease lies in the surface blemishing rather than any loss in yield,

although deeply scabbed tubers cause wastage in peeling. Badly scabbed tubers do no sell well, and will be still more difficult to dispose of with the increasing use of prepackaging in the retail trade. Another aspect of the disease which makes it more serious is that it is usually worse in a dry season when the crop is likely to be poor anyway.

Detailed surveys of common scab incidence have been made in the United Kingdom. Results published for 1952 and 1953 (Large and Honey, 1955) show that if tubers with one quarter of the surface scabbed are considered substantially scabbed, then when June and July are dry, 4 per cent. of the potato crop for that year will be in this category, and when wet, the figure will be about 2 per cent. This also showed the highest incidence of scab to be in the east and west midlands and parts of Yorkshire, and lowest in Scotland, most of Lancashire and the Fens.

The disease is of world-wide occurrence but is often no problem in the best potato growing areas. The survey already mentioned suggests that the disease is worse on light, sandy or gravelly soils and this is borne out by experience in other countries, it being particularly bad in the sandy soils of east Holland (Labruyere, p.c. 1965) and in the reclaimed areas of the Negev desert (Volcani, 1962).

The literature on common scab is well known for the

variability of results and contradictions which occur on almost every aspect of the disease. The fact that the pathogen is soil-borne with the variabilities inherent in working in this medium partially explains this. The scab organism exists in a wide range of physiological forms and it is not always possible to identify those that are pathogenic by classical techniques. Corbaz (1964) identified only 70 per cent. of a range of pathogenic streptomycetes as S.scabies, and Harrison (1962) described "potato russet scab" as being due to species other than S.scabies, developing more readily on warmer moister soils than those favouring common scab. Hoffmann (1958) gave results differing from those of Corbaz. Of 20 identified species, only S.scabies was pathogenic and he considered this to be the only cause of potato scab.

Attempts have been made to use serological methods for identifying pathogenic streptomycetes but Douglas (1953) concluded that no clear-cut division into scab-producers and saprophytes was possible at that time. Ten years later, Bowman and Weinhold (1963) concluded that the pathogenic streptomycetes were closely related serologically, but a few saprophytes were not separable, and the use of serology, therefore, was not entirely satisfactory. Actinophages active against S.scabies have been reported (Newbould, 1953;

Newbould and Garrard,1954). Their use in identification of S.scabies is not yet developed, although a close serological relationship between many phages active against S.scabies has been demonstrated (Corke, 1963).

It may not be safe to consider common scab as caused by a specific organism - S.scabies - unless one accepts that a main criterion for so naming an organism be that it exhibits pathogenicity, whatever its morphological and cultural characteristics. At present, one can say no more than that there is a range of normally saprophytic actinomycetes which under certain conditions infect suitable hosts.

Common experience and documented evidence agree on the soil factors favouring scab development. It is more prevalent in soils of pH 6.0 and above (Generaux, 1947; Richardson and Heeg, 1954). In a field in which plots had been amended with lime or sulphur to give a pH range of 4.5 to 6.5, Steinmetz (1946) found that over ten years, the increase in scab was directly related to the increase in pH. An exception to this general rule was the report by Boysen (1932) that the disease was prevalent in more acid soils of pH 4.5, the soils being of a very poor sandy heathland type.

A second factor favouring scab is dryness of the soil. The survey of Large and Honey (1955) showed scab to be twice as bad in a dry as in a wet season, confirming earlier

reports that soil moisture is an important factor in disease development (Sanford, 1923, 1945; Noll, 1939; Generaux, 1947). Dippenaar (1933) showed that increasing soil moisture over a range of soil temperatures between 13 and 25°C. decreased incidence of scab and increased yields. Experiments of Lutman (1941), on the other hand, gave different rports because he found maximum infection on areas that retained most moisture after rainfall.

Irrigation during dry weather gives some control of the disease (Peeler, 1966) provided it is applied before or during tuber initiation (Llewelyn, 1963; Lewis, 1962; Labruyere, 1965). Starr, Cykler and Dunnewald (1943) failed to check the disease by irrigation, but Lapwood (1966) suggests that this may be because they applied the water too late. In two experiments he found that there was an increase in scab when a dry period co-incided with active growth of the tubers. Irrigation to field capacity at this time largely prevented scab. In another experiment, Lapwood and Dyson (1966) found that increasing use of a nitrogen fertiliser resulted in an increase in scab. They thought this to be most likely due to the fact that the nitrogen fertiliser delayed tuber formation so that it occurred in a dry period with resultant increase in scab.

The scab organism will persist in the soil for a number

of years. Hooker (1955) observed a marked reduction after three years, particularly in plots left fallow. The value of any particular crop rotation in cutting down scab incidence is also related to changes in soil pH following the cultivation of a particular crop (De Bruyn, 1943). For example, it is common practice to lime fairly heavily for barley, and this is likely to increase scab incidence in crops grown subsequently.

Infection

Tuber infection is primarily through lenticels but direct penetration between cells can occur in young tubers (Lutman, 1941). Infection only occurs in actively growing tubers (Hooker and Page, 1960; Richardson, 1952; Lewis, 1962) although the pathogenicity test suggested by Lawrence (1956) contradicts this since he reported infection on small detached tubers.

Under field conditions, Schaal (1934) found that larvae of the potato flea beetle, Epitrix cucumeris, carried S. scabies internally and externally and may cause infection on tubers on which they feed. Scab lesions are frequently made worse by attack by other insects (Granowsky and Peterson, 1942).

Cooper et al. (1954) established that tubers in which

the periderm persists as a living tissue throughout development were scab resistant, whereas tubers in which the periderm is covered by collapsed dead cells are susceptible. These results were confirmed by Emilsson and Heikan (1956).

Johnson and Schaal (1952) were able to correlate resistance with a high concentration of chlorogenic acid, particularly in the periderm around lenticels and injuries. Tests with S.scabies in agar culture (1954) showed that inhibition of growth by chlorogenic acid depended mainly on formation of quinones by enzyme action in the tuber, because inhibition in culture is more pronounced at a higher pH when autoxidation of the phenolic compounds increases production of quinones. Schaal (1955) later showed that caffeic acid, catechol, and tetrahydroxy benzoin had a similar effect to chlorogenic acid on S.scabies in pure culture. Johnson and Schaal (1957) went on to show that o-dihydricphenols as well as chlorogenic acid were present in periderm of resistant tubers in much higher concentrations than in the periderm of susceptible tubers. A closely related mechanical/chemical defence system therefore seems possible.

Lawrence and McAllan (1964) reported the isolation of an inhibitor from culture filtrates of S.scabies which acts only on the pentose phosphate pathway or its terminal oxidation in the potato tuber. The activity of glucose-6-

phosphate dehydrogenase was reduced, and that of cytochrome oxidase strongly inhibited.

Chemical control of common scab

Two approaches have been tried for the chemical control of common scab; treatment of seed potatoes and of the soil.

The use of infected seed may contaminate clean soil but this is no longer considered to be important in the spread of the pathogen. In fact, common scab often occurs on newly cultivated land. However, a number of tuber treatments have been used repeatedly for their effect both on scab and on black scurf (caused by Rhizoctonia solani). Those most commonly used have been mercury compounds (Cross, 1925; Martin, 1929; Beare, 1944; Schaal, 1946; Dantas, 1954; Netzer et al., 1962). Other treatments known to be effective are hot formaldehyde (Rose, 1926; Vaughn, 1926; Goss, 1929), thiram (Dembskaya, 1944) and zinc oxide - alone and with mercury compounds (Beare, 1944).

Direct chemical treatment of soil has also been tested often. Sulphur has been used at rates of up to 2,500 lb/acre with some success (Reddy et al., 1946; Terman et al., 1949; Hooker and Kent, 1950; Oswald and Wright, 1952; Oswald, 1954; McAllister, 1963), the aim being to increase soil acidity. Ammonium sulphate is stated to be effective as well.

Pentachloronitrobenzene (PCNB, terraclor, Bayer-P, quintozene), at rates varying between 10 and 500 lb/acre has also been tested extensively. A general picture of fairly good control at 50 to 100 lb/acre emerges (Gram, 1944; Hooker, 1954; Oswald, 1954; Fink, 1956; Menzies, 1956; Nugent, 1956; Houghland and Cash, 1957; Van Emden and Labruyere, 1958; Van Doorn, 1959; Erickson, 1960; Dingler *et al.*, 1960; Metzer and Dishon, 1962; Gustafsson, 1964).

Against this, Potter *et al.* (1959) did not get good control except on a very sandy soil, and Rosser (1960) reported that PCNB gave significant control at 100 lb/acre when scab incidence was low but in a year when there was high scab incidence there was no control.

Van Emden (1958) and Houghland (1957) both reported effects lasting more than one season, but the effect of PCNB on yield is the subject of contradictory results. Hooker (1954) reported a 20 per cent. increase in yield following a furrow application at 50 lb/acre. Dingler (1960) found no effect on yield, but other workers have reported retardation of growth and loss in yield (Van Emden, 1958; Houghland, 1957; and Gustafsson, 1964).

A number of other fungicides are known to control scab. These include urea formaldehyde formulations (Bartz and Berger, 1958; Busch, 1961; Busch and Ashton, 1964; Schultz

et al., 1960; Schultz, 1962; Weinhold et al., 1964), mercury compounds (Martin, 1932; Emilsson and Gustafsson, 1954), pentachlrorophenoxyacetic acid (Garber, 1951; Emilsson and Gustafsson, 1954), thiram (Popkova et al., 1965), Calcium cyanamide (Rang, 1932; Bolley, 1926), "DAC-649" (3,3,4,4,-tetrachlorotetrahydrothiophene-1-1-dioxide) (Potter et al., 1961; Cetas and Jones, 1962).

Urea formaldehyde with aldrin (Busch, 1964; Schultz, 1962), mercurous chloride with sulphur (Generaux, 1947) or with dinitrorhodanebenzene (Popkova et al., 1965) are combinations which have also been tried successfully.

Control with major and minor elements

A number of investigators have studied the effect of various elements on the pathogen and on the disease.

Blodgett and Cowan (1935) considered that lime had no direct effect on the disease except by increasing soil pH but calcium oxide at the very high concentrations of 1 and 2 per cent. reduced infection considerably. Schroeder and Albrecht (1942) found that calcium and potassium added to the soil in equal amounts gave the best crop yields and least scab and suggested that the relation of calcium to potassium was synergistic rather than antagonistic. Cook and Houghland (1942), however, concluded that calcium had no

effect on disease incidence which was only indirectly related to the calcium content of the fertiliser in so far as this changed the soil reaction. Thus a neutralized kieserite fertiliser resulted in a less acid reaction and consequently more scab than a one-third neutralized one. Odland and Allbritten (1950) confirmed a lack of any direct effect of calcium on the disease, but the work of Horsfall, Hollis, and Carter (1954) was at variance with this. They concluded that the amount of scab was directly related to the calcium content of the tuber which in turn is regulated by the content of replaceable calcium in the soil, this being governed by hydrogen ion concentration and calcium dosage.

Houghland and Cash (1956) found that the total calcium content of tubers was not related to scab incidence but that periderm of scabbed tubers had twice the amount of calcium found in that of clean tubers. This was considered to be a result rather than a cause of infection. Phosphorus showed a similar but less marked pattern and with potassium it was still less marked. They also found that the calcium/potassium ratio in potato haulms and tubers bore no relation to the susceptibility of tubers to scab. Turner (1957) was also unable to establish any relation between calcium/potassium ratio and scab incidence, a result confirmed by Doyle and MacLean (1960). The situation was yet more

complicated by the results of Eichinger (1958) who suggested that deposits of calcium cations on the tuber surface were positively related to scab incidence, and that oxalic acid produced by the plant was the strongest inactivator of these ions, the degree of resistance of a potato variety being determined by its capacity to form oxalic acid. He recommended, therefore, exclusion of synthetic fertilisers containing magnesium and sodium on the grounds that these ions would compete with those of calcium for oxalic acid.

Nader and Nader (1937) reported that Bordeaux mixture at a rate of 75 lb/acre $CuSO_4$ markedly reduced scab. The treatment retarded flowering and increased yields. Similar results were reported by Dorozkein (1955). Gries (1950) showed that aluminium in acid and alkaline culture media was toxic to S.scabies and would control scab in muck soils. This work was partially verified by Houghland (1956) who found that aluminium as an organic salt inhibited growth at 100 ppm, and as aluminium chloride at 160 ppm, but at rates of up to 800 lb/acre aluminium sulphate did not control scab, neither did it alter the soil pH.

In 1955, Spatz published results of control of manganese deficiency of potatoes by application of manganese sulphate at 6 kg./ha. Incidental to these, he found that the percentage of badly scabbed tubers was reduced from 70

to 20. The extensive trials of Guntz and Copper (1957) examined the effect of a number of soluble salts on scab incidence and the results are summarised in Table 1.

Table 1. Results of Guntz and Coppenet for scab control

Soluble salt	Application (kg./ha.)	% marketable tubers
Untreated	0	1.6
Sodium borate	15	3.0
Cobalt nitrate	5	1.6
Manganese sulphate	500	24.0
Ammonium molybdate	2.2	4.3
Zinc sulphate	100	5.0
Magnesium sulphate	1,000	6.6
Sulphur	500	7.9

Turner reported that zinc compounds had no effect on scab at 12.5 and 25 lb/acre.(1957). Malenev (1959) found that scab resistance was increased 2 to 3 times by dipping seed tubers in 0.02 per cent. solutions of copper, boron and manganese salts. Mygind (1961) reported aluminium sulphate to be effective in field trials but not at economic levels, and manganese sulphate was found to have an erratic

effect unless large quantities were used.

Mortvedt et al. (1960) found that scab decreased as manganese concentration increased over a range of 0 to 20 ppm in pot culture. Further experiments (Mortvedt et al., 1961) gave significant reduction at 5 and 10 ppm and the suggestion was made that a high concentration of soluble manganese could explain the tendency for scab to be less prevalent in highly acid soils. Field trials gave no evidence of control at 50 and 150 lb/acre $MnSO_4$. The manganese content of the tuber epidermal tissue was increased but there was no effect on tuber parenchyma tissue. Later trials (Mortvedt, 1962) showed that manganese sulphate at 150 and 450 lb/acre was effective against scab; broadcasting it in 12 inch bands near the tubers was more effective than placing it in the fertiliser band at 150 lb/acre. Further greenhouse experiments did not relate periderm manganese content to scab incidence, suggesting that the effect was not systemic. This also applied to copper, magnesium, calcium and potassium. Copper sulphate at 20 and 50 lb/acre significantly reduced the disease, but was toxic at high levels in greenhouse trials.

McGregor and Wilson (1964, 1966) confirmed the effect of $MnSO_4$, stating that it gave fair control at 56 lb/acre,

mixed with a compound fertiliser in the drill before planting, but other workers have found little effects at these rates (Watson p.c., 1965).

MATERIALS AND METHODS

A. THE PATHOGEN

1. Isolation of the pathogen

A slightly modified version of the technique described by KenKnight and Muncie (1939) was used. A scabbed tuber was washed in tap water, surface sterilised in a 1 per cent. available chlorine solution for 5 minutes and washed in sterile water. A scab lesion with underlying tissue was removed with a flamed scalpel and comminuted in 10 ml. of water. The suspension was diluted 750 or 1,500 times and plated on to Czapek-Dox agar. Many actinomycete colonies appeared after 7 days incubation at 30°C.

2. Stock cultures

Three methods were used for maintaining stock cultures of Streptomyces scabies.

a) Cultures on PDA slopes in McCartney bottles were kept at 2°C. and at room temperature, 18 to 22°C. Cultures remained viable for up to one year but changes in cultural characteristics sometimes developed.

b) Cultures were stored at room temperature on PDA slopes in McCartney bottles under mineral oil.

c) The best method for maintaining stock cultures was to keep them in sterile soil. 10 g. samples of soil were put into McCartney bottles and autoclaved for 30 minutes at 15 p.s.i. A five day old liquid shake culture of the isolate in Czapek-Dox solution was then added to the soil at the rate of 1 ml. per bottle. The bottles were screwed up lightly and dried out at 2°C. for 2 weeks and then stored at room temperature. The actinomycete spread through the soil and in some cases, aerial mycelium was visible. To sub-culture, a few grains of soil were spread on to a suitable agar or added to liquid medium. Using this method, stock cultures maintained their cultural characteristics.

3. Nutrient media

A) Agar media

All agar media were steamed for 30 minutes after mixing of constituents and then autoclaved at 15 p.s.i. for 15 minutes unless otherwise stated.

(a) Czapek-Dox agar (CD)

Sucrose	30.0 g.
$NaNO_3$	3.0 g.
K_2HPO_4	1.0 g.
$MgSO_4.7H_2O$	0.5 g.
KCl	0.5 g.

$FeSO_4.7H_2O$	0.01 g.
Yeast extract	1.0 g.
Agar	15.0 g.
Minor elements	1.0 ml./100 ml. medium
Water	1,000 ml.

(b) Richards' agar

Sucrose	50.0 g.
KNO_3	10.0 g.
KH_2PO_4	5.0 g.
$MgSO_4$	2.5 g.
$FeCl_3$	0.1 g.
Agar	20.0 g.
Water	1,000 ml.

The KH_2PO_4 was autoclaved separately.

(c) Glycerol asparagine agar (GA)

Glycerol	10 ml.
Asparagine	1.0 g.
K_2HPO_4	1.0 g.
$CaCO_3$	3.0 g.
Agar	15.0 g.
Water	1,000 ml.

This medium was selective for actinomycetes.

(d) Potato extract agar (PEA)

Potatoes	200 g.

Agar	20 g.
Water	1,000 ml.

Potatoes were scrubbed but not peeled, cut into c.1 inch cubes and boiled in 1,000 ml. of water for one hour. The liquid was strained through muslin and made up to 1,000 ml. before adding the agar.

(e) Potato dextrose agar (PDA)

Potatoes	200 g.
Dextrose	20 g.
Agar	20 g.
Water	1,000 ml.

The procedure was the same as for PEA with the dextrose added after straining.

(f) Caseine tyrosine agar (CT)

Sodium caseinate	25.0 g.
$NaNO_3$	10.0 g.
L-tyrosine	1.0 g.
Agar	15.0 g.
Water	1,000 ml.

Casein hydrolysate could be used in place of sodium caseinate. This was dissolved in water at 60°C. and autoclaved separately at 10 p.s.i. for 20 minutes. This medium was partially selective for plant pathogenic streptomycetes (see page).

(g) V8 juice agar

V8 juice	75 ml.
Agar	20 g.
Water	925 ml.

(h) Modified Czapek-Dox agar

This was made up in the same way as Czapek-Dox agar with the addition of Rose Bengal at 33 ppm and streptomycin at 30 ppm, both additives being autoclaved separately.

B. Minor element solution

This was added to Czapek-Dox agar and solution at the rate of 1 ml. per 100 ml. of medium.

$FeSO_4.7H_2O$	0.125 g.
$ZnSO_4.7H_2O$	0.11 g.
$CuSO_4.5H_2O$	0.02 g.
$MnSO_4.4H_2O$	0.02 g.
$Na_2MoO_4.2H_2O$	0.025 g.

A trace of H_2SO_4 was added to clarify the solution if necessary.

C. Liquid culture solutions

(a) Nutrient broth

"Lab-Lemco" Beef Extract	1.0 g.
Yeast extract (Oxoid L20)	2.0 g.

Peptone (Oxoid L37)	5.0 g.
NaCl	5.0 g.
Water	1,000 ml.

(b) Richards' solution

This had the same constitution as Richards' agar but without the agar.

(c) Czapek-Dox solution

This had the same constitution as the solid medium but without agar. The concentrations of metals in Czapek-Dox solution are given in Table 2.

Table 2. Concentrations of metals in Czapek-Dox solution.

Element	Compound	Concentration of compound - g./litre	Weight of element - g./litre	PPM of element
Na	$NaNO_3$	3.0	0.81176	811.81
Na	$Na_2MoO_4.2H_2O$	0.0005	0.00005	
K	K_2HPO_4	1.0	0.22414	484.10
K	KCl	0.5	0.26	
P	K_2HPO_4	0.5	0.17812	178.16
Mg	$MgSO_4.7H_2O$	0.5	0.04858	48.58
Fe	$FeSO_4.7H_2O$	0.0125	0.00251	2.51

Element	Compound	Concentration of compound - g./litre	Weight of element - g./litre	PPM of element
Zn	$ZnSO_4.7H_2O$	0.0022	0.00049	0.49
Cu	$CuSO_4.5H_2O$	0.0004	0.0001	0.1
Mn	$MnSO_4.4H_2O$	0.0004	0.00009	0.09
Mo	$Na_2MoO_4.2H_2O$	0.0005	0.00009	0.09

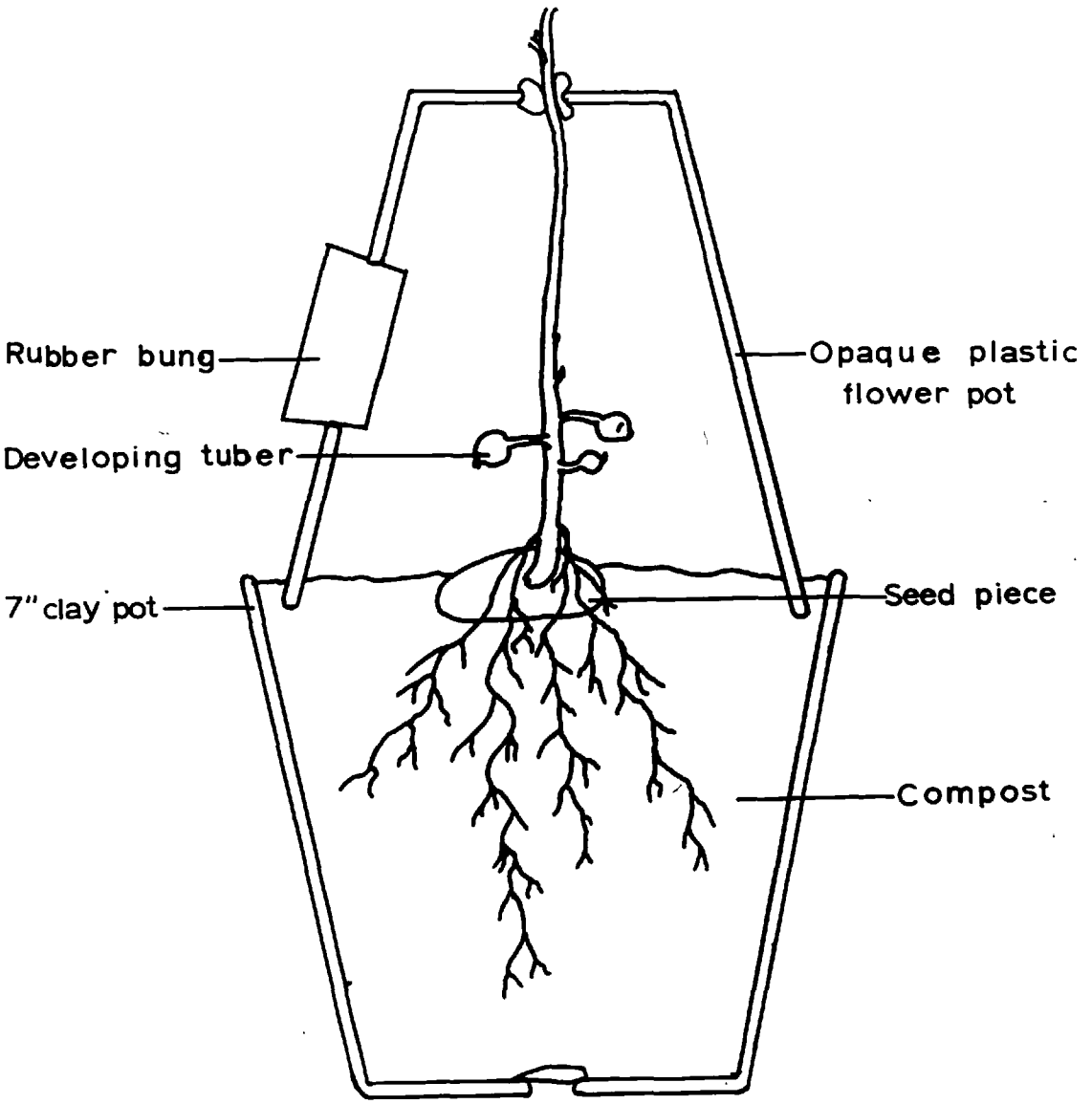
4. Pathogenicity tests

Three tests were used:-

(1) Using the method of Lawrence (1956), slightly modified by available apparatus, small tubers (variety Majestic) about 1 inch in length, were surface sterilised, dipped in spore suspension of the isolate and placed on a 2 inch deep bed of moist sterile vermiculite in sterile 3 by 6 inch screw top jars. The spore suspension was obtained from a two week old culture of the isolate on Czapek-Dox agar. Tubers were incubated for up to four weeks at 30°C. and were then examined for lesion formation.

(2) Using a modification of Hooker's method (1950), Majestic seed pieces were planted on the surface of vermiculite in 7 inch clay pots and the stems of the plants covered 3 weeks later with 5 inch opaque plastic pots (figure 1).

Figure 1 Pathogenicity test (after Hooker)



Plants were watered with Long Ashton nutrient solution. Stolons and developing tubers which formed under the opaque plastic pots were coated with spore suspensions of the isolates being tested and then observed for lesion formation over a period of six weeks.

(3) The two tests described are designed to take up to, at most, 8 weeks. A longer but simpler pathogenicity test was employed when time was not important. Majestic seed pieces were planted in John Innes number 2 compost to which had been added an equal volume of washed sand and a spore/hyphae suspension of the isolate at a dry weight concentration of 250 ppm. The plants were grown under greenhouse conditions with minimal watering for 12 weeks, and the developing tubers were then examined for lesions.

5. Rotary shaker

This was constructed in the workshops of the Imperial College Mechanical Engineering Department. It had a capacity of 72x500 ml. conical flasks mounted on a black plasticised rubber deck, and held in place by rubber clips. Four inch and three inch angle iron was used for the frame. The shaker was powered by a 230 volt DC shunt motor developing 0.5 horse power at 1,425 r.p.m. and the deck speed was adjustable over the range 150 to 200 r.p.m. The deck had

a horizontal throw of 2.5 cm. and the whole shaker was kept in a constant temperature room. This was kept at 29°C., the highest temperature at which it could be set and as near as possible to the optimum temperature of S.scabies which is 36°C.

6. Bulk culture of S.scabies

A ten litre fermentor was assembled (figure 2). It consisted of two 5 litre side arm flasks containing culture medium, through which were passed streams of sterile air, the whole being in an incubator maintained at 35°C. Air was sterilised by passing it through a filter consisting of two 24 inch lengths of ½ inch diameter glass tubes filled with tightly packed cotton wool, the filter being sterilised by autoclaving at 15 p.s.i. for 30 minutes. The fermentor produced a maximum of 2 g. dry weight of hyphae per litre of medium after 6 days incubation. Oleic acid at 0.1 ml./ litre was used as an anti-foaming agent and the medium used was Czapek-Dox solution, with yeast extract and minor elements.

B. THE HOST

1. Potato plants

Grade "A" Majestic seed was used throughout, this being the principle maincrop variety in England and Wales and one

Figure 2. 10 litre fermentor

that is susceptible to common scab. Arran Victory Grade "A" seed was used as a marker plant in field trials. Its foliage differed from Majestic, and the purple tubers made it easy to determine the boundary of a plot at the time of harvest.

All seed tubers were obtained from Carter's Ltd., of Raynes Park, London S.W.19.

2. Plant nutrient solution.

This was based on Hewitt's (1952) nutrient solution and the constituents of the six stock solutions are given in Table 3.

Table 3. Constituents of Stock Nutrient Solutions

Number	Constituents	Concentration g./litre
1	$Ca(NO_3)_2.4H_2O$	118.0
2	KNO_3	50.5
3	$MgSO_4.7H_2O$	37.0
4	$NaH_2PO_4.2H_2O$	20.8
5	FeEDTA stock solution	
	$FeCl_3.6H_2O$	1.35
	Na_2EDTA	3.7

Table 3. contd.

6	Micronutrients	
	$MnCl_2.4H_2O$	0.198
	$CuCl_2.2H_2O$	0.017
	$ZnCl_2$	0.014
	H_3BO_3	0.136
	Na_2MoO_4	0.610

One litre of nutrient solution contained 10 ml. of each stock solution. The six stock solutions were always added to at least 500 ml. of water to prevent them interacting to form precipitates. Distilled water was used throughout.

To make up the FeEDTA stock solution, a concentrated solution of $FeCl_3$ was added slowly to a very dilute solution of Na_2EDTA with constant shaking. The FeEDTA solution was adjusted to pH 5.5 by the addition of 10 to 11 ml. of N/1 NaCH per litre of solution.

3. Greenhouse - South Kensington.

Plants were grown in the rooftop greenhouse at South Kensington for pathogenicity tests. They were grown either in vermiculite and watered with nutrient solution every other day, or else in a compost/sand mixture. The temperature in the greenhouse ranged between 18 and 28°C. Daylight was

supplemented for 14 hours each day with batteries of five fluorescent tubes placed 4½ inches apart and 2 feet 6 inches above the bench. The light intensity at pot level was 900 lumens per square foot.

4. Greenhouse - Chelsea Physic Garden.

Pot trials were at the Chelsea Physic Garden. In order to shorten the growing period of the potato plants and also to enable control of watering, two greenhouses were erected. These were Crane Mk. II, type 6 Dutch glasshouses, with base dimensions of 20 feet by 8 feet. One inch deep beds of gravel were laid inside each glasshouse, except for a path down the middle, and each house had a floor area of 130 square feet of pot space. Since the glasshouses were of Dutch type construction, light intensity was high.

5. Disease assessment of scabbed tubers.

The degree of scab infection of tubers harvested from pot and field trials was estimated by the method of Large and Honey (1955). This made use of diagrams showing 1/16, 1/8, 1/4, and 1/2 of the tuber surface covered by scab lesions. Using these diagrams, the numerical scoring devised by McKee (1961) was employed. Thus five classes of infection were recognised and numerical scores directly related to the proportion of surface affected were allocated

to each class so that a mean score could be calculated for each sample. The scoring range is given in Table 4.

Table 4. Scoring system used in scab assessment.

Class	Proportion of surface covered by scab			Score
A	0	to	1/16	1
B	1/16	to	1/8	3
C	1/8	to	1/4	6
D	1/4	to	1/2	12
E	1/2	to	1	24

All tubers from each treatment were washed and assessed for scab. In pot trials, those below 2 cm. in length were discarded, and in field trials the minimum length was 2.5 cm. The weight of tubers in each class was recorded.

C. SOIL MICROFLORA

A method similar to that of Timonin (1940) was used for estimating numbers of soil bacteria, actinomycetes and fungi. This employed the dilution plate method and this cannot be relied upon to give an accurate indication of numbers of microorganisms, its use here being confined to giving an indication of relative size of populations.

The soil sample to be examined was passed through a 3 mm. sieve and 25 g. of sieved soil was placed in 250 ml. of sterile water in a 500 ml. medical flat. This was shaken for 1 hour on a reciprocating shaker at 100 shakes per minute. 5 ml. of the resultant suspension were then transferred with a sterile pipette to a 200 ml. medical flat containing 45 ml. of sterile water. This was shaken before transferring 5 ml. to another 45 ml. of sterile water. This procedure was continued to give a range of dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. One ml. of the required dilution was spread over the bottom of a sterile petri dish and to this was added 10 ml. of the appropriate agar at 45°C. The petri dish was shaken so that the soil suspension was thoroughly mixed with the agar. Five plates were used for each estimate of population.

Bacteria were estimated using Czapek-Dox agar, colonies being counted after 4 days incubation at 25°C., before actinomycete colonies were visible. If the plates were examined after a further 11 days incubation, the Czapek-Dox medium also gave an indication of actinomycete populations since these colonies were easily distinguishable from bacterial colonies. However, since the latter tended to overgrow the smaller actinomycete colonies, a selective medium for actinomycetes, glycerol-asparagine agar, was used

as an alternative medium.

Fungal populations were estimated using a modified Czapek-Dox agar containing 33 ppm of Rose Bengal and 30 ppm of streptomycin. These additives largely inhibited growth of bacteria and actinomycetes and fungal colonies could be counted after 5 days incubation at 25°C.

An approximate indication of numbers of pathogenic streptomycetes was obtained using caseine tyrosine agar, the actinomycete colonies which formed brown pigment on this medium being counted after 15 days incubation at 25°C. This is considered to separate scab-producing actinomycetes from non-scab-producing ones on the basis of the former producing a brown pigment on protein medium in the presence of air (Taylor and Decker, 1947; Vaisey et al., 1955). The brown pigment production is thought to be due to the action of tyrosinase on tyrosine, leading to the formation of melanin pigments (Skinner, 1938). Gregory and Vaisey (1956) have shown that tyrosinase does not seem to be associated with virulence of S.scabies and the "brown ring test" only gives a rough idea of number of pathogenic actinomycetes. Thus Taylor and Decker (1947) were able to correlate brown ring formation with pathogenicity in the case of all the 137 isolates they studied, but Vaisey et al. (1955) could

get a positive correlation in only about 90 per cent. of cases.

D. MATERIALS

1. Grass meal

This was obtained from W.A.Lidstone of Slough, Berks.

2. Glass frits

These were obtained from Ferro-Enamel Ltd., through the Tenant Trading Company of London, E.C.3.

3. Rayplex Manganese

This was obtained through the London office of Rayonier Inc., of New York.

4. Chelating agents

The chelating agents and metal chelate complexes used in this work were all obtained from Geigy Ltd., of Manchester, with the exception of $Na_2MnDTPA$ which was made up in the laboratory using Na_5DTPA and $MnCl_2$.

5. Insecticides

"Murfume" and "Systemic Insecticide" (Rogor/DDT) were both used to control greenfly in the greenhouses at Chelsea, both being products of the Murphy Chemical Company, Wheathampstead, Herts.

PART I. CHEMICAL CONTROL OF COMMON SCAB

EXPERIMENTAL

Preparation of stock cultures

Samples of badly scabbed tubers were received from Mr.E.Lester of the National Agricultural Advisory Service at Reading, Berkshire. A number of actinomycetes were isolated from these tubers and six isolates were selected for identification because of cultural similarities to Streptomyces scabies. The six isolates were designated S1a, S1e, S1f, S2a, S2b and S2c and they were submitted to standard tests to see if they conformed to the description of Streptomyces scabies given by Waksman (1961). The results are given in Table 5 and suggest that isolates S2a, S2b and S2c conformed to the description slightly more than the others although this was not considered proof of identification without a pathogenicity test.

Two sets of pathogenicity tests were carried out, one using Lawrence's method and the other Hooker's method. In addition to the three isolates, culture IMI 99049 of S.scabies from the Commonwealth Mycological Institute, originating in Israel in 1959, was tested.

Table 5. Identification tests for Streptomyces scabies

Test	S1b	S1e	S1f	S2a	S2b	S2c
Cell morphology	+	++	++	++	++	++
Nutrient agar	+	+	+	++	++	++
Gelatin stab	++	++	++	++	++	++
Nitrate reaction	+	+	+	+	+	+
Starch agar	++	++	++	++	++	++
Richard's agar	-	-	-	-	-	-
PDA	-	-	-	+	+	+
Tyrosine reaction	-	+	+	++	++	++
Litmus milk	++	++	++	++	++	++

Key:- ++ = Agreement with Waksman's description
+ = Partial ,, ,, ,, ,,
- = No ,, ,, ,, ,,

No lesions developed from any inoculation of the three isolates with either method. The CMI culture did not produce lesions with Hooker's method but produced occasional small lesions with Lawrence's method. It appeared, therefore, to have largely lost its capacity to infect tubers and it, together with the three isolates were of no use as stock cultures for the programme of work.

Following this attempt to get pathogenic cultures, five pathogenic cultures were obtained which had been isolated and tested for pathogenicity by Dr.R.McKee of the John Innes Institute. These were designated 2.2, 2.5, 5.5, 5.9 and 5.13, isolate 2.2 being used in laboratory work and all five cultures being used in pot trials. The retention of pathogenicity of these stock cultures was confirmed 12 months later.

Liquid culture of Streptomyces scabies

The testing of fungicides against S.scabies has rarely been attempted in pure culture, and then only with agar media, no quantitative results being reported. The use of liquid culture allows quantitative assessments of toxicity so it is surprising that it does not seem to have been used in the past.

To test the rate and form of growth of this organism in liquid culture, 150 ml. conical flasks containing 25 ml. of Oxoid nutrient broth (pH 7.4) were inoculated with 1.0 ml. of a spore/hyphal fragment suspension of S.scabies grown on Czapek-Dox agar. Flasks were incubated at 25 and 35°C., three replicates per treatment. After 30 days, growth was negligable, a thin diffuse sedimentary mat being

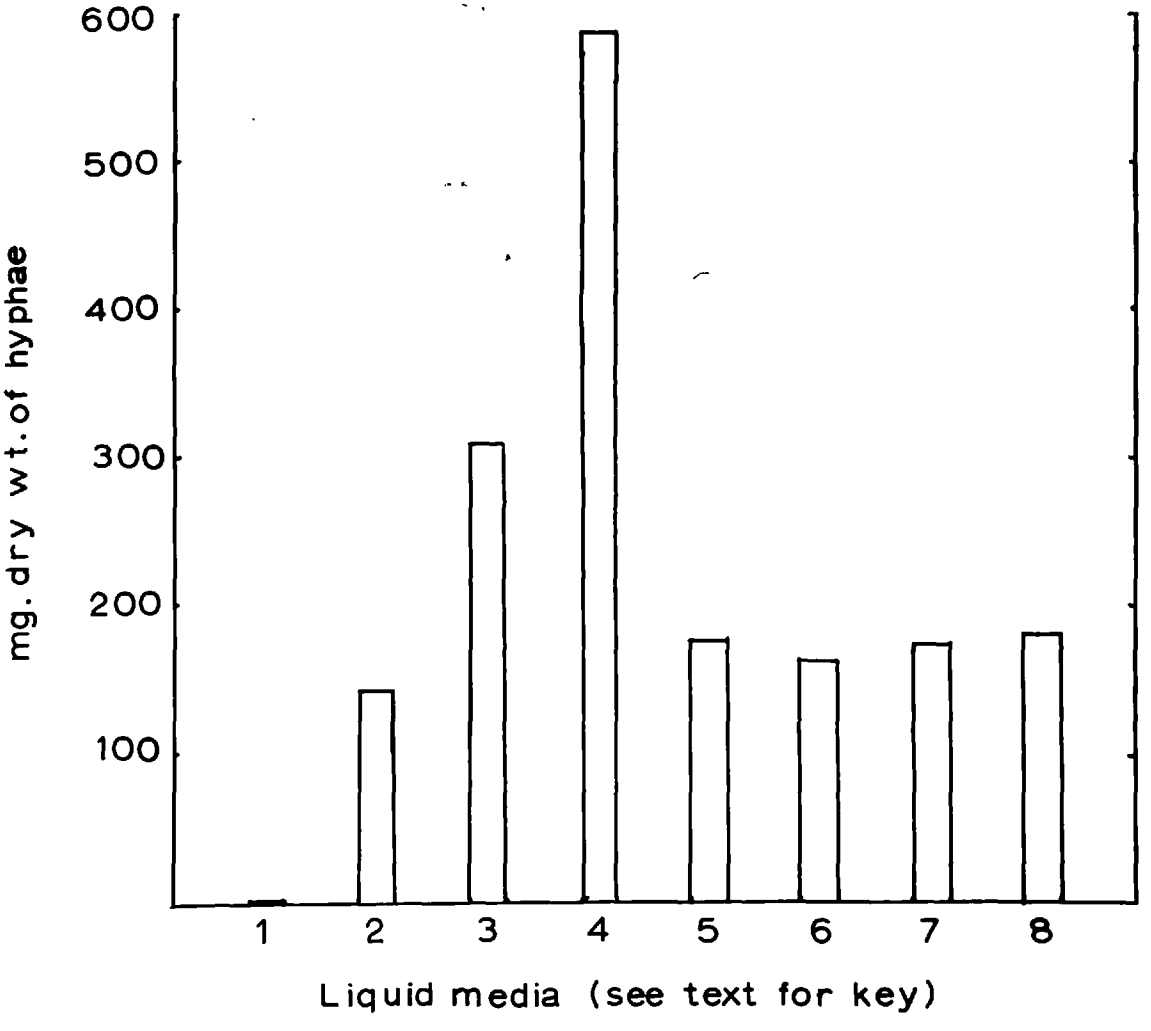
formed. Growth at 35°C. was only slightly better than at the lower temperature.

The slow growth rate precluded the use of standing liquid culture methods, and shake culture methods were then tried on the assumption that S.scabies, being a strong aerobe, would have a substantially higher growth rate under these conditions. Waksman (1959) recommended Czapek-Dox and Richards' media as suitable for general use with actinomycetes.

Richards' medium, Czapek-Dox medium with two modifications, and four forms of potato dextrose medium were used to determine their suitability as media for growth of S.scabies. 500 ml. conical flasks containing 100 ml. of medium were inoculated with a spore/hyphal fragment suspension of S.scabies and incubated on the rotary shaker for 7 days. Each treatment was replicated three times. The results are shown on Figure 3 with the following key:-

1. Richards' medium.
2. Czapek-Dox medium.
3. Czapek-Dox medium with 0.1 per cent. yeast extract.
4. Czapek-Dox medium with yeast extract and minor elements.
5. Potato dextrose agar made from peelings of Majestic tubers.
6. Potato dextrose agar made from peeled Majestic tubers.

Figure 13 Growth of S.scabies in various liquid media



7. Potato dextrose agar made from peelings of King Edward tubers.
8. Potato dextrose agar made from peeled King Edward tubers.

The King Edward tubers were used in addition to those of Majestic since they are more resistant to scab and it was of interest to see whether the resistance persisted in a sterilised extract. The peelings used were 2 mm. thick.

The results showed that Czapek-Dox medium modified by the addition of 1 g./litre of yeast extract and minor elements gave the best growth of S.scabies. There was very little difference in the growth of S.scabies in the various potato dextrose media. This would suggest that if there is any chemical basis for resistance to S.scabies, then this chemical resistance is rendered ineffective by crude extraction and autoclaving.

Following these tests, the following procedure was developed for liquid culture tests of the effect of chemicals on growth of S.scabies. 500 ml. conical flasks containing 100 ml. of the modified Czapek-Dox medium were used with three replicates per treatment. 10 ml. of an 8 day old culture of S.scabies in Czapek-Dox medium containing c.0.05 g. dry weight of mycelium were diluted with 20 ml. of water in an 8 by 1¼ inch boiling tube containing

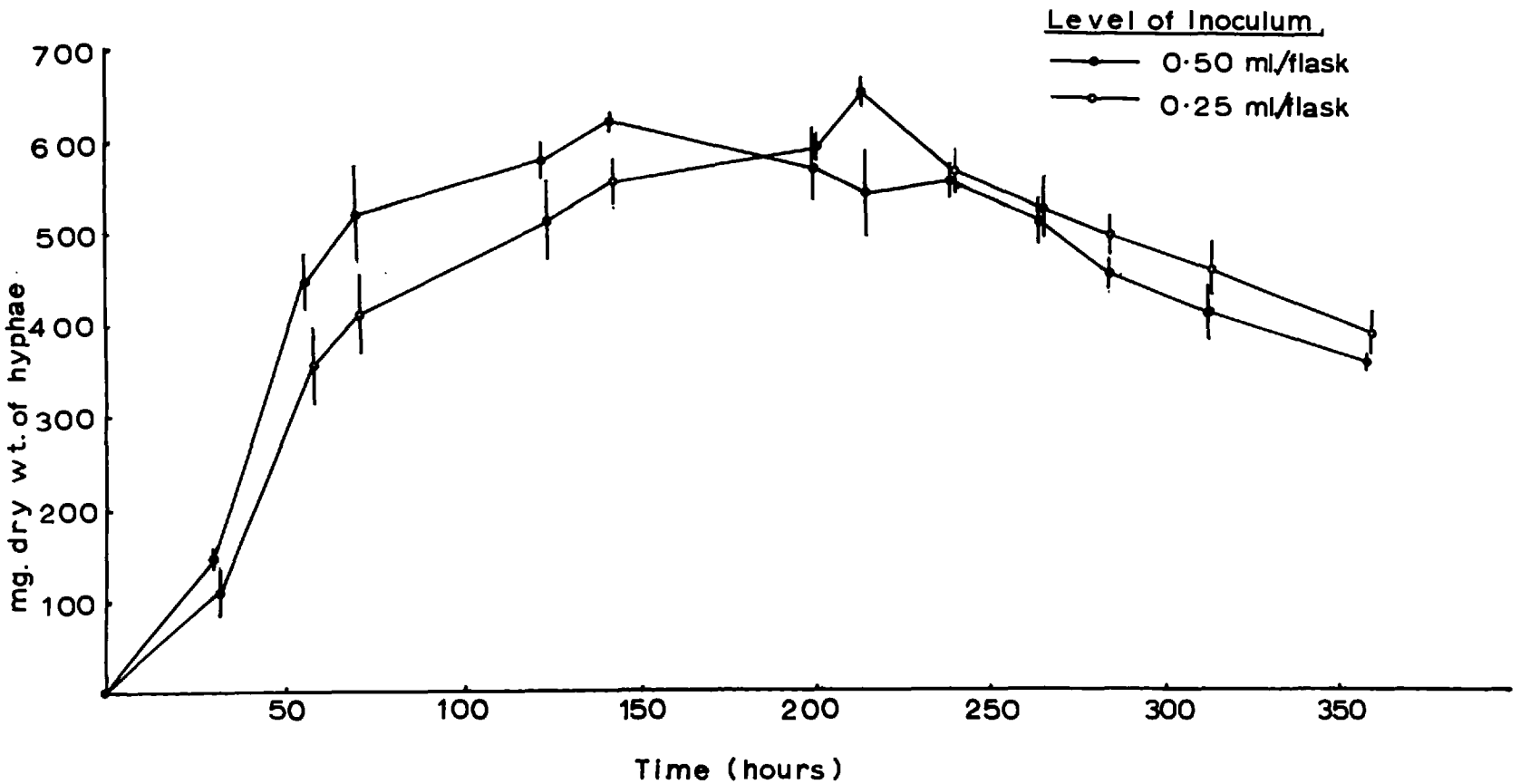
5 mm. ballatini to a depth of 1 inch and were shaken on a Griffin Flask Shaker for 30 minutes. This broke up and dispersed the colonies to give a spore/hyphal fragment suspension.

Flasks were then inoculated at the rate of 0.5 ml. of this suspension per flask and incubated on the rotary shaker at 200 rpm at 29°C. After incubation, cultures were filtered through muslin, dried in aluminium foil trays for 48 hours at 70°C. cooled in a desiccator and weighed.

A preliminary experiment established the growth pattern of the organism under these conditions. Since it is not easy to standardise inoculum between experiments, the effect of level of inoculum on subsequent growth was also measured. 36 flasks were inoculated at the rate of 0.5 ml. of a spore/hyphal fragment suspension per flask and 36 flasks at a rate of 0.25 ml. per flask. The flasks were then incubated on the rotary shaker and three flasks at each inoculum level were harvested approximately every 24 hours for the next 15 days.

The results, with standard deviations, are shown in Figure 4, and show that the lag phase of growth is very short. The level of inoculum did not greatly affect the rate of increase as expressed by dry weight measurements. It would seem that if cultures are harvested between 150

Figure 4 The growth of S.scabies in liquid culture



and 200 hours, then there would be little variation between untreated cultures in different experiments, even with differences in the amount of inoculum used.

The foregoing procedure was adopted for liquid culture work with S.scabies. In the work with minor elements, concentrations are expressed as parts per million. In some cases, probit transformations of data were made in order to determine levels of additives inhibiting growth of S.scabies by 50 per cent., details being given in Appendix 3.

Effect of manganese on growth of S.scabies

The evidence that toxicity of manganese to S.scabies is important in determining the incidence of common scab made it desirable to investigate the effect of various forms of manganese on the growth of S.scabies in liquid culture.

For the control of scab in the field, manganese has been applied as manganese sulphate. The only widely used manganese fungicide is maneb, manganous ethylenebisdithiocarbamate containing 16.5 per cent. manganese. Its use for the control of potato blight, caused by Phytophthora infestans, involves applications of sprays or dusts at rates

of 1½ or 2 lb./acre of active ingredient. Up to three applications per crop in a season could give some accumulation of maneb in the soil, and if it were toxic to S.scabies, some effect on scab incidence might result.

A liquid culture test was made with manganese applied as manganese sulphate over a 5 to 100 ppm range, and maneb (using Manzate, 80 per cent. active ingredient) over a range of 5 to 100 ppm of total maneb. The results are given in Figures 5 and 6 and show that in the case of maneb, there was some stimulation of growth at 5 and 10 ppm but complete inhibition at 100 ppm., although in terms of manganese in the maneb, the level inhibiting growth was 16 ppm. A probit transformation showed that growth was inhibited by 50 per cent. at 50.2 ppm of total maneb.

It would seem that concentrations of total maneb of over 50 ppm in the tuber forming zone of a potato crop would be needed before there was likely to be any effect on scab. Since maneb applications for blight control are only made after tuberisation when scab infection will have already started, it is unlikely that use of this fungicide has any effect on scab incidence.

The effect of soluble manganese, causing a 50 per cent. inhibition of growth at 38.0 ppm is less marked than would be expected following Mortvedt's report (1961) of control

Figure 5 The effect of Maneb on growth of S.scabies

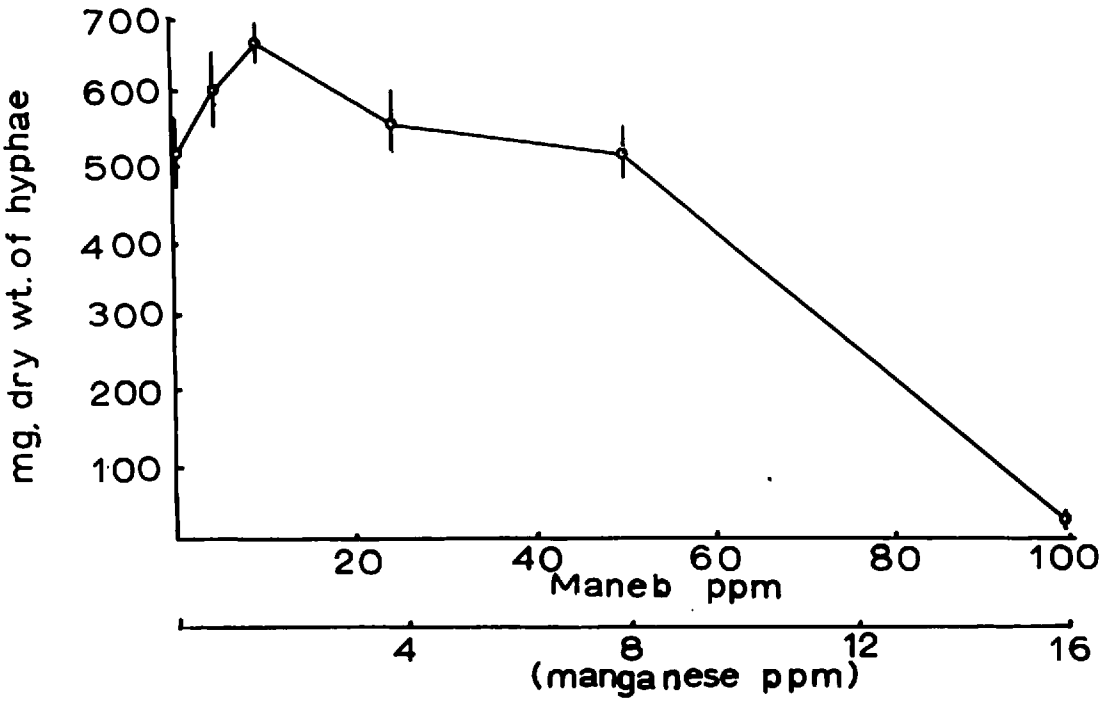
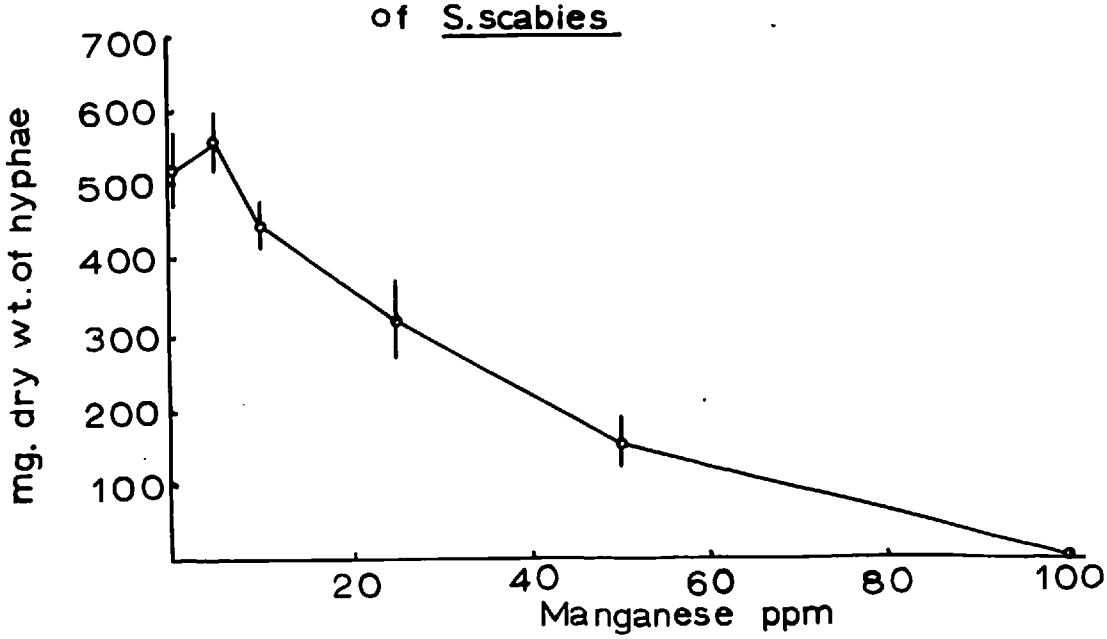


Figure 6 The effect of Manganese on growth of S.scabies



of scab at 20 ppm of soluble manganese added to soil in pot culture of potatoes.

A major difficulty in attempting to control scab with soluble manganese is that many scab infested soils are alkaline and soluble manganese is quickly oxidised to unavailable and, therefore non-toxic forms. A number of forms of manganese were therefore tested for their effect on S.scabies in the hope that a form could be found which would not be subject to oxidation in the soil.

1. Rayplex manganese

This is one of a number of metal complexes which have recently been marketed as being effective for curing trace element defficiencies. It is described as being modified polyflavonoid copolymers derived from a selective chemical extraction of hemlock bark. The total manganese in the complex is 9.6 per cent. and a solution was made up containing 1.042 g./litre of Rayplex Managanese which contained 1,000 ppm of manganese. This was used to test the effect of Rayplex Manganese on S.scabies in liquid culture at concentrations of 10 to 100 ppm of manganese in complex form.

The results are given in Figure 7 and show that

Rayplex Manganese did not inhibit growth of S.scabies over this range. There was actually a slight increase in growth.

2. Manganese frit

A rather different approach to the problem of adding manganese to soil in a form toxic to S.scabies would be the use of a manganese glass frit. Metal-containing glass frits have been used successfully in the treatment of soil for rectifying trace element deficiencies (Wynd, 1951), and zinc frit has been used to control crook root disease of watercress caused by Spongospora subterranea (Wallr.) Lagerh. f.sp.Nasturtii Tomlinson (Tomlinson, 1958). A glass frit containing 40 per cent. manganese as manganese dioxide was obtained from Ferro Enamel Limited and the following experiment was done to determine if manganese from the frit was released in culture solution in quantities sufficient to inhibit growth of S.scabies. Five concentrations of frit were used in three series. In the first, the frit was added immediately before inoculation, and in the other two series it was added to the culture solution and shaken for three and twelve days before inoculation.

The results are given in Figure 8 and show that there was no marked effect of the frit on the growth of S.scabies,

Figure 7 The effect of Rayplex manganese on growth of S.scabies

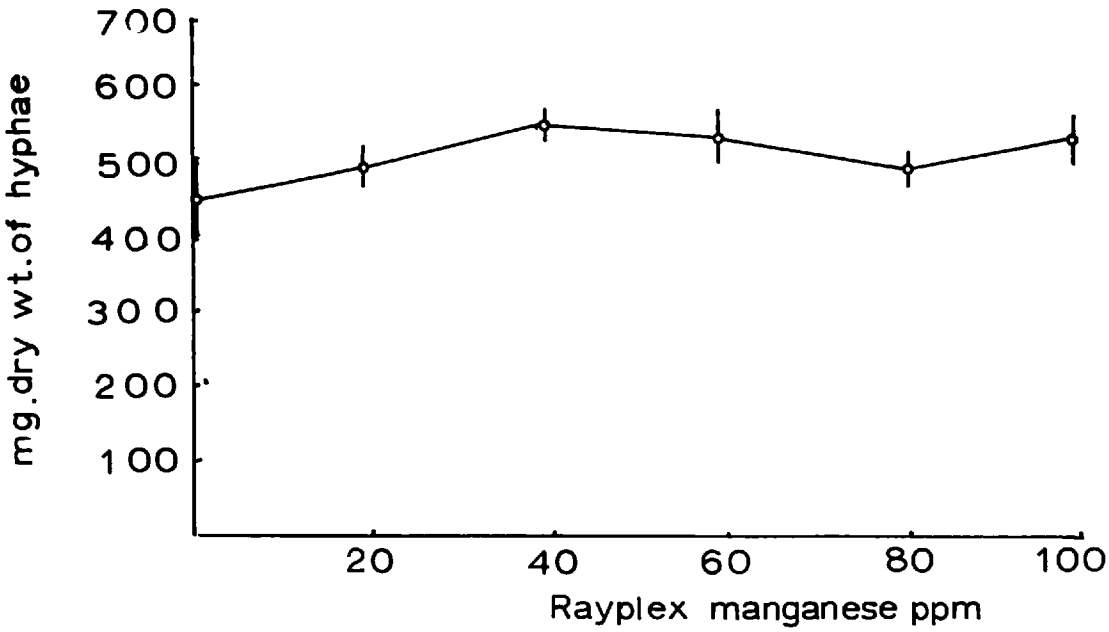
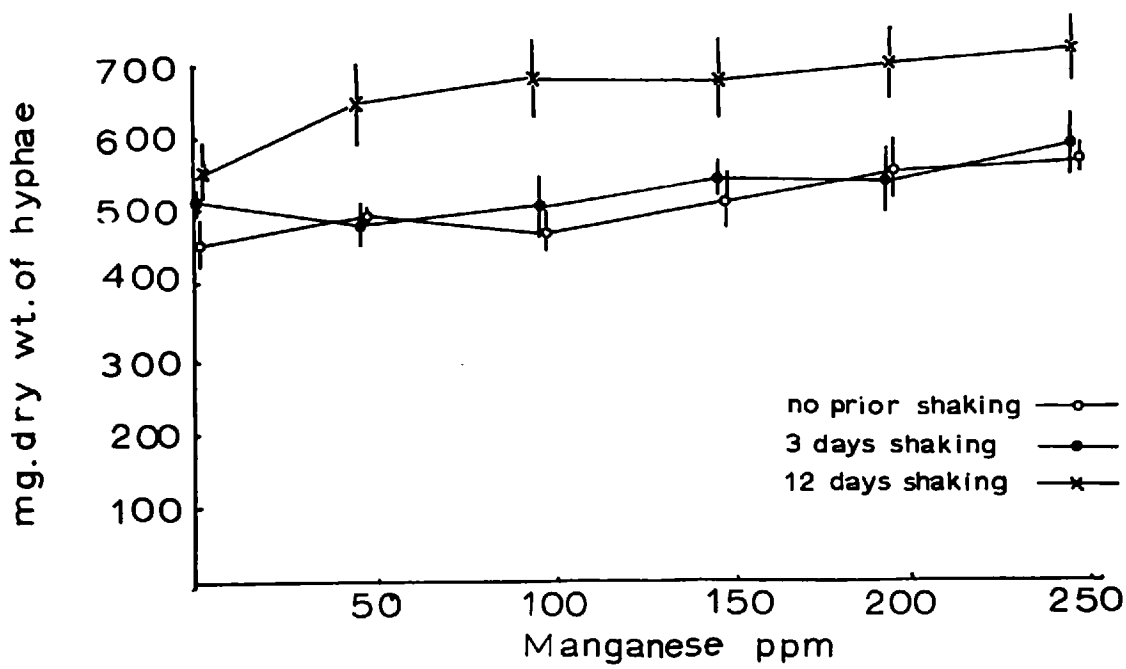


Figure 8 The effect of Manganese frit on growth of S.scabies



although there was some stimulation of growth when the frit was added to the culture medium 12 days before inoculation. No figures are available for the release of manganese from a frit, but a 750 ppm suspension of a zinc frit containing 23 per cent. zinc released 0.21 ppm of zinc when shaken for 24 hours and released 3.68 ppm of zinc when there was carbon dioxide present in the solution at 16 ppm. (Tomlinson, 1958).

If the rate of release of manganese from a frit is similar to this then concentrations of up to 250 ppm of manganese in the form of a frit would release at most 4 ppm of manganese after 12 days shaking. Since 5 ppm of soluble manganese was shown to stimulate growth of S.scabies (see Figure 6), this might account for the slight stimulation of growth which occurred after the manganese frit had been shaken for 12 days.

The liquid culture tests showed that the frit did not inhibit growth of S.scabies in liquid culture. The rate of release of manganese from the frit would therefore have to be much higher in soil than in liquid culture medium for the frit to have any effect on scab.

3. Manganese chelate complexes

A third form of manganese to be investigated was

chelated manganese, since it was hoped that manganese chelate complexes might be sufficiently stable to prevent oxidation of the manganese in the soil.

The ferric chelate with EDTA (ethylene diamine tetra-acetic acid) is a particularly stable metal chelate complex and as "sequestrene iron" is widely used for correcting iron deficiencies in soils. No other metal/EDTA complex is in general use, there being a tendency for displacement of the metal cation to occur, the extent of this displacement being dependent on the stability of the metal concerned.

Table 6 shows the stability constants of a number of metals (Geigy, 1959) and this shows that manganese has a relatively low stability constant. For this reason it is not normally used for correction of trace element deficiencies. The use of chelated manganese for control of scab would depend, at least, on a) its being toxic to S.scabies, and b) its remaining stable in the soil sufficiently long to affect populations of the scab organism.

The effect of chelated manganese, $Na_2MnEDTA$, on growth of S.scabies in liquid culture was tested at a range of concentrations of 5, 10, 25, 50 and 100 ppm. Growth was inhibited at 50 ppm and very much reduced at 10 ppm, and a second test was made using a lower range of concentrations up to 25 ppm. The results, which are shown in

Table 6. Stability constants of some metals

Divalent metals		Trivalent metals	
Metal	Stability constant (log K2)	Metal	Stability constant (log K2)
Ba	7.99	Ce	15.98
Sr	8.63	Al	16.31
Mg	8.69	Ga	20.27
Ca	10.59	Fe	25.10
Mn	14.00		
Fe	14.45		
Co	16.10		
Cd	16.47		
Zn	16.90		
Pb	18.40		
Ni	18.62		
Cu	18.80		
Hg	21.80		

Figure 9, showed that manganese in chelated form was far more toxic to S.scabies than manganese applied as a soluble salt, growth being inhibited by 50 per cent. at 7.8 ppm.

There was a possibility that the chelate itself was

Figure 9. The effect of Na_2Mn EDTA on growth of S.scabies

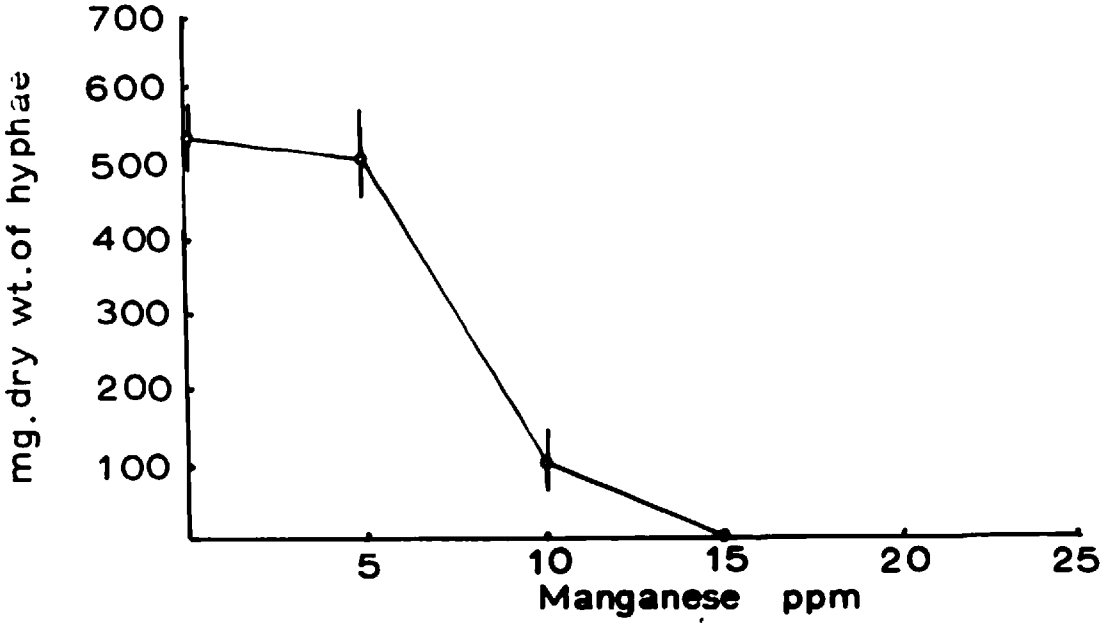
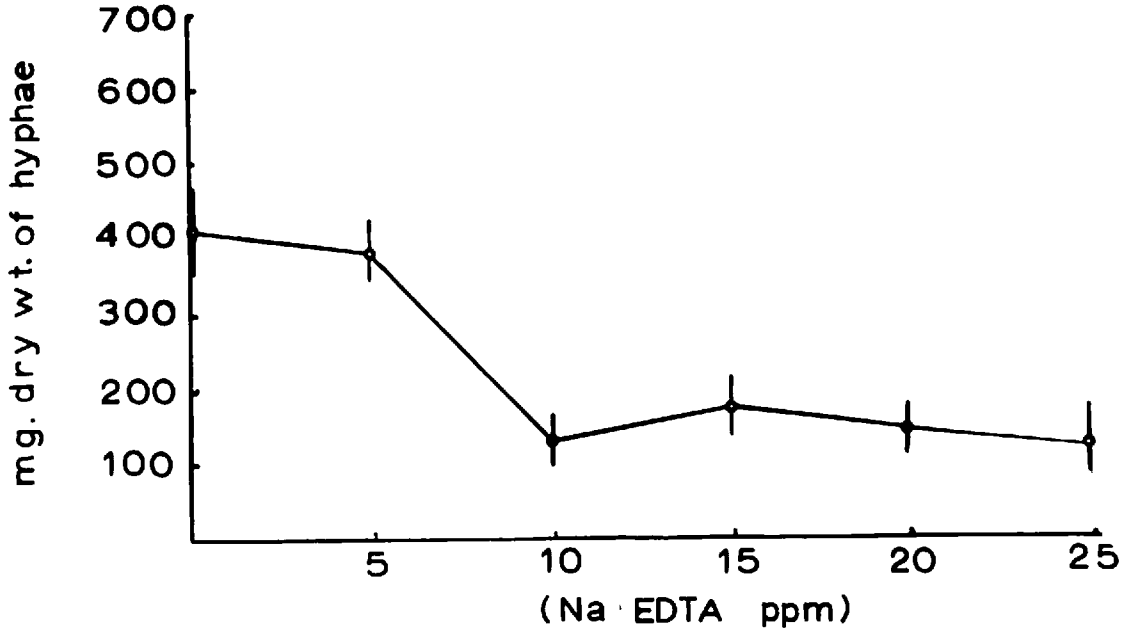


Figure 10. The effect of Na EDTA on growth of S.scabies

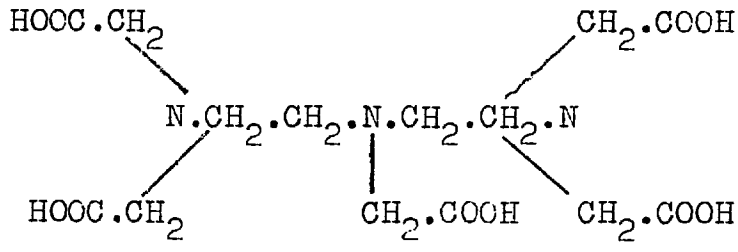


toxic to S.scabies and the chelating agent was therefore tested on its own with the results given in Figure 10. The concentrations of chelate given in Figure 10 are in terms of the concentrations of chelating agent required to chelate 5, 10, 15, 20 and 25 ppm of manganese and the results are therefore directly comparable to those obtained using the chelated manganese.

The chelate on its own reduced growth at 9.3 ppm by 50 per cent. but this degree of inhibition did not increase greatly with the increase in chelate concentration up to 25 ppm. It is possible that the chelate is not directly toxic to S.scabies at these concentrations, but reduces growth by chelating with micronutrients in the culture medium, such as iron. Such complexes may be less readily available to S.scabies and the poor growth at 10 to 25 ppm of the chelate could be due to a deficiency in micronutrients.

A second chelating agent was used, the manganese complex of which, unlike $Na_2MnEDTA$, is not fixed by clay. This is DTPA (Di-ethylene triamine penta acetic acid). It forms rather more stable chelate complexes with metals than does EDTA, log K2 of the ferric chelate being 27.8 compared with 25.1 for the ferric chelate of EDTA. The

structure of DTPA is:-



A 30 per cent. w/w solution of the pentasodium salt was used to make up a solution of the Na_2MnDTPA complex. This was tested for its effect on S.scabies in liquid culture over a 5 to 25 ppm range. The results are given in Figure 11 and show that this complex was rather more toxic than Na_2MnEDTA to S.scabies (50 per cent. inhibition at 3.8 ppm). The effect of DTPA alone on S.scabies was then established and this gave rather different results to those obtained using EDTA, the results being shown in Figure 12. The yields in this test were low because of early harvesting due to a mechanical fault in the rotary shaker. 13.8 ppm of DTPA inhibited growth by 50 per cent. and this increased at 15 and 20 ppm to complete inhibition at 25 ppm, suggesting direct toxicity of DTPA to S.scabies.

Results of the liquid culture tests of manganese compounds suggested that Na_2MnEDTA and Na_2MnDTPA both showed promise for control of scab. Since Na_2MnEDTA was readily

Figure 11. The effect of $Na_2MnDTPA$ on growth of S. scabies

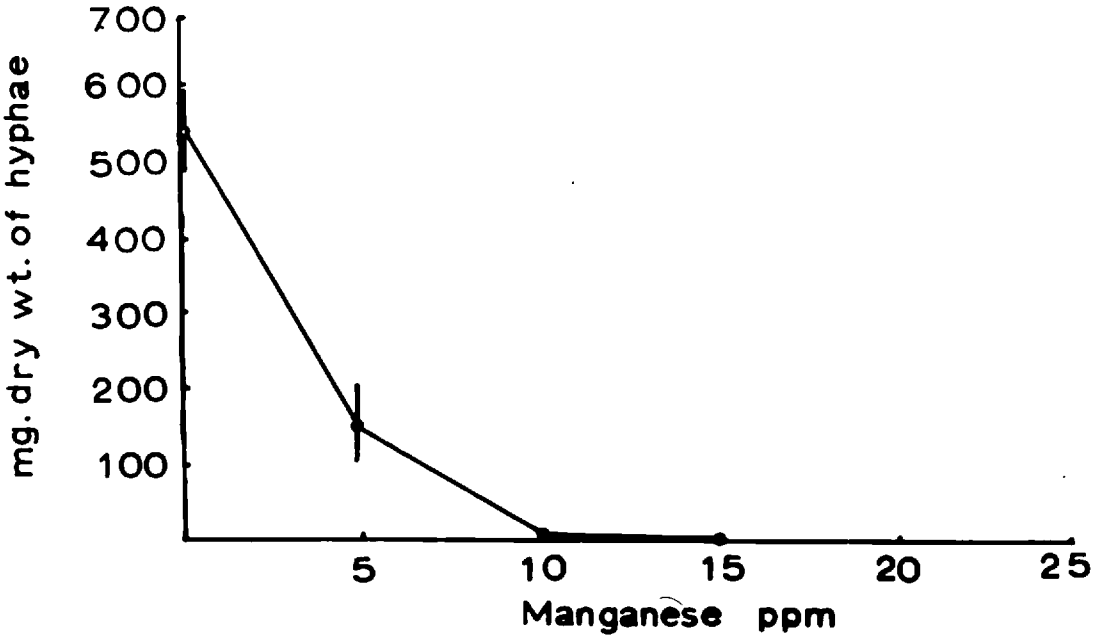
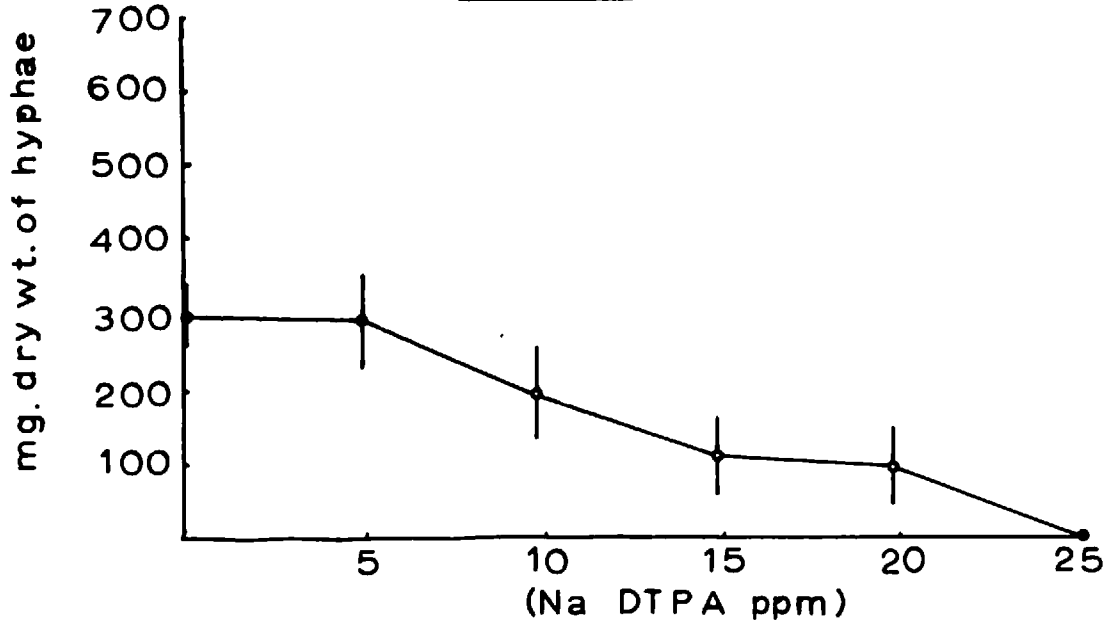


Figure 12. The effect of Na_5 DTPA on growth of S. scabies



available as a complex in powdered form whereas $Na_2MnDTPA$ was not, the former was used primarily in subsequent pot and field experiments.

Greenhouse pot trials

The liquid culture assessment was intended to be an initial screening test for minor elements of possible use in scab control, and pot culture was intended to provide an intermediate test before embarking on field trials. A series of pot trials was therefore set up to test a range of compounds containing manganese for their effect on scab.

The three soil conditions which tend to favour the occurrence of scab are high pH, a sandy soil and a low soil moisture content. All these were taken into account when setting up the pot trials. Thus the low soil moisture content was maintained by growing the potatoes under glass with minimal watering. Good ventilation was necessary to prevent heat damage to the potato plants but the temperature was generally above the outside temperature and this would also tend to favour scab (Dipennaar, 1933). The other two conditions, high pH and a sandy soil were provided by using a sandy limed compost of the following composition:-

5 bushels	-	light loam
2 bushels	-	sieved Irish peat
3 bushels	-	washed sand
6 pints	-	lime
1½ pints	-	fertiliser

pH = 7.0 to 7.5

The fertiliser was Fison's "Growmore" with the following composition:

Nitrogen (N)	7 per cent.
Phosphoric acid (P_2O_5)	
water soluble	6 per cent.
insoluble	1 per cent.
Potash (K_2O)	7 per cent.

Potatoes were grown in 50 by 45 cm. 200 gauge polythene bags, each having ten 7 mm. diameter drainage holes at the base. A bag filled to a depth of 30 cm. contained 20 kg. of compost and one "mix" of compost provided sufficient compost for 14 bags.

Two methods of inoculating the compost with S.scabies were used.

a) This followed the method of McKee (1963). Five lots of each of the five stock cultures were grown for 14 days in 10 oz. medical flasks containing 30 ml. of PDA which had

been allowed to set along one side of each flat. The surface mat of S.scabies in each flat was transferred into 50 ml. of sterile water and the 25 lots of 50 ml. spore/hyphal fragment suspensions were mixed together and made up to 1,500 ml.

This suspension was added to vermiculite at the rate of 50 ml. of suspension per 250 cc. volume of vermiculite, each 250 cc. volume having previously been moistened with 75 ml. of water. Each 250 cc. volume of vermiculite thus inoculated, provided the inoculum for one bag of compost (20 kg.).

b) A similar general method was used but instead of cultures being grown on PDA in medical flats, 6 day old liquid cultures, grown in Czapek-Dox solution, were used, the final rate of application being the spore/hyphal suspension from one 100 ml. culture per bag of compost.

Majestic Grade "A" seed was chitted for 30 days at 20°C. before planting. Chemicals and inoculum were mixed by hand with the compost before planting seed pieces 25 cm. deep. Each treatment was replicated five times. Plants were watered sparingly during the summer to keep soil moisture low but care was needed to avoid wilting. The greenhouses were fumigated with "Murfume" every 3 to 4 weeks and plants

were occasionally sprayed with a rogor/DDT systemic insecticide, both measures being intended to control aphids which tended to build up in numbers very quickly. Tubers were harvested in mid-October when the haulms had died down. Scab incidence was estimated visually using the key of Large and Honey (1955). Manganese sulphate and $Na_2MnEDTA$ were applied at concentrations from 5 to 100 ppm of manganese. In addition maneb was applied at the rate of 50 ppm total maneb, and manganese sulphate and $Na_2MnEDTA$ were applied as soil soaks in 500 ml. of solution per bag of compost, the solutions containing sufficient manganese to give final concentrations in the compost of 25 and 50 ppm respectively. Finally, manganese sulphate was applied as a foliar spray one month after planting at a total rate equivalent to 10 ppm of manganese in the compost, including run-off from sprayed foliage.

The results are given in Table 7. Adopting the definition of a "substantially affected" crop in use for surface diseases of tubers in administering a Domestic Plant Health Order, Large and Honey considered batches of tubers with a scab rating over 2.0 to be in this class, but with common scab, they considered this to be a low rating and an "unmarketable crop" was considered to be one scoring 6.0 or more.

Table 7. Pot trials with manganese compounds for the control of common scab

Compound	Form of inoculum	Concentration of manganese (ppm)	Scab score
$MnSO_4$	a) Cultures grown on PDA	0	1.1
		5	1.3
		10	1.0
		25	1.1
		50	1.1
		100	1.2
$Na_2MnEDTA$	b) Liquid culture	0	2.4
		5	1.2
		10	1.1
		25	1.1
		50	1.0
		100	1.1
Maneb	b)	0	1.1
		50	1.1
$MnSO_4$	b)	0	1.6
		10 (foliar spray)	1.4
		25 (soil soak)	1.2

Table 7 (contd.)

$Na_2MnEDTA$	b)	0	1.0
		50 (soil soak)	1.0
Untreated and uninoculated soil			1.0

On this basis, the incidence of scab in these trials was not high enough to give any indication of the effect of the treatments on the disease.

There are two possible explanations for the low level of scab incidence. The first is that the cultures used were no longer pathogenic. This was shown to be untrue by a pathogenicity test carried out on the stock cultures three months after the end of the pot trials. Plants were grown in the greenhouse at South Kensington in compost which had been heavily inoculated with the stock cultures. Tubers harvested three months later were heavily infected with scab. The second possibility is that the level of inoculum was too low. This is more likely although the inoculum level used was similar to that used successfully by McKee (1963).

Field Trials for the control of common scab - 1966

Although the pot trials did not give conclusive results for the effect of various manganese compounds on scab incidence, the initial liquid culture tests had suggested that chelated manganese might be of value in scab control.

There is a difficulty in undertaking field trials on common scab in that the occurrence of the disease is not predictable. To get over this difficulty, three trials were set up in the South of England, two of which tested the effect of $Na_2MnEDTA$ on scab incidence, and the third tested its effect on yield as well. In addition, two trials were set up in Scotland by Mr.D.Watson of the North of Scotland College of Agriculture, and these are described in Appendix 2.

South of England Trials

1. Silwood

This was at Silwood Park, Ascot, Berkshire on a sandy soil which had been fallow the previous year. It was designed primarily to test the effect of $Na_2MnEDTA$ on yield although the site chosen had a previous history of scab.

A randomised block design of six blocks of four plots was used. Each plot measured 34 feet by 10 feet and

contained four 28 inch rows of thirty setts, 13 inches apart, with marker plants at either end. The outer two rows of each plot were guard rows. Majestic Grade "A" scab-free tubers were used as seed and Arran Victory Grade "A", producing a purple tuber, was used as a marker plant.

Seed was chitted in a greenhouse at the Chelsea Physic Garden for 7 weeks in 3 feet by 2 feet chitting trays, each holding about 40 lb. of seed placed rose end uppermost. A 2½ Kw. fan heater was set at 38°C. to prevent frost damage; it also circulated the air. The trays were moved around so that all seed was periodically exposed to daylight. 7 cwt. of Majestic and ½ cwt. of Arran Victory were chitted, sufficient for this and the other two trials.

The chelated manganese was applied at the rates shown in Table 8. The chelate complex was spread in 6 inch bands

Table 8. Application rates of $Na_2MnEDTA$

Treatment	Wt./plot	Equivalent wt./acre
A	0	0
B	136 g.	35 lb.
C	272 g.	70 lb.
D	545 g.	140 lb.

along the line of each row and then mixed with the soil with a front mounted rotor tiller. The trial was laid out in early April, the crop being planted on April 26th and 27th. During the summer the crop was assessed for emergence (after 5 weeks), weeded, ridged, and sprayed against blight with maneb at 1½ lb./acre from the middle of July. Heavy rain at the time of planting resulted in partial waterlogging of the crop which gave rise to poor emergence. This is shown in Table 9 which includes a plan of the trial.

Table 9. Percentage emergence after 5 weeks

	5				6		
D	C	B	A	C	B	A	D
25	30	25	20	15	10	50	90
	3				4		
B	A	C	D	C	A	D	B
75	85	95	95	95	80	95	90
	1				2		
C	B	A	D	B	D	A	C
90	95	95	90	95	95	95	95

The mean emergence per treatment was:-

A = 71 per cent.

B = 65 per cent.

C = 70 per cent.

D = 82 per cent.

An angular transformation of the data was made (Appendix 1) and an analysis of variance of the transformed data showed that there were no significant differences between treatments, although differences between blocks were significant at the 0.1 per cent. level.

There was a very low level of emergence in blocks 5 and 6 and blocks 3 and 4 were also affected to some extent. This, combined with moderate blight infection in August, in spite of protective spraying, and magnesium deficiency in the crop, rendered the experiment useless as a yield trial.

Because of the possibility of there being natural scab infection, 3 yard sample lengths were lifted at random from each row of blocks 1 to 4 in early September when the haulms had died back. There was no scab present above a trace and the tubers were not assessed.

Although the experiment gave no results for the effect of $Na_2MnEDTA$ on yield, it had no significant effect on emergence.

2. Binstead

This trial was undertaken at West Court Farm, Binstead,

Hampshire, on a field with a previous history of scab, the aim being to test the effect of $Na_2MnEDTA$ on scab incidence. The trial area measured 84 feet by 9 feet 4 inches, forming part of a potato field. The soil was a light sandy loam, pH 6.5, overlying upper greensand.

Each plot consisted of 10 plants with marker plants at each end, and the trial contained six blocks of four randomised plots. Row width was 28 inches and seed and chitting methods were the same as for the Silwood trial. Soil treatment and planting were done when the rest of the field was planted and the application rates are given in Table 10. Each application was spread in a 6 inch band

Table 10. Application rates of $Na_2MnEDTA$ at Binstead

Treatment	Weight/plot	Equivalent wt./acre
A	0	0
B	11.3 g.	25 lb.
C	22.7 g.	70 lb.
D	45.4 g.	140 lb.

along the line of each row and dug in well with a fork to a depth of 4 inches. Seed was planted 6 inches deep and the rows were then ridged by hand, this being done on April

13th. Post planting cultivation was carried out by the farmer as part of the field as a whole. Emergence was assessed five weeks after planting and appearance of blackleg and other diseases noted during the summer. The crop was harvested when all the haulms had died down in early October. Tubers under 2.5 cm. in length (rose/heel) were discarded and the rest were washed, weighed and assessed for scab. The results are given in Table 11.

Table 11. Effect of $Na_2MnEDTA$ on scab incidence and yield

Treatment	Mean scab score	Mean yield g./plot	Number of tubers per plot	Average tuber wt. (g.)
A	1.09	3,916	56.7	68.7
B	1.17	5,308	59.0	91.1
C	1.20	3,355	38.3	87.2
D	1.26	5,620	59.2	90.3

Emergence was approximately 98 per cent. after 5 weeks with no difference between treatments. During the summer, 2 per cent. of the crop succumbed to potato blackleg.

An analysis of variance was done on yield (Appendix 1) which showed no significant difference between treatments but significant differences between blocks at the 20 per

cent. level. Scab incidence was too low for any conclusions to be drawn on the effect of $Na_2MnEDTA$ on the disease.

3. Crowthorne

A third trial was done at a smallholding at Crowthorne, Berkshire, on a sandy soil of pH 6.5. The land had had a history of severe scab infection when susceptible Majestic potatoes had been grown four years previously, and more recently, even scab-resistant King Edward tubers had been markedly affected. The methods used in this trial were similar to those used at Binstead but the blocks were half of the previous size due to shortage of space and only one application of $Na_2MnEDTA$ was used: 45.4 g./plot, equivalent to 140 lb./acre.

The crop was planted on April 18th and harvested on September 12th when scab was assessed as before. Emergence five weeks after planting was about 98 per cent. with no differences between treatments, but after 10 weeks, about 10 per cent. of the crop had been affected by potato root eelworm. The results of the trial are given in Table 12.

There was heavy scab infection on this site. An analysis of variance was carried out on the results for mean scab score and mean yield. This showed that the

Table 12. Effect of $Na_2MnEDTA$ on scab incidence and yield

Treatment	Mean scab score	Mean yield g./plot	Number of tubers per plot	Average tuber weight
Untreated	10.75	3,143	77.0	41.5
$Na_2MnEDTA$	11.57	2,540	69.8	36.7

chelate slightly increased scab and decreased yield, but these effects were not significant at the 20 per cent. level.

Field Trial - Crowthorne 1967.

A trial was set up on the same smallholding at Crowthorne that was used in 1966, the aim being to test the effect of manganese frit on scab incidence. The rate used was 50 lb./acre and the treatment was included in a trial, the principle function of which was to determine the effect of dried grass meal on soil manganese, soil microflora and scab incidence. The details of the trial are described on page and the results for the manganese frit treatment are given in Table 13. An analysis of variance was done on the results and showed that the mean scab score and average tuber weight were not significantly affected by the

Table 13. Effect of Manganese frit on scab incidence and yield

Treatment	Mean scab score	Mean yield g./plot	Number of tubers per plot	Average tuber weight
Untreated	17.18	2,624	65.0	40.70
Mn frit	17.48	3,560	80.3	43.75

manganese frit treatment but that there was an increase in yield significant at the 20 per cent. level, and in average tuber weight significant at the 5 per cent. level.

Table 14 summarises results of the field trials for control of scab using manganese compounds.

Effect of other minor elements on growth of S.scabies in liquid culture.

Manganese is not the only element to be tested for the control of scab and the results of other workers are summarised in Table 15. The wide range of rates of applications used by the various authors makes it difficult to determine the relative effectiveness of these metals. The liquid culture method used to assess the effect of various

Table 14. Summary of field trials on common scab.

Site	Treatment	Rate (lb/acre)	Result
Scotland:			
Linkwood	$MnSO_4$	56	No control
	$Na_2MnEDTA$	70	No control
	Mn frit	25	Reduction in scab but not statistically significant.
Sanquhar Mains	$MnSO_4$	56	No scab
	$Na_2MnEDTA$	70	
	Mn frit	25	
England			
Binstead	$Na_2MnEDTA$	35	No scab and no effect on yield
		70	
		140	
Crowthorne	$Na_2MnEDTA$	140	No control and no effect on yield
Silwood	$Na_2MnEDTA$	30	No scab
		70	
		140	
Crowthorne (1967)	Mn frit	50	No control but significant yield increase

Table 15. Effect of minor elements on growth of S.scabies and control of scab

Source/test	Compound	Rate	Effect
Mader 1937 Field trial	$CuSO_4$	75 lb/ acre	Marked reduction in scab
Houghland 1956 Solid media	Organic Al	100 ppm	Growth inhibited
	$AlCl_3$	160 ppm	Growth inhibited
Houghland 1956 Field trial	$Al_2(SO_4)_3$	800 lb/ acre	No control of scab
Guntz 1957 Field trial	$Na_2B_4O_7$	15 Kg/Ha	1.4 } Increase in %
	$Co(NO_3)_2$	5 ,,	0 } marketable
	$(NH_4)_6Mo_7O_{24}$	2.2 ,,	2.7 } tubers
	$ZnSO_4$	100 ,,	3.4 } (control = 1.6)
Turner 1957 Field trial	Various Zn compounds	12.5 } lb/ 24.0 } acre	No effect on scab
Malenev 1959 Seed dips	Soluble Co salts: B	0.02 % solution	2 - 3 times increse in resistance
Mygind 1961 Field trial	$Al_2(S)_4)_3$	Effective levels	but not at economic
Mortvedt 1961 Pot trial	$CuSO_4$	20 } lb/ 50 } acre	Decrease in scab but with some phytotoxicity

Table 16. Effect of metals on growth of S.scabies in liquid culture

Element	Salt	Effect on S.scabies
As	As_2O_3	No growth
Sb	$Sb_2(SO_4)_3$	Very little growth
Al	$Al_2(SO_4)_3 \cdot 18H_2O$	Slight decrease in growth
Ba	$Ba(C_2H_3O_2)_2 \cdot 2H_2O$	No effect
B	H_3BO_3	No effect
Cd	$(CdCl_2)_2 \cdot 2H_2O$	No growth
Ca	$CaCl_2 \cdot 2H_2O$	No effect
Cr	$Cr_2(SO_4)_3 \cdot 15H_2O$	No effect
Co	$CoSO_4 \cdot 7H_2O$	No growth
Cu	$CuSO_4 \cdot 5H_2O$	No growth
Pb	$PbNO_3$	Slight decrease in growth
Mo	H_2MoO_4	No effect
Ni	$NiSO_4 \cdot 7H_2O$	Slight decrease in growth
Ag	$AgNO_3$	No growth
Sr	$SrCL_2 \cdot 6H_2O$	No effect
Sn	$SnCl_2 \cdot 6H_2O$	Slight decrease in growth
Zn	$ZnSO_4 \cdot 7H_2O$	Slight decrease in growth
Hg	$HgCl_2$	No growth
Mg	$MgSO_4 \cdot 3H_2O$	No effect
K	KCl	No effect

manganese compounds on growth of S.scabies was therefore used to give a quantitative assessment of the effect of these and a number of other metals. Soluble salts of all the metals were tested initially at a concentration of 50 ppm of the metal using the usual methods. The results are given in Table 16 (page 81).

Of the metals tested, barium, boron, calcium, chromium, molybdenum, strontium, magnesium and potassium had no effect on the growth of S.scabies at 50 ppm. Of these, mention may be made of magnesium and potassium since these were already present in the culture medium at levels of 48 and 484 ppm respectively. No effect at the increased levels of 98 and 534 ppm would be expected and they were only tested in view of the fact that they had been the subject of previous work. The large body of literature on the effect of calcium and potassium on scab has already been reviewed (page) and shows some variation of opinion as to whether these two elements have any effect on scab. The fact that neither affects growth of S.scabies in liquid culture at these levels suggests that if they do have any effect on scab incidence, then it is not by any direct effect on S.scabies.

Aluminium, lead, nickel, tin and zinc all inhibited growth of S.scabies slightly at 50 ppm but as the effect

was not marked, no further tests were done on these metals since they were unlikely to be of use in scab control.

The remaining metals, arsenic, antimony, cadmium, cobalt, copper, silver and mercury were all tested at lower concentrations in order to get a more accurate idea of their effect on S.scabies. The results are given in Figures 13 to 19. These show that arsenic and silver had similar effects on S.scabies. There was marked inhibition of growth at 10 ppm but some growth persisted up to 25 ppm. Arsenic inhibited growth by 50 per cent. at 6.3 ppm and silver at 7.8 ppm. With antimony there was no effect at 10 ppm but 50 per cent. inhibition at 15.2 ppm. Mercury had a similar effect except that there was slight growth stimulation at 10 ppm with 50 per cent. inhibition at 16.0 ppm. Cobalt was rather less toxic than these four since in this case, growth was decreased by half at 29.5 ppm.

Copper, on the other hand, was rather more toxic with 50 per cent. inhibition at 6.6 ppm. There was a similar effect to that of manganese in that there was some stimulation at low concentrations (2 and 4 ppm in the case of copper) followed by inhibition which increased with increase in concentration. Cadmium had a very similar effect on S.scabies to that of copper, except that in this case, the

Figure 13 Effect of Arsenic on growth of S.scabies

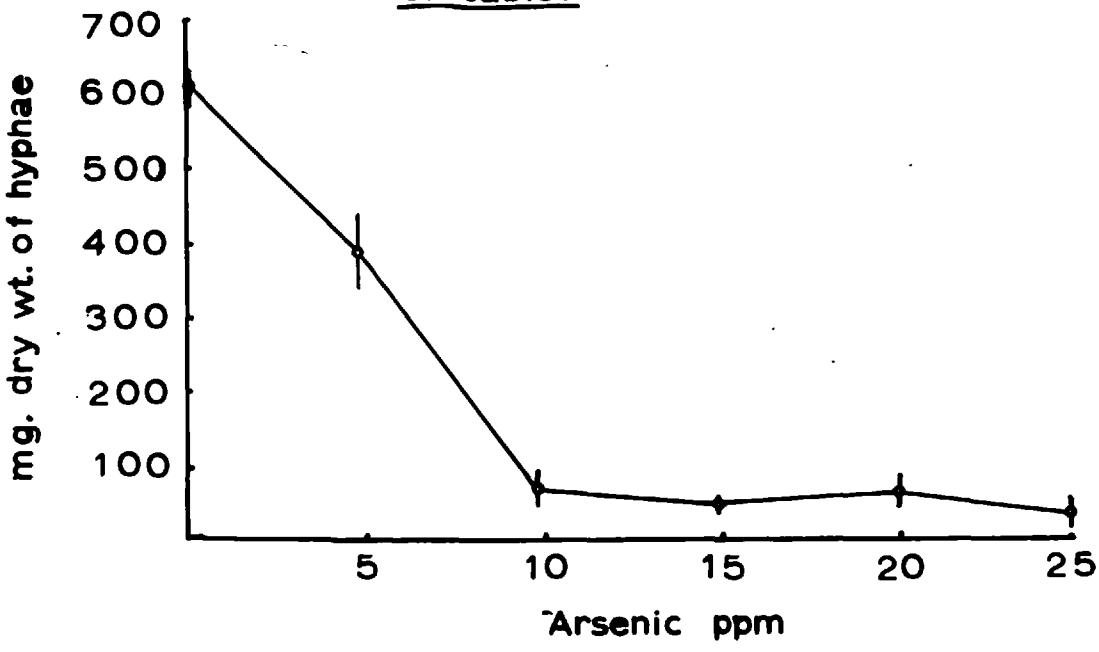


Figure 14 Effect of Antimony on growth of S scabies

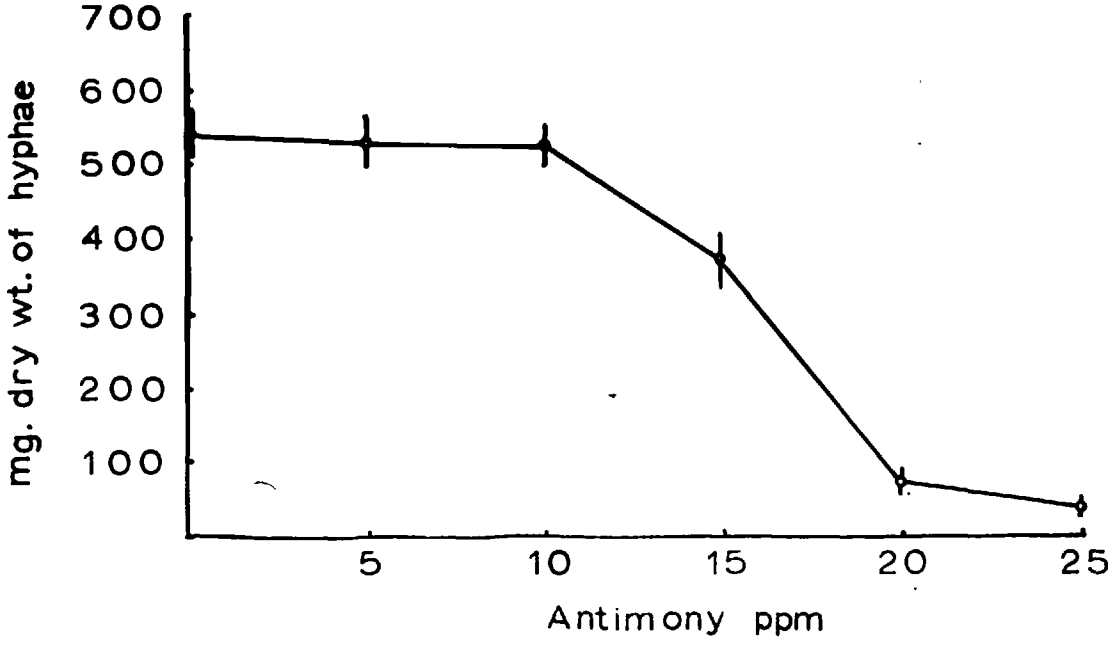


Figure 15. The effect of Cadmium on growth of S.scabies

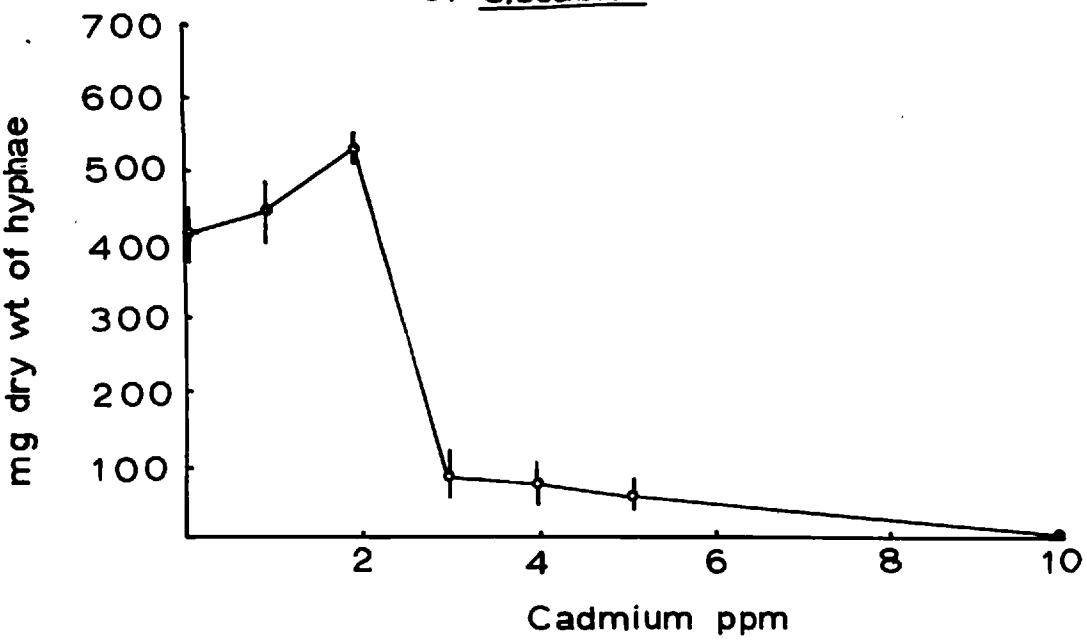


Figure 16 The effect of Cobalt on growth of S scabies

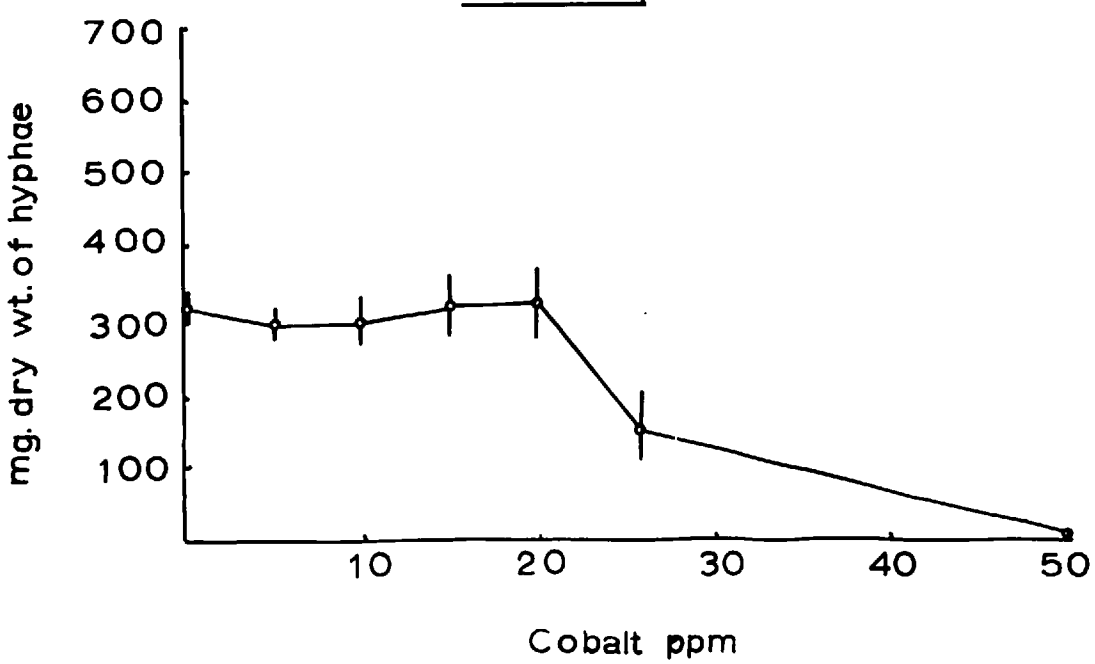


Figure 17. The effect of Copper on growth of S.scabies

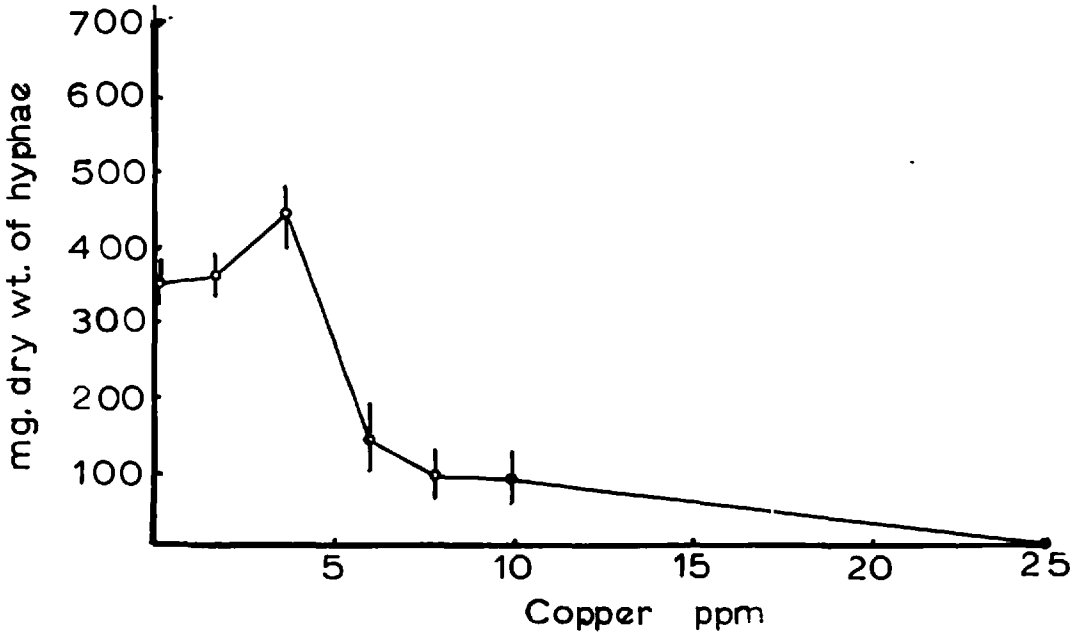


Figure 18. The effect of Silver on growth of S.scabies

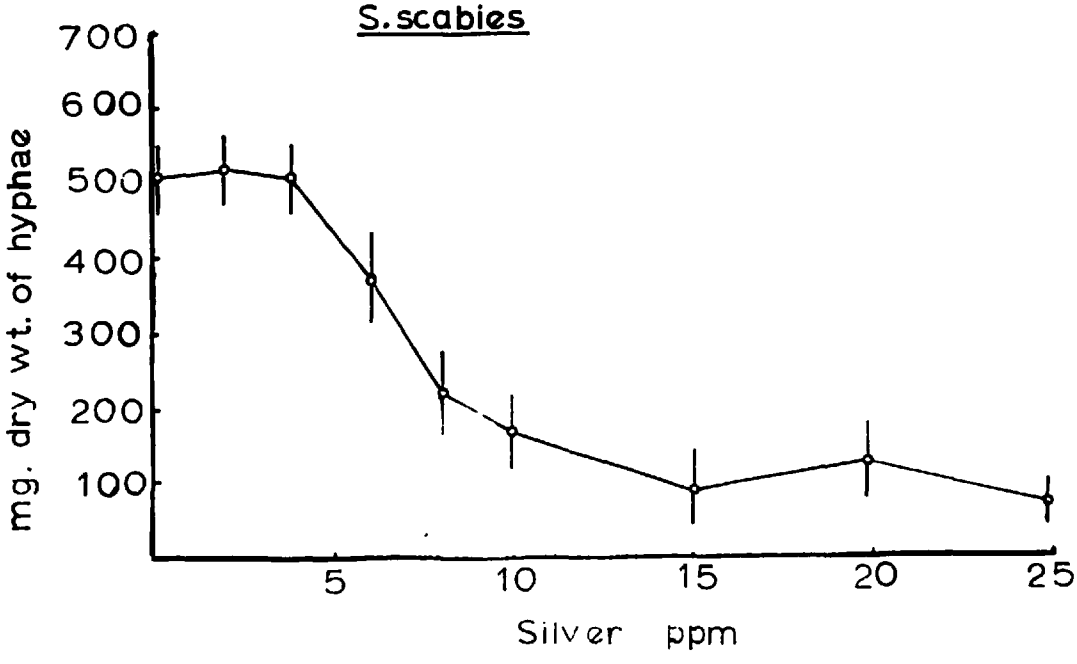
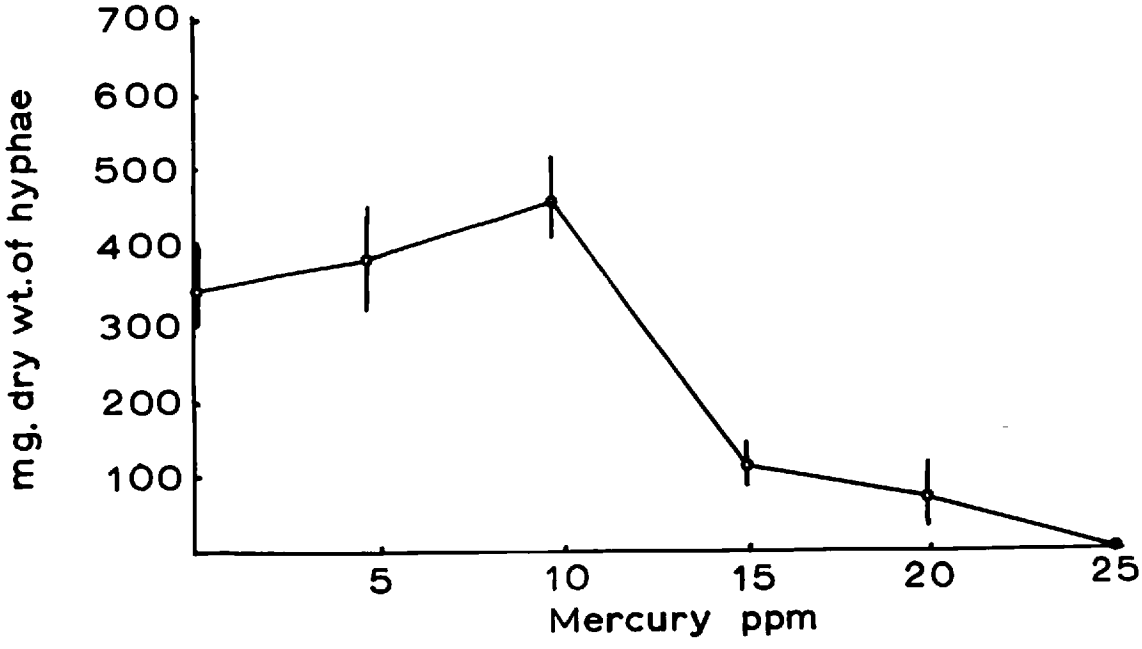


Figure 19. The effect of Mercury on growth of S.scabies



concentrations required were much lower. Thus growth was stimulated at 1 and 2 ppm but this was followed by 50 per cent. inhibition at 3.2 ppm and no growth at all at 10 ppm.

General conclusions are that of the 20 metals tested, six metals, cadmium, copper, arsenic, antimony, mercury and silver were all sufficiently toxic to S.scabies for them to have potential as control measures against scab.

The effect of copper frits on the growth of S.scabies in liquid culture

Copper has been used as a treatment for the control of common scab and the liquid culture test showed that it was toxic to S.scabies at concentrations above 5 ppm. Its use on a commercial scale for scab control may be restricted by the fact that it has been shown to be phytotoxic at rates of 20 to 50 lb./acre. Although manganese frit was not successful in controlling scab, there was a possibility that copper may be effective, if applied as a frit, without being phytotoxic.

As an initial test, the effect of a copper frit containing 26.6 per cent. of copper on growth of S.scabies in liquid culture was tested. This experiment was done in a similar way to that involving the manganese frit (page 55),

five concentrations of frit being used - 50 to 250 ppm of copper in fritted form. Three series were set up. In the first, the frit was added to the culture solution at the time of inoculation, and in the other two series it was added to the culture solution and shaken for 3 and 12 days before inoculation. The results are shown in Figure 20 and show that the copper frit was considerably more toxic than the manganese frit with a marked reduction of growth of S.scabies at below 100 ppm. A further experiment was done at a lower range of concentrations of copper, the frit being added at the time of inoculation. The results (Figure 21) showed that copper affected growth at between 60 and 80 ppm with a 50 per cent. reduction in growth at 68.4 ppm. Since soluble copper had previously been shown to limit growth at 6 ppm (page 83), this would suggest that a release of copper from the frit of an order of 10 per cent. of the total copper in the frit had occurred.

To test whether this high rate of release occurred in other media, the experiment was repeated with the difference that the medium used was potato dextrose solution. The results are shown in Figure 22 and show that the toxicity of the copper frit was much lower in potato dextrose solution than in Czapek-Dox solution, no effect on growth taking place below 250 ppm. This suggests that the rate of release of

Figure 20 The effect of Copper frit on growth of S.scabies (i)

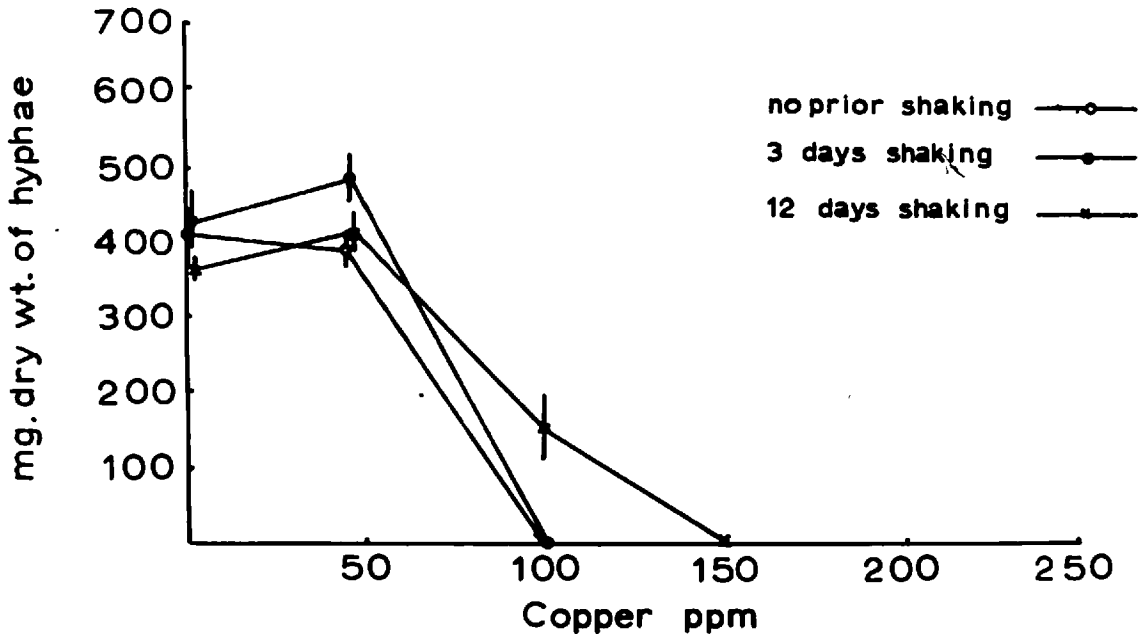


Figure 21 The effect of Copper frit on growth of S.scabies (ii)

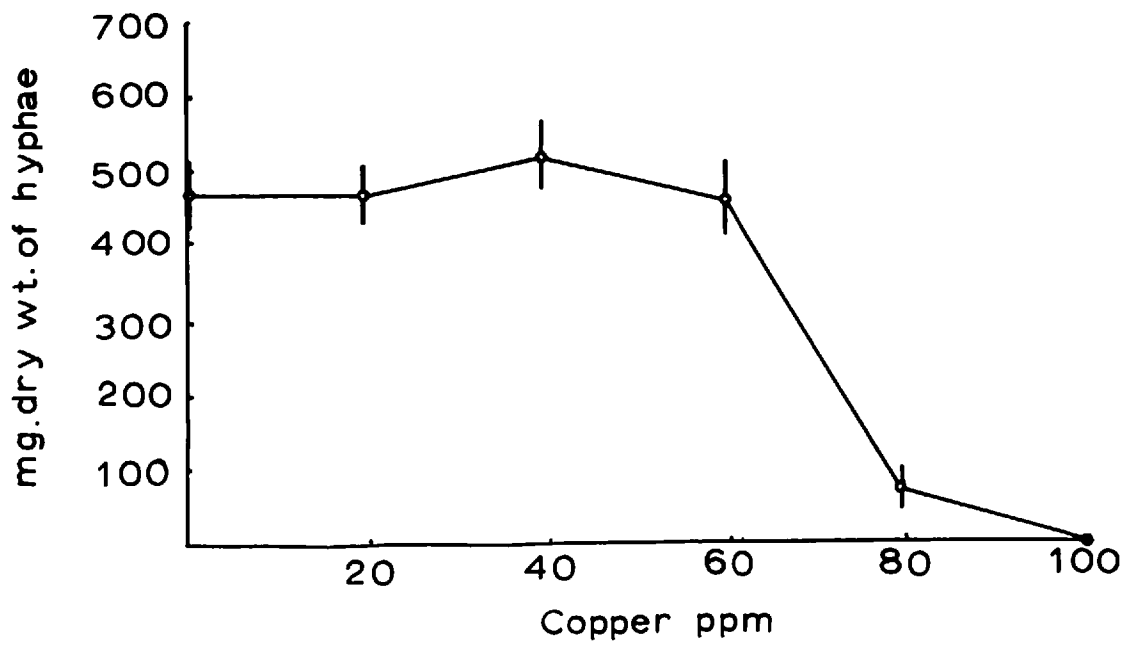


Figure 22 The effect of Copper frit on growth of S. scabies (in potato dextrose soln.)

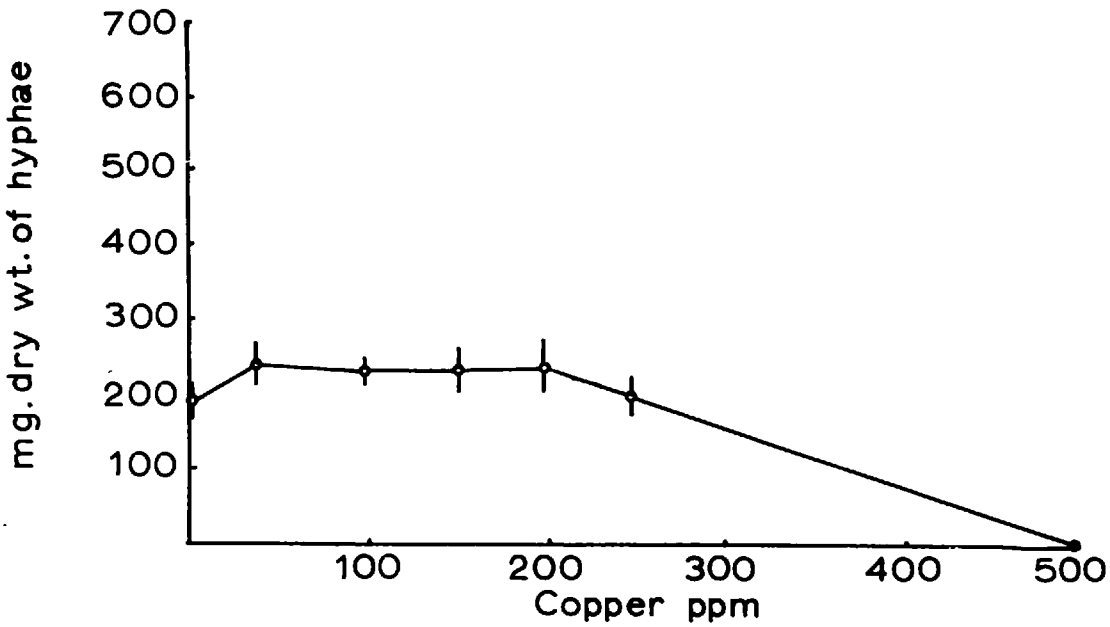
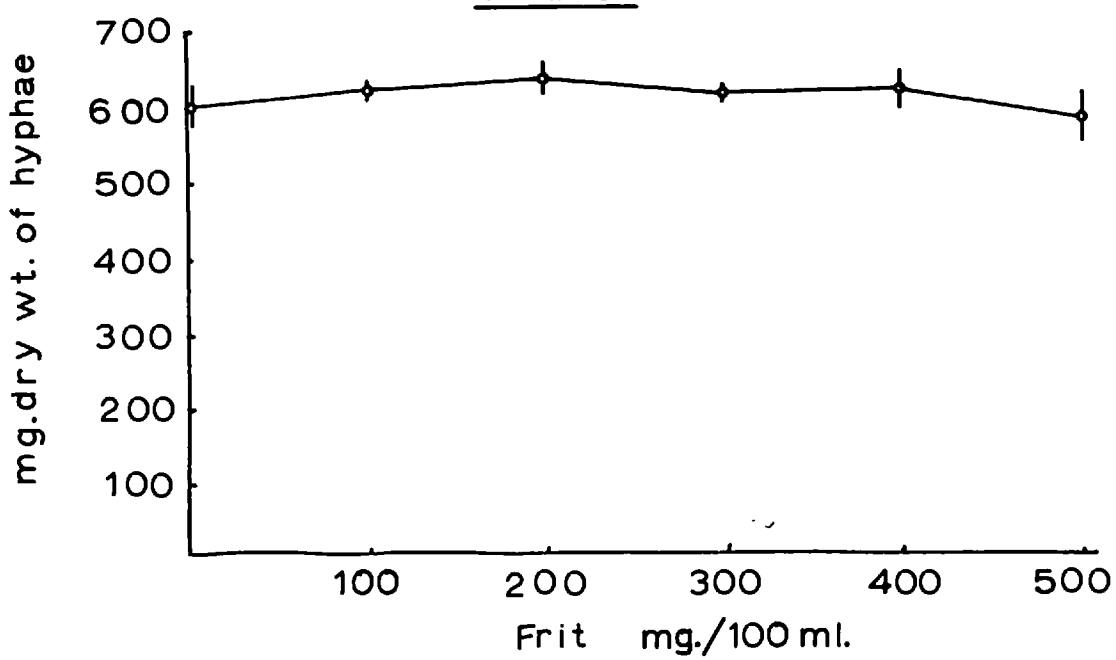


Figure 23 The effect of Frit 235a on growth of S.scabies



copper from the frit varies with the culture medium used.

A second frit was tested for its effect on S.scabies since it contained copper and manganese, both toxic to S.scabies. It was used at 0.1, 0.2, 0.3, 0.4 and 0.5 g. per 100 ml. of culture solution and the concentrations of elements at these rates are given in Table 17.

Table 17. Composition of Frit No. 253 A.

Wt. of frit (g.) per 100 ml. of medium	Concentration of elements (ppm in culture medium)					
	Cu	B	Fe	Mn	Mo	Zn
0.1	20	20	120	50	1.3	20
0.2	40	40	240	100	2.6	40
0.3	60	60	360	150	3.9	60
0.4	80	80	480	200	5.2	80
0.5	100	100	600	250	6.5	100

The results are given in Figure 23 and show that at these concentrations, the frit had no effect on the growth of S.scabies. This suggests that the rate of release of copper from this frit is lower than that from the copper frit, and the frit is unlikely to be as effective as the copper frit in controlling scab.

The effect of metal chelate complexes on S.scabies in liquid culture

The work with $Na_2MnEDTA$ showed that chelated manganese was considerably more toxic to S.scabies than manganese as a soluble salt. In view of this, the question arose as to whether this applied to other metals. To investigate this, the EDTA chelate complexes of cobalt, nickel, copper, zinc, lead, cadmium, aluminium and chromium were tested for their effect on S.scabies, each at a concentration of 50 ppm. The results of the test are given as a histogram in Figure 24 and were surprising. Cobalt, nickel, zinc, cadmium and chromium all had no effect on the growth of S.scabies even though cobalt and cadmium were toxic as soluble salts. Copper and lead both slightly inhibited growth. In the case of lead there was a similar effect to that of the soluble salt, whereas copper was far less toxic in chelated form than as a soluble salt. NaAlEDTA was the only chelate complex of the eight tested which markedly inhibited growth of S.scabies, and the results of its application at concentrations from 5 to 25 ppm are given in Figure 25. Yield was decreased by half by a level of 10.5 ppm of chelated aluminium. These results suggest that chelated metals are not therefore likely to be of use in controlling scab.

Figure 24 The effect of chelated metals on growth of S. scabies

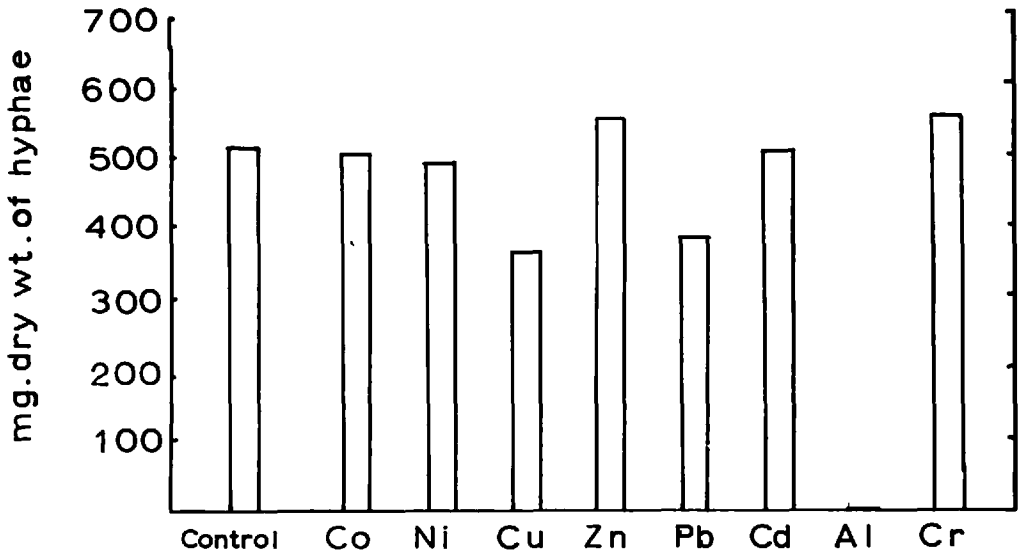
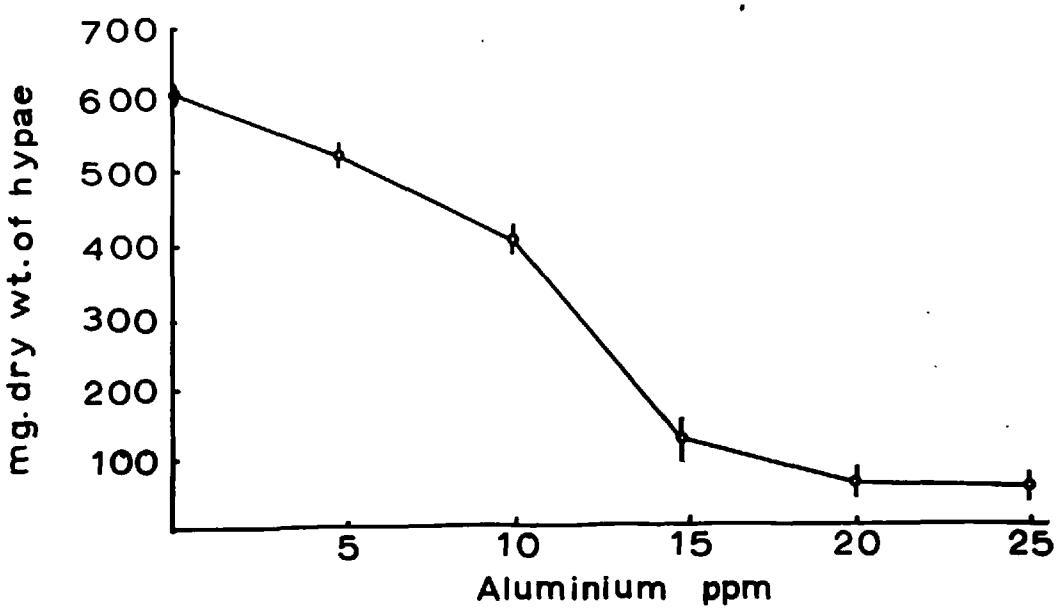


Figure 25 The effect of NaAl EDTA on growth of S.scabies



Greenhouse pot trials

In addition to the pot trials conducted at the Chelsea Physic Garden on the effect of various manganese compounds on scab incidence, a number of trials were set up using minor elements as chelates and as soluble salts. The treatments are summarised in Table 18.

Table 18. Pot trials using minor elements for scab control

Element	Compound	Concentration (ppm)
Zn	$ZnSO_4 \cdot 7H_2O$	25
Mg	$MgSO_4 \cdot 7H_2O$	25
Al	$Al_2(SO_4)_3 \cdot 18H_2O$	25
Ca	$CaCO_3$	25
K	K_2SO_4	25
Ni	Na_2NiEDTA	50
Pb	Na_2PbEDTA	50
Cd	Na_2CdEDTA	50
Co	Na_2CoEDTA	50
Zn	Na_2ZnEDTA	50
Cu	Cu frit (26.6% Cu)	100
Cd	$CdCl_2$	10, 25 and 100
Cd	Na_2CdEDTA	50) Applied as a
Sb	$SbCl_2$	50) soil soak.

As with the trials using manganese compounds, there was not sufficient scab to make it possible to determine the effect of the minor elements on scab incidence. The only treatments which affected the potato plants were those using cadmium compounds. There were marked signs of cadmium toxicity in plants growing in compost containing 25 and 100 ppm of cadmium but this was less evident in plants growing in compost treated with 50 ppm of cadmium as a chelate.

DISCUSSION

Three groups of manganese compounds were tested for their effect on the growth of S.scabies in liquid culture. Of these, Rayplex manganese had no effect on growth and no further tests were made on it. Although a glass frit containing manganese had no effect on S.scabies in liquid culture, its effect on scab incidence was tested at 25 lb./acre at two centres in Scotland in 1966. There was a low level of scab on one site, and although plots treated with manganese frit had less scab than untreated plots, this was not statistically significant. The other site did not produce any scab.

At Crowthorne in 1967, on a heavily scab-infested site, a 50 lb./acre application of manganese frit did not affect scab incidence, but there was a significant increase in yield and tuber number. During the growing season, the level of soluble manganese in the untreated plots varied between 0.6 and 1.35 ppm and that of exchangeable manganese between 2.5 and 4.8 ppm. The levels of exchangeable manganese were therefore only a little above the 2 ppm level which is generally considered to indicate manganese deficiency (Alexander 1965). It is therefore possible that there was some release of soluble manganese from the frit, this

being sufficient to increase yield and tuber numbers, but not sufficient to control scab.

Chelated manganese was shown to be considerably more toxic to S.scabies than was manganese in the form of a soluble salt, when tested in liquid culture. In a series of field trials, however, the chelated manganese had no effect on scab.

The chelated manganese was mixed with the soil before planting the potatoes, that is, about six weeks before tuber initiation and subsequent scab infection. It is possible that the manganese chelate complex broke down in the soil, the manganese being displaced by other metals having higher stability constants (see Table 6, page 59). This would result in an increase in soluble manganese in the soil, but this would be quickly oxidised to tetravalent insoluble forms.

One possible way of overcoming this might be to apply the chelated manganese as a soil soak to the tuber forming zone of a potato crop a little before the commencement of tuberisation. In this case, the chelated manganese would be able to affect the populations of the scab organism and decrease incidence of scab.

The effect of the nine metal chelate complexes on growth of S.scabies in liquid culture is difficult to

explain. Only manganese and aluminium chelates were markedly toxic at 50 ppm. Their stability constants (log K2, see Table 6) are 14.00 and 16.31 respectively and cadmium, zinc, lead, nickel and copper all have higher constants than this. A possible explanation could therefore be that the lower the stability constant, the greater the toxicity to S.scabies, but this is rendered unlikely by the fact that cobalt has a lower stability constant than aluminium but was considerably less toxic to S.scabies.

A number of metals were tested for their effect on S.scabies in liquid culture, and six depressed growth by at least 50 per cent. at concentrations less than 25 ppm, and might therefore hold some promise for the control of scab. The results conformed well with published results of field trials. Thus, copper was highly toxic and aluminium slightly so, and boron, molybdenum and zinc had no effect below 50 ppm.

Published results of trials with cobalt (Guntz, 1957) deal only with a rather low rate of 5 kg./ha., this having no effect on scab. The figure of 50 per cent. inhibition at 29.5 ppm in liquid culture did not, therefore, contradict this field results.

Antimony, arsenic, cadmium, copper, silver and mercury were all shown to be highly toxic to S.scabies. Of these,

four must be dismissed as soil fungicides for practical reasons. Antimony, arsenic and mercury would be too hazardous, and it is unlikely that silver could be an economical means of control, even at rates as low as 25 lb/acre.

This leaves cadmium and copper. The former was the most toxic of all metals tested but there are two factors which prevent its development as a means of control of scab. A cadmium chloride/urea solution has, in the past, been used to control dollar spot disease of turf. Recently, cadmium has been found to be highly toxic to mammals and because of its alleged carcinogenic properties, the Sports Turf Research Institute at Bingley, Yorkshire, no longer recommend its use. In view of this, control of scab using cadmium must also be ruled out. The second factor is that the pot trials at Chelsea showed that cadmium was toxic to the potato plant at levels below 25 ppm.

Copper has already been shown to control scab in pot trials (Mortvedt, 1962) but it is also toxic to potatoes at rates of 20 lb./acre and this may limit its use. It may be possible to apply copper frit at a rate which would give a release of copper sufficient to control scab but not sufficient to harm the potato crop. Pot trials at Chelsea did not indicate whether a 200 ppm application of copper

frit would control scab. Although the trials were not designed to test the effect of the frit on yield, they did show that the frit did not have any observable toxic effect on the general growth of the plant at this level.

In liquid culture, 100 ppm of copper in the form of a frit prevented growth of S.scabies in Czapek-Dox solution. No figures are available for the rate of release of copper from a frit in soil and the results with liquid culture cannot be assumed to indicate the effect of copper frit on scab, particularly as copper frit is less toxic to S.scabies when the latter is grown in potato dextrose solution than when grown in Czapek-Dox solution.

It might be possible that copper frit applied at a rate of about 1 cwt./acre to the tuber forming zone of a potato crop would give a release of copper sufficient to control scab without affecting the potato plant. Certainly this is the aspect of this present work which warrants further investigation.

PART II. BIOLOGICAL CONTROL OF COMMON SCAB

INTRODUCTION

There has been increasing interest in recent years in the biological control of crop pests. This interest has, in the main, been confined to insect pests since these more easily succumb to biological control and this provides an alternative to insecticides which include those pesticides most toxic to man and other animals.

Although fungicides are not generally as toxic to mammals as insecticides, the soil fungicides and seed dressings are more toxic than most fungicides and this makes biological control of some soil-borne diseases desirable. Also, soil fungicides are generally used at rates far higher than those of foliage protectants. The latter are applied at rates of the order of 1 to 3 lb./acre whereas soil fungicides are often used at over ten times this rate.

Soil fungicide treatment for potato scab control has not so far proved economical and the work reported here has shown that, with the exception of copper, minor elements are not likely to be of use.

The biological control of scab has already been the subject of considerable work. The standard control for

scab as recommended in gardening books, is the application to the soil of grass mowings or some other green manure. This practice is occasionally employed by potato growers, in the form of ploughing in a cover crop. The comprehensive studies of Millard et al. (1922, 1923, 1926, 1927) showed that applications of green manure gave considerable control, even to the extent of offsetting heavy applications of lime. These effects were not observed in pot trials unless the saprophytic actinomycete Streptomyces praecox was introduced simultaneously. Millard suggested that the addition of organic matter to the poor soils in which scab often occurs would enable saprophytes to increase, and, by using up the available food supply and possibly by toxin production, prevent multiplication of, or even eliminate parasites such as S.scabies.

Sanford (1926) failed to control scab by applications of green manure but concluded that this could have been due to the absence of suitable antagonistic actinomycetes, or their inability to multiply under acid conditions. Goss (1937) did not confirm Millard's results with S.praecox, nor did he find any antagonism against S.scabies by a range of penicillia, actinomycetes or fungi. He found no effect on the disease in the field with green manure, but ordinary

manure, sterile or unsterile, reduced scab.

Daines (1937) found that Trichoderma lignorum was antagonistic to S.scabies, and gave some control in the field when added as a suspension in furrows about the developing tubers. KenKnight (1941) failed to get control using green manures of lucerne and bluegrass (Poa pratensis); bulk inoculation of scab infested soil by a wide range of microorganisms also had no effect. Kent et al. (1945) found that intercropping with rye increased yields and decreased scab, as did autumn compared with spring ploughing.

Sanford (1946) in another series of experiments, was able to confirm Millard's early work with rye and clover green manures. He considered an antagonistic microflora to be responsible for disease control. Sethofer and Kraal (1949) found that use of badly stored manure increased scab incidence considerably. Rouatt and Atkinson (1949 1950) published results of comprehensive pot trials which showed that addition of 6 week old soybean crops markedly decreased disease incidence, but rye or clover had little effect.

Clover and soybean both considerably increased populations of bacteria, fungi and actinomycetes, particularly fungi with soybean, but rye only increased the bacterial populations. Green manuring with soybean lowered the soil pH from 6.4 to 5.0.

De Haan (1955) found that municipal refuse applied at a rate of 50 tons/acre slightly increased scab, but considerably less than would be expected on the basis of the alkaline reaction induced by the treatment. De Boer (1962) found that 2 per cent. maize flower, usually combined with 3 ppm "Trichoderma dust" reduced incidence of both scurf (Rhizoctonia solani) and scab. McAllister (1963) reported that ploughing in a light crop of Italian rye grass had no effect on disease incidence.

Weinhold et al. (1964) confirmed Rouatt's results with soybean. Under continuous potato culture, scab increased to a maximum in 8 years. A Canadian pea cover crop had no effect. Barley doubled scab incidence but soybean prevented any increase in disease incidence although it failed to reduce it once the pathogen was established.

Lochead (1949), and Peterson (1953) each tested large numbers of actinomycetes known to be antagonistic to S. scabies for cross-antagonism. This was prevalent and suggests that any one type antagonistic to S.scabies is unlikely to dominate in a mixed population, so that a complex effect is more likely, if indeed direct antagonism is why some soil amendments cut down incidence of scab.

Menzies (1959) obtained the interesting results that soil from fields free of scab suppressed the disease in

pot trials over five years, whereas with virgin soil, the disease increased. Scab was controlled in infested soil by mixing the scab-free soil with it in a 1:1 ratio but this did not work if the scab-free soil was first steamed. Even as little as 1 per cent. scab-free soil and 1 per cent. lucerne gave good control when mixed with the infested soil although neither was consistently effective alone. These results suggest that a biological factor is responsible.

Although these published experimental results show some variation, there has been considerable success in control of scab using organic soil amendments, although the mechanism is not understood.

McGregor and Wilson (1966) have recently suggested that available soil manganese may largely influence scab incidence and that organic amendments may affect scab incidence by increasing the level of available manganese in soil.

Manganese exists in the soil in several oxidation states of varying availability to plants. These forms are the bivalent ion - existing in the soil solution, or as an exchangeable ion, or in a non-exchangeable form - and the tetravalent insoluble higher oxides, minerals and organically combined forms, these being not available to plants. The insoluble higher forms include trivalent oxides but whether these are available to plants is not clear.

For the purpose of soil analysis, four classes of soil manganese may be considered:

1. Water soluble. This is obtained by a water extract (soil : liquid ratio - 1 : 10) and values lower than 1.0 ppm are common.

2. Exchangeable. This is given by a normal neutral ammonium acetate extract (soil : liquid ratio - 1 : 10) and values below 2 ppm are generally indicative of deficiency.

3. Reducible. Soil previously extracted for exchangeable manganese is re-extracted with normal neutral ammonium acetate containing 0.2 per cent. hydroquinone (soil : liquid ratio - 1 : 10). This may be classified as being the "reserve" of manganese in the soil which can be reduced to a form available to plants, its reduction depending largely on soil pH. The level of reducible manganese frequently exceeds 100 ppm.

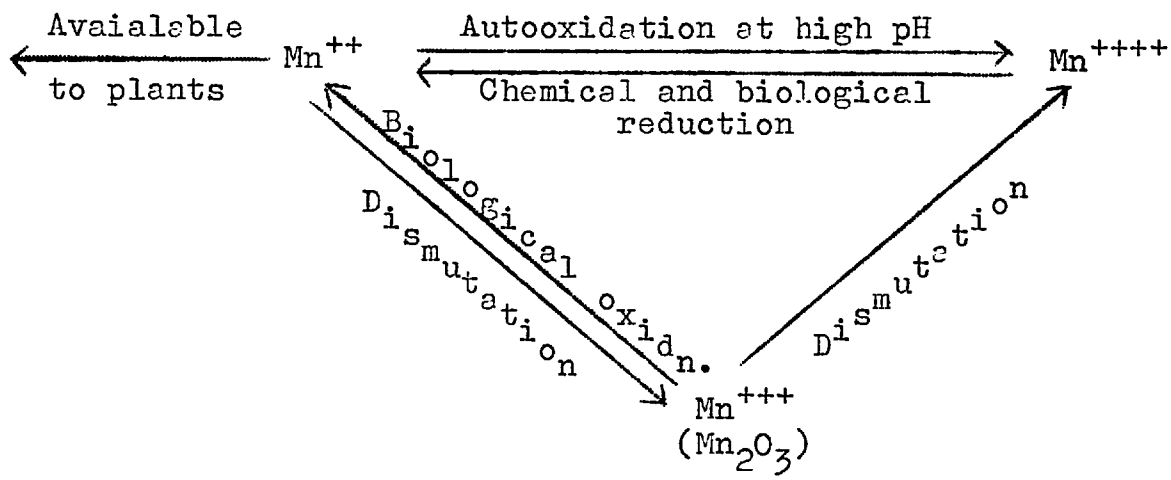
4. Total. This varies in level between 50 and 3,000 ppm. (Alexander, 1965; McGregor and Wilson, 1966).

Alexander (1965) states that of the two major forms (divalent and tetravalent), the ion that predominates depends on pH. At reactions more acid than pH 5.5, manganese is present largely as exchangeable Mn^{++}. At reactions more alkaline than pH 8.0, Mn^{++} is unstable and is oxidised to manganic oxides. Because manganic oxides are not taken

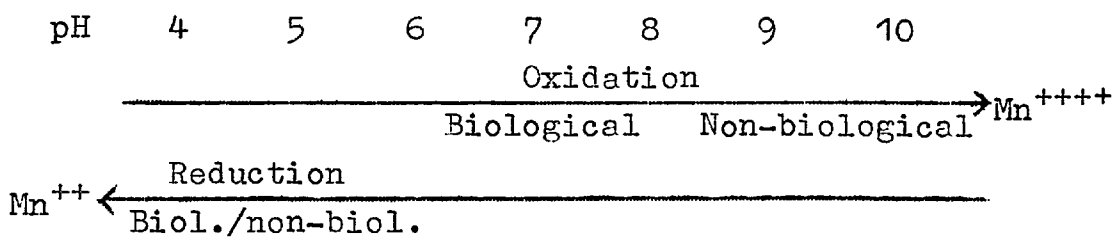
up appreciably by plants, alkaline conditions are frequently associated with deficiencies of the element. Below pH 8.0 there is little chemical oxidation of divalent manganese and reduction takes place,

$$MnO_2 + 4H^+ + 2e^- \underset{\text{alkaline}}{\overset{\text{acid}}{\rightleftharpoons}} Mn^{++} + 2H_2O$$

In the intermediary ranges, between pH 5.5 and 8.0, the prominence of microbiological phenomena becomes evident. This may be summarised:-



This can be simplified and referred to a pH range:-



It is the reduction of manganese and factors which affect it that are of interest in common scab (McGregor and Wilson, 1966) and these may be considered in four sections:

1) Christensen, Toth and Bear (1950) showed that decomposition of organic matter in soil results in an increase in exchangeable manganese and they suggested that this could be due to direct reduction of manganese by organic compounds produced during decomposition and by reduction of pH by organic acids present. They found that in heavily limed soils, this reduction did not persist after decomposition was completed since the reduced manganese was oxidised back to oxidised forms, whereas in more acid soils, the effect of organic matter on manganese availability was more long lasting.

Kosegarted (1957) showed that soil organic matter can effect reduction of tetravalent manganese without direct biological intervention. This reduction can also be partially biological, since Clarke et al. (1956) and Mann and Quastel (1946) have established that addition of glucose to a well-drained soil results in a decrease in manganic oxides, the accumulation of divalent manganese exhibiting a logarithmic transformation suggestive of microbial action.

2) Sulphur applications may have a direct effect in reducing manganese. Vavra and Frederick (1952) determined the effect of bacterial oxidation of sulphur upon the release of soluble manganese from manganese dioxide. Using the soil perfusion technique, it was found that the oxidation of elemental sulphur or sodium thiosulphate applied to soil, resulted in a release of soluble manganese accompanied by a lowering of pH. Addition of calcium carbonate caused a decrease in the amount of soluble manganese released, although the amount of sulphur formed was not changed significantly.

In pure culture studies on synthetic media, ten times more soluble manganese was formed from MnO_2 when sulphur was oxidised by Thiobacillus thioxidans than when the same acidity was produced by the addition of sulphuric acid. Separation of the manganese dioxide by a collodion membrane did not prevent the reduction of manganese.

Starkey (1950) considered that manganese dioxide could be utilised as a hydrogen acceptor in place of atmospheric oxygen by autotrophic bacteria when oxidising sulphur:-

$$3\ MnO_2 + 4H + S \longrightarrow 3\ Mn^{++} + SO_4^{--} + 2\ H_2O$$

3) Reducing conditions in poorly aerated soils caused by

high soil water content will increase the level of exchangeable manganese in soil (Clarke et al., 1957; Conner, 1932; Gooden et al., 1928; Metzger, 1930).

4) Acid soils favour reduction of manganese to divalent soluble forms.

All these conditions favour a low incidence of common scab and lend strong support to the ideas expressed by McGregor and Wilson (1966) and by Mortvedt et al. (1961) that manganese availability is an important factor in the occurrence of common scab.

The effect of the addition of organic matter on manganese availability could provide an explanation for the variable results obtained in attempts to control scab by green manuring. The green manuring could stimulate biological and non-biological reduction of manganese which could then depress growth of actinomycetes causing scab. Failure of green manures to control scab in some experiments could be due either to a low level of easily reducible manganese in the soil treated, or else to alkaline soil conditions which would enhance immediate oxidation of reduced manganese back to insoluble forms.

EXPERIMENTAL

Isolation of manganese-reducing microorganisms from soil.

Any attempt to relate control of common scab by use of organic amendments to reduction of insoluble manganese requires that there be methods available for making a quantitative estimation of this reduction. Soil analysis of water-soluble and exchangeable manganese will give a general indication, and an estimate of manganese-reducing microorganisms would give an indication of the importance of biological reduction in this process.

According to Alexander (1964) "in pure culture, many bacteria reduce MnO_2 in the presence of oxidisable organic nutrients. MnO_2 may serve here as an electron acceptor for respiratory enzymes, replacing molecular oxygen in this regard.

$$RH_2 + MnO_2 \longrightarrow R + Mn(OH)_2 \text{ "}$$

A dilution series of a soil sample from a flower bed was made over the range 10^{-3} to 10^{-6} using a medium containing manganese dioxide with sucrose as the carbon source. The manganese was in the form of a very finely divided black powder and this formed a dense black suspension.

The composition was:-

Sucrose	30 g.
MnO_2	2 or 5 g.
Yeast extract	1 g.
Agar	15 g.
Water	1,000 ml.

Three replicates were made at each dilution on each medium (2 and 5 g. of MnO_2) and the plates were incubated for 4 days at 25°C. No evidence of manganese reduction was visible after 4 days although many colonies had formed at the lower dilutions. Fortunately, the plates were not discarded and examination a few days later showed that a number of colonies of bacteria and fungi had reduced the manganese dioxide, with clear zones around the colonies. The medium containing 5 g./litre showed this clearing far better than the medium with the lower concentration, where the plates were not entirely opaque.

A number of the manganese-reducing colonies were isolated onto the MnO_2 agar and after 10 days showed the reduction of manganese clearly (figure 26). A number of these isolates were tested in liquid culture using the MnO_2 medium less agar, and were incubated at 25°C. at 200 rpm on the rotary shaker. Reduction of manganese was shown by

Figure 26 Reduction of manganese dioxide by fungi growing on medium containing 5·0 g./l. MnO_2

Figure 27. Reduction of manganese in liquid culture

Key:

1 A Czapek-Dox solution.

1 B Czapek-Dox solution with 5 days growth of Penicillium sp.

2 A MnO_2 solution (2 g./l. MnO_2)

2 B MnO_2 solution with 5 days growth of Penicillium sp.

3 A MnO_2 solution (5 g./l. MnO_2)

3 B MnO_2 solution with 5 days growth of Penicillium sp.


14
2
2
5
5
1a
1b
2a
2b
3a
3b

complete clearing of the MnO_2 suspension which occurred within 4 days. (figure 27).

Although shake culture shows the ability of an organism to reduce tetravalent manganese more quickly than does agar culture, the former method is less adaptable to making an estimate of the numbers of organisms in soil.

To test dilution plate methods, and get an idea of numbers of manganese-reducing microorganisms present in soil, dilution series were made from two samples of soil. These were taken from adjacent flower beds, but one of these had recently been limed. Counts were made of bacteria and actinomycetes, fungi, manganese-reducing bacteria and actinomycetes and manganese-reducing fungi. No attempt was made to separate bacterial and actinomycete populations since in practice very few manganese-reducing actinomycetes were found.

Results are given in Table 19. These show that there were about ten times as many manganese-reducing organisms in the unlimed soil as in the limed soil, although total microflora populations were almost the same. In both samples, the proportion of fungi capable of reducing manganese was about ten times the proportion of bacteria capable of reducing manganese.

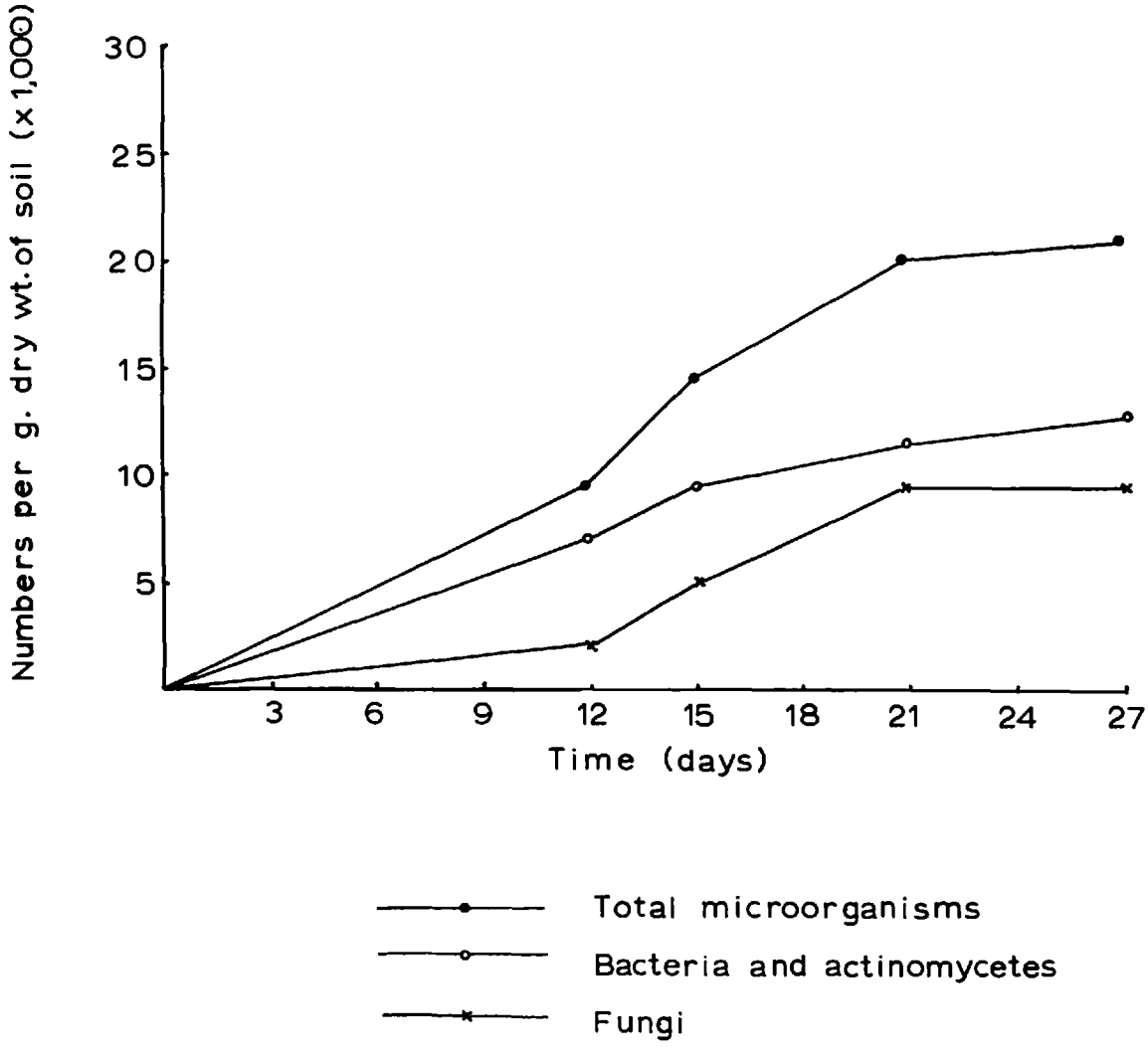
In this experiment, numbers of manganese-reducing

Table 19 Numbers of manganese-reducing microorganisms in soil

Organism	1st sample (unlimed)	2nd sample limed
Total bacteria/actinomycetes x 10^5	109.0	98.0
Total fungi x 10^5	4.8	6.3
Total microorganisms x 10^5	113.8	104.3
Manganese-reducing bacteria and actinomycetes x 10^3	14.0	1.5
Manganese-reducing fungi x 10^3	7.0	1.2
Total manganese-reducing microorganisms x 10^3	21.0	2.7
Manganese-reducing bacteria and actinomycetes as percentage of total bacteria/actinos.	1.3	0.15
Manganese-reducing fungi as a percentage of total fungi	14.6	1.9
Manganese-reducing microorganisms as percentage of total.	2.0	0.26

organisms which could be observed of the manganese dioxide agar were counted after 12, 15, 21 and 26 days. The purpose of this was to give an idea of how long it took

Figure 28 Manganese-reducing microorganisms on dilution plates – increase in numbers with time



for manganese reduction to become visible. The results for the sample from the limed soil are shown in Figure 28. This shows that after 21 days, there was little further increase in numbers and this period of incubation should be sufficient for assessing numbers of these organisms.

The effect of grass meal on numbers of manganese-reducing organisms in soil.

It was proposed to undertake a field trial in 1967 to determine the relationship between the effect of organic amendments of soil on control of scab and reduction of available manganese. The preliminary work on populations of manganese-reducing organisms in soil was done in the autumn of 1966, and in the intervening months two pot experiments were done.

Experiment 1.

The pot trials at Chelsea Physic Garden in 1966 had included the effect of grass meal on common scab of potatoes grown in pots. Concentrations of grass meal of 0.5 and 1 per cent. were used, equivalent to approximately 5 and 10 tons per acre, but there was not sufficient scab on the tubers to make assessment possible.

In spite of this, the pot trials did make it possible

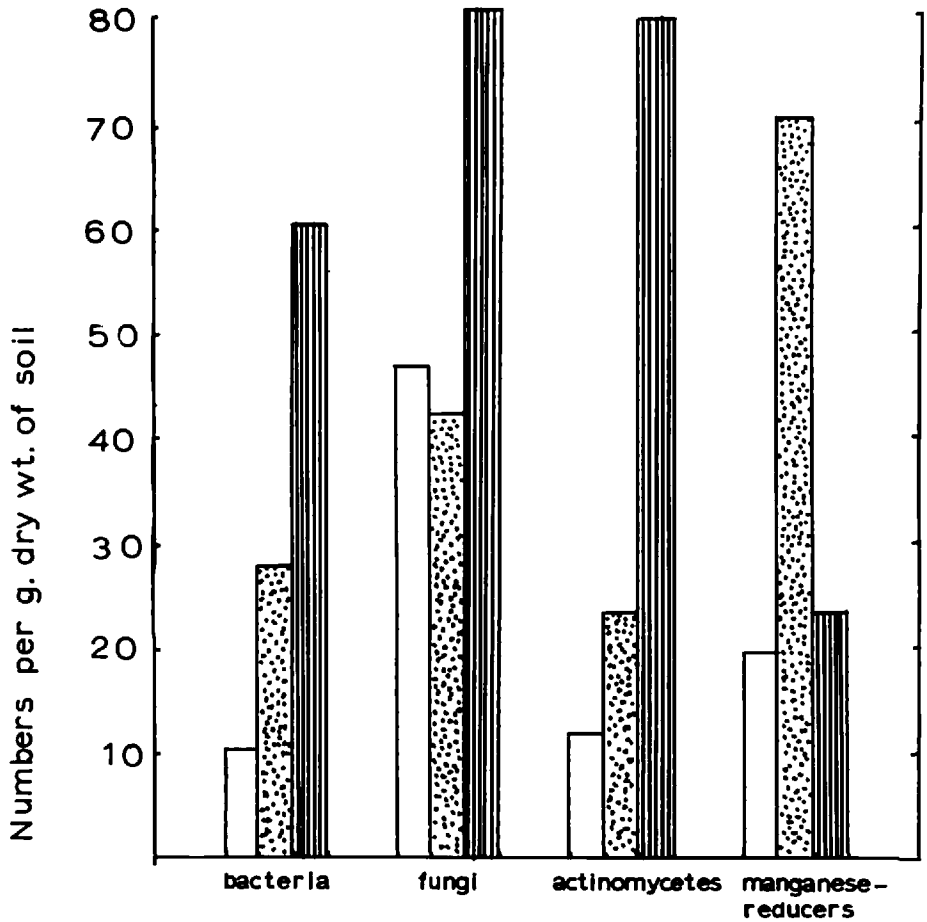
to determine whether any effect on the soil microflora had persisted for the 5 months from the time of planting to harvesting. Composite soil samples were made consisting of two samples from each of the six replicates in each treatment. The samples from each treatment were mixed and a dilution series made, the samples having been collected 2 weeks after the tubers had been harvested. Diluted suspensions were plated on to selective media to determine numbers of bacteria, fungi, actinomycetes and manganese-reducing organisms. The results are given in Figure 29.

These show that in soil amended with grass meal, some effect on microflora persisted for up to 5½ months. With bacteria, fungi and actinomycetes, the 1 per cent. level had the greatest effect. Populations of bacteria and actinomycetes were 5 times those in unamended compost and populations of fungi were twice as large. The 0.5 per cent. level of grass meal had a much less noticeable effect except with the manganese-reducing organisms where populations were three times as large as those of unamended compost. The 1 per cent. grass meal amendment had no effect on the manganese-reducing organisms.

Experiment 2.

A sample of soil was taken from the site of the field trial at Crowthorne, Berkshire in 1966, from an area known

Figure 29 The effect of grass meal on numbers of soil microorganisms



Dilutions:

Bacteria - x 100,000

Fungi - x 1,000

Actinomycetes- x 10,000

Manganese-reducers - x 1,000

Treatments:

Untreated compost -

0·5% grass meal amendment -

1% grass meal amendment-

(sampled 5 1/2 months after mixing)

Figure 30 Changes in numbers of soil microorganisms 10 days after amendment with grass meal

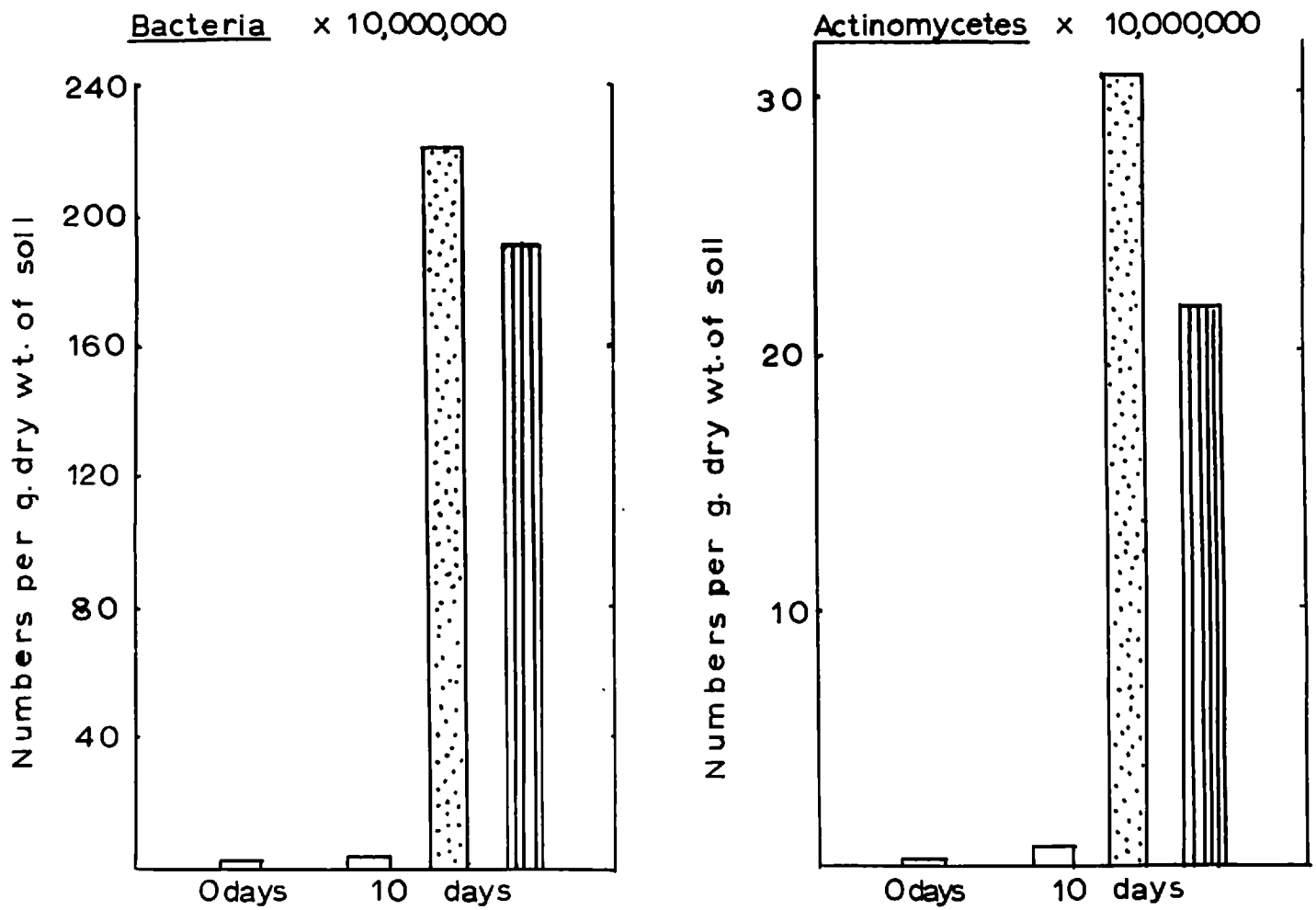
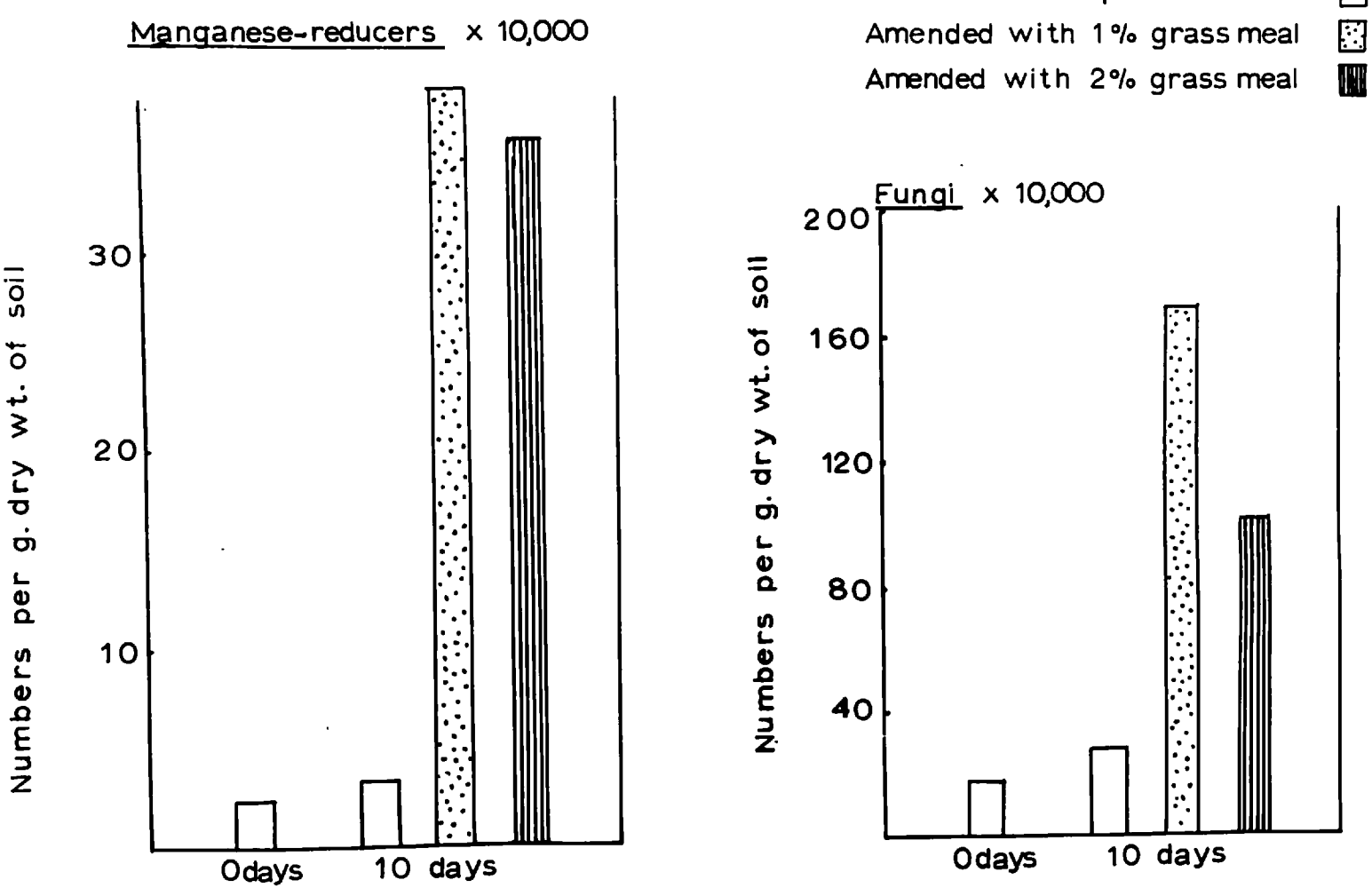


Figure 30 contd.



to have a high level of scab infestation. It was sieved through a ¼ inch sieve, watered lightly and left overnight. Nine five inch pots were filled with the soil and treatments were 0, 1 and 2 per cent. grass meal mixed thoroughly with the soil, with three replicates per treatment. The pots were placed in a moist chamber to avoid water loss. Dry weight determinations and estimates of microflora numbers were made on the original soil sample, 24 hours after sieving and watering, and on unamended and amended soil after 10 days. Samples were collected by taking 3 by 0.5 inch plugs of soil from each pot with sterile 0.5 inch diameter test tubes and mixing these plugs to get a composite sample.

These soil samples were then used to prepare dilution plates on selective media in order to estimate numbers of bacteria, fungi, actinomycetes and manganese-reducers in the soil samples. The results are given in Figure 30. Table 20 gives the ratios of numbers of microorganisms in amended soil to those in unamended soil.

There were large increases in numbers of microorganisms in the amended soil. The 1 per cent. amendment led to a greater increase in populations after 10 days than did the 2 per cent. amendment, except in the case of the manganese-reducing organisms where the populations in the soil amended with 1 and 2 per cent. grass meal were similar.

Table 20. Ratio of microorganism numbers in grass meal amended soil to those in unamended soil - after 10 days.

Organisms	1 per cent. grass meal	2 per cent. grass meal
Bacteria	60.5	52.0
Actinomycetes	28.0	19.9
Fungi	6.0	3.8
Manganese-reducers	10.8	10.2

The greatest increase in populations after amendment with 1 per cent. grass meal was by a factor of 60 for bacteria and the least by a factor of 6 for fungi.

The two experiments gave results which suggest that an amendment of soil by 1 per cent. of grass meal would lead to a marked increase in numbers of microorganisms within 10 days, and that some increase in numbers would be likely to persist for up to 5½ months.

Field Trial at Crowthorne - 1967.

The aim of this trial was to determine the effect of dried grass meal on incidence of common scab and to attempt

to relate any effect to changes in soil microflora and in available manganese.

The site was again on the smallholding, Sulby Croft, near Crowthorne and adjoined the plot used in the previous year. The 1966 plot was not used again in order to avoid an increase in potato root eelworm. The layout was a randomised block design with six blocks of four plots. Blocks measured 9 feet 4 inches by 14 feet each. Each plot was a row of ten setts, 14 inches apart, with marker plants at each end. Grade "A" Majestic seed, chitted for six weeks, was used. This trial included a treatment to test the effect of manganese frit on scab incidence, the results of which have been discussed on page 77.

The four treatments were:-

A. Untreated.

B. Manganese frit at 16.2 g./plot = 50 lb./acre.

C. Dried grass meal at 725 g./plot = 1 ton/acre.

D. Dried grass meal at 1,450 g./plot = 2 tons/acre.

Dried grass meal was used as the organic amendment because it had been used by Okpala (1966) a previous worker in the Department who had investigated its use in control of a number of seedling diseases and a body of data concerning its effect in this context had been accumulated.

The grass meal was applied in 18 inch wide bands along

the line of planting. It was dug in by hand and mixed thoroughly with the soil to a depth of 4 to 6 inches. Seed was then planted at a depth of 4 inches and the soil was ridged. This procedure insured that the grass meal was dispersed in the tuber forming zone. Soil treatment and planting were done on April 27th.

Soil samples for manganese and microflora analysis were collected on April 27th, May 8th and 19th, June 7th and 23rd, July 20th and September 12th. On each occasion, the soil sample from each treatment was a composite one made up of six approximately 100 g. samples, two taken from each of three replicate plots, each small sample being taken from the middle of a ridge. Dilution plates were prepared as previously described and selective media were used to estimate numbers of bacteria, actinomycetes, fungi, manganese-reducers and pigment-producing actinomycetes.

For manganese analysis, soil samples were air-dried at 35°C. for 48 hours, passed through a 3 mm. mesh sieve and the fraction of particle size less than 3 mm. dried again for a further 12 hours. Water content was measured by drying 10 g. samples of the air-dried soil for 72 hours at 100°C. The analysis of the soil samples for manganese content was done by the Imperial College Analytical Services Laboratory and results had a maximum error of $\pm$ 5 per cent.

The crop was assessed for emergence after 5 weeks and for potato root eelworm periodically during the summer. Spraying for blight was not necessary but the crop developed magnesium deficiency symptoms in early July and was treated with a foliar application of magnesium sulphate. The haulms dies down towards the end of August and the crop was dug up on September 12th. All tubers were washed, weighed and assessed for scab, tubers under 2.5 cm. in length being discarded.

There was 100 per cent. emergence after 5 weeks and a maximum of 5 per cent. attack by potato root eelworm during the season. Results for scab incidence, total weight of tubers per plot, number of tubers per plot and average tuber weight are given in Table 21 as means of six replicates.

Table 21. Effect of grass meal on scab and yield of potatoes

Treatment	Scab incidence	Yield g./plot	Number of tubers	Average tuber wt.
A. Untreated	17.2	2,624	65.0	40.7
C. Grass meal 1 ton /acre	15.8^{nsd}	4,218*	97.3**	42.5^{nsd}
D. Grass meal 2 tons /acre	14.1**	5,840***	90.2**	62.8***

Levels of significance:-

nsd = no significant difference

* = significant at 2 per cent. level.

** = significant at 1 per cent. level.

*** = significant at 0.1 per cent. level.

Results for levels of water-soluble and exchangeable manganese are given in Table 22.

Table 22. Effect of grass meal on soil manganese - ppm in air-dried soil

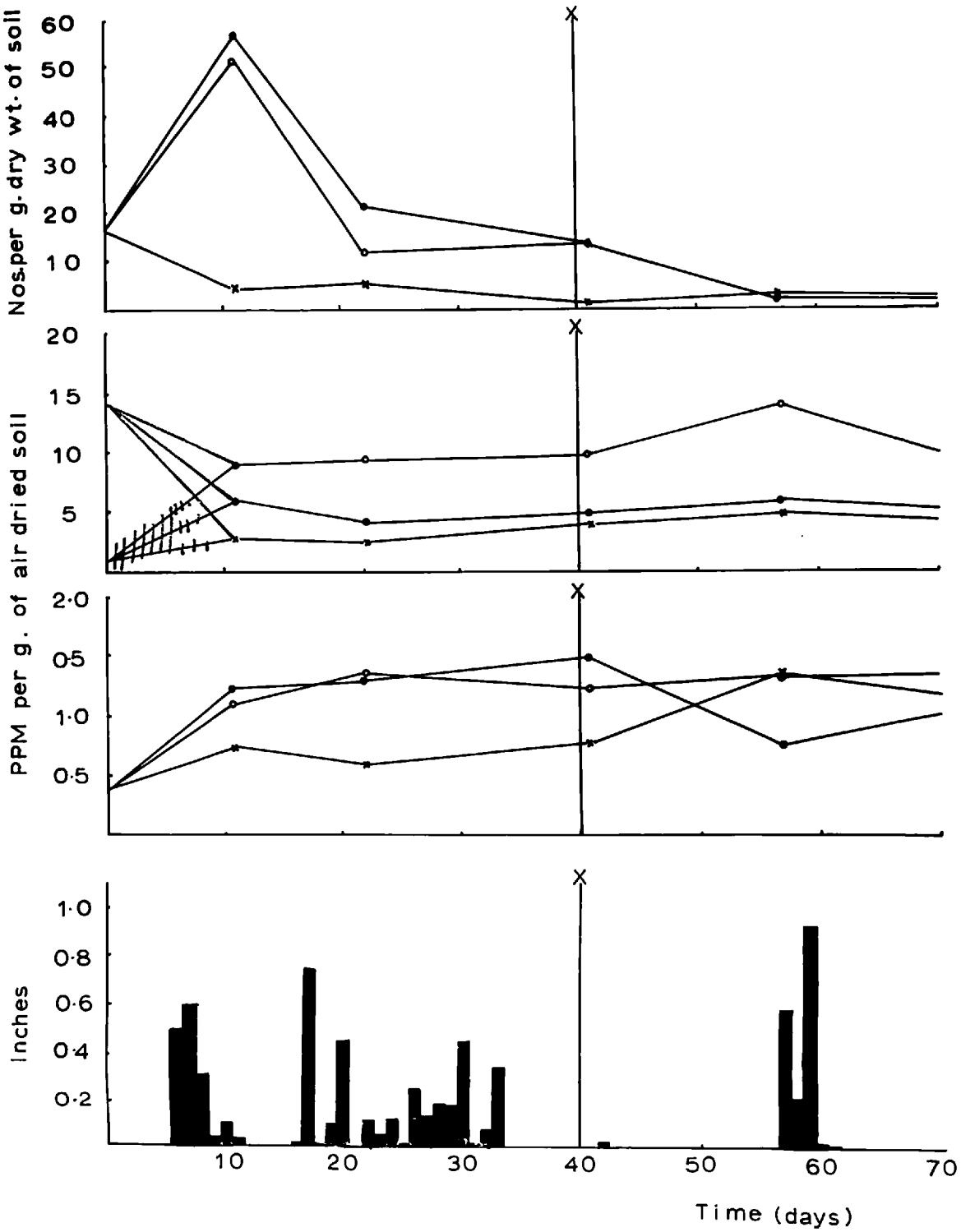
Form of manganese and treatment.		Days after planting						
		0	11	22	41	57	84	138
Soluble	A	0.55	0.75	0.6	0.75	1.35	1.0	0.35
	C		1.1	1.35	1.25	1.35	1.35	0.5
	D		1.25	1.3	1.5	0.75	1.3	0.4
Exchangeable	A	14.6	3.0	2.5	4.0	4.8	3.6	2.4
	C		9.0	9.8	14.0	5.7	9.3	3.2
	D		6.0	4.2	4.8	6.0	4.2	3.0

Results for changes in the numbers of soil microorganisms are given in Table 23 and in Figure 31. The latter also gives rainfall figures. These were obtained by taking

Table 23. Effect of grass meal on soil microflora

ORGANISM	CONC.	TREATMENT	DAYS 0	11	22	41	57	84	138
Bacteria	10^6	A	55.5	47.0	42.2	37.7	59.7	33.7	25.6
		C		592.6	1130.7	134.7	79.7	55.3	36.7
		D		706.3	2045.2	214.5	97.0	57.8	30.0
Actino-mycetes	10^6	A	13.0	11.8	6.2	4.0	7.8	7.3	4.4
		C		106.6	116.9	28.5	9.3	5.9	6.7
		D		115.2	108.3	34.9	11.9	7.6	8.6
Fungi	10^4	A	25.6	12.9	13.4	9.5	8.2	12.4	10.5
		C		57.0	38.2	47.9	23.1	17.4	9.2
		D		94.2	54.1	42.9	20.3	14.5	9.7
Manganese reducers	10^4	A	16.6	4.3	5.2	0.7	3.1	2.6	1.3
		C		53.3	11.9	13.7	2.5	1.9	1.8
		D		57.0	21.6	13.7	2.2	1.9	4.3
Pigment-producing actino-mycetes	10^5	A	21.0	5.0	29.0	13.0	4.4	11.0	8.8
		C		49.0	119.0	114.0	16.0	13.0	15.0
		D		74.0	72.0	45.0	45.0	22.0	22.0

Figure 31 Effect of grass meal on soil microorganisms



and soil manganese

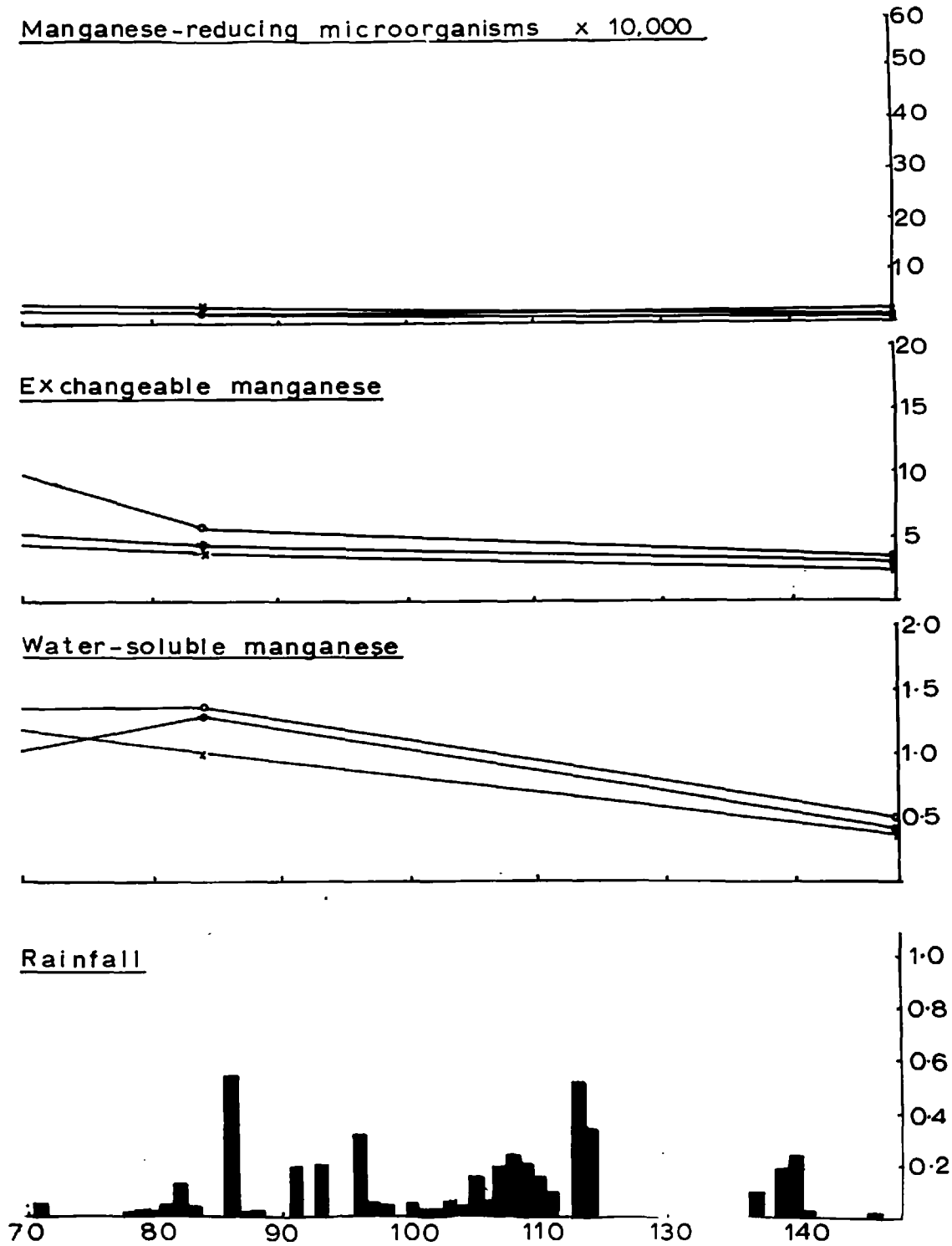
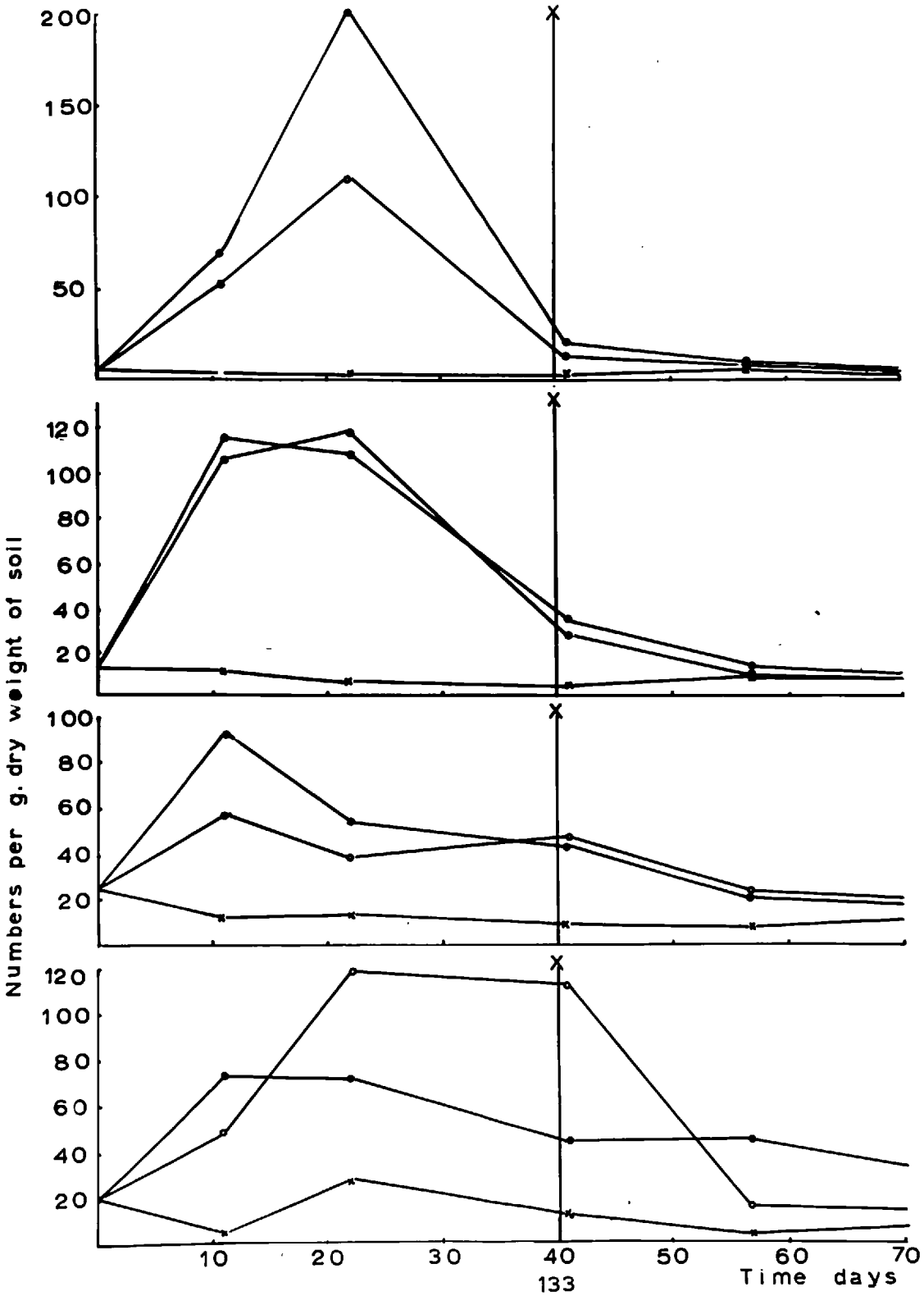
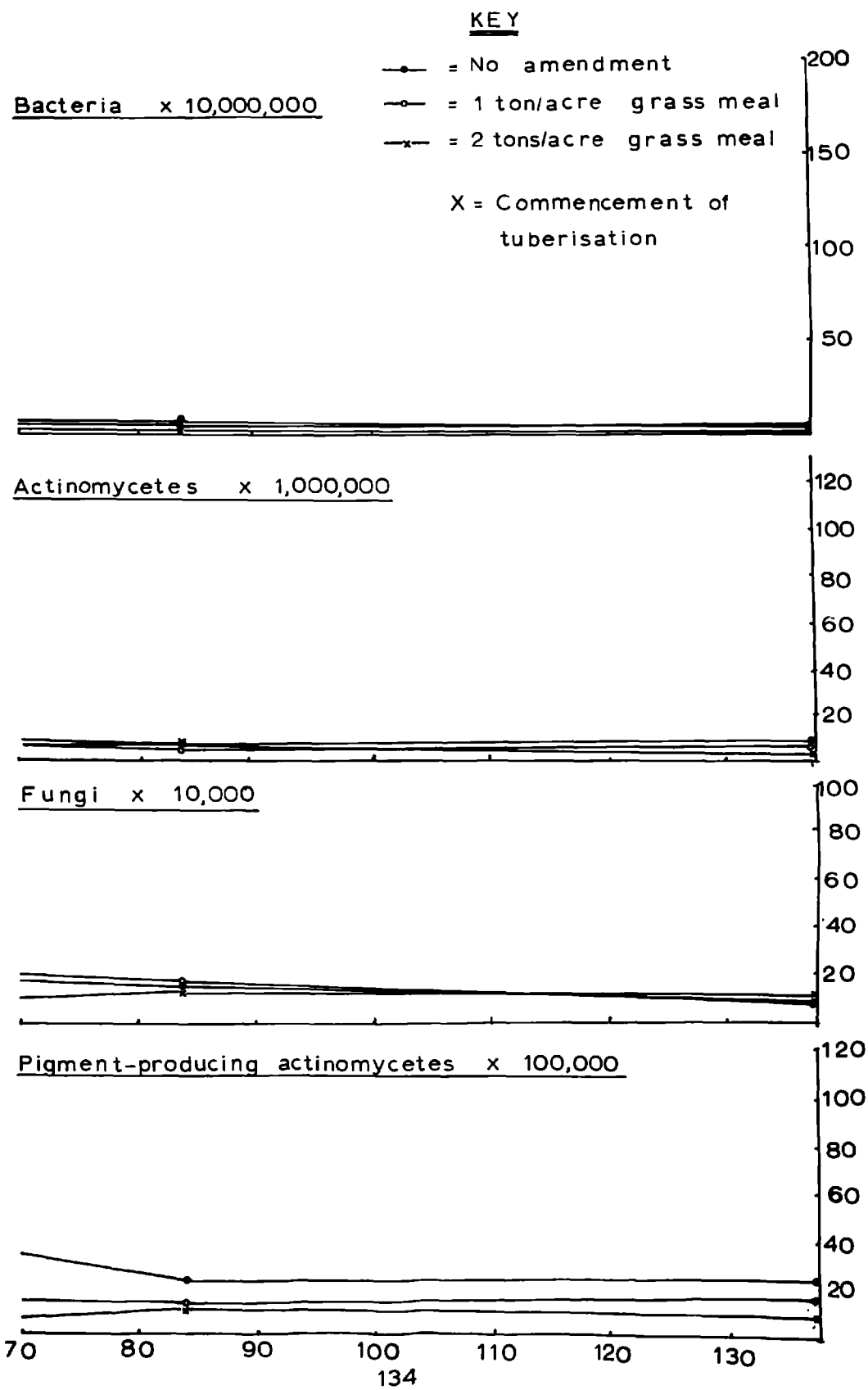


Figure 31 (contd.)




KEY
= No amendment
= 1 ton/acre grass meal
= 2 tons/acre grass meal
X = Commencement of tuberisation
Bacteria x 10,000,000
200
150
100
50
Actinomycetes x 1,000,000
120
100
80
60
40
20
Fungi x 10,000
100
80
60
40
20
Pigment-producing actinomycetes x 100,000
120
100
80
60
40
20
70
80
90
100
110
120
130

the mean of figures from three nearby meteorological stations. These were Wokinghm UDC to the north, Wellington College to the south-west and Broadmoor Hospital to the south-east. All three stations were within 1½ miles of the Crowthorne site and the figures should give a good indication of rainfall at the site since the surrounding area is not hilly and there should be little rain shadow effect.

In addition to estimating the concentrations of water-soluble and exchangeable manganese during the season, estimates were made of concentrations of easily-reducible and total manganese at the start of the season. The level of total manganese was 60 ppm and of this 20.4 ppm was in easily reducible form.

Table 24 gives the populations of microorganisms in amended soil as ratios to the populations in unamended soil.

Table 24. Effect of grass meal on soil microflora expressed as the ratio of populations to those of unamended soil. Grass meal at 1 ton/acre.

Organisms \ Days	11	22	41	57	84	138
Bacteria	11.3	26.8	3.6	1.3	1.6	1.4
Actinomycetes	9.0	18.8	7.1	1.2	0.8	1.5
Fungi	4.4	2.8	5.0	2.8	1.4	0.9
Manganese-reducers	12.4	2.3	19.5	0.8	0.7	1.4
Pigment-producers	9.8	4.1	8.8	3.6	1.2	1.7

Grass meal at 2 tons/acre

Organism \ Days	11	22	41	57	84	138
Bacteria	15.0	48.5	5.7	1.6	1.7	1.2
Actinomycetes	9.7	17.4	8.7	1.6	1.0	1.9
Fungi	7.3	4.0	4.5	2.5	1.2	0.9
Manganese-reducers	13.2	4.1	19.5	0.7	0.7	3.3
Pigment-producers	14.8	2.5	3.5	10.2	2.0	2.5

Tuberisation had commenced at the time of the fourth sampling after 41 days. At this time, approximately half of the stolons of a random selection of plants examined had tips swollen to twice the diameter of the stolon.

The dry period between June 3rd and 23rd occurred at the time of tuberisation and scab incidence increased to a higher level than in 1966 when early June had been wet, the levels of scab in untreated plots being 17.2 for 1967 compared with 10.8 for 1966.

Grass meal at 2 tons/acre decreased scab by an amount significant at a 1 per cent. level. At 1 ton/acre the grass meal had no significant effect on scab or average tuber weight but increased yield and tuber number by an amount significant at 2 and 1 per cent. levels respectively. The 2 tons/acre treatment increased yield and average tuber

weight by an amount significant at the 0.1 per cent. level and tuber numbers by an amount significant at the 1 per cent. level.

The grass meal led to an increase in numbers of all microorganism groups. The increase was greatest for bacteria but the populations later decreased faster than in any other group. In the bacterial, actinomycete and manganese-reducing groups, population levels had returned almost to normal after 57 days, numbers of fungi and pigment-producing actinomycetes remaining high for up to 84 days. Bacterial populations increased considerably more after 2 tons/acre amendment than after 1 ton/acre amendment. Actinomycete and manganese-reducer populations were stimulated almost equally by either level and the numbers of pigment-producing actinomycetes increased more at the lower level of amendment.

For fungi, the higher rate of application gave a larger increase but the increase only lasted about 40 days, both rates of application thereafter giving the same numbers of fungi. Numbers of both manganese-reducers and fungi reached a peak within 10 days and then dropped off.

The level of exchangeable manganese in unamended soil was 15 ppm at the time of planting but dropped sharply within 11 days to 3 ppm and remained at about this level for the rest of the season. In soil amended by an application

of 1 ton/acre grass meal, the level of exchangeable manganese remained at between 9 and 16 ppm for most of the growing season, about 6 to 9 ppm above that of the unamended soil.

The 2 tons/acre amendment had a much smaller effect, the levels of exchangeable manganese being only 1 to 3 ppm above that of unamended soil during the growing season. Both levels of grass meal caused an approximately 1 ppm rise in water-soluble manganese but this persisted for only 50 days.

With the start of tuberisation, 40 days after planting, bacteria, actinomycete and manganese-reducer populations had fallen considerably from the levels reached at 11 to 22 days. Fungal populations had dropped to a much smaller extent and numbers of pigment-producing actinomycetes were only just beginning to fall from their peak levels. Levels of exchangeable manganese did not begin to fall until at least 20 days after the start of tuberisation.

DISCUSSION

The aim of this work was to investigate the effect of organic amendment of soil on incidence of common scab disease in relation to soil manganese. The hypothesis has been that the organic amendment leads to an increase in reduction of insoluble tetravalent manganese to the divalent soluble form which in turn is toxic to *S.scabies* and brings about a decrease in scab.

Soil conditions which tend to allow an increase in manganese reduction are also unfavourable to scab. These are high organic content, high soil moisture content, acidity and addition of sulphur to soil. There is thus some circumstantial evidence for a relationship between amount of soluble manganese and incidence of scab.

This work has investigated only one of the factors, organic amendment, and although conclusive results were not obtained, a number of points may be made.

The soil on which the field trial was carried out had a total manganese content of 60 ppm. Of this, 15 ppm was water-soluble or exchangeable and a further 20 ppm was in an easily reducible form. Although Mortvedt (1960) has obtained control of scab in pot culture of potatoes with levels of 20 ppm of manganese added to the soil, the liquid culture

tests with S.scabies described on page 51 suggest that a total soluble manganese content of at least 30 ppm would be needed to control scab. On this basis, almost complete reduction of the easily reducible manganese in the Crowthorne soil would have been necessary for control of scab. That this did not happen is shown by the figures for manganese in soil samples taken during the growing season. The exchangeable manganese level in unamended soil remained at under 5 ppm for most of the growing season. The grass meal treatment at 1 ton/acre increased this by only 9 ppm but the 2 tons/acre treatment caused even less of an increase.

Since the decrease in scab was significant at the 2 tons/acre level but not at the 1 ton/acre level, it is clear that the increase in soluble manganese brought about by the grass meal was not responsible for the decrease in scab. Numbers of manganese-reducing organisms decreased after the initially large increase in numbers recorded 11 days after soil treatment and since there was an increase in reduced manganese much later on, non-biological reduction of manganese must have been significant.

These results do not make it necessary to abandon the idea that organic amendment prevents scab by the mechanism of manganese reduction. In the case of this field trial, the level of easily reducible manganese in the soil was too

low for there to be sufficient manganese reduction to affect scab.

An unexplained results is why grass meal at 1 ton/acre should have more effect on manganese reduction than at 2 tons /acre. It is known that organic amendment will increase numbers of organisms capable of oxidising manganese as well as those capable of reducing it. Whether the oxidisers or the reducers are favoured by organic amendment will depend on soil pH, and possibly soil moisture content. Thus if an acidic soil of about pH 5.0 is amended, reducers will increase whereas in a more alkaline soil oxidisers will increase. The soil at Crowthorne had a pH of 6.0.

The 2 tons/acre amendment caused a slightly larger increase in numbers of manganese reducers than the 1 ton/ acre amendment, yet there was less manganese reduction. This suggests that manganese oxidisers increased in numbers to a greater extent in the 2 tons/acre treatment than in the 1 ton/acre treatment. To get sufficient data to explain the results of this trial, it would have been necessary to have made more frequent estimates of microflora populations and to have estimated numbers of manganese oxidisers as well as reducers.

The field trial gave two interesting results.

1. Applications of dried grass meal increased the level of

divalent manganese in the soil. It is possible that in some soils, this increase would be sufficient to control scab.

2. Scab was significantly reduced by the 2 tons/acre application of grass meal. Competition of other organisms with the scab organism may not be the only reason for this, for, by the time tuberisation had begun, populations of bacteria and actinomycetes had fallen considerably from the very high levels attained following soil amendment. Even so, the populations were still considerably higher than in unamended soil. Competition may therefore be important, especially if this is viewed in terms of competition for the infection sites, lenticels, of developing tubers rather than in terms of general competition in the soil.

GENERAL DISCUSSION

Lapwood, Lewis and others have shown that scab may be effectively controlled by irrigating the potato crop at, and immediately after tuber initiation. The degree to which this method of control can be applied in England and Wales is not clear at present. Potatoes respond very well to irrigation but it is seldom undertaken until about a month after tuberisation has begun, July and August being the important months as far as increasing yield by irrigation is concerned. In fact, in some varieties, irrigation before this time results in an increase in haulm and decrease in yield (MAFF, 1962), a definate drawback as far as scab control by irrigation is concerned.

According to the report published by the Office of the Minister for Science (1962), the practical limit of irrigation development in England and Wales would be of the order of 48 per cent. of the total acreage of maincrop potatoes. An accurate picture of scab incidence is not clear but the published results of Large and Honey (1955) suggested that scab was worse in parts of Yorkshire and the East and West Midlands. These are areas which are amongst those most likely to be irrigated in the future.

The acreage of potatoes at present irrigated is far below the 48 per cent. which could be irrigated. Actual figures are not known but the maincrop survey conducted by the Potato Marketing Board in 1963 covered 905 farms totalling 545,000 acres and according to this, between 5 and 10 per cent. of the farms had irrigation facilities. The actual percentage area of potatoes irrigated may be lower than this since the 1965-66 Quality Assessment Survey states that only 3 per cent. of the 568 farms surveyed irrigated in this period.

These figures suggest that irrigation is unlikely to provide a general answer to scab in the near future. The other method for scab control now available is treatment with manganese sulphate, but this will only work on soils in which the manganese is not liable to oxidation.

The present work has shown that other forms of manganese such as manganese chelates are unlikely to be of any more value than manganese sulphate. Other minor elements do not, with one exception, hold out much promise, the exception being copper, particularly if applied as a frit.

Organic amendment of soil for control of scab provides some interesting results in relation to soil manganese, but the practical potantial for this line of work in scab control

is difficult to assess at present.

It is not known what acreage of potatoes subject to scab could be successfully treated by irrigation or manganese sulphate. If a survey could be conducted which would show this then it would be possible to determine the potential value of copper or organic amendments for scab control.

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APPENDIX 1

SOUTH OF ENGLAND FIELD TRIALS 1966

Silwood Park

Analysis of Variance:- Emergence

The data in the form of percentage emergence was first transformed to angles.

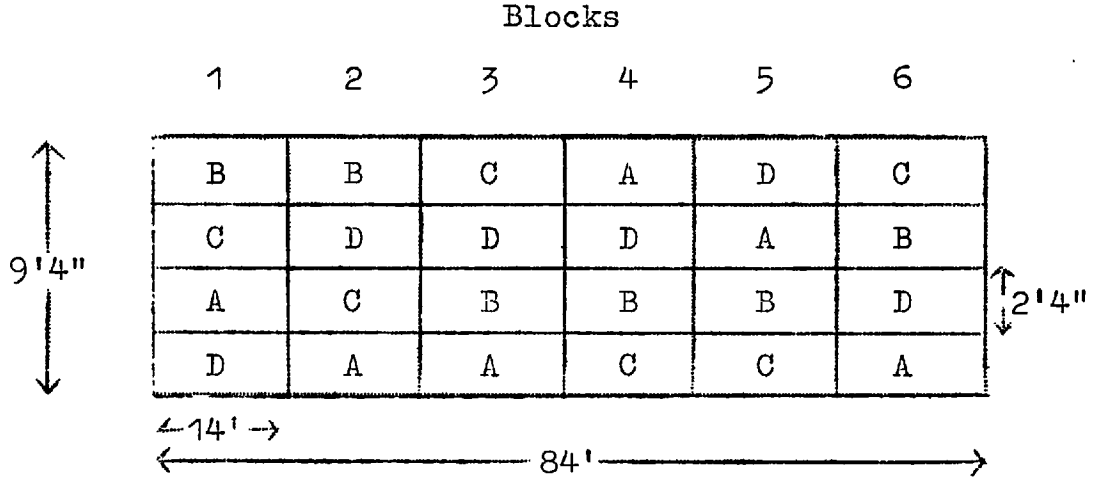
Source of Variation	Sum of Squares	°F	Variance	Variance ratio	F(Tables) 20·5	1.0	0.1
Total	10,139.1	23					
Blocks	7,942.5	5	1,588.5	14.26	1.7	2.9	4.6	7.6
Treatments	526.1	3	175.4	1.57	1.8			
Error	1,670.5	15	111.4					

Blocks significant at 0.1 per cent. level.

Treatments not significant.

Binstead

Layout



Analysis of variance:- Yield

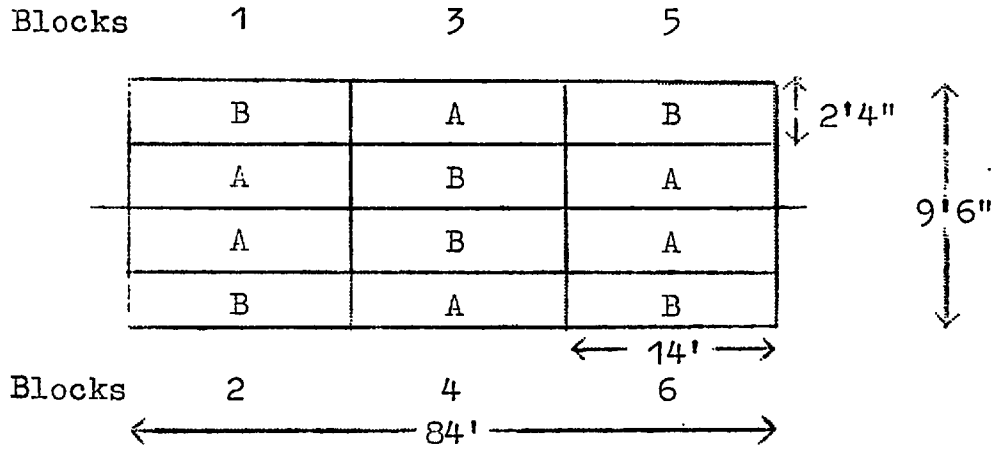
Source of variation	Sum of Squares	°F	Variance	Variance ratio	F (Tables) 20	5	1.0	0.1
Total	178,945,866	23						
Blocks	63,486,824	5	12,697,364	2.02	1.7	2.9		
Treatments	21,293,406	3	7,097,802	1.13	1.8			
Error	94,165,636	15	6,277,709					

Blocks significant at 20 per cent. level.

Treatments not significant.

Crowthorne

Layout



Analysis of variance:- Scab incidence

Source of Variation	Sum of Squares	°F	Variance	Variance ratio	F (Tables) 20	5	1	0.1
Total	59.61	11						
Blocks	33.51	5	6.70	1.39	2.2			
Treatments	2.00	1	2.00	0.414	2.2			
Error	24.10	5	4.82					

Blocks not significant

Treatments not significant

Crowthorne

Analysis of variance:- Yield

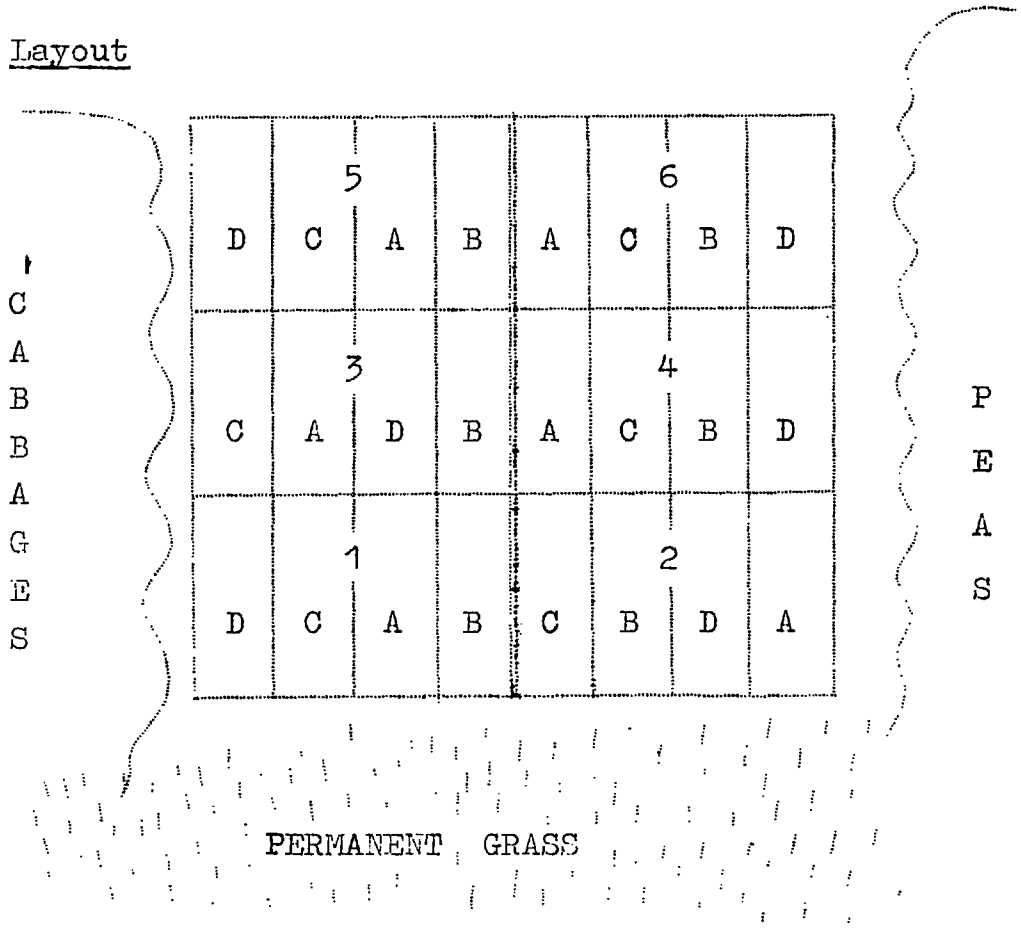
Source of Variation	Sum of Squares	°F	Variance	Variance ratio	F (Tables) 20	5	1.0	0.1
Total	5,298,270	11						
Blocks	1,308,876	5	261,775	0.451	2.2			
Treatments	1,090,740	1	1,090,740	1.88	2.2			
Error	2,898,654	5	579,731					

Blocks not significant

Treatments not significant

Field trial 1967: Crowthorne

Layout



Teatments:

A No treatment

B Manganese frit - 50 lb./acre

C Grass meal - 1 ton/acre

D Grass meal - 2 tons/acre

Field trial 1967: Crowthorne

Analysis of variance 1) Scab Assessment

Source of Variation	Sum of Squares	^{o}F	Variance	Variance ratio	F(Tables) 20	5	1
Total	156.316	23					
Blocks	13.925	5	2.8785	0.423	1.7		
Treatments	43.569	3	14.5230	2.204	1.8	3.3	
Error	98.822	15	6.5881				

Blocks not significant

Treatments significant at 20 per cent. level

$$LSD = T\sqrt{\frac{Ve}{n}}$$

$$= T \times 1.04$$

Thus LSD 0.1% 4.23

1.0% 3.06

2.0% 2.70

20.0% 1.39

D - A = -3.103 significant at 1 per cent.

C - A = -1.403 significant at 20 per cent.

B - A = 0.29 not significant.

Field Trial 1967: Crowthorne

Analysis of Variance . 2) Yield

Source of Variation	Sum of Squares	°F	Variance	Variance ratio	F (Tables) 20	5	1	0.1
Total	81,874,410	23						
Blocks	19,156,121	5	3,831,122	1.93	1.7			
Treatments	33,039,447	3	11,013,149	5.56	1.8	3.3	5.4	9.3
Error	29,687,842	15	1,978,589					

Blocks significant at 20 per cent. level

Treatments significant at 1 per cent. level

$$LSD = T\sqrt{\frac{Ve}{n}}$$

$$= T \times 573.8$$

Thus LSD at 0.1% 2,337

1.0% 1,694

2.0% 1,493

20.0% 969

D - A = 3,226.3 significant at 0.1 per cent.

C - A = 1,594.8 significant at 2.0 per cent.

B - A = 936.3 significant at c. 20 per cent.

Field Trial 1967: Crowthorne

Analysis of Variance 3) Average tuber weights

Source of Variation	Sum of Squares	°F	Variance	Variance ratio	F (Tables) 20	5	1	0.1
Total	5,519.86	23						
Blocks	1,429.94	5	285.98	1.88	1.7			
Treatments	1,816.26	3	605.42	3.99	1.8	3.3	5.4	9.3
Error	2,273.66	15	151.57					

Blocks significant at 20 per cent. level

Treatments significant at 5 per cent. level

$$LSD = T.\sqrt{\frac{Ve}{n}}$$

$$= T \times 5.03$$

Thus LSD at 0.1% 20.48

1.0% 14.84

20.0% 6.74

D - A = 22.1 significant at 0.1 per cent.

C - A = 1.80 not significant

B - A = 3.05 not significant

Field Trial 1967: Crowthorne

Analysis of Variance 4) Tuber number

Source of Variation	Sum of squares	°F	Variance	Variance ratio	F (Tables) 20	5	1	0.1
Total	9,074	23						
Blocks	1,759		351	1.39	1.7			
Treatments	3,526	3	1,175	4.66	1.8	3.3	5.4	9.3
Error	3,789	15	252					

Blocks not significant

Treatments significant at 5 per cent. level

$LSD = T.\sqrt{\frac{Ve}{n}}$

$= T.x\ 6.48$

Thus LSD at 0.1% 26.4

1.0% 19.1

2.0% 16.8

5.0% 13.8

10.0% 11.3

D - A = 25.2 significant at 1.0 per cent.

C - A = 32.3 significant at 0.1 per cent.

B - A = 15.3 significant at 5 per cent.

APPENDIX 2.

SCOTTISH FIELD TRIALS: 1966

1st trial

Centre:	Linkwood Farm, Elgin, Morayshire.
Treatments:	C = Control - no manganese
	I = Inorganic manganese - $MnSO_4$ at 56 lb/acre
	CH = Manganese chelate ($Na_2MnEDTA$) at 70 lb/acre
	F = Manganese frit at 25 lb/acre
Experimental design:	Randomised block: 5 blocks
Size of plots:	15 yards x 4 drills.
Soil:	Light sand.
Manuring:	10cwt./acre 12 : 10 : 18.
Previous crop:	Lea
Sowing date:	April 19th 1966.
Results:	There was no scab present above a trace when the tubers were harvested and no scab assessment was made.

2nd trial

Centre:	Sanquhar Mains, Forres, Morayshire.
Treatments:	C = Control - no manganese
	I = Inorganic manganese - $MnSO_4$ at 56 lb/acre
	CH = Chelated manganese - $Na_2MnEDTA$ at 70 lb/acre
	F = Manganese frit at 25 lb/acre
Experimental design:	Randomised blocks: 5 blocks.
Size of plots:	15 yards x 4 drills.
Soil:	Sandy loam.
Sowing date:	April 23rd 1966.
Results:	The layout is given in Table A and this includes the scab scores for each plot. The mean scab score is given in Table B and an analysis of variance was done on the scab scores. Although there was less scab on tubers grown in plots treated with the manganese frit, this decrease in scab was not significant

Table A. Plot layout and scab scores - Sanquhar Mains

BLOCKS					
B	1	I 1.74	F 1.74	C 1.74	CH 1.78
L	2	CH 2.06	C 1.56	F 1.20	I 1.76
O	3	F 2.16	I 1.70	CH 1.84	C 2.18
C	4	I 1.36	F 1.64	CH 1.50	C 1.74
K	5	I 2.86	F 1.48	C 2.18	CH 3.52
S					

Table B. Mean scab scores per treatment

Treatment	C	F	CH	I
Scab score	1.88	1.62	2.14	1.82

Analysis of Variance: Scab score

Source of Variation	Sum of squares	°F	Variance	Variance ratio	F (Table) 20	5	1
Total	5.2739	19					
Blocks	2.3172	4	0.5793	2.736	1.8	3.3	
Treatments	0.4156	3	0.1385	0.0654	1.8		
Error	2.5411	12	0.2177				

Blocks signicifant at 5 per cent level.

Treatments not significant.

APPENDIX 3.

Probit transformation of liquid culture data

Varipus chemicals were tested for their effect on S.scabies in liquid culture and in a number of cases, the data obtained made it possible to determine the concentrations which inhibited growth of S.scabies by 50 per cent. To do this, yields at the concentrations tested were expressed as percentage inhibition and transformed to probits and plotted against log dose, regression lines then being calculated. The regression lines for metals are given in Figure A and for chelated metals, chelates, frits and maneb in Figure B. Table C gives levels of the chemicals tested which gave 50 per cent. inhibition:-

Metal	PPM giving 50% inhibition	Compound	PPM giving 50% inhibition
Silver	7.86	Copper frit	68.4
Cobalt	29.50	Maneb	50.2
Mercury	16.00	$Na_2MnEDTA$	7.8
Antimony	15.20	Na_4EDTA	9.3
Copper	6.61	$Na_2MnDTPA$	3.8
Manganese	38.00	Na_5DTPA	13.8
Cadmium	3.15	$Na_2AlEDTA$	10.5
Arsenic	6.2		

Figure A Effect of metals on growth of S. scabies

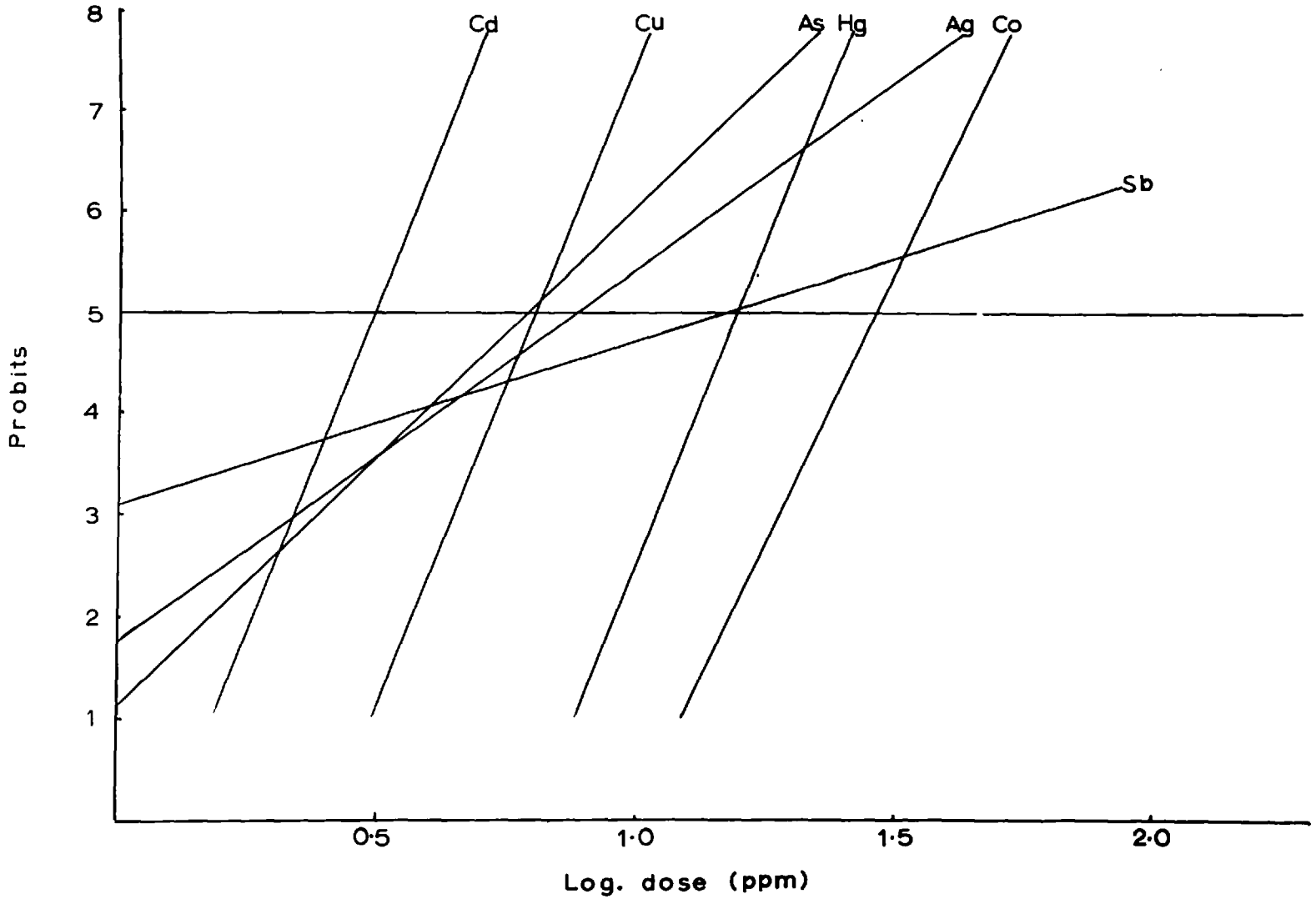
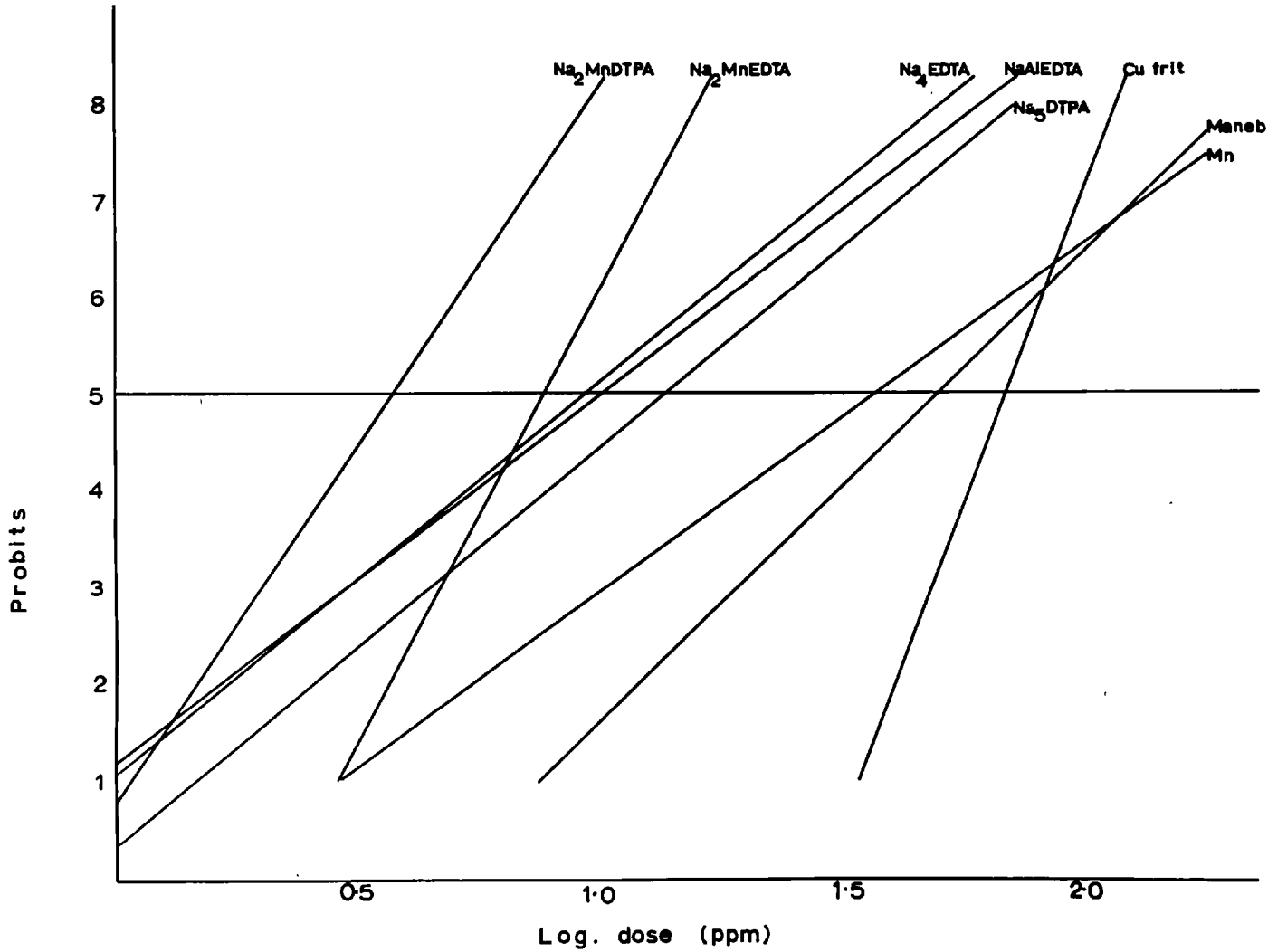


Figure B Effect of chelated metals, chelates, copper frit, manganese and maneb on growth of S. scabies



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CORRECTIONS AND ADDITIONS