SOIL COPPER IN RELATION TO CEREAL CROPS

by

E. Jean Mackenzie

Thesis presented for the Degree of
Doctor of Philosophy of the University of Edinburgh
in the Faculty of Science.

February 1966

Plate 1. Withertip in oats, Hexpath 1962.

Plate 2. Young copper deficient oat plant.

Abbreviations and Symbols

E.D.T.A.	Ethylene diamine tetra acetic acid.
L.S.D.	Lowest significant difference between two means.
p.p.m.	parts per million.
*	significant at $P < 0.05$.
**	significant at $P < 0.01$.

INTRODUCTION

When plant tissues are analysed chemically, it is often found that carbon, hydrogen and oxygen together make up about 90% of the weight of the living material. Most of the remaining 10% is accounted for by the "major" elements absorbed from the soil: nitrogen, phosphorus, potassium, magnesium, calcium and sulphur. Plant material has also, however, been shown to contain very many of the other elements at concentrations lower than 1%. These are described as "trace elements", and are usually present in such small proportions that, where it is possible to determine them quantitatively, their concentrations are expressed in parts per million of dry matter more conveniently than in percentages. Of the trace elements, some, such as tin and aluminium have no known function in the plant, others, such as sodium, chlorine and silicon have been shown to be beneficial to growth in some cases, and a few, the "micro nutrients", have been proved to be essential for the normal life cycle of plants. As techniques improve more elements may also be proved indispensable, but, at present, the recognised micro nutrients are: iron, manganese, copper, boron, zinc and molybdenum. Tiny quantities of these micro nutrients are essential to plants because they are required for the activation of some enzyme systems, but larger concentrations are usually toxic. If a micro nutrient is present in excess, the normal delicate equilibria of the metabolic reactions in which the element takes part are upset, and other enzyme systems may be inhibited by the replacement of the usual activator.

Copper was first used in agriculture not as a micro nutrient, but as a fungicide and a weed killer. In some parts of the world, however, it has also been used to overcome copper deficiency conditions in plants and animals. Thus in Australia, "falling" disease of cattle, and enzootic ataxia and "straight wool" in sheep, have been prevented by suitable doses of copper. In Britain, swayback of lambs has been prevented by giving supplementary copper to the ewes during pregnancy, and molybdenum induced scouring on "teart" pastures has been cured by increasing the copper intake of affected animals.

About forty years ago some plants in certain areas of Europe and America were found to benefit from copper application more than could be accounted for by fungicidal effects. Among the many crops found to respond to applied copper on certain soils were; citrus trees, apple trees, peas, tomatoes, onions, subterranean clover, and the cereals, wheat, barley and oats. The copper deficiency diseases which have been cured are exanthema in fruit trees and the condition of cereals known variously as reclamation disease, heathermoor disease, white tip, withertip or blind ear. These names were first used to describe a disorder which was seen in oats on reclaimed moorland in Holland and northern Germany, and was characterised by white twisted leaf tips and by low grain yields. At first it was thought that there was a toxic substance in peaty soils which caused the disease and which was neutralised by copper. After copper had been proved essential for the life cycle of green plants by Sommer (1931) and Lipman and Mackinney (1931), however, and this disorder had been produced, not only on peaty soils, but also on sands and in pure aqueous cultures, it was recognised as a copper deficiency condition. Thus the fact that some humus compounds can cause the disease, is now generally assumed to be due, not to their toxicity, but to their ability to render copper unavailable to plants. Reclamation disease has since been reported in many parts of the world, and the losses of yield in oats, barley and wheat caused by it make the disorder and its prevention and cure economically important in affected areas.

REVIEW OF THE LITERATURE

COPPER NUTRITION OF CEREALS

(1) Soil Copper

(a) Soil Type

Goldschmidt (1954) estimated that there are approximately 70 p.p.m. copper on average in the whole of the earth's crust. It is very unevenly distributed, however, and acid igneous rocks and granites contain less than basalts. Copper is usually found as a sulphide or mixed sulphide with iron, but it is very readily complexed with hydroxyl, carbonate or organic radicles, and may replace other ions in the silicate and ferromagnesian crystal lattices. It is comparatively easily leached away from arenaceous sedimentary rocks, but is often deposited in the slates and shales laid down under reducing conditions. The oxidation of the sulphide minerals in rocks takes place most readily when the climate is hot and dry. The products of the reaction are more soluble compounds which are washed away if the environment becomes wetter, leaving particularly deficient sandstone deposits. During soil formation, detritus from several varying rock types may mix to give normal copper contents, or further extreme weathering such as carriage by water or ice may leave a very deficient soil. Thus the type of soil formed, which is partly governed by its derivation and partly by climate, drainage and the flora it supports, is correlated with its total copper content. (Wells 1957, Coppenet and Calvez 1955). Copper deficiency is therefore usually found on specific types of soil, and has been reported on fluvio-glacial soils derived from sandstones in Scotland (Mitchell, Reith and Johnston 1957(1), Purves and Ragg 1962), on sandy soils in Sweden (Lundblad, Svanberg and Ekman 1949) and South Africa (Perold 1951), on calcareous blown sands in South Australia (Piper 1942(1)), and on granitic soils in Brittany (Coppenet and Calvez 1955).

Biological, as well as mechanical and chemical activity may influence the distribution of some elements in the soil. Goldschmidt (1954) has stated, "Copper may concentrate in forest litter and the uppermost layers of forest humus if the tendency is not counteracted by leaching", while Mitchell (1955) has reported that under Scottish moorland conditions in uncultivated soils, copper is among the elements with which the surface horizons are enriched. During the reclamation of heathland in North West Europe, however, these natural conditions are radically changed through cultivation, drainage, liming and fertilization. For one or two seasons the surface concentration of micro nutrients, such as copper, may be sufficient for the production of crops, but these reserves are soon liable to be exhausted. Thus copper deficiency in cereals has been reported frequently from the reclaimed podzolised peaty moorland soils of North Germany, Holland and Denmark. The soil type producing crops showing the most severe symptoms has been generally a sandy humus soil, "black sand", which consists of a mixture of quartz crystals and amorphous humic compounds. (Steenbjerg 1940, Hoffmann 1952). Copper deficiency diseases in plants have also been seen on deep peat soils in U.S.A. (Harris 1948), reclaimed rendzina soils in England (Davies 1962), peat soils in Russia (Ivanov 1950), and bog peat soils in Ireland (Fleming and Delaney 1961), Norway (Sorteberg 1962) and England (Batey 1961). Some arable land in Europe has been cultivated so intensively that the low original content of copper has been depleted to a deficient level. (Fritzsch 1958, Henkens 1957).

(b) Subsoil

The foregoing remarks on the derivation of the soil apply equally to top soils and subsoils, which may be rich or poor in copper according to the minerals they contain, and the conditions of weathering and soil formation which they have undergone. The effect of cultivation will, however, be less on the subsoil, which may form a useful reservoir of copper in regions where the removal of heavy crops has depleted the surface supplies of the element. In other areas, however, the subsoil may contain less copper than the top soil, because of enrichment of the surface layer by the Goldschmidt concentration effect, or as a result of top dressings of copper containing fertilizers or pesticides.

(c) Copper Availability

The total copper content of the soil to plough depth is of primary importance in problems of copper deficiency in plants (Lundblad, Svanberg and Ekman 1949, Scharrer and Schaumloffel 1960) because, if it is at the extremely low level of 0 - 1 p.p.m., healthy growth in susceptible plants is unlikely. Copper availability can also have an effect, however, for it has been reported that the total copper level in soils associated with copper deficiency problems is occasionally higher than that in other soils producing healthy crops. (Hoffmann 1952, Piper 1942(1), Leeper 1951, Steenbjerg 1940, Gilbert 1957). Thus, although the total copper content of a soil has been found to be correlated with the copper concentration in plants growing on it, (Wells 1957, Mitchell Reith and Johnston 1957(1)), many workers have attempted to assess the level of available copper in soil. Methods involving the analysis of plants, (Piper and Walkley 1943), the appearance and growth of the micro organism Aspergillus Niger (Mulder 1950), or the analysis of chemical extracts, (Cheng and Bray 1953), have been employed for this purpose. E.D.T.A., which is among the many chemical reagents which have been tried as soil extractants, has been used frequently in recent years. "E.D.T.A. should extract at least a portion of the organically complexed copper as well as the colloidally bound fraction." (Mitchell Reith and Johnston 1957(1)).

The availability of the copper in a soil depends to a large extent on how it is linked with soil compounds. Roots are unlikely, for example, to be able to assimilate much of the copper from intact mineral crystal lattices, while, on the other hand, any of the metal in the soil solution would normally be easily absorbed. Mitchell Reith and Johnston (1957(1)) have found very little copper in aqueous soil extracts, and so the concentration in the soil solution is probably usually very low. Although the level of copper in solution is likely to be maintained because of an equilibrium between free ions and those adsorbed on soil surfaces, it seems probable that, in normal soils, plants are also able to utilise some of the other copper present. It has been shown that barley can take up the metal in a chelate molecule (Beringer 1963) and that some of the copper complexed with the organic fraction of the soil is available to oats (Ennis and Brogan 1961). Other likely sources of supply are the ions adsorbed on, or complexed with, clay minerals (Leeper 1952).

It has been found that the ratio of available to total copper varies from soil to soil. (Mitchell Reith and Johnston 1957(1), Henkens 1961). The causes of some of this variation have been examined because, when a soil contains about 1 - 5 p.p.m. copper, availability of the metal may determine whether a crop is healthy, slightly stunted, or severely diseased.

(d) Conditions Influencing Copper Availability

(i) Humus

Vermaat and Van der Bie (1950) have shown that, among the tropical soil types they examined, the fraction of the total copper which was easily extracted chemically was higher in those soils containing little organic matter. It has also been reported that, in peat soils, much of the total copper present is often rendered unavailable by humus fixation (Leeper 1951, Scharrer and Schaumloffel 1960, Steenbjerg and Boken 1950, Hoffmann 1952), and the figures of Lundblad Svanberg and Ekman (1949), if converted to p.p.m. copper in soil, demonstrate that, in general, copper in peat soils is less available to plants than that in mineral soils. This binding of copper to humic compounds has been demonstrated chemically (Dawson and Nair 1950, Lees 1950) and physiologically, in pot experiments using copper treated humic acid (Ennis and Brogan 1961). Humus extracts from various soils have been shown to bind copper to an extent characteristic of the soil (Martin and Lavollay 1950), and Hoffmann (1952) has reported that only fixation to certain kinds of humus causes unavailability of copper. Hoffmann (1952) has also shown that the addition of small quantities of humic acid to healthy cultures of oats in sand causes reclamation disease. Recently, Sorteberg (1962) has grown healthy oats in pots of unclaimed peat, and oats showing copper deficiency symptoms in pots of the same virgin peat with the addition of small amounts of reclaimed peat soil. Perhaps the processes of reclamation may be accompanied by the development of different humus compounds which bind the depleted copper more tightly, or by the emergence of a different bacterial population which competes more successfully for the copper with the plants.

(ii) Soil pH.

The effect of soil reaction on copper availability is not so marked as is the case with manganese, and conflicting results have been reported. Increasing acidity below pH 5.5 has been found to give rising availability of copper. (Steenbjerg 1940, Scharrer and Schaumloffel 1960, Piper 1942(1)). This greater mobility is not always advantageous, however, because it leads to more rapid leaching, which can cause depletion of low reserves in the top soil. (Peech 1941, Lundblad Svanberg and Ekman 1949). At pH levels between 5.5 and 6.5, copper availability has been found by Steenbjerg (1940) to be at a minimum, and it has also been reported that less of the metal is taken up by plants at pH 6.0 than at pH 5.4 or 6.4. (Mitchell Reith and Johnston 1957(1) and (2)). As the alkalinity of the soil rises above pH 6.5, fixation of copper has been observed by Steenbjerg (1940) to decrease, by Scharrer and Schaumloffel (1960) to increase, and by Piper (1942(1)) to remain fairly constant. No general correlation between copper uptake and soil reaction has been found by Lundblad Svanberg and Ekman (1949), Piper and Beckwith (1951), Wells (1957), Blevins and Massey (1959), Pack Toth and Bear (1953), or Williams and Moore (1952). Soil pH, therefore, although of great importance as part of the plant environment, does not have a consistent influence on copper availability.

(iii) Soil Bacteria

Mulder (1950) has shown that copper is necessary for the efficient functioning of at least some micro organisms and that "copper precipitated by hydrogen sulphide producing bacteria is unavailable to both Aspergillus Niger and higher plants". On the other hand, the copper in mineral copper sulphide has been stated to be available to plants (Mitchell 1955). It thus seems that micro organisms and plants are in competition for the copper in the soil, and that some organisms can render the metal unavailable to plants. It has been shown (Piper 1942(1), Steenbjerg 1940) that partial sterilisation of the soil increases copper uptake by plants, and a similar effect has been observed in the field when healthy crops are grown after a fire (Piper 1942 (1)). This phenomenon in peat soils has been attributed by Hoffmann (1952) to a change in the structure of humic colloids during heating, but Sorteberg (1962) has demonstrated that formaldehyde and heat treatments both reduced copper fixation to a similar extent.

(iv) Previous Crop

On copper deficient land, the crop grown before cereals are sown may have a big effect on the cereal yield, even apart from such obvious enrichment of the soil copper content as will result from the spraying of potatoes with copper containing fungicide. Mitchell Reith and Johnston (1957(1)) have found that oats are more likely to show the symptoms of copper deficiency when they follow turnips than when they come after potatoes. These authors demonstrated that, although no copper had been applied to either preceding crop, and there was no difference in the total copper content of the soil after the two crops had been harvested, the availability after potatoes, as measured by an E.D.T.A. extraction, was over double that after turnips. It has been suggested (Hoffmann 1952) that aeration of the soil mobilises copper, and so it may be that the greater soil cultivation necessary with potatoes, and their more extensive root system, render the copper more available.

It is common experience in South East Scotland that in an agricultural rotation, a cereal following ley is less likely to develop reclamation disease than one following any other crop, (Buckpitt and Purves 1960), and Steenbjerg (1940) has demonstrated that soil under long established lawn contains more copper than fallow soil. This enrichment of the top soil may be the result of copper being extracted by the grass roots and deposited in the surface litter. Under ley this process would continue undisturbed for three or four years, giving enough time for an appreciable effect.

(v) Climate

The climate of an area, the weather in any season, and the microclimate round any individual plant, greatly influence its growth and development. When reports of the effects of weather conditions on a crop are compared, however, the great differences between the normal climates in various countries must be taken into account. A "dry" season in Ireland, for example, might have more rainfall than a "wet" season in Western Australia, while a "hot" summer in Norway might seem cool in Ohio. Climatic conditions in various areas also determine winter or spring sowing practice, lengths of growing seasons, and varieties planted, and these factors may mask any direct relationship which might exist between the weather and the ability of the plant to utilise copper.

It has, however, been remarked that reclamation disease is not observed to the same extent in cool wet summers as in hot dry ones, (Piper 1942(1), Hoffmann 1952, Lucas 1945, Harmer 1945), and Steenbjerg (1940) has observed that, in

Denmark, severe copper deficiency in oats occurs in those years when the early summer is dry and the later summer wet and warm. It has been shown that, in general, the best yields of healthy oats are produced in years when there is a warm dry spring and early summer, followed by a wet spell about the beginning of July. (Roberts 1928). Thus it seems, according to Steenbjerg, that reclamation disease is worst in those years when the best yields of oats are expected. Hallsworth, Greenwood and Auden (1957) have stated that deficiency symptoms are more marked when plants are growing vigorously.

Weather is the condition of the atmosphere with regard to temperature, light, humidity and wind. Although wind damage to young cereals may make the symptom of "withertip" more difficult to recognise, no reference to any specific effect of wind on copper deficient plants has been seen. The tendency for copper deficiency to be worse in hot dry summers might, however, be the result of the influences of stronger light, higher temperatures or less moisture working in conjunction or separately.

It has been shown in a pot experiment that the symptoms of reclamation disease in oats appear sooner, and at an earlier stage of growth, under spring daylight conditions than under less intense artificial illumination during the winter. (Hagin 1960). Strong light should enable plants to outgrow copper reserves more quickly than is possible under dull conditions. In the field, intensity of illumination has not, however, been reported to affect the severity of copper deficiency symptoms, unlike those of zinc deficiency. (Trumble 1951). It has been reported that day length can influence the appearance of symptoms in clover plants (Hallsworth, Greenwood and Auden 1957) or their response to copper (Ferres 1951). Copper deficient and healthy oats in the field have been shown to exhibit some of the anatomical differences usually found between shade and full light plants respectively (Ford 1940).

Piper (1942(1)) and Wood and Womersley (1946) have suggested that copper is more available to plants at higher temperatures, but Ferres (1951) has found that a decrease in the summer soil temperature causes no change in the response of clover to copper.

There are many reports on the subject of soil moisture in relation to copper deficiency. Hoffmann (1952) has stressed that, in a dry season, copper deficiency diseases in cereals are seen in the field, while very few symptoms are noticed in wet years, and yet in pot experiments on soils containing little copper, the symptoms appear however profusely the pots are watered. The results of a pot experiment performed by Piper (1942(1)) on oats in a greenhouse substantiated these views, in that the degree of saturation of the soil with water between 45% and 90% appeared to have very little effect on the onset of copper deficiency symptoms. In a pot experiment in the open air on barley, however, Steenbjerg and Boken (1949) found that a wet season, when the soil was at more than 40% moisture capacity, resulted in much less severe symptoms in the control plants, conforming more to the field experiment results. These differences between the findings of outdoor investigations and greenhouse experiments could perhaps be explained if rain water contained copper. Very little data is available on this point, but it does not seem likely except near heavy industrial works. Hewitt (1952) has reported that rain water collected off a glasshouse roof near Bristol contains an average of 0.027 p.p.m. copper, and that the level varies according to the season and to the rainfall. Mitchell Reith and Johnston (1957(1)) have estimated that 0.025 p.p.m. copper is removed from the soil each year of cropping and, under the conditions near Bristol of 36" rain a year, it follows that there would be an annual net gain of about 0.075 p.p.m. copper to the top soil. This might accumulate to make a profound difference to the copper status of the land.

The main reason for the disparity between results obtained in the field and in the greenhouse, however, may be that, in wet years, the plant roots can tap a far larger volume of soil solution in the field than in a pot, and so obtain sufficient copper. Drainage conditions may also affect results. In pot experiments on cereals, even if the soil is kept at a high moisture level, drainage may be assumed to be free, while many fields are imperfectly drained. In Scotland, Mitchell Reith and Johnston (1957(1)(2)) have shown that copper is more available in imperfectly drained than in well drained soil. Beeson and Matrone (1950), however, have found that copper uptake in natural vegetation is not influenced by drainage conditions. In hot dry summers, there is a possibility that reclamation disease may have been wrongly diagnosed in some fields. (Steenbjerg and Boken 1950). Confusion may have arisen because of the similarity of some copper deficiency and drought symptoms. (Gilbert 1952, Piper 1942(1), Andrew 1963, Wood and Womersley 1946, Brown Tiffin and Holmes 1958).

(2) Availability of Applied Copper

Once copper has been applied to deficient soil, the extent to which it remains available is of interest. This has been reported to vary markedly on different soils. In general, copper appears to be fixed quickly in heavy peat soils and more slowly in heavy loams, while in light sands it remains mobile (Piper 1942(1), Lundblad Svanberg and Ekman 1949, Scharrer and Schaumloffel 1960). Humus in the soil has been stated to have a large influence in rendering both native and applied copper unavailable to plants. (Gilbert 1952, Steenbjerg and Boken 1950). Some effects of soil pH have also been reported and, in general, the availability of applied copper appears to increase with increasing soil acidity below about pH 6, and to remain fairly constant as the reaction becomes more alkaline above this level. (Piper 1942(1), Peech 1941, Piper and Beckwith 1951). It has been claimed that the response to copper fertilization in Denmark is higher when the rainfall during May is above average. (Steenbjerg and Boken 1950).

The residual effect of copper application appears to be substantial, for an E.D.T.A. extraction of a mineral soil nearly two years after copper treatment, has been reported to show a recovery of over 50%. (Mitchell Reith and Johnston 1957(1)). Increases of yield caused by a moderate dressing of copper have been proved to persist under field conditions for eight to ten years by Hoffmann (1952), five years by Mitchell Reith and Johnston (1957(1)), and at least four years by Steenbjerg and Boken (1950) and Gilbert (1957). Rises in the copper content of grass after fertilization with copper have also been shown to continue for at least five years. (Lundblad Svanberg and Ekman 1949). Copper applied to the soil surface has been found to be more available to the crop grown in the succeeding year. (Steenbjerg and Boken 1950, Trumble 1950, Davies 1962). Possible causes of this delayed effect are microbiological activity (Riceman 1951), and the immobility of applied copper under many conditions (Henriksen 1959). The element has been shown to remain confined to the top inch of soil for three months by Henriksen (1959), and in the top two inches of soils under grass for as long as six years by Lundblad Svanberg and Ekman (1949).

(3) Interactions of Copper and Other Nutrients

Healthy nutrition involves a balance where all the elements needed by the plant are available at requisite levels and in certain proportions to one another, but the optimal levels and proportions may be different in various species and even for one plant during changing stages of the life cycle. Too much or too little of one nutrient may profoundly affect the uptake of another, and any consequent increase or decrease in growth can give misleading figures in comparative plant analysis.

Hagin (1960) has shown that symptoms of reclamation disease appear earlier in oats which are better supplied with other nutrients. Plants deficient in both copper and nitrogen have been stated to be miniatures of healthy plants, with no symptoms except stunting (Mulder 1950), but the application of nitrogen under copper deficiency conditions has been reported to make symptoms worse in wheat (Fleming and Delaney 1961, Mulder 1950) and in oats. (Harris 1948, Wood and Womersley 1946, Hagin 1960, Rademacher 1940). Lack of copper may lead to higher nitrogen levels in oat plants (Lucas 1948, Wood and Womersley 1946, Ennis and Brogan 1961), which perhaps explains why the first sign of copper deficiency is often a dark green colour of the leaves. (Gilbert 1952).

Neither phosphorus nor potassium dressings appear to alter the uptake of copper in pasture plants (Hemingway 1962), and the copper content of rice plants has been shown to be unaffected by applications of phosphates. (Brown *et alia* 1955). Very high copper treatments have been reported to lower the phosphorus and potassium contents of oats (Forster 1954), but at more normal copper levels the uptake of phosphorus in oats was found to be correlated with the growth of the plant and with the uptake of copper and zinc. (Piper and Walkley 1943).

After an application of copper, the appearance of iron deficiency chlorosis has been observed (Willis and Piland 1936, Sommer 1945, Gilbert 1952, Hagin 1960, Forster 1954, Brown *et alia* 1955), and manganese deficiency symptoms may also sometimes occur. (Gilbert 1952, Steenbjerg and Boken 1950). Under some circumstances, when the availability of iron or manganese is undesirably high, a dressing of copper can be of value in correcting their excessive absorption. (Willis and Piland 1936). The relationship with zinc seems similar, in that copper curtails too great an uptake under luxury conditions, but may cause deficiency when little zinc is available. (Gilbert 1952, Smith 1962). Forster (1952) has reported that cobalt application slightly reduces the copper concentration in oats. In some parts of Australia and Scotland, soils which are copper deficient tend also to be poor in zinc or cobalt (Trumble 1950, Leeper 1951, Mitchell Reith and Johnston 1957(2)), and in these areas balanced fertilization is particularly important.

(4) Copper Effects on Different Species and Varieties of Plants

Although copper is now assumed to be essential for the life cycle of all green plants, yet different species require and take up varying quantities of the element. This has been demonstrated by Piper and Walkley (1943) who have reported that the coefficient of variation of the copper contents of Algerian oats grown on very different soils was ±45%, while for different species of plant on the same soil it was ±62%. Also, Mitchell Reith and Johnston (1957(1)) have shown that an application of copper sulphate to the soil raises the concentration of the metal in clover by 500%, but in grasses by only 30%.

Not all cereal crops are equally susceptible to copper deficiency disease. Rye, for instance, can apparently take up more copper than other cereals (Piper and Walkley 1943) and grow well (Piper 1942(1), Gilbert 1952, Coppenet and Calvez 1955) where oats, barley and wheat fail to survive, and wheat has been found to be more prone to copper deficiency disease than oats (Henkens 1961). The order of decreasing susceptibility of cereals to the disease has been stated to be: barley, wheat, oats, and rye (Batey 1961). The responses of barley and oats to added copper have been found to be similar (Henriksen and Jensen 1958) while that of wheat appears to be greater. (Harmer 1945). In pot experiments, however, Scharrer and Schaumloffel (1960) have found that barley assimilated applied copper more readily than oats.

Wheat and oats have also been shown to exhibit varietal differences in response to copper. Rotenburger black oats, which is resistant to reclamation disease, was found by Rademacher (1940) to take up more copper from the soil in the early stages of growth than the more susceptible Victory oats, and a varietal difference in the time at which symptoms of withertip appeared in oats has been demonstrated by Hagin (1960). Williams and Moore (1952) have discussed the possibility that distribution of copper within the plant differs in Mulga and Algerian oats. The copper content of wheat grain grown on a single soil was found by Greaves and Andersen (1936) to differ by as much as threefold between varieties.

(5) Copper in the Plant

The enzymes laccase, tyrosinase and ascorbic acid oxidase have copper as their prosthetic group, (Arnon 1950, Neilands and Stumpf 1958), and either the cupric ion itself or one of these enzymes has been found to be necessary for the respiratory system in the higher plant (Waygood 1950, Arnon 1950, Steinberg 1950). The presence of copper has also been shown to be essential for photosynthesis (Arnon 1950) and it has been suggested that copper is involved in the synthesis of chlorophyll (Dawson 1950, Neilands and Stumpf 1958).

The symptoms seen in copper deficient cereals, however, are not all easily associated with these particular metabolic functions. Reclamation disease of oats has been described similarly both in Australia and North West Europe as a condition in which the younger leaves have pale edges and white twisted or rolled tips. The plants are stunted, with white heads containing little or no grain, the shoot is surrounded by many tillers, none of which produces grain, and the straw remains green instead of maturing to yellow. (Piper 1942(1), Mitchell Reith and Johnston 1957(1), Wood and Womersley 1946, Wallace 1961, Hagin 1960). The patchy appearance of the disease in the field has been remarked more often in Europe, while the "drought" symptoms of limpness and lack of turgor in the leaves, even under ample soil moisture conditions, has been noticed more in Australia and U.S.A. It has been suggested that lack of copper may have an adverse effect on the translocation of water within the plant (Piper 1942(1)) and the copper deficient oat plant has been shown to contain unlignified narrow vascular bundles, a poorly developed cuticle in the leaves and a thin walled epidermis on the stem. (Hagin 1960, Ford 1940). All of these features, especially in warm dry conditions, would make a good water balance in the plant difficult. Wood and Womersley (1946), however, found no difference between the degree of lignification in copper deficient and healthy oats grown in the greenhouse, but it may be that, in this protected environment, lignification would not normally have occurred. Where lack of lignification has been noted in copper deficient oats, it may be a sign of immaturity associated with the failure to set grain, rather than a primary symptom of deficiency. The greenhouse oats grown by Hagin (1960) produced normal roots with very little copper, and it has been suggested that when there is a lack of copper, the needs of the root are satisfied first. (Riceman and Jones 1958, Hagin 1960). In the field, however, Teakle, Turton and Throssell(1940) and Buckpitt and Purves (1960) have reported that copper deficient oats have a poorly developed root system. Hagin (1960) has also mentioned the abnormal formation of adventitious roots from the node above a tiller in copper deficient oats grown in a greenhouse.

The symptoms of copper deficiency in wheat and barley seem similar to those in oats (Piper 1942(1), Brown Tiffin and Holmes 1958). Davies (1962), however, has reported that wheat grown under mildly deficient conditions developed normally until after "heading", when the grain did not swell and a purple black pigment was often produced at the nodes, below the ears and on the glumes. Brown Tiffin and Holmes (1958) have shown that, in copper deficient wheat, the sugars and organic acids do not undergo the usual changes in concentration during internode elongation, but that the formation of grain is not necessarily linked with these changes.

During development, the copper concentration in the dry matter of whole oat plants has been shown to decrease, at first quickly, and then more slowly as the plant grows, until, during late maturity, the much lower copper content varies very little with time. (Williams and Moore 1952, Piper 1942(1), Piper and Walkley 1943). The importance of a satisfactory early uptake of copper has been stressed, (Rademacher 1940, Hagin 1960, Hoffmann 1952) and Piper (1942(1)) has demonstrated that copper absorbed by oat seedlings enables them to withstand later, deficient conditions to an extent depending on the length of time the copper has been available to them. It has also been reported, however, that plants which show severe withertip symptoms throughout early growth, can produce healthy tillers yielding grain if given sufficient copper (Piper 1942(1), Hagin 1960). The rise in the copper content of cereals after copper treatment has been stated to be small except when very heavy doses are used, (Piper 1942(1), Mitchell Reith and Johnston 1957(1), Scharrer and Schaumloffel 1960, Ennis and Brogan 1961) and sometimes the grain may not show any increase in concentration. (Mitchell Reith and Johnston 1957(1)).

(6) Copper Toxicity

The natural occurrence of copper toxicity has not been reported in Britain, but Vermaat and Van der Bie (1950) found injurious concentrations of copper in the latex of rubber trees in Java, and deduced that the metal was available in harmful quantities in the soil. Human activity, however, may raise the copper content of soils very considerably through industrial fall out, the prolonged use of copper-containing fungicides, and the mistaken application of copper to soils in an effort to cure a supposed deficiency. Gilbert (1957) has commented that there is very little danger of damage to plants from over dressing with copper, and has claimed that, on a peat soil, an application of 10,000 pounds per acre copper sulphate leads to only temporary injury. Piper (1942(1)), however, has reported that optimum yields are obtained from a copper deficient sandy soil after treatment with less than ten pounds per acre copper sulphate.

It has been found that the total copper concentration in soil samples taken from areas of one square foot in a field in Scotland showed a ten fold variation (Purves and Ragg 1962), and Mitchell (1963) has advised caution in micro nutrient fertilization, especially in Britain, where soils can be so different within such small areas. Mitchell (1963) has pointed out the "needless expense" involved in over application of trace elements and has stated, "It is generally much more difficult to deal with toxicity arising from excess of a plant food required in trace amount than to remedy a deficiency".

Decreases in the growth of oat plants in a greenhouse have been reported after the addition of about 3,750 pounds per acre copper sulphate to a sandy soil, (Forster 1954) and applications of 750 pounds per acre have been shown to cause diminution of the growth of barley and rye plants. (Forster 1952). Forster (1952) has also demonstrated that barley is more susceptible to copper toxicity than rye. Oats have been studied in aqueous cultures over a range of copper concentrations, the highest of which have given plants with poor growth and development. Hagin (1960) has found that more than 30μg copper per oat plant depressed growth, while the concentration of copper in the nutrient solution which causes stunting and persistent diminution of yield has been reported by Piper and Walkley (1943) to be 8.8 p.p.m. and by Piper (1942(1)) to be 3 p.p.m.

CONDITIONS IN SOUTH EAST SCOTLAND

The work for this thesis was carried out in fields in South East Scotland and on a local soil in the greenhouse. Although trouble in stock had been reported, and cured by administration of copper, on certain farms in this area, no copper deficiency in field crops was observed before 1953. During the next ten years, however, low grain yields of cereals, sometimes accompanied by chlorosis, curling of leaf tips and severe stunting, were observed in cereals, especially oats, on some farms. In 1958 these symptoms were cured in a micro plot within a badly affected patch of a field of oats by spraying with copper sulphate solution before ear emergence. (Purves and Ragg 1962). It was also found that whole untreated plants from affected fields contained very little copper, and that the total copper in the soil to plough depth was nearly always less than 4 p.p.m. on an oven dry basis. This may be compared with an average of about 15 p.p.m. obtained from 100 random soil samples from South East Scotland (Purves and Ragg 1962), and with a range of 3 - 100 p.p.m. which has been said to cover the usual total copper contents in mineral soils (Mitchell 1955). It was therefore deduced that the symptoms seen in cereals were caused by copper deficiency and, when the positions of fields in which the disease had occurred were plotted on a soil series map, a very obvious relationship was evident, in that very nearly all reported occurrences were on the Eckford Soil Series (Purves and Ragg 1962).

A description of the soils in this part of Scotland has been given by Ragg (1960). The Upper Old Red Sandstone rocks of the area have given rise, among others, to the Eckford and Hobkirk Soil Associations. The Eckford Association soils are fluvioglacial sands, of which the Eckford Series is a freely drained brown forest soil of coarse texture, while the Hexpath Series is an iron podzol which underlies heathland, and changes after reclamation until it is very similar to the Eckford Series. A similar relationship exists in the Hobkirk Association between the brown forest soil of Hobkirk Series and the peaty podzol of Faw Series, both of which are developed on till of sandy loam texture. The total copper concentrations in 20 Eckford Series surface soils have been found to be in the range 1.2 to 6 p.p.m., while 11 Hobkirk Series surface soils contained 4.5 to 31.9 p.p.m., only one soil being below the Eckford maximum. (Purves and Ragg 1962). Some of the Hobkirk values were influenced by the presence of basic igneous rocks, which are relatively rich in copper. In general, it may be that weathering during transport by river, lake or glacier has removed copper from the Eckford parent material, while the Hobkirk soils, some of which have been derived from the decay of underlying rock, have been subject to far less leaching. In fields where withertip is seen, the problem is therefore mainly lack of total copper on the Eckford Series, while, on the Hobkirk Series, the availability of the element may be of more importance.

It was found from the analysis of a random sample of 100 arable soils from the South East of Scotland, that 14% contained less than 4 p.p.m. total copper and that 4% contained less than 2 p.p.m. (Purves and Ragg 1962). It has been observed (Fritzsch 1958, Scharrer and Schaumloffel 1960, Trumble 1950) that copper deficiency in cereals may cause subclinical or "latent" disease, which, because its only symptom is a loss of yield, may not be obvious without comparison with copper treated plants. The low soil copper concentrations in South East Scotland showed that there was a definite possibility that "latent" copper deficiency was lowering grain yields on some farms in the area.

Field experiments were conducted on soils which contained low levels of copper and which were mostly in the Eckford or Hobkirk Series. Oats and barley, which are the cereals normally grown in the area, were treated with copper. The objects were to find out whether withertip symptoms on these crops could be prevented, and grain yield raised by copper application, and, if a favourable response was obtained, to determine how much copper would be required to give maximum yields. The residual availability of applied copper after various lengths of time was also investigated and some varietal and climatic effects on copper availability and uptake were studied during field trials and greenhouse work.

ANALYTICAL METHODS

(1) Copper

In determinations of trace elements, particularly copper, there is always a possibility of contamination at every stage. It was found, however, that, with care, reproducible results could be obtained in a normal laboratory with brass fittings.

The water used in all analytical work had been single distilled in a pyrex glass "Scorah" still. The apparatus which contained solutions during copper analyses, and the stoppers, were made either of pyrex glass or heavy polythene so as to avoid contamination picked up from the walls of vessels. Stopcocks were greased with a little vaseline. All apparatus was cleaned very carefully at first with detergent and plenty of water. It was then washed with dilute nitric acid, distilled water, dilute E.D.T.A. solution, and finally rinsed about six times with more distilled water. The apparatus was subsequently used solely for copper determinations. It was washed with nothing but dilute E.D.T.A. solution and distilled water between samples; tap water was unsuitable because the laboratory supply flowed through copper pipes and was found to contain up to 0.8 p.p.m. copper. Analyses were completed as quickly as possible, but when samples had to be left on a bench for any length of time during a determination, they were protected with polythene sheeting from dust or splashing.

(a) Copper in Herbage

It was desirable that many samples of herbage should be analysed for copper, and so a quick reliable method of determining this element was sought. First, the spectrochemical procedure of Farmer (1950), which is in routine use for advisory samples at the Edinburgh School of Agriculture, was examined. This method involves the dry ashing of one or two grams of the plant sample and the excitation in a carbon arc of a mixture of one part ash, one part potassium sulphate and two parts carbon powder. The carbon powder contains silver which serves as an internal standard. The spectrum produced, modified by the use of a step sector, is recorded on a photographic plate. From the density of the appropriate lines read on a microphotometer, the ratio of the intensity of light emitted by copper to that emitted by silver during the burn is calculated, and hence, from standard curves, the concentration of copper in the plant ash is determined. The reproducibility of the spectrographic part of the method was tested using a sample of copper deficient oat plant. The herbage was ashed as usual but filled into six electrodes. The results obtained are shown in Table 1. The high coefficient of variation would probably not have been found at more normal copper levels (above 1.5 p.p.m.), where the standard curve is steeper.

Table 1

Reproducibility of the spectrographic part of Farmer's method for copper in herbage

p.p.m. copper found:	0.38	0.73	0.19	0.50	0.38	0.50

Average = 0.45 p.p.m., standard deviation = ± 0.18 p.p.m., coefficient of variation = ± 40%

It was anticipated, however, that many copper deficient samples would be analysed during the present work, and so the variation given by a method at copper concentrations of under 1 p.p.m. in herbage was important. An advantage of Farmer's method is that, if strontium is mixed into the carbon powder as an additional standard, the iron and manganese concentrations in a sample can be determined from the same photographic plate as copper. It has been found, however, that an experienced worker requires at least two days to complete twelve samples for copper, and this time would only be justified if manganese and iron were to be determined also, or if no other quicker method was as good.

As an absorptiometer was available, references to colorimetric methods were examined. Copper forms coloured complexes very readily and so there are very many reagents which can be used in its determination. The ideal compound would be specific for copper and not subject to interference or inhibition from other elements. It would also be very sensitive at low concentrations of copper and the optical density of the coloured complex would obey Beer's law. No such perfect compound has yet been described, and new reagents are constantly being tested. The best compromise for use on plant tissues had therefore to be decided.

Sandell (1959) has described many methods for the colorimetric determination of copper with various reagents. Dithizone has been used by Butler and Newman (1956), but was not seriously considered during the present investigation because it was felt that the advantages of its great sensitivity do not compensate for the disadvantages arising from its lack of specificity and stability. Methods with rubeanic acid have been utilised (Johnson 1955, Baroccio and Saponaro 1960), but these require very carefully controlled conditions because the reagent is not very specific. Rubeanic acid, like biscyclohexanone oxalyldihydrazone, which has been called cuprizone, forms a copper complex which is insoluble in common organic solvents. Because of this insolubility, the colour of the cuprizone copper complex is measured directly in aqueous solution, giving simple but comparatively insensitive methods. (Williams and Morgan 1954, Johnson 1955, Russell and Hart 1958, Unicam 1961). The formation and stability of the cuprizone copper complex are affected by pH, ammonium ion concentration and temperature levels below 15°C (Thompson and Ravenscroft 1960), but Somers and Garraway (1957) have used the reagent successfully with a borate buffer in a method for herbage samples. Interference from Co, Fe^{++}, Cr and Ni at levels above 10 mg. have been stated to take place (Johnson 1955).

2,2 biquinoline, a derivative of 2,2 bipyridine, has been chosen for the determination of copper in gelatin (Russell and Hart 1958) but, although its specificity is reported to be good, its sensitivity has been found to be much lower than many other reagents. (Sandell 1959, Borchardt and Butler 1957). After a comparison of many methods, Borchardt and Butler (1957) have recommended bathocuproine, 2,9 dimethyl 4,7 diphenyl 1,10 phenanthroline, for the analysis of paper. The derivatives of 2,2 bipyridine and 1,10 phenanthroline have been described as expensive (Sandell 1959, Russell and Hart 1958, Borchardt and Butler 1957) and, for herbage samples, their greater specificity does not appear to warrant their extra cost.

The most popular reagent for the determination of copper has been sodium diethyl dithiocarbamate. It has been recommended for herbage after a preliminary extraction with dithizone (Piper 1942 (2), A.O.A.C. 1960). The A.O.A.C. method has been reported to be dependable (Beeson 1953) but the reagent has disadvantages of instability and unspecificity. The colour of the copper complex in carbon tetrachloride has been found to be unstable in daylight (Sandell 1959, Russell and Hart 1958). The use of E.D.T.A. to reduce interference from iron, manganese, nickel and cobalt has been suggested (Forster 1953, Sandell 1959) but Russell and Hart (1958) have found that this is not entirely effective. If more compounds are added to the sample solution in order to increase the specificity of the copper reaction, the method probably becomes less sensitive. No advantages have been reported in copper analyses when sodium diethyl dithiocarbamate is replaced by diethyl ammonium diethyl dithiocarbamate (Johnson 1955).

The reagent chosen for further investigation was zinc dibenzyl dithiocarbamate. This compound has the big advantage that its copper complex is formed in acid solution. If an acid digestion of plant material is performed, no buffering or neutralisation is therefore required, and small blanks should be obtained. Zinc dibenzyl dithiocarbamate is more specific than sodium diethyl dithiocarbamate (Sandell 1959) and more sensitive than bathocuproine (Borchardt and Butler 1957). The yellow colour of the copper complex in carbon tetrachloride has been reported to be stable in bright daylight (Sandell 1959, Madan 1958, Martens and Githens 1952) but Russell and Hart (1958) have stated that it fades very rapidly in sunlight. Borchardt and Butler (1957) have found that the reagent has advantages over other compounds which they investigated, but that "the precision of results using this method left something to be desired".

Methods which can be used to determine copper in herbage samples with zinc dibenzyl dithiocarbamate have been described by Sandell (1959), Johnson (1955), Andrus (1955), Borchardt and Butler (1957) and Schuurmans and Steiner (1958). In all these procedures, the acid digest of herbage is diluted and shaken with a solution of zinc dibenzyl dithiocarbamate in carbon tetrachloride. The optical density of the solvent layer is read at 435 or 440 mμ, and the copper concentration calculated from a calibration curve. The lack of precision reported by Borchardt and Butler (1957) may have been caused by insufficient or inconsistent dilution of the acid digestion mixture. Some effects of the presence of other ions on the method have been reported. Borchardt and Butler (1957) have found no interference from ferric iron, manganese, mercury or nickel, and Andrus (1955) has reported that the combined effect of 1 mg. iron, 1 mg. manganese and 0.1 mg. cobalt is negligible. Johnson (1955) and Schuurmans and Steiner (1958) have shown that up to 0.5 g. of aluminium, arsenic, cadmium, calcium, chromium, lead, manganese, magnesium, potassium, sodium or zinc can be present in the sample solution without causing any interference.

Some elements have, however, been reported to affect the method, and they are listed in Table 2. When these elements are present in the sample solution in amounts less than the limiting weights shown in the table, they have been stated to cause no interference. In Table 2 these limiting weights have also been expressed as concentrations of the elements in oven dry herbage, assuming that 2 g. of plant material is digested to give the sample solution. When the limiting concentrations are compared with typical and abnormally high levels which have been found in herbage, it is seen that interference under normal conditions is negligible.

Experiments on the zinc dibenzyl dithiocarbamate method were carried out to check the claims made for it. 2 ml. sulphuric acid and aliquots of a standard copper solution were diluted to 50 ml. in separating funnels and extracted with 10 ml. of a solution of zinc dibenzyl dithiocarbamate in carbon tetrachloride. The optical density of the solvent layer was read in a Unicam S.P. 600 spectrophotometer against a blank extracted from 2 ml. sulphuric acid and 48 ml. water. It was found that reproducible results, over the range 0-40μg copper, were obtained only when the concentration of the zinc dibenzyl dithiocarbamate solution was 0.03% and shaking was continued vigorously for 1½ minutes or longer. The spectral absorption curve obtained with 10μg. copper was shown to have a peak at 438 mμ (see graph 2), and so all readings for the determination of copper were taken at this wavelength. Graph 1, which was drawn from results with 1 cm. and 2 cm. cells, demonstrates that Beer's law is obeyed over the range 0-40μg. copper. The

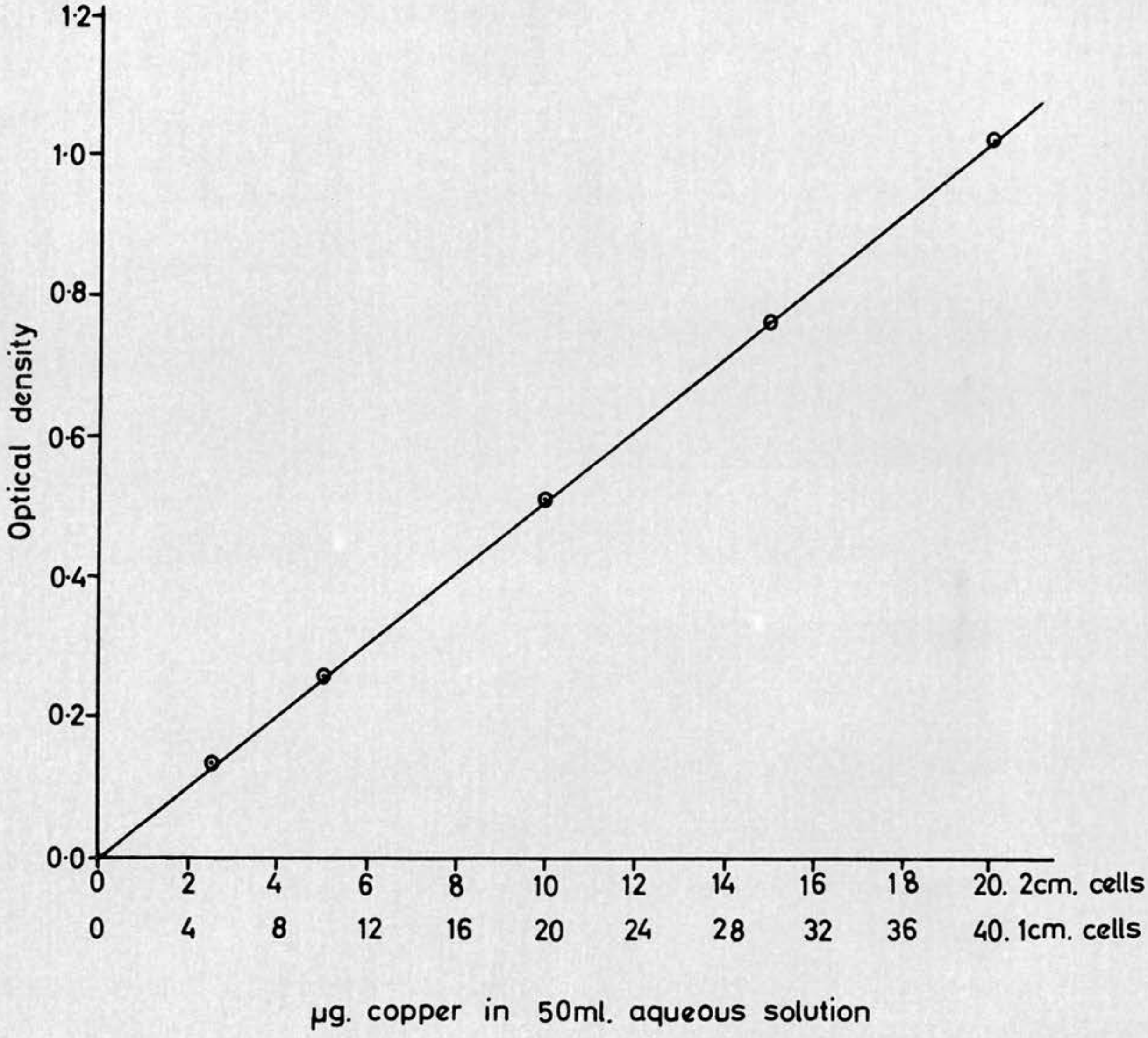
Table 2.

Elements interfering in the zinc dibenzyl dithiocarbamate method for herbage

Element	Limiting values below which no interference occurs		Values found in oven dry herbage (p.p.m.)	
	Weight in sample solution (mg.) ≡	Concentration in oven dry herbage (p.p.m.)	Typical concentrations in oat grain	Exceptionally high concentrations in various plants
Sb	"few milligrams" (a)	circa 2,500		
Bi^{+++}	0.005 (b)	2.5		
Co^{++}	0.114 (b)	57	0.05 (e)	1 (e)
	10 (a)(c)	5,000		
Fe^{+++}	50 (a)	25,000	76 (e)	$<$ 10,000 (e)
	100 (b)	50,000		
Hg^{++}	0.044 (b)	22		
	0.1 (a)(c)	50		
Mo	0.25 (b)	125	0.2 (e)	100 (e)
Ni^{++}	0.125 (b)	62	3.2 (e)	$<$100 (e)
	10 (a)	5,000		
	100 (c)	50,000		
Se	0.1 (b)	50	10 (d)	5,000 (d)
Ag^{+}	0.1 (a)(c)	50	$<$0.1 (e)	$<$1 (e)
Sn	0.1 (b)	50	$<$1 (e)	
V^{+++++}	1 (b)	500	0.06 (e)	$<$10 (e)
W	1 (b)	500		

References:
(a) Sandell 1959
(b) Schuurmans and Steiner 1958
(c) Johnson 1955
(d) Mitchell 1957
(e) Mitchell 1954.

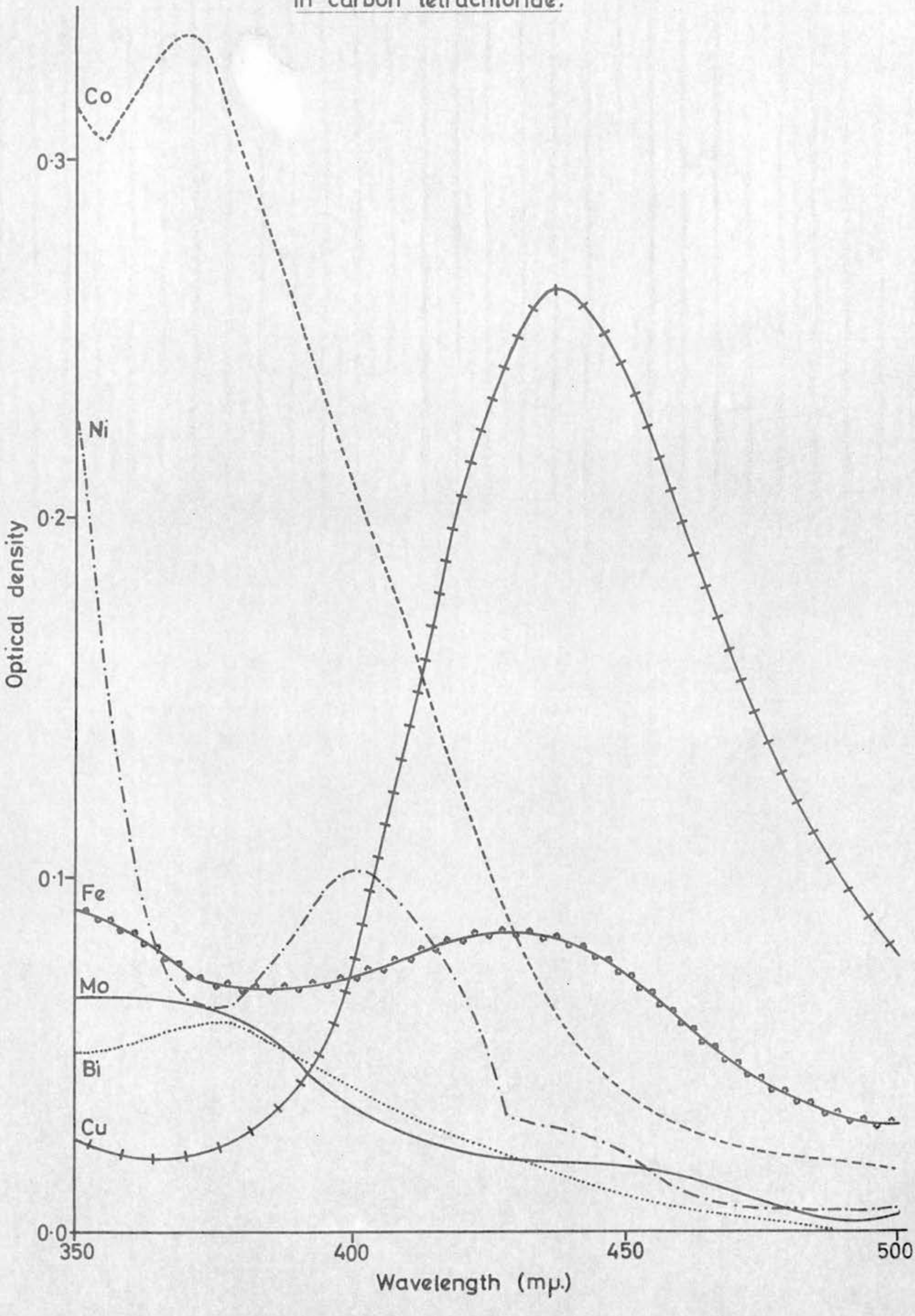
Diagram 1. Optical density of copper dibenzyl dithiocarbamate in carbon tetrachloride at 438 mμ against a blank.



colour of the reagent-copper complex was found to be stable for at least four hours in darkness and at least two hours in bright daylight. The presence of 2 ml. perchloric acid and 3 ml. sulphuric acid in the 50 ml. sample solution was found to have no influence on the results, so long as the blank solution also contained the extra acid. The acid concentration seems therefore to be less critical than reported by Johnson (1955).

It has been reported that the complexes formed with elements which might cause interference in the method have distinctive spectral absorption curves. (Madan 1958, Johnson 1955). It was felt that a knowledge of these curves would be useful in checking at any time whether interference was occurring, and so graph 2 was prepared. In this graph are shown the absorptions, over a range of wavelengths, given by 0.01 mg. bismuth, 10 mg. cobalt, 10μg. copper, 200 mg. ferric iron, 0.5 mg. molybdenum and 20 mg. nickel, when each was extracted with 10 ml. 0.03 % zinc dibenzyl dithiocarbamate in carbon tetrachloride, from 50 ml. of an aqueous solution containing 2 ml. sulphuric acid, and read against a blank in a 1 cm. cell. It is seen that the ferric iron and copper curves are similar while all other curves are distinctive. An extra check was made on the interference of iron, and it was found that 0.2 mg. Fe^{+++} in the sample solution had no effect, while 20 mg. Fe^{+++} gave readings equivalent to about 0.4μg. copper. These values for ferric iron are equivalent to 100 p.p.m. and 1% respectively in the herbage, and so (see Table 2) no interference is expected during the analysis of plant material. Interference from iron was found to increase very much with rising

Diagram 2. Spectral absorption curves of dibenzyl dithiocarbamate complexes in carbon tetrachloride.



concentrations of hydrochloric acid in the aqueous solution.

These investigations show that zinc dibenzyl dithiocarbamate is a suitable reagent for copper determination in herbage.

It was decided to use an acid digestion of the plant material, for convenience with this colorimetric method, and as dry ashing has been reported to give erratic results (Borchardt and Butler 1957) or incomplete recovery of copper (Sandell 1959). The details of the method used for herbage are given in appendix 1. When standards and blanks were taken through this procedure, the results were the same as those obtained using the colorimetric method alone, and the curve in graph 1 was therefore used for the calculation of results. Thus the only effect of the acid digestion was to raise the blank very slightly, so that its reading was equivalent to about 0.2μg. copper. During a determination, three blanks are run through the method with the samples. The average absorption of these blanks is subtracted from the absorption of each sample to give the figure on which calculation is made. Thus there is a constant check on all reagents. If the agreement of the blanks is not good, the analysis of the batch of samples is repeated. With the method described in appendix 1, twenty-one samples a day can be analysed for copper. The reproducibility of the method was checked by analysing a sample of young oats nine times. The results are given in Table 3. Twenty samples of young oats were analysed by two workers, one using the method in appendix 1, and the other using Farmer's spectrographic method. The results, which are given in Table 4, show generally good agreement between the two methods.

Table 3

Reproducibility of the zinc dibenzyl dithiocarbamate method for copper in herbage

p.p.m. copper found:	0.74	0.74	0.74	0.72	0.72	0.74	0.72	0.72	0.74

mean = 0.73 p.p.m., standard deviation = ± 0.011 p.p.m., coefficient of variation = ± 1.44%

Table 4

Comparison of the method in appendix 1 and Farmer's method for copper in herbage

Sample	1	2	3	4	5	6	7	8	9	10
p.p.m. copper found by Farmer's method	2.23	2.17	1.73	1.42	1.45	2.14	2.00	2.07	1.92	1.54
p.p.m. copper found by colorimetric method	2.14	2.24	1.69	1.26	1.58	2.06	1.95	2.02	2.00	1.70
difference	0.09	-0.07	0.04	0.16	-0.13	0.08	0.05	0.05	-0.08	-0.16

Sample	11	12	13	14	15	16	17	18	19	20
p.p.m. copper found by Farmer's method	1.50	1.54	1.08	1.28	1.46	1.51	1.90	2.01	1.55	2.25
p.p.m. copper found by colorimetric method	1.45	1.68	1.06	1.29	1.48	1.47	2.09	1.86	1.65	2.54
difference	0.05	-0.14	0.02	-0.01	-0.02	0.04	-0.19	0.15	-0.10	-0.29

(b) Total Copper in Soils

It was also desired to analyse soils for copper. In 1962, the method in routine use in the Edinburgh School of Agriculture was Farmer's spectrographic procedure (Farmer 1950), modified by the use of calibration standards in a soil base instead of a plant ash base. By this method, which has given useful results for advisory purposes, the total copper content of a soil is determined. In Table 5A are listed the results obtained when one ignited soil sample was arced six times. The author sometimes, however, found good reproducibility impossible to obtain, and Table 5B shows the results of an experiment to determine at which stage in the method variation was occurring.

Table 5

Reproducibility of Farmer's total soil copper method

A:

p.p.m. copper found:						
	2.7	3.0	1.5	2.2	2.4	10.6

If the last result is ignored; mean = 2.4 p.p.m., standard deviation = ± 0.57 p.p.m., coefficient of variation = ±24%.

B:

p.p.m. copper found

electrode filling mixture	1		2		3		4	5	6
ignition A	9.9	2.8	17.2	5.4	10.6	59.0	23.4	10.4	5.6
ignition B	3.0	4.1	7.7	6.7	4.6	3.8	12.2	6.6	7.8

One soil was ignited in duplicate. Six weighed subsamples from each ignition were added to the requisite weights of carbon powder and potassium sulphate, and ground together to give the mixtures to be filled into the electrodes. Half of these mixtures were arced as usual, and half were arced in duplicate.

The results show poor agreement even between the duplicate arcings, and it was thought that the brass parts of the stand, in which the electrodes were clamped during arcing, might have caused some contamination. No explanation was found, however, as to why agreement was sometimes good and sometimes poor. All reports of total soil copper concentrations in the present work are the averages of concordant duplicate analyses.

(c) Available Copper in Soils

Many methods of assessing the level of available copper in soil have been reported. Lundblad, Svanberg and Ekman (1949), Wells (1957) and Pack, Toth and Bear (1953) have analysed plants growing on the soil in the field. This method is difficult to standardise and use, however, because the concentration of copper in a plant varies according to its variety, stage of growth and general nutrition. Piper and Walkley (1943) have recommended a modified Neubauer method, using the analysis of young oat plants grown on the soil under controlled conditions, but this would require much time, space, and the sustained use of rigourous techniques. The micro organism Aspergillus Niger has been widely used (Stiles 1961, Wallace 1961, Mulder 1950) to measure available copper in soil, but this method is also time consuming.

A variety of chemical reagents have been tried as soil extractants for copper, and Swaine (1955) has summarised the results obtained by many workers. In this review, references are given to extractions with water, dilute hydrochloric, sulphuric, nitric, citric and acetic acids, and solutions of magnesium sulphate, ammonium nitrate, ammonium oxalate and ammonium acetate. Where concentrated acids were used, the results refer more to the total than to the available copper content of the soil. More recently, comparisons, some of which are listed in Table 6, have been made between various chemical and biological methods of soil extraction. These studies have demonstrated that E.D.T.A. is a suitable reagent except with soils of high pH (Neelkantan and Mehta 1961, Viro 1955). As the copper level in the soil increases, the Aspergillus Niger method becomes less sensitive, while the E.D.T.A. method is unchanged. (Henriksen and Jensen 1958, Baroccio and Saponaro 1960).

It was therefore decided that E.D.T.A. solution would be used for the extraction of copper in the present work. The method of Henriksen and Jensen (1958), which is similar to that of Cheng and Bray (1953), was adopted. Thus, 10g. of air dry soil was shaken for one hour with 100 ml. 0.02M disodium E.D.T.A. at its natural pH and the suspension allowed to stand for a further hour before being filtered. Henriksen and Jensen (1958) have stated that a normal extraction with sodium diethyl dithiocarbamate in carbon tetrachloride can be carried out on an aliquot of the filtrate, while Cheng and Bray (1953) and Blevins and Massey (1959), using the same colorimetric reagent, have found that direct extraction of the E.D.T.A. soil suspension gives good results. Other workers have ignited the filtered E.D.T.A. soil extract before starting copper analysis. (Mitchell Reith and Johnston 1957(1), Henkens 1961, Viro 1955, Baroccio and Saponaro 1960). In the present work, zinc dibenzyl dithiocarbamate was chosen for the colorimetric analysis of soil

Table 6

Comparison of methods of determining available copper in soils

Author	Methods of Extraction: Biological	Methods of Extraction: Chemical	Remarks
Cheng and Bray 1953	-	1% E.D.T.A., 0.1N hydrochloric acid	Correlation between E.D.T.A. and HCl methods
Westerhoff 1955	*Aspergillus Niger*	3% nitric acid, hydrochloric acid	Similar results from all three methods
Mitchell, Reith and Johnston 1957(1)	flowering red clover	0.05M E.D.T.A., 2.5% acetic acid, water	E.D.T.A. and acetic acid methods correlated to clover method
Henriksen and Jensen 1958	*Aspergillus Niger*	0.02M E.D.T.A., hydrochloric acid at pH 2.	Correlation between E.D.T.A. and HCl methods.
Blevins and Massey 1959	millet	0.05M E.D.T.A., dithizone in carbon tetrachloride	Both chemical methods correlated to millet method
Baroccio and Saponaro 1960	*Aspergillus Niger*	0.05M E.D.T.A., 10% acetic acid, ammonium acetate, hydrochloric acid	Correlation between E.D.T.A., and biological methods at low copper levels
Neelkantan and Mehta 1961	sorghum	0.05M E.D.T.A., N and 0.5N nitric acid, N and 0.1N hydrochloric acid, Morgans universal solution, ammonium acetate	Ammonium acetate method correlated to sorghum method
Henkens 1961	*Aspergillus Niger*	1% E.D.T.A., 3% nitric acid	All methods diagnostic of copper deficiency

extracts. When, however, an acidified aliquot of the filtered E.D.T.A. soil extract was shaken with the carbon tetrachloride solution of the colorimetric reagent, emulsification took place, and separation of the solvent layer was impossible. It was proved that 0.02M E.D.T.A. solution does not affect the determination of copper and it was thought that emulsification was caused by humus. Methods of destroying the humus were therefore examined, and potassium periodate, potassium permanganate and hydrogen peroxide were all shown to be satisfactory for this purpose. The most convenient reagent, hydrogen peroxide, was also found to give the smallest blanks and to cause no interference in the colorimetric reaction even when present in excess.

The possibility of elements in the soil extract interfering in the zinc dibenzyl dithiocarbamate method was investigated. Table 7, like Table 2, shows the elements which have been reported to affect the method, and the weights of them, in the aqueous solution for analysis, below which no interference is said to occur. These limiting weights have been converted into p.p.m. in the air dry soil, assuming that when 10 g. soil has been shaken with 100 ml. E.D.T.A. solution, an aliquot of 40 ml. is taken for analysis. Also listed in Table 7 are the total concentrations of these elements which have been found in soils. As only a portion of the total concentration of any element in a soil is extracted by E.D.T.A., it is seen that iron is the only likely source of interference. Viro (1955) has reported that the average concentration of iron extracted by E.D.T.A. at pH 7 from six soils was only 240 p.p.m. This level of iron would certainly not cause interference, but a method of reducing the effect of iron was nevertheless devised as a check. It was found that, when 10 ml. of a freshly prepared solution, containing 5% each of citric acid, sodium pyrophosphate and sodium hexa metaphosphate, was mixed with the aqueous layer about five minutes before the colorimetric extraction, interference by 200 mg. ferric iron dropped from the equivalent of 4.5 μg. to 0.7 μg. copper.

Table 7

Elements interfering in the zinc dibenzyl dithiocarbamate method for soil extracts

Element	Limiting values below which no interference occurs		Total values found in air dry soil (p.p.m.)	
	weight in sample solution (mg.)	= concentration in air dry soil (p.p.m.)	normal concentrations	exceptionally high concentrations
Sb	"few milligrams" (a)	circa 1,250		
Bi^{+++}	0.005 (b)	1.2	probably <1 (d)	
Co^{++}	0.114 (b)	28	1-40 (d)	300 (d)
	10 (a) (c)	2,500		
Fe^{+++}	50 (a)	12,500	7,000-40,000 (e)	800,000 (e)
	100 (b)	25,000		
Hg^{++}	0.044 (b)	11	0.03 (d)	
	0.1 (a) (c)	25		
Mo	0.25 (b)	62	0.2-5 (d)	
Ni^{++}	0.125 (b)	31	5-500 (d)	5,000 (d)
	10 (a)	2,500		
	100 (c)	25,000		
Se	0.1 (b)	25	0.1-2 (d)	
Ag^{+}	0.1 (a) (c)	25	<1 (d)	"few p.p.m." (d)
Sn	0.1 (b)	25	<10 (d)	
V^{+++++}	1 (b)	250	20-500 (d)	
W	1 (b)	250		

References:
(a) Sandell 1959
(b) Schuurmans and Steiner 1958
(c) Johnson (ed.) 1955
(d) Swaine 1955
(e) Lawton 1955 (pages 66-68)

Table 8

Comparison of methods of analysing E.D.T.A. soil extracts

concentrations of extractable copper found (p.p.m.)

Sample / Method	1	2	3	4	5	6
A	0.86	0.84	0.36	1.16	0.84	1.11
B	0.87	0.84	0.37	1.18	0.84	1.11
C	0.86	0.83	0.35	1.16	0.83	1.10
D+	0.88	0.88	0.44	1.12		

\+ results of analyses by Mr. Voss, West of Scotland Agricultural College.

Table 8 gives results obtained during the investigation of the method for determining extractable soil copper. In methods A, B and C, the soil was extracted with 0.02M disodium E.D.T.A. solution and copper was determined with zinc dibenzyl dithiocarbamate. In methods A and B, the humus in an aliquot of the E.D.T.A. soil extract filtrate was destroyed by treatment with nitric and sulphuric acids. The acid solution was treated like the acid digest in the method for herbage. In method A the iron complexing mixture was added before the colorimetric determination. Hydrogen peroxide was used in method C to oxidise the humus in an acidified aliquot of the E.D.T.A. filtrate. Method D, carried out by Mr. Voss at the West of Scotland Agricultural College, involved an extraction of the soils with 0.05M E.D.T.A. at pH 7, the ignition of an aliquot of the filtered extract, and the determination of copper by a porous cup spectrographic spark technique. As there was good agreement between the results from these methods, the pH and concentration of the E.D.T.A. solution apparently have little effect on the quantity of copper extracted, at least from soils of low copper status. The results also show that a hydrogen peroxide oxidation is as effective as an acid digestion treatment for the destruction of humus, and that E.D.T.A. does not extract enough iron from these soils to interfere with the method. In all, 21 representative soil samples from different fields have been analysed both with and without the iron complexing agent, and no evidence of any interference by iron has been found.

Slight contamination, giving large blanks, was traced to the filter paper. Table 9 lists the results of copper analyses of the first 40 ml. of 0.02M E.D.T.A. solution passing through a filter paper. There thus seemed to be no advantage in using acid washed papers. As number 3 papers give a more uniformly bright filtrate more quickly than other grades, it was decided to use them, but to rinse them with E.D.T.A. solution before filtration.

The details of the method adopted for determining extractable copper are given in appendix 2. When in routine use, 21 samples a day can be analysed by a worker experienced in the method. Standard copper solutions were twice taken through the entire method. The results, which are shown in Table 10, are slightly lower than those found in the colorimetric procedure alone, because of the moisture retained in the filter papers after rinsing. The standardisation curve is a straight line and results were calculated from it. To check the reproducibility of the method, a soil sample was analysed nine times. The results are shown in Table 11.

Table 9

Contamination of E.D.T.A. solution by filter papers

Whatman number	3	30	40	44	540	542
Size of paper	15 cm.	15 cm.	15 cm.	12.5cm.	12.5cm.	15 cm.
Number of determinations	13	2	2	1	2	6
Mean μg. copper found in first 40 ml. filtrate	1.05	1.21	0.60	3.60	0.34	1.16

Table 10

Standardisation of extractable soil copper method

μg. copper	5	10	15
optical density read at 438mμ in 2 cm. cells against blank	250	498	755
optical density read at 438mμ in 2 cm. cells against blank	250	499	747
average	250	498	751

Table 11

Reproducibility of method in appendix 2

p.p.m. available copper found:	1.72	1.69	1.65	1.70	1.66	1.71	1.70	1.62	1.66

mean = 1.679, standard deviation = ± 0.033
coefficient of variation = ± 1.96%

Table 12

Reproducibility of Farmer's method for manganese

p.p.m. manganese found:	27.9	27.5	28.3	29.7	28.8	27.9

mean = 28.35, standard deviation = ± 0.79,
coefficient of variation = ± 2.80%

Table 13

Reproducibility of Farmer's method for iron

p.p.m. iron found:	58.2	56.5	59.8	63.7	60.9	60.9

mean = 60.0, standard deviation = ± 2.48
coefficient of variation = ± 4.14%

Table 14

Reproducibility of chlorophyll method

p.p.m. chlorophyll found in herbage

Sample	7	8	9	11	13	14	16	18	20
Analysis 1	61	80	88	71	75	72.5	82	84	67
Analysis 2	63	76	88	69.5	67	70	83	86	65
Analysis 3		78			73				

(d) Copper in Water

The water sample was thoroughly mixed and an aliquot, containing, if possible, between 4 and 12μg. copper, was evaporated to 50 ml. This concentrate was treated in a kjeldahl flask in the same way as filtered E.D.T.A. soil extracts.

(2) Manganese and Iron in Herbage

The method of Farmer (1950) was used for the determination of manganese and iron in herbage. The reproducibility of the spectrographic part of the procedure was tested by the analysis of six portions of one ignited oat sample. The results are shown in Tables 12 and 13 for manganese and iron respectively.

(3) Chlorophyll

The "official photoelectric colorimetric method for total chlorophyll only", in the A.O.A.C. methods of analysis, (1960 page 92) was used. The chlorophyll was extracted by grinding 1 g. herbage with 0.1 g. sodium carbonate, 3 g. sand and a little 85% acetone in a glass mortar. Extraction was complete after 2 minutes grinding had been repeated eight times, the residue being leached with 85% acetone between periods of maceration. The acetone solution of chlorophyll was made up to 200 ml. and the optical density was read in a 1 cm. cell against 85% acetone at 665 mμ. The A.O.A.C. procedure for the standardisation of the method was followed exactly, in triplicate. The mean calibration curve was found by averaging the optical densities given by the three experimental curves at each of four levels of chlorophyll, and plotting these average optical densities against mg. chlorophyll in 200 ml. dilution. It is a straight line through the origin. Table 14 shows the results obtained when samples were analysed more than once within a month.

(4) Total Nitrogen in Herbage

Determinations of total nitrogen were carried out by the method described in the Fertilisers and Feeding Stuffs Regulations (1960, 8th schedule), except that selenium was used as a catalyst instead of mercury.

(5) Potassium in Herbage

Potassium in herbage was determined using a Unicam S.P. 900 flame photometer. The plant material was dry ashed. A diluted hydrochloric acid extract of the ash was atomised in an air-acetylene flame and the resulting emission read at 766.5mμ.

The method was calibrated by running a series of standards immediately before each batch of sample determinations. (Mitchell 1964).

(6) "Available" Potassium and Phosphorus in Soils

Soil samples were extracted with an ammonium acetate-acetic acid solution. An EEL flame photometer was used in the determination of potassium in the soil extract, while a molybdate colorimetric procedure was carried out for phosphorus. These methods, which are in routine use for advisory work in the Edinburgh School of Agriculture, have been described by Alston (1964).

(7) Soil pH

The pH of a 1:2.5 suspension of soil in water was measured using a glass electrode.

(8) Bushel Weights of Grain

The grain was poured into a 250 ml. conical beaker of known volume until it overflowed. The beaker was tapped three times on the bench and again filled to overflowing. The collected grain was then weighed. This was repeated, if possible using a different part of the grain sample, until results agreeing within one gram were obtained. The figures were then converted from grams/ml. to lb./bushel.

FIELD EXPERIMENTS

(1) 1962

(a) Aims

(i) To determine whether lack of copper is lowering cereal yields on soils containing low levels of total copper. The four fields in which experiments were carried out were chosen so as to cover a range of total soil copper values below 4 p.p.m.

(ii) To ascertain, where a response to copper was found, how much copper is required to give good yields.

(iii) To compare the immediate effects of seedbed and foliar treatments on the yield and copper content of cereals, and their residual effects on the copper status of the soil.

(b) Statistical Design

At all four sites the treatments were:

A: control

B: 5 lb. copper oxychloride in 100 gal. water per acre applied as a foliar spray

C: 10 lb. copper oxychloride in 200 gal. water per acre applied as a foliar spray

D: 10 lb. copper oxychloride in 50 gal. water per acre applied as a soil dressing

E: 20 lb. copper oxychloride in 100 gal. water per acre applied as a soil dressing

A randomised block design with four replicates was used, and the layouts are detailed in appendix 4. The analysis of variance for data from the experiments, including a sub-division of the treatment sum of squares when there were significant treatment differences, (Cochran and Cox 1960 p.61) is given in Table 15.

Table 15

Analysis of variance of data from field experiments in 1962

Factor	Degrees of freedom
Total	19
Replicates	3
Treatments	4
Error	12
Splitting of treatment sum of squares:	
Treatments	4
Control versus copper treatments	1
Soil versus foliar treatments	1
"B" versus "C"	1
"D" versus "E"	1

(c) Description of Sites

The sites at which experiments were carried out are described in Table 16. The figures given for pH and "availability" of P and K in the soil within the trial area, were averaged from analyses of the plot seedbed samples from Oldcastles and Rosehill. At the other sites, however, combine drilling had taken place before the seedbed was sampled, and so the figures are averages of the levels found in the harvest samples. Representative field samples were taken in the autumn of 1961 and analysed for total and E.D.T.A. extractable copper. The trial fields were chosen on a basis of these copper values, which are shown in Table 16. Withertip had previously been seen only at Hexpath, although yields of cereals at Oldcastles had sometimes been lighter than expected. Swayback in lambs had been severe in some years at Hexpath, and had occurred in neighbouring fields at Rosehill. Some unthriftiness and unusual pigmentation in cattle had been caused by copper deficiency at Oldcastles and Billie Mains.

Table 16

Field Experiment sites 1962

	Site 1	Site 2	Site 3	Site 4
Farm	Hexpath	Billie Mains	Oldcastles	Rosehill
Field	Bog	Castle Shotts	Craigielaw	No.3.
Elevation in feet	575	300	350	930
Soil series	Eckford	Eckford	Eckford	
Surface soil texture	sandy-loam	sandy-loam	sandy-loam	clay-loam
Average % loss on ignition in soil	varying from 5.8 to 9.2	5.8	3.7	12.6
Average pH of soil	6.2	6.5	5.9	5.8
Average "availability" of P in soil	moderate	high	high	moderately low
Average "availability" of K in soil	moderate	moderate	high	very high
p.p.m. total copper in field soil sample	less than 1	2.0	3.7	3.9
p.p.m. E.D.T.A. extractable copper in field soil sample	0.40	0.86	0.87	1.14
Drainage	free	free	free	poor
Previous crops:				
1961	swedes	barley	barley	oats
1960	oats	oats	turnips	grass
Experimental crop	oats	barley	barley	oats
Experimental variety	Forward	Freja	Maythorpe	Blenda
Manuring: rate in cwt/acre	3	2	2	2
fertilizer	6-15-15	13-13-20	16-9-9	13.5-13.5-13.5
If undersown	yes	yes	yes	no
Date sown	23rd April	11th April	22nd March	28th April
Date of foliar spraying	31st May	23rd May	29th May	15th June
Date of first sampling of plants	12th July	4th July	20th July	27th July
Date harvested	8th October	4th October	21st Sept.	8th November

(d) Experimental Methods

(i) General

The dimensions of the trials were planned in consultation with the farmers so that, at harvest, one run of the combine down the middle of a plot would cut 1/40 acre. A treated margin of just under half the combine width was left at both sides of each plot so as to minimise contamination from neighbouring treatments, and to make the harvesting of margins as simple as possible. In early spring, suitable trial sites in the fields were chosen with as great a uniformity as possible of soil type, drainage, aspect, slope and history of cropping and manuring. The farmer then cultivated, sowed and fertilized the whole field, including the trial area. About the same time as the seed was sown, the trial site was measured accurately and aligned, if possible, so that the length of the plots was at right angles to the drill direction. In order that the experimental area could be found precisely in the field, wired stones were buried at the corners of the trial, and the distances between these corners and white stubs at the edge of the field were measured. Soil samples were then taken with trowels as described below. The "D" and "E" plots and margins were delineated with string, and the treatments were applied with a knapsack sprayer. The young cereal plants were sprayed in the same way later with the "B" and "C" treatments. Copper oxychloride was used in preference to copper sulphate because it causes less corrosion of spraying equipment, and can be applied at a higher rate on herbage without causing damage.

The sites were visited every ten or fourteen days during the growing season, and observations of the condition of the crop and any treatment effects were recorded. Samples of herbage were taken at the time when the ears were emerging. There were a number of reasons for choosing this stage of growth:- (1) It has been reported that any differences in copper concentration are larger in young tissues than in harvested grain and straw. (Piper 1942 (1), Rademacher 1940). (2) Ear emergence occurred long enough after the foliar spraying to ensure that the copper salts had been assimilated or washed away, and were not contaminating the surface of the leaves. (3) At this stage of growth it is easier to walk among the plants without causing damage than it is later. (4) Ear emergence is a stage of development which is easily recognised.

At harvest, usually after the rest of the field had been cut, the yields of the plots were measured. The grain from each plot was collected in a sack, weighed and sampled. From the moisture content of the samples, the yields of the plots were converted to a uniform 18% moisture level. The length and breadth of each plot cut by the combine harvester were measured, and from these dimensions the yield in cwt. per acre was calculated. At the end of every plot, the combine harvester was run for about a minute until the grain of the preceding plot was cleared. When time and the weather permitted, straw yields were measured. The conversion to cwt. per acre of oven dry straw was made using factors derived from the dimensions of the plots and the moisture levels found in the straw samples. Soil samples were also taken from the plots at harvest.

(ii) Sampling

In order to minimise the effects of variation caused by the sampling techniques of different individuals, each worker sampled complete replicates of any experiment, and routine sampling was standardised as much as possible. Growing plants were cut with clean stainless steel secators about one and a half inches above the soil surface. The sampler walked in zig-zag fashion once down a plot which was differentiated by two canes topped by brightly coloured socks. Three plants were cut from each of sixteen different locations spaced out along his way, and two plants from one other place. Any obviously dirty plant was not taken, but, otherwise, sampling of a species was at random at the seventeen locations. Grain from plots cut by combine harvester was sampled by taking nine small handfuls from places as far apart as possible in the sacks. The straw sampler walked immediately behind the harvester and picked up nine handfuls spaced at random along the plot. The very beginning of the cut, however, where straw from the preceding plot might still be ejected, was avoided. Obviously earthy or weedy patches were not sampled. All herbage samples were placed immediately in clean, labelled polythene bags which were closed with elastic bands and transported together in a box, away from any soil. In order to avoid the risk of soil contamination, herbage was never, under any circumstances, collected by anyone after soil sampling.

Plant samples were taken to the laboratory as soon as possible. They were examined, and any dirty plants, or any of another species, were discarded. Each sample was then weighed, spread on a tray lined with clean greaseproof paper and dried in an oven at 90°C overnight, or to constant weight. If other herbage was also in the oven, samples from copper trials were loosely covered with clean paper to minimise contamination. Dried straw samples were milled through a screen with 1 mm. perforations, but were usually then too bulky for convenient storage, and so had to be subsampled after thorough mixing. Other herbage went through a 2.45 mm. sieve after milling, but in the case of grain, a screen with 4 mm. holes was found to be more suitable. The only mills used were those in which all brass parts had been replaced by stainless steel. Samples were stored in securely closed glass bottles or polystyrene pots which had been carefully washed out with distilled water.

No duplicate samples were taken of any plants from the same plot, and so the sampling error is not known. All experiments had four replicates of each treatment, however, and so in the statistical analysis of data, any sampling error was included in the general experimental error.

Soil was sampled from the field experiments by the worker walking in zigzags down a plot and taking nine augerfuls of soil at sites spaced out at random along his path. The soil was emptied from the auger, the screw of which was 8" long, into a numbered stout paper bag lined with greaseproof paper. If a very dry soil or a seedbed was being sampled the technique was similar, but trowels instead of augers were used. In this case, a hole 8" deep was made with the trowel and a slice down the side of it taken as one of the nine subsamples. The soil samples were collected in a box for transport back to the laboratory, where they were dried by being spread for three or four days at about 30°C on trays lined with brown paper. They were then mixed and ground with stainless steel rollers through stainless steel screens with 2 mm. perforations, and stored in clean glass bottles or in the original lined paper bags.

As the condition of the soil governed whether auger or trowel should be used, no samples were taken by both methods at the same time. Data from samples taken at various times from the same plot indicate, however, that seedbed trowel sampling gives equivalent results to auger sampling. On two occasions the untreated seedbeds of experiments were sampled in duplicate by one worker using a trowel. The resulting two samples from each of the twenty plots at both sites were analysed for E.D.T.A. extractable copper. The duplicates did not usually differ much from one another, as shown in Table 17. Only four control plots of one experiment were sampled in duplicate with an auger, and the E.D.T.A. extractable copper values for these soils are listed in Table 18. The sampling error, as with the data on plants, was always included statistically in the random error when the significance of the results of any experiment was considered.

Table 17

Duplication of trowel soil sampling

p.p.m. E.D.T.A. extractable copper in air dry soil

Site A.

Plot	1	2	3	4	5	6	7	8	9	10
Duplicate 1	0.40	0.38	0.56	0.40	0.44	0.64	0.53	0.49	0.51	0.54
Duplicate 2	0.42	0.39	0.49	0.44	0.46	0.72	0.51	0.51	0.82	0.54

Plot	11	12	13	14	15	16	17	18	19	20
Duplicate 1	0.40	0.41	0.44	0.46	0.48	0.46	0.56	0.52	0.50	0.42
Duplicate 2	0.51	0.47	0.46	0.48	0.52	0.52	0.57	0.44	0.57	0.46

Site B.

Plot	1	2	3	4	5	6	7	8	9	10
Duplicate 1	1.98	1.92	1.80	1.78	1.74	1.66	1.74	1.63	1.77	1.87
Duplicate 2	1.96	1.88	1.85	1.68	1.77	1.70	1.72	1.62	1.70	1.71

Plot	11	12	13	14	15	16	17	18	19	20
Duplicate 1	1.86	1.89	1.72	1.52	1.39	1.47	1.35	1.52	1.45	1.31
Duplicate 2	1.68	1.78	1.65	1.55	1.48	1.35	1.33	1.43	1.31	1.34

Average difference between duplicates: Site A: 0.055 p.p.m.,
Site B: 0.072 p.p.m.

Table 18

Duplication of auger soil sampling

p.p.m. E.D.T.A. extractable copper in air dry soil

Replicate (Plot)	1	2	3	4
Duplicate 1	0.52	0.42	0.45	0.38
Duplicate 2	0.40	0.42	0.46	0.43

Average difference between duplicates: 0.045 p.p.m.

(e) Observations and Comments

(i) Weather

Although the winter was milder than usual, March and April were very cold. The first six months of 1962 were abnormally dry, and the rainfall in June was particularly low. All the summer months were cool, and a very wet September gave a protracted harvest. Strong winds were unusually frequent throughout the year.

(ii) Hexpath

Although the most uniform part of the field was chosen, two replicates were higher, sloped more steeply, and contained less humus than the rest of the trial. Early growth was poorer on the lower half of the experiment. Bad wind burn was observed on the top leaves of all the plants during all of the month of June. The crop treatments were sprayed when the oats were about 4½ to 8" high, and caused a negligible degree of scorching. By the end of June, the growth was showing uneveness, and some of the smaller plants were slightly chlorotic, especially at the edges of the leaves. Immediately before the "heading out" stage, withertip developed in patches (see plate 1) in all the controls but in none of the treated plots. The plants showing withertip (see plate 2) were generally stunted compared with healthy plants. The roots were not extensive and the first and second leaves were green with no sign of senescence. Younger leaves were paler green, except for the tips, which were white and formed spirals. The main stems were surrounded by many small green or white tillers and, in severe cases, the whole of the centre growing shoot was white.

Plate 1. Withertip in oats, Hexpath 1962.

Plate 2. Young copper deficient oat plant.

By the beginning of August, there was a general difference in height of about six inches between the untreated and treated oats. From August until harvest, the control plants remained obviously less mature than treated plants. In the centre of plate 3, for example, in the middle distance beyond the two white flags, is a green control plot outlined by yellowing treated plots. The oats were attacked by Helminthosporium Avenae equally throughout all treatments. In September "blind ear", in which some ears were empty, white and papery, was seen where withertip had appeared earlier. Each affected plant had many small immature tillers. At harvest, the treated plots all looked yellow when seen from above, while the control plots looked white because of the empty ears. Plate 4 shows part of the end of a control plot, with a treated plot to the right of the cane. When the trial was all cut, it could be seen that the straw from all the control plots was still green, while that from three of the four "C" treatment plots was bright yellow. The other straw was intermediate in colour.

In 1963 the field was grazed, and in 1964 a hay crop was grown. At no time were any treatment differences in growth, colour or development observed in either clover or grass.

(iii) Billie Mains

The growth of the barley was very uniform throughout the season and no treatment differences were observed. In 1963, when the reliability of the method for estimating extractable soil copper had been proved, it was discovered that the whole field, including the trial, had an initial E.D.T.A. extractable copper level of about 5 p.p.m. The experiment was therefore

Plate 3. Copper trial at Hexpath, August 1962.

Plate 4. Control plot at harvest, Hexpath 1962.

not a copper deficiency trial as intended, but some of the results are included as they show that there were no toxic effects caused by the addition of 20 lb. per acre copper oxychloride to this soil. As the undersowing was as high as the barley in places, no straw yields were measured.

(iv) Oldcastles

During the application of copper to the seedbed, a strip diagonally across two plots was mistakenly treated. To rectify this, both of the half treated plots were uniformly sprayed, so that a double plot resulted. The results from the two plots were averaged to give data to be used in statistical analysis. The barley was sown under cold conditions and grew very little during the first month. At the beginning of May, when it was about 6" high, the field was harrowed and undersown. The harrowing was very harsh and uneven, and some of the barley was half buried or half uprooted. The crop was not sprayed with copper until the plants had recovered from this, when they were about 12" high and at the 5th leaf stage. After the spraying, there was a spell of dry weather, and the plants which had been given foliar treatments were distinctly scorched. During ear emergence the plants were affected by drought, and later some lodging and mildew was observed. No clear treatment differences were seen, but the plants in the control plots seemed stronger than those in the treated plots to two independent workers at the end of July. Because of weather conditions, and the late foliar applications, the barley was sampled about a month after ear emergence.

(v) Rosehill

The layout was different from that on other farms because the field was too small for the square design. The soil was reclaimed heather moor overlying clay, and the field drainage, which was at right angles to the length of the treated plots, was very variable. The manuring, applied parallel to the plots, was also uneven, with a frequent area of overlap which had been fertilized twice. Thus growth was very patchy. A great variety of strong weeds were seen throughout the season, and in many places were taller and stronger than the oats. The foliar treatments were sprayed when the oats were about 4 to 12" high, and caused slight scorch. No treatment responses were ever observed. The soil was very dry during June, but the oats were flattened under wind and rain in September. The harvest was not made until November, when conditions, although drier than previously, were still wet. As the harvester broke down before all the plots had been cut, no accurate yield data was obtained, and, because all the straw and grain was very muddy, no useful analyses could be made on the harvest samples of plant material.

(f) Results

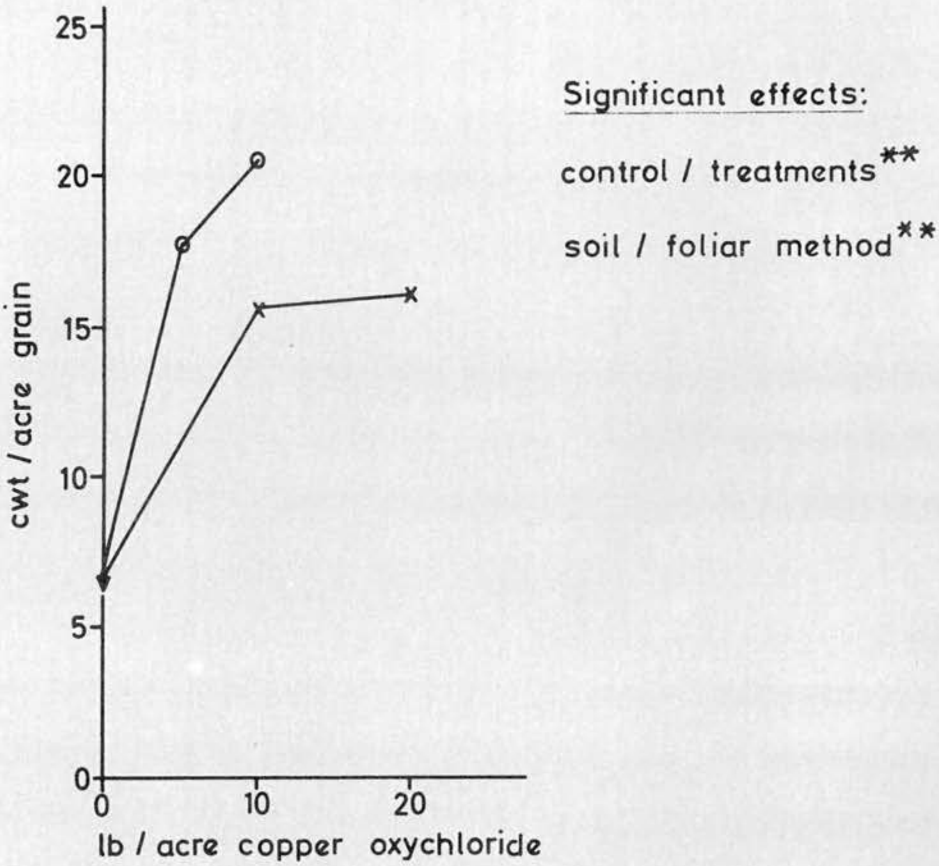
The detailed results from each site are shown in appendix 5.

All the copper treatments increased the yield of the oat plants sampled at the "heading out" stage at Hexpath, but had no measurable effect at the other sites.

Grain yields were measured at three sites, but only the oats at Hexpath showed a response to the copper treatments. The yields at Billie Mains were remarkably uniform, and so there was no diminution of yield caused by the addition of even the highest treatment to this soil. The barley at Oldcastles showed no response to the treatments, and so there was apparently enough native copper to satisfy the needs of the crop under the conditions there. At Hexpath during harvesting, it was found, after completion of the third plot, that the riddle in the combine had slipped, so that only a very small weight of large grain had been collected. The tiny yield of the previous plot, which was a control, was also suspected, and so neither weight was used in the evaluation of the results. The missing plot technique described by Paterson (1939 p.182), was used for the estimation of the missing data, and for the amendment of the usual statistical analysis. In spite of this incompleteness in results, the response to all the copper treatments at Hexpath was highly significant (see diagram 3). The yield of grain was more than doubled by the seedbed dressings, and trebled by the foliar treatments. The level of application had no significant effect.

The straw yields measured at Oldcastles showed no response to the treatments. At Hexpath, however, the production of dry straw in the two replicates growing on the lower, blacker soil was much less than in the other half of the experiment. When allowance was made in the analysis of variance for the interaction, which was very nearly significant, between the effects of copper and position, it was found that the "C" and "D" treatments gave a significantly smaller yield than the "A", "B" and "E" treatments (at P less than 0.05). Thus there was no difference between the general effects of the foliar and seedbed treatments.

Diagram 3. Effect of treatments on yield of grain at Hexpath 1962.



Note. In diagrams 3-9

o = foliar treatment

x = soil treatment

∇ = control

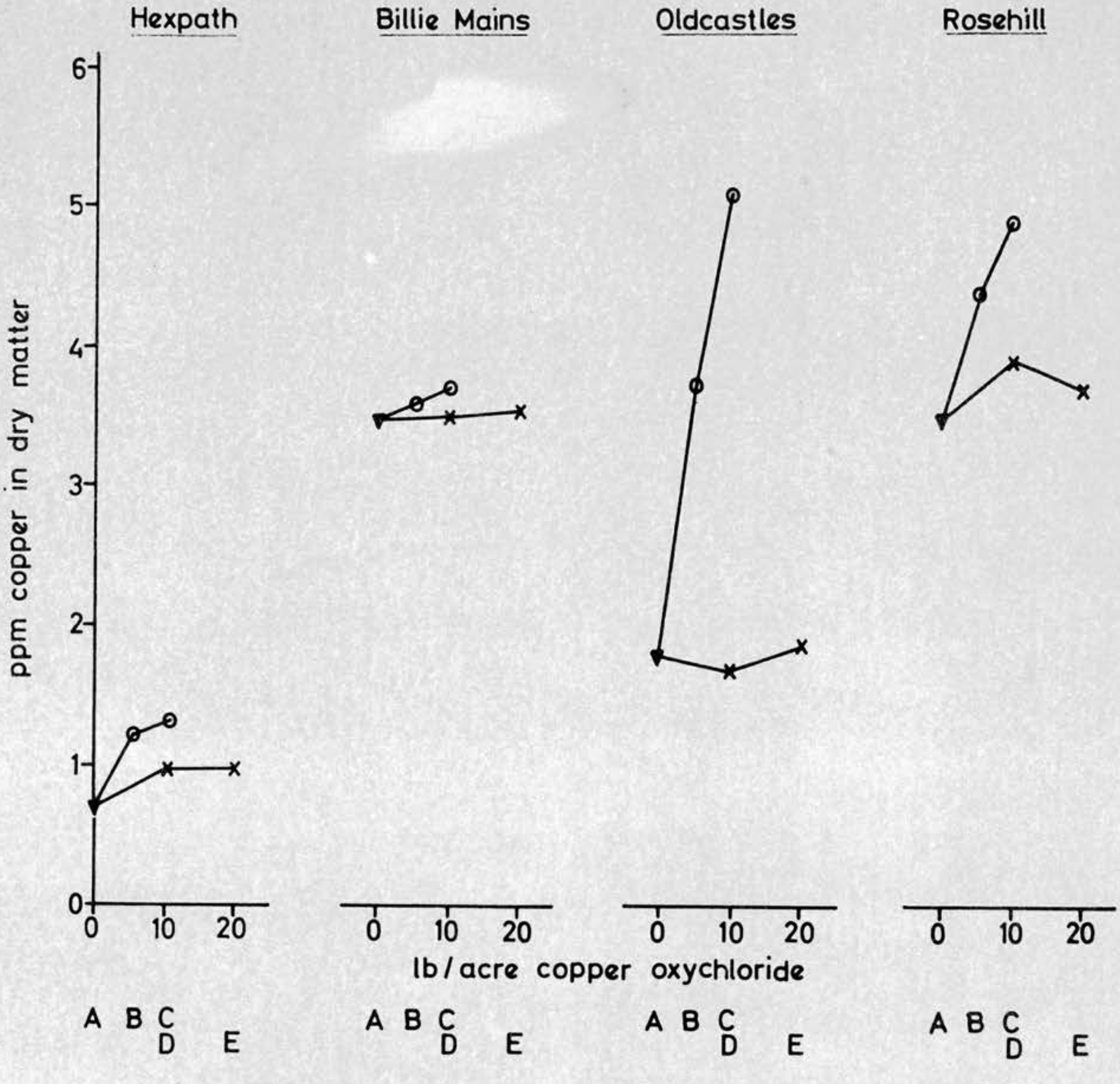
Mitchell Reith and Johnston (1957(1)) have reported that a foliar application of copper sulphate may slightly reduce the straw yield, while a seedbed dressing can cause a small increase. The figures for the percentage of oven dry grain in the total harvest of dry matter showed no significant treatment differences at Oldcastles. At Hexpath, however, a very large and similar increase in the percentage of grain was caused by all the copper treatments. The effect of copper in raising the grain rather than the straw yield was expected from the results of Mitchell, Reith and Johnston (1957(1)), and from the reports that one of the first results of copper deficiency is a lowered grain yield, not necessarily accompanied by a reduced production of straw. (Piper 1942(1), Wallace 1961, Hoffmann 1952).

At Hexpath, in both grain and straw, the moisture level in the control plants was very significantly higher than that in the copper treated oats. This difference may be a result of the observed immaturity of the untreated plants, as is their high proportion of green grain (see appendix 5). There were no differences between the moisture contents of grain from the various copper treatments, but straw from the plots given a seedbed dressing contained more water than that from the foliar treatments. The bushel weights of grain were measured on fresh material. Although, therefore, it is very probable that the control grain was far lighter than the treated grain at Hexpath (see appendix 5), this difference was not proved because there was also a disparity in moisture contents.

At Hexpath, Rosehill and Oldcastles, the concentrations of copper in the samples cut at ear emergence were raised significantly by the foliar treatments but not by the seedbed dressings. Diagram 4 illustrates the results at all four sites. At Oldcastles, the high values obtained for the foliar treatments, and the comparatively large difference between them, may be a result of their application at a later stage of growth than elsewhere. The differences between the copper levels in the control plants at the four sites were obviously not caused by the variation in soil copper status alone. The different varieties and conditions of growth must also have had an effect. The treatments raised the copper concentrations in the grain and straw only at Oldcastles, where the foliar treatments produced higher levels of copper than either the soil applications or the control. Thus the foliar treatments raised the copper concentration in the oats at ear emergence much more than in the mature plants. This difference in response as the oats matured may be related to the length of time between copper application and sampling, or may be an effect of the development of the plant. Piper (1942(1)) and Rademacher (1940) have reported that a similar reduction in response with age occurs after a soil dressing. In spite of the effect on yield at Hexpath, insufficient of the copper applied to the soil was apparently available to raise the concentration in the herbage. Rises obtained by Piper (1942(1)) and Mitchell Reith and Johnston (1957(1)) followed heavier copper fertilization.

The samples taken at ear emergence from Hexpath were also analysed for chlorophyll, nitrogen, manganese and iron. The chlorophyll results showed no significant differences, which was surprising, as Arnon (1950) has proved that copper plays a part

Diagram 4. Effects of treatments on copper concentration in plants at ear emergence 1962.



Significant differences between treatment means

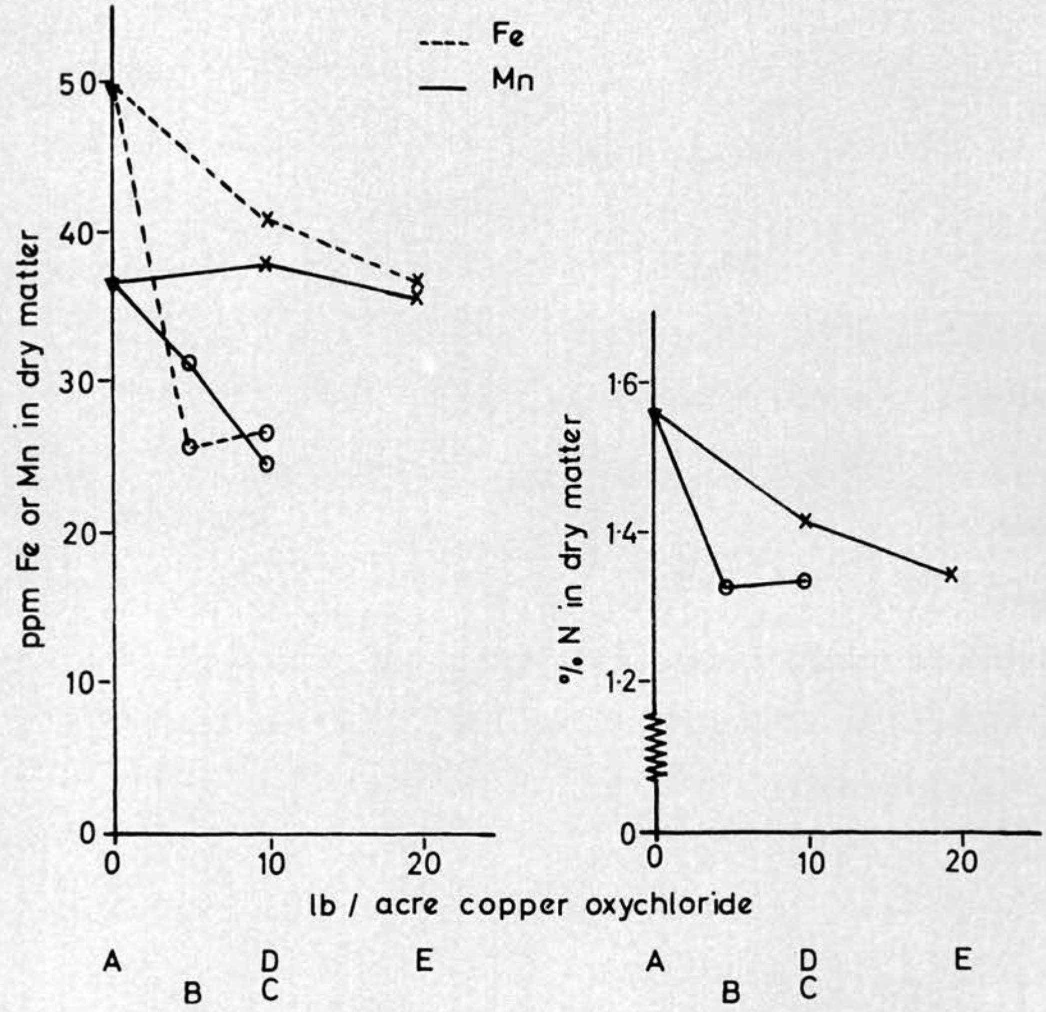
Hexpath	Billie Mains	Oldcastles	Rosehill
C / A, D and E**	none	B / A, D and E**	C / A, D and E*
A / B**		C / A, D and E**	A / B*
A / E*		B / C*	soil / foliar**
soil / foliar**		soil / foliar**	

in the synthesis of chlorophyll. The nitrogen and iron levels in the control plants were higher than those in any of the treatments, as shown in diagram 5. The concentrations of both iron and manganese were higher in the plants given seedbed dressings than in those in the foliar treatments. Calculation of the total uptake per plant, from the average yield per plant sampled, showed no differences in the nitrogen contents. The foliar treatments, however, caused a very significant drop in the uptake of iron, while the seedbed dressings caused a rise in the total content of manganese per plant. Thus the considerable effect of copper on iron uptake apparently takes place within the plant, while the smaller effect on manganese uptake is more related to the copper concentration in the soil than to that in the plant.

The level of nitrogen in the Hexpath grain was highest in the wettest, low lying replicate, but there were no significant treatment differences. The nitrogen concentration in the control straw, however, was greater than that in the straw from any of the copper treatments, and the straw from the foliar treatments contained a lower level of nitrogen than that from the seedbed treatments. These differences, which were similar to those between the corresponding uptakes, were probably connected with the variation in maturity observed at harvest.

Soil samples from the plots at seedtime and harvest were analysed for E.D.T.A. extractable copper. There was some variation between the levels found in the seedbed plot samples and those found in the field samples taken in October 1961 (see Table 19). The variation at Billie Mains was thought to

Diagram 5. Concentrations of Fe, Mn and N in oats at ear emergence, Hexpath 1962.



Significant differences

Fe: soil / foliar,** A / B, C and E,** D / B and C,** B / E,** A / D,* E / C.*

Mn: soil / foliar,** C / A, D and E** B / C and D.*

N: A / B, C, D and E.**

be due to some gross error in the field sample, and the differences at the other sites may have been caused by uneven distribution of copper in the field, as reported by Purves and Ragg (1962). It seems odd, however, that there should have been more copper in the trial area than in the rest of the field at all three sites where comparison can be made. There may have been some seasonal influence, causing higher results in the spring than in the autumn.

Table 19

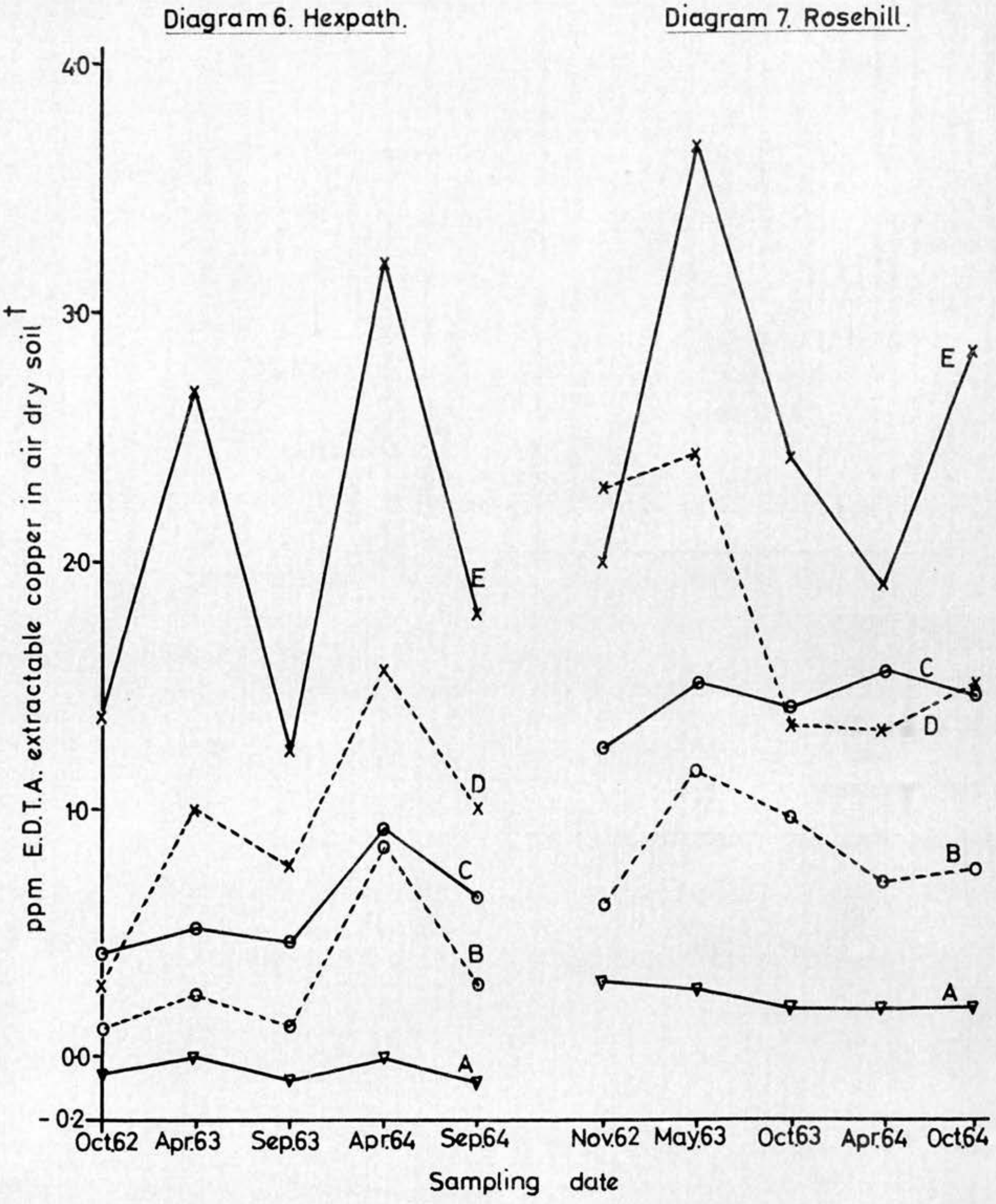
Comparison of the copper levels in field samples taken in October 1961 and plot samples taken in April 1962.

p.p.m. E.D.T.A. extractable copper in air dry soil.

Site	Hexpath	Billie Mains	Oldcastles	Rosehill
Representative field sample	0.40	0.86	0.87	1.14
Average of plot seedbed samples	0.50	5.27	1.08	1.65

The residual effect of the treatments in the soil was measured by subtracting the seedbed concentration of E.D.T.A. extractable copper from the harvest level for each plot, and carrying out a statistical analysis of variance on these differences. There was no significance between the residual effects at Billie Mains. At the remaining three sites, however, the copper treatments all gave higher results than the controls, and the foliar applications gave lower figures than the seedbed dressings. Only at Oldcastles was there significance at the 1% level. Diagrams 6 and 7 summarise the results, at Hexpath and Rosehill

Effects of treatments and sampling dates on residual extractable copper in soil.



Significant differences: Hexpath	Rosehill
E / A,B,C and D,** A / C and D,** B / D,** A / B,* C / D,* Apr.64 / all other dates,** Apr.63 / Oct.62,** Apr.63 / Sep.63.*	E / A,B,C and D,** A / B,C and D,** B / C and D,** May 63 / Oct.63 and Apr.64,** May 63 / Nov.62 and Oct.64.*

† Results after subtraction of the concentration in the untreated seedbed.

respectively, of spring and autumn samplings during 1962, 1963 and 1964. At Hexpath, all the differences between treatment means were significant at the 5% level except that between the averages for "B" and "C", while at Rosehill, the difference between the means of "C" and "D" was the only one which was not significant at the 1% level. Thus, as 10 lb. copper oxychloride per acre was applied to both treatments "C" and "D", the method of application had a significant effect at Hexpath but not at Rosehill.

Diagrams 6 and 7 also show variations between the results from individual sampling dates at both sites. These differences in the levels of extractable copper were not caused by any change in technique in the laboratory, as they were confirmed by the analysis, in one batch, of samples taken at various dates. At Hexpath, the average for April 1964 was very significantly higher than any of the other date means, and the figure for April 1963 was higher than that for either October 1962 or September 1963. This gave an obvious seasonal rise and fall to diagram 6 at all treatment levels. The pattern from Rosehill in figure 7 was different, in that, although the mean for May 1963 was higher than any of the other averages, there was no corresponding rise in the April 1964 samples. A possible explanation for this difference between sites is that, while the Bog field Hexpath was undersown in 1962 and remained under grass in 1963 and 1964, the cropping of the site at Rosehill was more varied. The weedy stubble of the trial oats there was used as a poultry run in the winter 1962-1963. Turnips were grown during 1963, and the field was ploughed and sown with oats before the

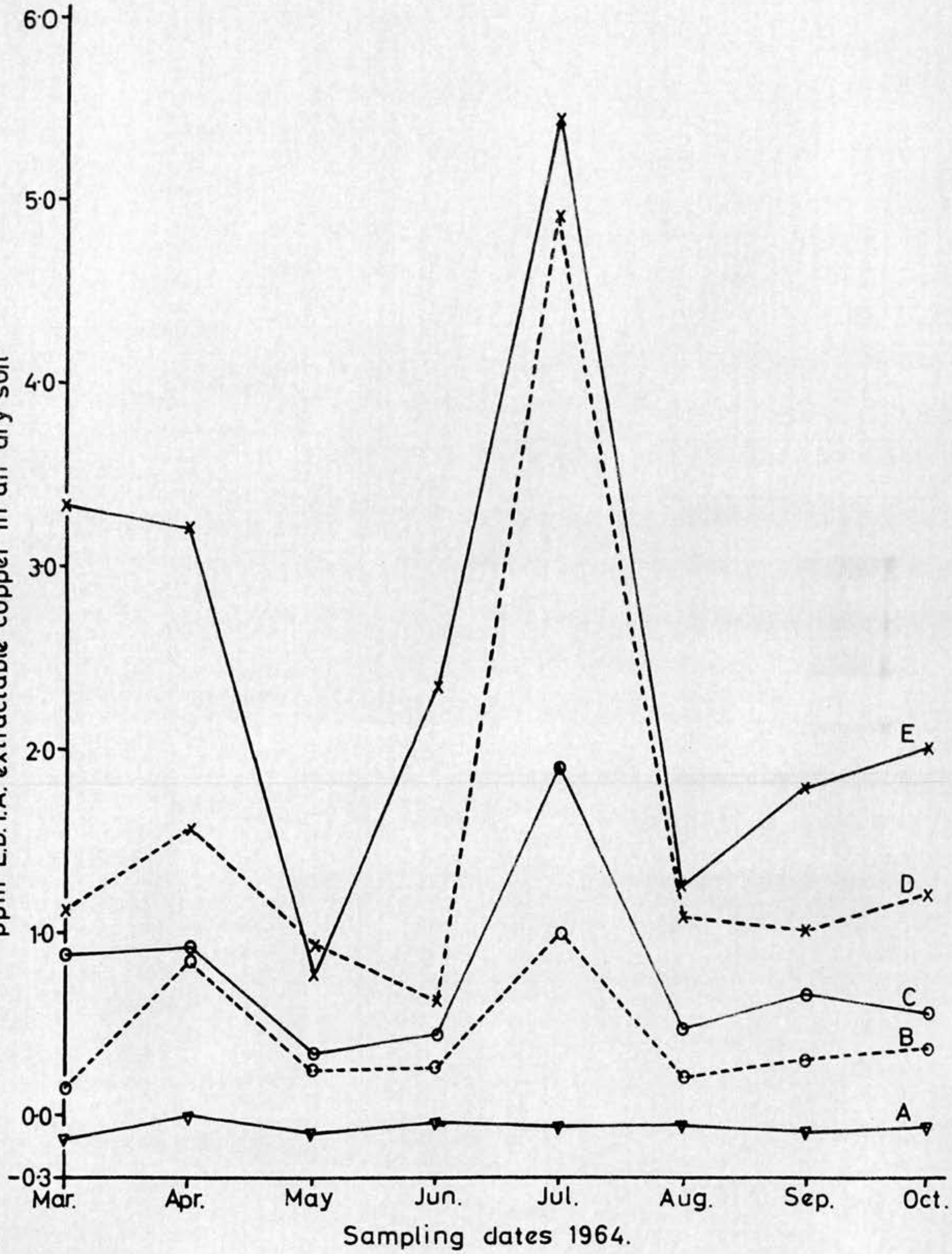
April 1964 samples were taken.

There was no evidence at either site of the applied copper becoming progressively fixed in a form not extractable by E.D.T.A., or being progressively lost in drainage.

Monthly soil samples were also taken from the plots at Hexpath during the growing season of 1964. The Bog field was not grazed in the early spring, and a hay crop was cut at the beginning of July. From July until October there were sheep in the field. The residual effects found in the soil samples are illustrated in diagram 8. All differences between treatments were significant except between "B" and "C", as noted from the spring and autumn samples from this site. The sampling dates had a marked effect on the results obtained. As is obvious from diagram 8, the July figures for all the copper treatments, but not for the control, were much higher than those from any other month. The ground was very hard and dry towards the end of July, and a higher proportion of the surface soil than normal was possibly present in the samples. Most of applied copper has been found by Lundblad Svanberg and Ekman (1949) to remain very close to the surface. There may also have been an effect from the change of crop. When the hay in the field was cut and removed, the soil was left more exposed. The physical, and possibly chemical and bacteriological conditions of the soil therefore changed during this period (Russell 1950 p.203).

The highly significant drop, between the extractable copper in April and that in May, cannot be explained by any difficulty in sampling at either date. The moisture content of the soil fell, however, so that the soil was described as "wet"

Diagram 8. Effects of treatments and sampling dates on residual extractable copper in soil. Hexpath (Bog field)



Significant differences: E / A, B, C and D,** D / A, B and C,** A / B and C,* Jul. / all other dates,** Apr. / Aug. and May,** Apr. / Sep. and Jun.*, Mar. / May.*

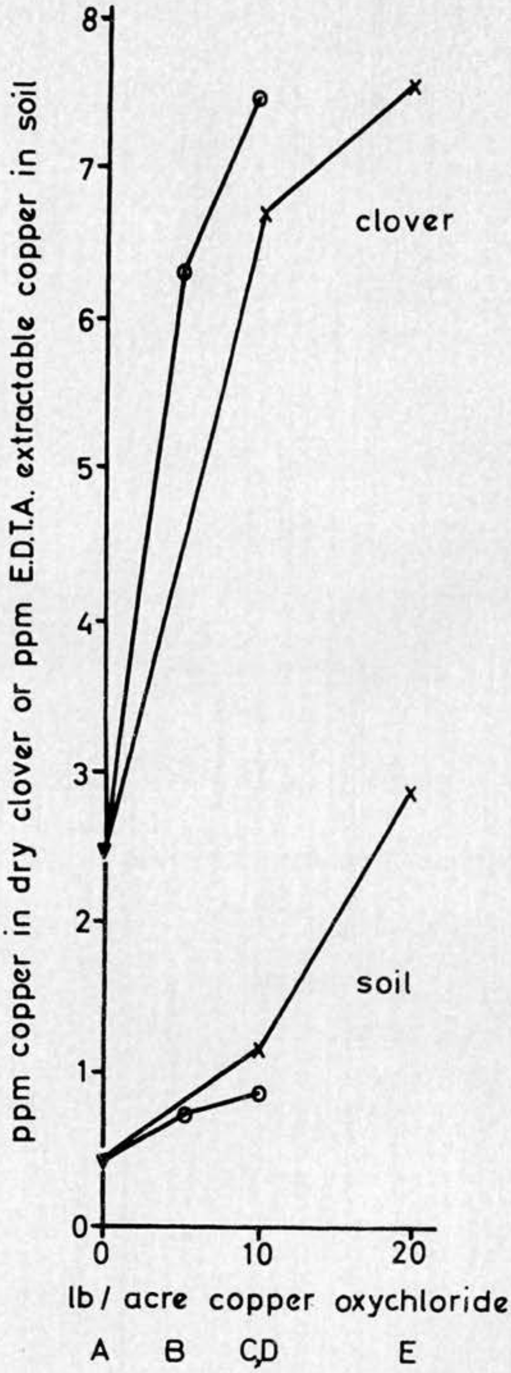
† Results after subtraction of the concentration in the untreated seedbed.

in April, but only "moist" in May. This change may have had an effect on availability. (Hoffmann 1952, Steenbjerg 1940). The other crop change in the year also occurred during this period, as the spring growth began after the April sampling, and the grass was about 12" high by 21st May. This growth will have removed some of the readily available copper along with other nutrients. There were no significant differences between the averages for May, June, August, September and October. The October sampling, however, was the only one which gave a mean not significantly different from those of March and April. Thus, if the July figures are discounted because of the difficulty of sampling and the crop change, there was a tendency for the high spring E.D.T.A. extractable copper values to fall as the growing season began, and to start rising again in the late autumn.

Another method of measuring the residual effect of the copper treatments is to analyse plants growing on the soil. In June 1964, the flowering clover at Hexpath was sampled on the same day as the soil. The results of the copper analyses of the oven dry herbage are shown in appendix 5. These figures, and those of the soil analyses, are illustrated in diagram 9. The most striking effect was the raising of the clover copper level by all the treatments. This was in contrast to the extractable soil copper figures (see diagram 9) which showed significance, with the "t" test, between the control and both the seedbed treatments, but not between the control and either of the foliar treatments. From diagram 9 it is also seen that the copper concentration of the clover from the "C" treatment was equal to that from "E", and higher than that from "D".

Diagram 9. Effects of treatments on copper concentrations in clover and soil.

Hexpath (Bog field) June 1964.



Significant differences

Clover: A / B, C, D and E,** B / C and E,** D / C and E*

Soil: E / A, B, C and D,** A / D,* soil / foliar**

Thus 10 lb. per acre copper oxychloride gave a higher residual availability when used as a foliar dressing than when applied to the soil. The soil analyses for the monthly samples, (see diagram 8) showed, on the other hand, that the soil dressings were more effective in raising the soil copper status than the foliar sprays.

Blenda oats were grown at Rosehill in 1964 and the trial plots were sampled at ear emergence. The results of the analysis of the plant material are given in appendix 5. There were no significant differences between the treatment means. Thus, although E.D.T.A. extracted different concentrations of copper from the soils in the various treatment plots, there was no difference in availability to the oats. As in 1962, the growth of the oats was very uneven because of drainage differences, and there were no visible treatment effects.

(2) 1963

(a) Aims

(i) To find the lowest rate of copper oxychloride which produces good yields when sprayed on young cereals growing on copper deficient land.

(ii) To compare the effects of different rates of copper application on the copper concentration in cereal plants and the extractable copper level in the soil.

(b) Statistical Design

At all three sites the treatments were:

F: control	∴ log (treatment + 1)	= 0.00
G: 0.8 lb. per acre copper oxychloride	∴ " " "	= 0.26
H: 2.3 " " " "	∴ " " "	= 0.52
J: 5.0 " " " "	∴ " " "	= 0.78
K: 10.0 " " " "	∴ " " "	= 1.04

A randomised block design, with four replicates of the five treatments, was again used. The detailed layouts are given in appendix 4. The analysis of variance is shown in the first part of Table 15. The treatment sum of squares could not be split as in 1962, but could be subdivided into linear, quadratic and cubic functions, using the coefficients of orthogonal polynomials given by Fisher and Yates (1957 p.90). This subdivision is possible (Steel and Torrie 1960 p.222) because the treatments are equally spaced, if they are expressed as log (lb. per acre copper oxychloride + 1) as shown above.

(c) Description of Sites

The sites at which experiments were carried out are described in Table 20, from which it is seen that only fields with low E.D.T.A. extractable copper levels were chosen. The figures for pH and "availability" of P and K for all three sites were averaged from determinations on the soil samples taken from the plots at harvest, because the seedbed had been fertilized just before the spring soil sampling. The seedbed of the rest of the field at Pirnie was dusted by a contractor with copper oxychloride and the E.D.T.A. extractable copper results from the plots before treatment indicate (see appendix 6) that there was surface contamination of some of the plots in the spring.

Table 20

Field experiment sites 1963

	Site 1	Site 2	Site 3
Farm	Pirnie	Upper Huntlywood	Choicelee
Field	Monkshott	West Knock	3rd Birks
Elevation in feet	400	700	700
Soil series	Eckford	Eckford	Hobkirk
Surface soil texture	loamy-sand	fine sandy-loam	sandy-loam
Average % loss on ignition in soil	4.5	4.2	5.9
Average pH of soil at harvest	6.5	7.2	5.8
Average "availability" of P in soil at harvest	moderate	high	low
Average "availability" of K in soil at harvest	moderately high	very low	low
p.p.m. total copper in untreated seedbed	less than 1	1.3	3.1
Average p.p.m. E.D.T.A. extractable copper in untreated seedbed	0.62+	0.65	0.49
Drainage	free	free	free
Previous crops: 1962	turnips	oats	potatoes
1961	oats	swedes	barley
Experimental crop	barley	oats	oats
Experimental variety	Ymer	Sun II	Forward
Manuring: rate in cwt per acre	2	3	3
fertilizer	15-10-10	10-10-18	15-10-10
If undersown	yes	yes	yes
Date sown	22nd April	23rd April	3rd April
Date of foliar spraying	28th May	11th June	30th May
Date of first sampling of plants	9th July	23rd July	9th July
Date harvested	30th Sept.	(23rd Sept.)	11th October

+ Average of uncontaminated samples only.

Withertip in oats had been seen in a neighbouring field at Choicelee, and had been suspected in the trial field at Upper Huntlywood. Swayback in lambs had occurred at Choicelee and Pirnie, and bone deformities in young horses on the latter farm were thought to have been caused by copper deficiency.

(d) Experimental Methods

Only differences in methods between 1962 and 1963 are described. As a check, a representative soil sample was taken from each site as soon as it was chosen, and analysed for E.D.T.A. extractable copper before the trial was laid down. All the treatments were foliar, and were applied at the same time. All the plots, including the controls, were sprayed at a rate of 100 gal. water per acre so as to avoid any confusion of water and copper effects.

(e) Observations and Comments

(i) Weather.

The winter was very severe indeed, and very cold weather continued until March. July and August were wet months, and lodging of cereal crops was widespread. Most cereals ripened later than normal under dull conditions.

(ii) Pirnie.

The treatments, which were applied when the barley was 6 to 7" high and in fourth leaf, produced no scorching. Several mole runs loosened the roots of plants in the trial, and couch grass grew strongly in all replicates. There was no withertip, and the only abnormality in growth was a spiralling and bleaching of some of the awns of heads containing little grain. This deformity occurred on scattered plants throughout the field, and not particularly in the control plots. At the "heading out" stage, two observers independently graded the plots according to strength of growth, and agreed that the plants in the highest treatment were stronger than the control plants in every replicate. This, however, was the only treatment effect seen during the season. Towards the end of July, severe lodging occurred in the first replicate and one other plot, and, by harvest time, secondary tillers had grown through the first growth, much of which had rotted. No yields were therefore recorded for these plots. In the remainder of the trial, lodging occurred to a smaller extent, and gales the week before harvest caused the shedding of some of the grain.

(iii) Upper Huntlywood.

At the beginning of June, the younger oat leaves were pale green at the edges. The weather was very wet and the first suitable day for the spraying of the treatments coincided with the time when weed control was applied by the farmer. As there was ample moisture in the soil, the controls were not sprayed with water. The plants were in fifth leaf, 8 to 11" high, and rather variable in growth. The treatments were applied on a hot morning and, contrary to a special forecast from the meteorological office, there was a heavy thunderstorm in the early afternoon. Very much of the dressing was thus probably washed off the leaves within about three hours of application. Three days later the plants were scorched in a complex way. In three replicates, the scorch followed the copper treatments, being most severe in the "K" plots, but, in the fourth replicate, damage caused by the weed control spray was also evident. In addition, many plants were beginning to show markings which were thought to be "grey speck" caused by manganese deficiency (Wallace 1961). It was difficult in some cases to know, however, whether either of the sprays or the deficiency was the main cause of the observed symptoms. By the end of June, the growth was very uneven, and it was discovered that the farmer had had trouble with the flow of fertilizer out of his combine drill. In the last week of July, the plants were very uneven, probably manganese deficient, and attacked by <u>Helminthosporium Avenae</u> and aphids. No treatment effects were observed, and it was decided that any yield response to copper would be masked by other variable factors. The yields were therefore not measured, and plants from the plots were cut with secators, in the same way as for the sampling at ear emergence, immediately before the field was harvested by a binder. The grain was separated from the straw by hand in the laboratory.

(iv) Choicelee.

The treatments were applied when the oats were 5 to 8" high and in fourth leaf. There were large pale blotches on the third leaf of very many plants. In some areas in the field, perhaps because of the placement of high concentrations of fertilizer close to the seeds, the oats were pale and stunted with darker striping down the leaves. The trial area was, however, not much affected. The plants appeared uniform by the middle of June, when, however, some of the oats were attacked by <u>Helminthosporium Avenae</u>. There was no scorching and no visible effect of the treatments at any stage. High winds in October, especially during the week between the cutting of the rest of the field and the harvesting of the trial, caused the shedding of some of the grain. The most exposed plot had lost much more grain in this way than the rest of the trial, and so the missing plot technique was used to estimate its yield.

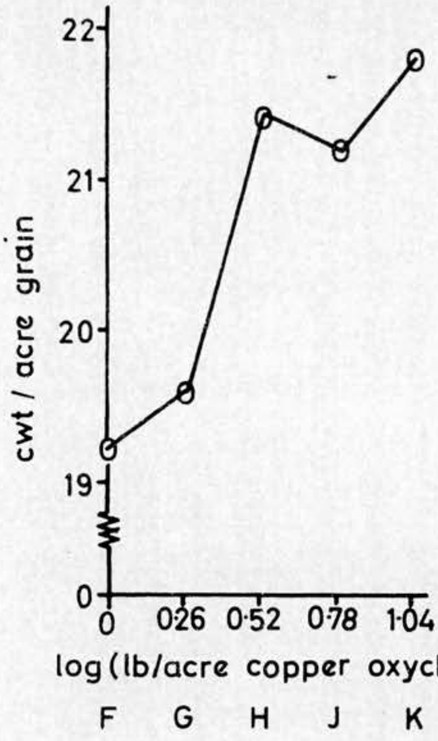
(f) Results

The detailed results are given in appendix 6.

There was no significant response to the copper applications in the grain yield at Pirnie. At Choicelee, although the differences between individual treatments were not significant, the response to the applications was linear (P less than 0.05) when the treatments were expressed as log (lb./acre copper oxychloride + 1), (see diagram 10). The straw yield at Choicelee did not respond to copper, and that at Pirnie was not measured because of the quantity of secondary growth present. There were no treatment differences, at either Choicelee or Pirnie, in the percentage of moisture in the straw or grain. The copper response curve of the bushel weights of dry grain from Choicelee is given in diagram 11. It was expected from the results at Hexpath in 1962 that this curve would have an upward slant, so that the lightest grain would be from the control. The opposite occurred, and the lightest grain was produced by the highest level of copper application. The percentage of grain in the harvest at Choicelee gave a linear response to the coded copper treatments, being lowest in "G" and highest in "K".

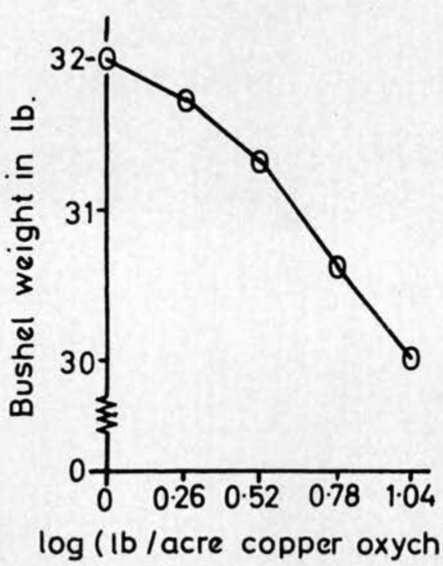
The results of the copper analyses of the cereal samples

Diagram 10. Effect of treatments on grain yield. Choicelee 1963



Significant effect: Linear response to coded treatments.*

Diagram 11. Effect of treatments on bushel weight of dry grain. Choicelee 1963

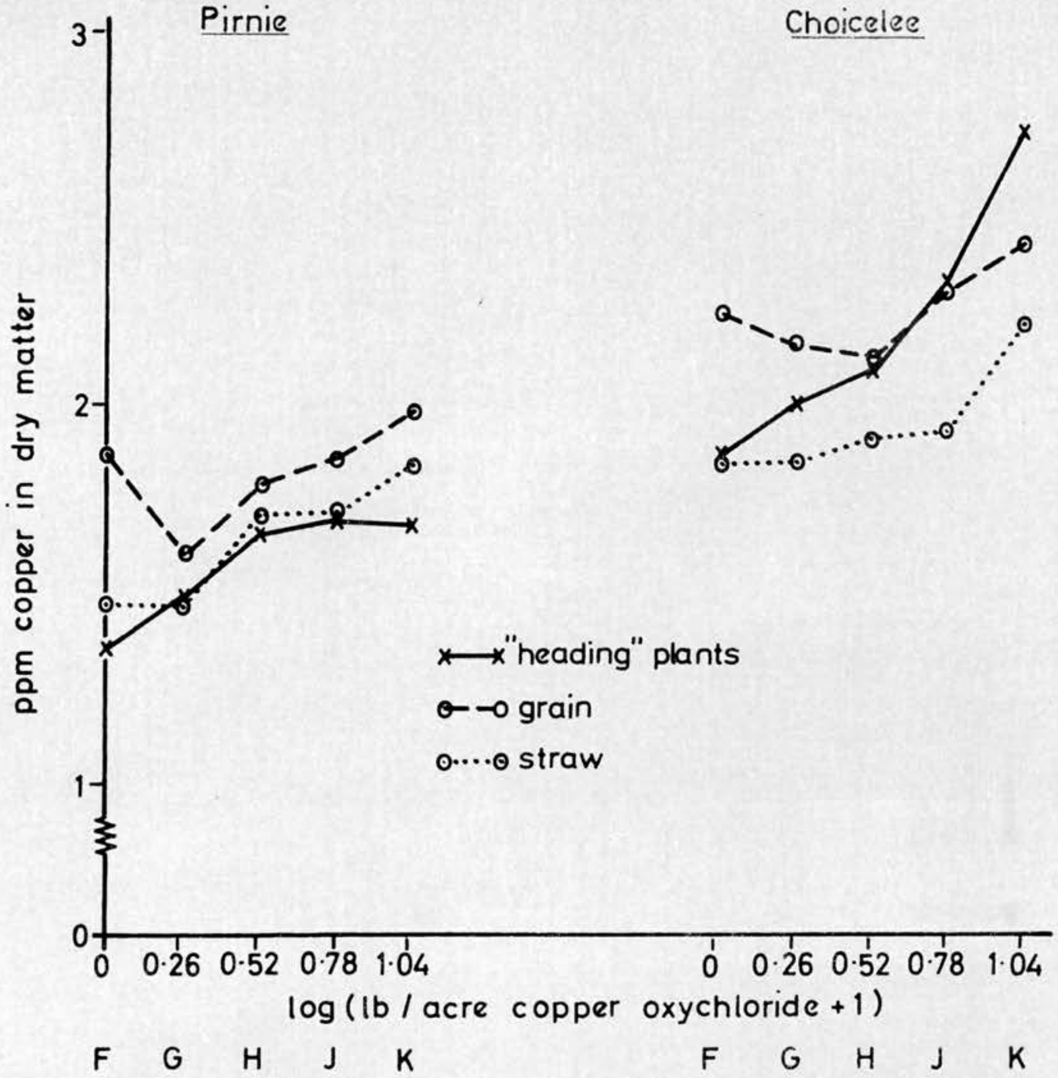


Significant effect: Linear response to coded treatments.*

are illustrated in diagram 12. At all three sites the two highest applications caused significant increases in the concentration at ear emergence and there was a linear response to the coded treatments at this stage. There were, however, no significant differences between the treatment means of the copper levels in the grain and straw from Upper Huntlywood. In the grain, there was a quadratic response at Choicelee, but no significance between individual treatments, while at Pirnie, application "G" resulted in a concentration of copper lower than that from any other treatment, even the control (see diagram 12). At Choicelee, the decrease in copper concentration in "G" and "H" was the result of the increase in grain yield, for the total uptake of copper per acre in the grain was similar from treatments "F", "G" and "H". At Pirnie, however, treatment "G" did not increase the grain yield. In the straw at both Pirnie and Choicelee, the linear response was highly significant, and there were two increases in copper concentration between neighbouring treatments, giving a stepped appearance to the response curves (see diagram 12). The copper concentrations in the control plants were higher at Choicelee than at Pirnie throughout the season. The results from Upper Huntlywood were similar to those at Pirnie, but the grain and straw data are not exactly comparable, as the Upper Huntlywood oats were cut before complete maturity.

The samples cut at ear emergence at Upper Huntlywood were analysed for manganese and iron as well as copper. The manganese values of about 6 p.p.m. were well below the deficiency

Diagram 12. Effect of treatments on copper concentration in plants. 1963.



Significant effects and differences

	Pirnie	Choicelee
at "heading":	Linear response,** F / K,** F / H and J.*	Linear response,** K / F, G, H and J,** F / J,** G / J.*
grain:	G / F, H, J and K.*	quadratic response.*
straw:	Linear response,** K / F and G,** F / H and J,* G / H and J.*	Linear response,** K / F and G.**

limit of 14 p.p.m. given by Goodall and Gregory (1947), and so confirmed the leaf diagnosis of "grey speck". There were no treatment differences in the iron and manganese levels, although the variation between replicates was very large.

Soil samples at sowing, at harvest, and in the spring and autumn of 1964, were taken and analysed. Residual effects were found by subtracting the seedbed levels from the figures at later samplings. At Pirnie, subtraction was from 0.62 p.p.m., which was the average result from uncontaminated plots. The linear response to the coded copper dressings was highly significant at all three sites at all sampling dates (see diagram 13). Both the "J" and "K" treatments raised the soil copper status at all sites. At Pirnie, the spring 1964 mean was higher than either of the autumn averages, but the differences between sampling dates were not significant at either Upper Huntlywood or Choicelee. It is obvious from diagram 13, however, that, at each of the sites, the control plots contained more extractable copper in spring 1963 than at any other sampling date. Although the Pirnie plots were probably slightly contaminated in spring 1963, this is very unlikely to have happened at either of the other sites as no copper treatments were applied at the time the samples were taken.

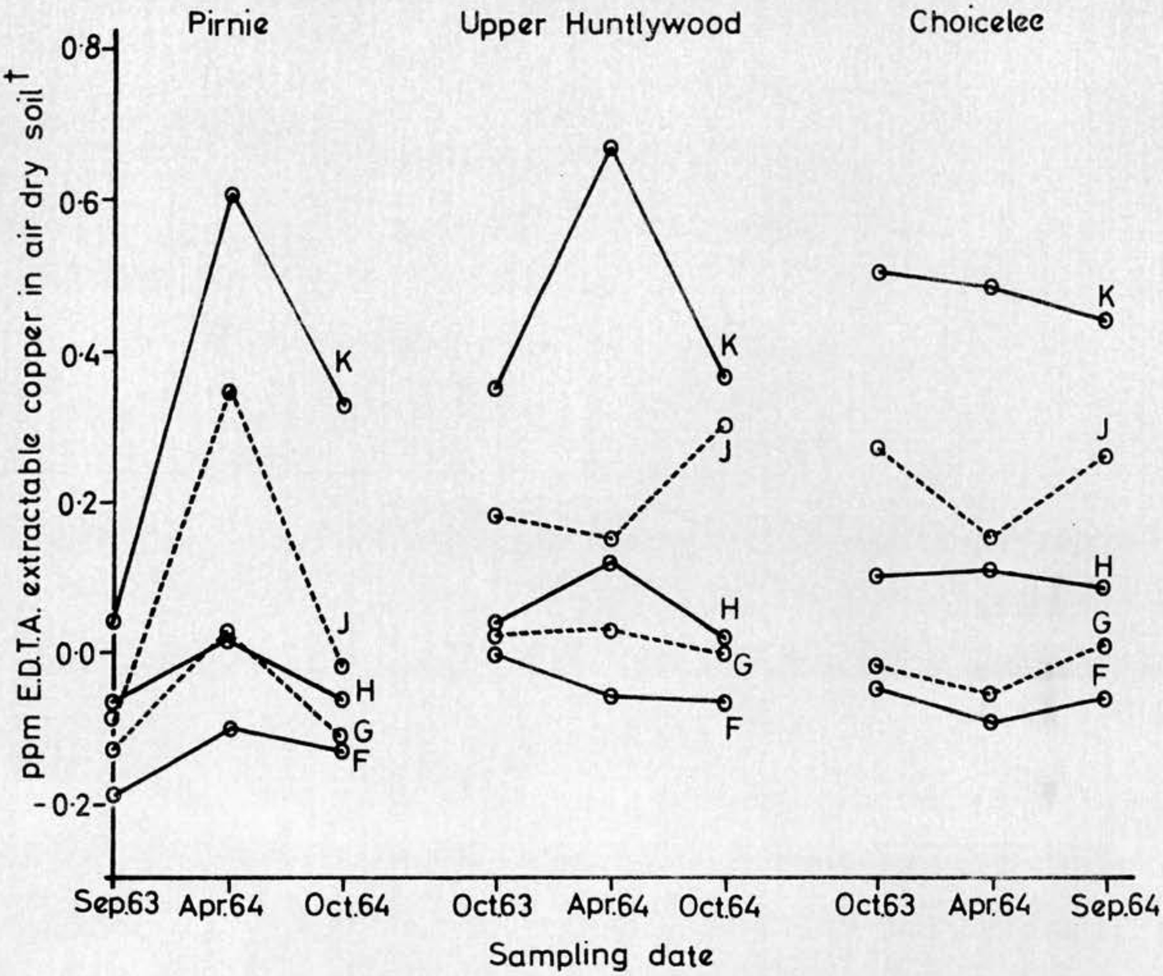
(3) 1964

(a) Aims

(i) Experiment 1. To repeat the 1963 experiment at two sites.

(ii) Experiment 2. To compare the effects of a foliar copper treatment on the yield and copper content of three

Diagram 13. Effects of treatments and sampling dates on residual extractable copper in soil, 1963 field experiment.



Significant effects and differences

Pirnie: Linear response to coded treatments,** K / F, G, H and J,** F / J,* Apr. 64 / Sep. 63 and Oct. 64.**

Upper Huntlywood: Linear response to coded treatments,** K / F, G, H and J,** J / F, G and H.**

Choicelee: Linear response to coded treatments,** K / F, G, H and J,** J / F and G,** F / H,** G / H,* H / J.*

† Results after subtraction of the concentration in the untreated seedbed.

varieties of oats and three varieties of barley.

(iii) Experiment 3. To measure the copper concentration in oat plants at various stages of development.

(iv) Experiment 4. To compare the copper concentrations in rain water collected in an urban and a rural area.

(b) Statistical Design

The design and analysis of variance of experiment 1 were those used in 1963. The detailed layouts at the two sites are given in appendix 4. Experiment 2 also had a randomised block design with four replicates, but each whole, varietal, plot was split into a control half and a copper treated half. The varieties tested were Astor, Blenda and Forward oats, and Freja, Maythorpe and Ymer barley. During the randomisation of the whole plots, all varieties, whether of barley or oats, were considered alike. Within each whole plot, the copper treatment was allotted at random to one subplot, without reference to the arrangement in any other whole plot. (Cochran and Cox 1960 p.297). The analysis of variance was therefore that of a split plot experiment and is shown in Table 21. The detailed layout is given in appendix 4. The measurements in experiment 3 were made in triplicate, so that some reliance could be placed on the results.

Table 21

Analysis of variance of data from field experiment 2, 1964.

Factor		Degrees of freedom
Whole plots:		
Total		23
Replicates		3
Treatments	Oats/barley	1
	Varieties (within cereals)	4
Whole plot error		15
Sub-plots:		
Total		47
Treatments	Copper	1
	Copper x cereals	1
	Copper x varieties (within cereals)	4
Sub-plot error		18

(c) Description of Sites

The two sites for experiment 1 and the single site for experiment 2 are described in Table 22. The oats for experiment 3 were sampled from areas very near the trial in First Moor, Choicelee, and initial conditions were similar for experiments 2 and 3. As there was no manuring at Langtonlees in 1964, the figures given for pH and "availability" of P and K were averaged from determinations on the seedbed samples. At Hexpath and Choicelee, however, the pH, P and K values are those from the harvest samples, so as to avoid recently fertilized soils. The trial site in Plantation field, Hexpath in 1964 was about 2¾ miles from the 1962 site in Bog field of the same farm, while the plots in First Moor at Choicelee were about 1¼ miles

Table 22

Field experiment sites 1964

	Experiment 1 Site 1	Experiment 1 Site 2	Experiment 2
Farm	Hexpath	Langtonlees	Choicelee
Field	Plantation	Under Back-hill South	First Moor
Elevation in feet	625	825	750
Soil series	Eckford/ Hexpath	Hobkirk	Hobkirk/Faw
Surface soil texture	sandy-loam	sandy-loam	sandy-loam
Average % loss on ignition in soil	11.1	8.5	8.0
Average pH of soil	5.8	6.6	6.3
Average "availability" of P in soil	low	moderately low	moderately low
Average "availability" of K in soil	moderate	low	very low
p.p.m. total copper in untreated seedbed	less than 1	3.0	2.4
Average p.p.m. E.D.T.A. extractable copper in untreated seedbed	0.55	0.50	0.51
Drainage	free	moderate	free
Previous crops: 1963	grass	rape	oats
1962	grass	oats	rape
Experimental crop	barley	oats	
Experimental variety	Ymer	Minor	
Manuring: rate in cwt. per acre	2½	nil	2½
fertilizer	13-13-20		18-9-9
If undersown	no	no	yes
Date sown	10th April	8th April	13th April
Date of foliar spraying	27th May	5th June	1st June
Date of first herbage sampling	3rd July	13th July	9th July
Date harvested	11th Sept.	13th Sept.	23rd Sept.

from those laid down in 1963 in Third Birks field. Swayback in lambs and withertip in oats had occurred at Hexpath, Langtonlees and Choicelee.

(d) Experimental Methods

Experiment 1 was carried out using the same methods as in 1963. For experiment 2, the seed grain was dressed with "Harvesan" and sown after the field had been fertilized. Six of the outer spouts of the drill were blocked so that the width of the sown plot would be within the width of the combine harvester cut. No margins of plants were provided, but a strip about 9" wide was left unsown between plots. The length of the whole plots was calculated so that each half plot would cover 1/40 acre. The treatment of 10 lb. per acre copper oxychloride was applied to the young cereals with knapsack sprayers as in previous years, except that the edges of the plots were so obvious that string was unnecessary (see plate 5). The control plots in the first and third replicates only, were sprayed with water. The sampling methods were those used in 1962 and 1963. The straw yields of only two replicates were measured because of lack of time.

In experiment 3, Sun II oat plants, growing in three areas of about 1/40 acre outside the trial at Choicelee, were sampled at intervals of approximately a fortnight. The plants were cut about one inch above the soil, and were picked from about 20 random positions along a zigzag walk through each area. Fifty plants were taken from each area on the first five sampling dates, and twenty five plants on the next four dates. Where possible, grain and straw were separated by hand in the laboratory.

Plate 5. Spraying copper treatment. Experiment 2 1964.

Plate 6. Healthy (left) and copper deficient barley, Hexpath 1964.

Rain water was collected, in experiment 4, beside the trial in the Plantation field, Hexpath, and in an Edinburgh garden. At each site, rain fell into a clean polythene funnel of diameter 17 cm. which drained into a clean, narrow necked polythene bottle of capacity 2 litres. The bottle at Hexpath was strapped to the top of three posts which were about four feet high, while the bottle in Edinburgh was placed securely on the roof of a small shed. The bottles were replaced once every fortnight or three weeks and the contents measured and analysed for copper. The bottles were cleaned thoroughly before being reused, but only leaves or other obvious debris were removed from the funnels.

(c) Comments and Observations

(i) Weather

After December, the winter was milder than usual. April and May were cool and dull, however, and there were strong winds in May. The year was abnormally dry, although not very sunny except for a spell in late June. Under fairly dry conditions, the harvest was generally a little earlier than in the previous two years.

(ii) Experiment 1, Hexpath

The early growth of the trial barley appeared to be uniformly good, and when the plants were about 8" high and in fourth leaf, the treatments were applied. The spraying caused only very slight scorching in the "J" and "K" plots. At the beginning of July, when the stems of the plants were elongating, and the ears were beginning to appear, withertip was seen in small patches in all the control plots and round the perimeter of the trial. Plate 6 shows a clump of withertip plants compared with a clump from the highest copper treatment. The symptoms, of stunting and of white twisted ends to the youngest and second youngest leaves, were similar to those seen in oats in the Bog field Hexpath in 1962. At this time, all the trial plants appeared to be a little limp, and many were attacked by aphids and mildew. Three weeks later wind burn disguised any withertip which might have been showing. In August, crinkling of the awns of some heads of barley round the untreated margin of the trial was seen. This effect was similar to that reported from Pirnie in 1963. The copper treated plants matured a little more quickly than the controls. Soon after the harvest had begun, the wind increased to a gale, and freshly cut straw was blown about 20' up in the air. No straw yields were therefore measured.

(iii) Experiment 1, Langtonlees.

The oats appeared healthy until the 1st of June, when the youngest leaves of the majority of the plants were pale at the edges. The third leaves in many plants were also brown and spotted, in some cases only the tip and the base being green. This appearance of the third leaves indicated that the plants were manganese deficient. (Wallace 1961 p.109). Manganese sulphate, at a rate of 10 lb. per acre, was therefore added to the copper treatments and applied to the whole trial, including the controls. The level of water application was 100 gal. per acre in all treatments. The plants were 9-10" high when they were sprayed, and the two highest copper treatments scorched the oats a little. Towards the end of June, withertip occurred throughout the control plot in the third replicate, and was severe at three edges of the trial area. Plate 7 shows a patch of these oats and demonstrates that, where the growth of the cereal was retarded, the weeds, mainly spurrey, throve. The appearance of the plants affected by withertip was exactly the same at Langtonlees in 1964 and at Hexpath in 1962. In mid-July, manganese deficiency spotting, or "grey speck", was showing on the flag leaves of the plants round the perimeter of the trial and in all the plots in the first replicate. At the beginning of September, when the treated plants were turning yellow, three of the control plots were greener than the rest of the trial. The control oats in the third replicate were especially immature and were about 24" high, while the treated plants averaged 30". Plate 8, in which the colour is not very clear, shows untreated plants compared with oats from a "J" plot. The control plants were stunted, with white "blind" heads, greenish straw and many green tillers, while the treated plants were maturing normally and producing grain. During the harvest, no yield was measured for one plot because the sack, in which the grain was being collected, split. The straw and stubble of the control plots in replicates 3 and 4 were very much greener than elsewhere in the trial.

(iv) Experiment 2. Choicelee.

The trial site was on a gentle slope (see plate 5), so that one sub-plot of each whole plot was higher than the other sub-plot. The barley grew more strongly at first than the oats, and was eaten preferentially by hares. This, however, did not

Plate 7. Withertip in oats, Langtonlees 1964.

Plate 8. Control (left) and copper treated oats, Langtonlees Sep. 1964.

appear to cause much lasting damage. On the 1st of June, when the copper treatment was applied, the oats and barley were all about 9-10" high in 5th leaf. The spraying caused scorching on all the barley, the effect on Ymer being especially bad. The Forward and Blenda oats were only slightly scorched and the plots of Astor showed no effect. At the beginning of July, the copper treatment had caused no visible difference in growth or development in the barley, but the control halves of the oat plots seemed slightly stronger than the treated halves. The Astor oats were not so tall, nor so thick, as the Blenda or Forward oats at any stage. Later in the season there was a tendency for the barley to lodge, especially the Maythorpe variety. Mildew attacked the Astor oat plants and "grey speck" affected many of the Forward oats and, to a lesser extent, the Blenda and Astor plants. The Freja and Maythorpe barley matured a day or two before the Ymer plots, and the Forward and Astor oats ripened a day or two after the Blenda plants.

(v) Experiment 3. Choicelee

The oats were sown on the 11th April and harvested on 21st September. Development was similar to that of the Blenda oats in experiment 2, and "grey speck", but no withertip, was observed.

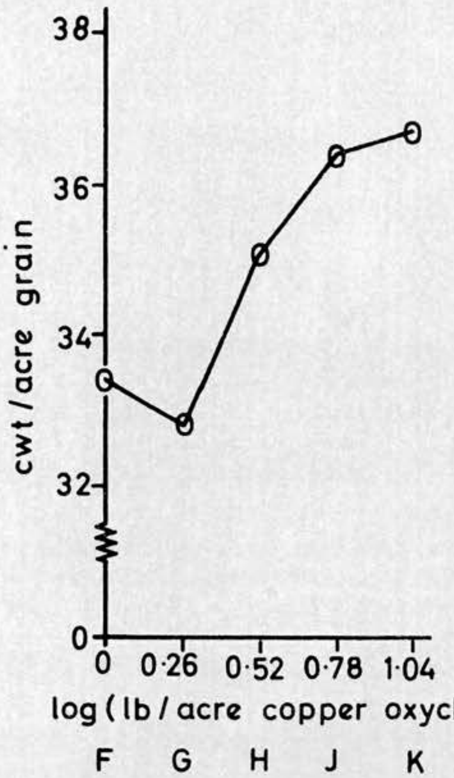
(f) Results

The detailed results are given in appendix 7.

(i) Experiment 1.

The linear response of the grain yield to the coded copper treatments at Hexpath is illustrated in diagram 14. The two

Diagram 14. Effect of treatments on grain yield at Hexpath. Experiment 1 1964.



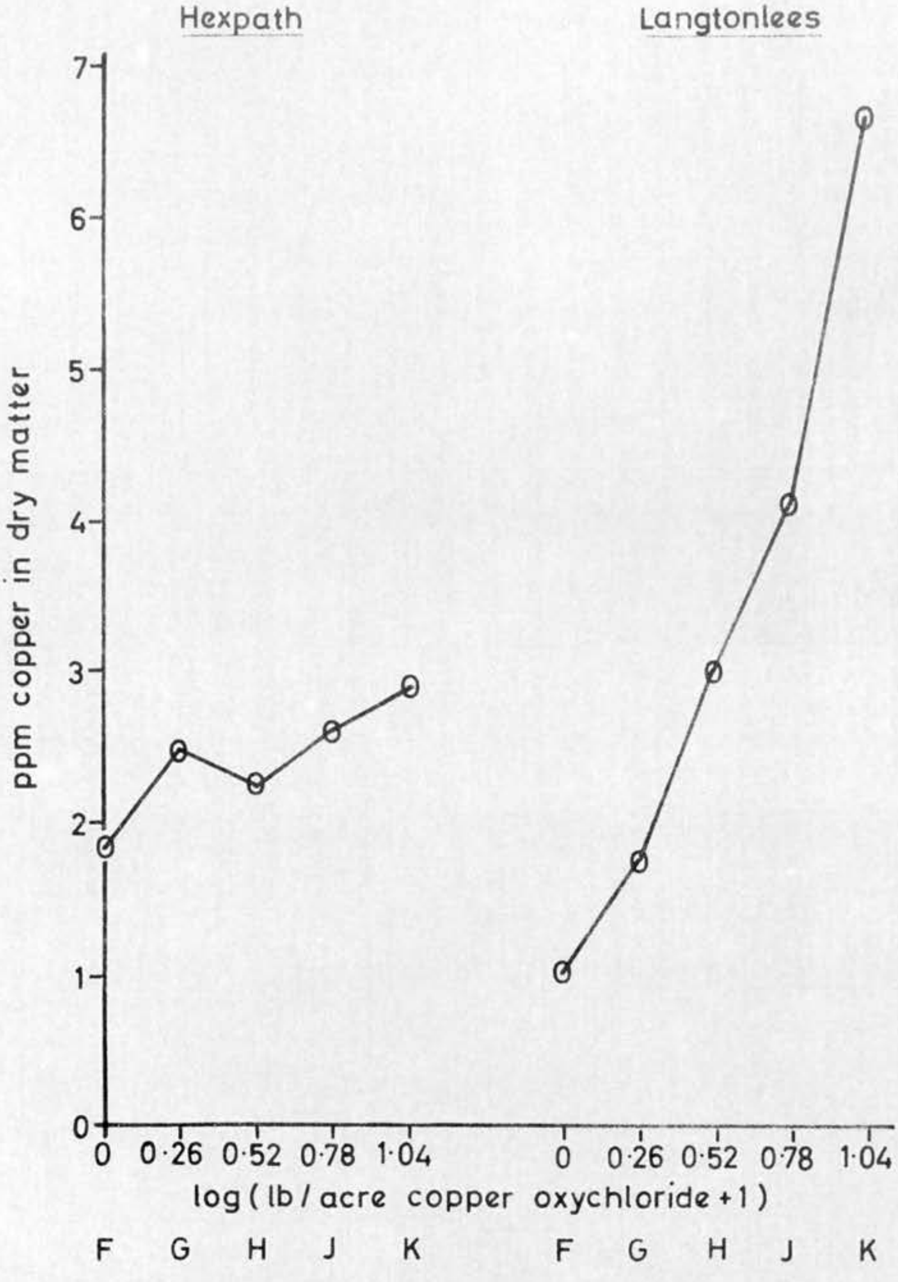
Significant effects and differences.

Linear response to coded treatments,** F / J and K,* G / J and K.*

highest treatments, "J" and "K", produced significantly higher yields than either the control or the lowest copper application, "G", although the increase caused by 10 lb. per acre copper oxychloride was not so large as in 1962 at the same farm. The grain yield results from Langtonlees were very disappointing. Only the third replicate control plot, where withertip was observed, gave a low value, and the results from each treatment were variable (see appendix 7). The probability that there was a linear response to the coded treatments, was only between 0.10 and 0.05, and so there were no conclusive findings. There were no significant differences between the treatment means in the data from Langtonlees of straw yields, of grain expressed as a percentage of total yield, or of moisture in grain or straw. The third replicate control plot did, however, produce considerably wetter straw and grain, a lower bushel weight and a higher percentage of green grain than any other plot (see appendix 7). The only significant differences in the figures for the moisture contents of grain and straw at Hexpath were between replicates. The bushel weights of grain from the treatments showed no significant differences at either Langtonlees or Hexpath.

The effects of the treatments on the copper concentrations in the samples cut at the "heading out" stage are illustrated in diagram 15. At Langtonlees, the large response may have been caused by the application of manganese with the copper (Younts 1964), the slightly delayed spraying, and the small production of dry matter by Minor oats. There was a linear response to the coded treatments in the copper concentration

Diagram 15. Effect of treatments on copper concentration in plants at ear emergence. Experiment 1 1964.



Significant effects and differences.

Hexpath: Linear response to coded treatments,** F / J and K,** F / G,* H / K.*

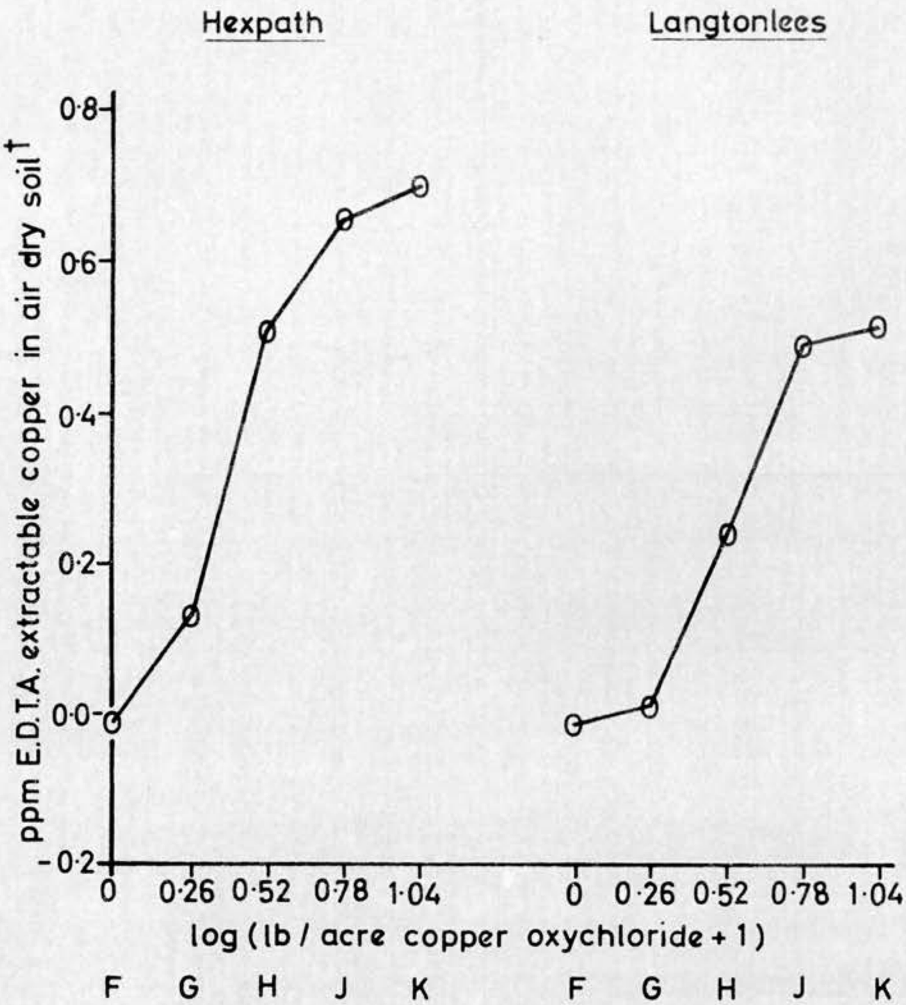
Langtonlees: Linear response to coded treatments,** K / F, G and H,** J / F, G and K.*

in grain at Langtonlees, but not at Hexpath. At neither site were there any significant differences between individual treatments. The copper level in the straw at both sites gave a linear response, the "H", "J" and "K" applications raising the concentrations significantly.

As in previous years, the soil samples taken in the spring and autumn were analysed, and the residual effects of the treatments were calculated. Figure 16 illustrates the linear response to the coded treatments, which was highly significant at Langtonlees and significant at the 5% level at Hexpath. The response curves are somewhat sigmoid, unlike those obtained after one season at Pirnie, Upper Huntlywood and Choicelee (Third Birks).

All the observations and harvest results from Langtonlees indicate that the whole area of the control plot in the third replicate produced copper deficient oats. The control plots in the first and second replicates, on the other hand, gave apparently healthy plants, and the only abnormality observed in the fourth replicate control, was the green colour of the straw. The reason for this difference in availability of copper is not known. The soil appeared more uniform at Langtonlees than at any other site, drainage seemed very even, and the E.D.T.A. extractable soil copper level was no lower in the third replicate control than in any other (see appendix 7). The pH results from the control soils, in both the spring and autumn samplings, were 7.0, 7.0, 6.7 and 6.1 in replicates 1, 2, 3 and 4 respectively, and so the pH level is unlikely to have caused a unique effect in replicate 3. As copper

Diagram 16. Effect of treatments on residual extractable copper in soil after one season. Experiment 1 1964.



Significant effects and differences.

Hexpath: Linear response to coded treatments.*

Langtonlees: Linear response to coded treatments,** F/J and K,* G/J and K.*

†Results after subtraction of the concentration in the untreated seedbed.

deficiency was severe round three edges of the trial site, the possibility of contamination of the plots cannot be dismissed. Precautions were taken against this, however, and the controls were sprayed from a cleaned polythene watering can before the copper treatments were applied. Any contamination which might have been in the manganese sulphate should have affected all plots alike.

(ii) Experiment 2.

When the harvest results were examined, it was found that the grain yields from the sub-plots which were higher up the slope were generally larger than those from the other half plots. This effect was highly significant, and masked any differences caused by the copper treatment. In the sub-plot analysis of variance, the sums of squares were therefore adjusted to take account of this position effect, and the response to copper was found to be significant at the 5% level of probability. After the elimination of the position effect, the estimated response to copper was 1.6 cwt. per acre of grain at 18% moisture. The barley produced more grain than the oats, but no interactions and no varietal differences were significant. As the straw yields of only two replicates were measured, and the statistical analysis of the grain yields had proved to be so complex, no calculations on the straw yields were attempted. It is obvious, however, that the oats produced more straw than the barley, and that there was no large effect produced by the copper treatment, (see appendix 7).

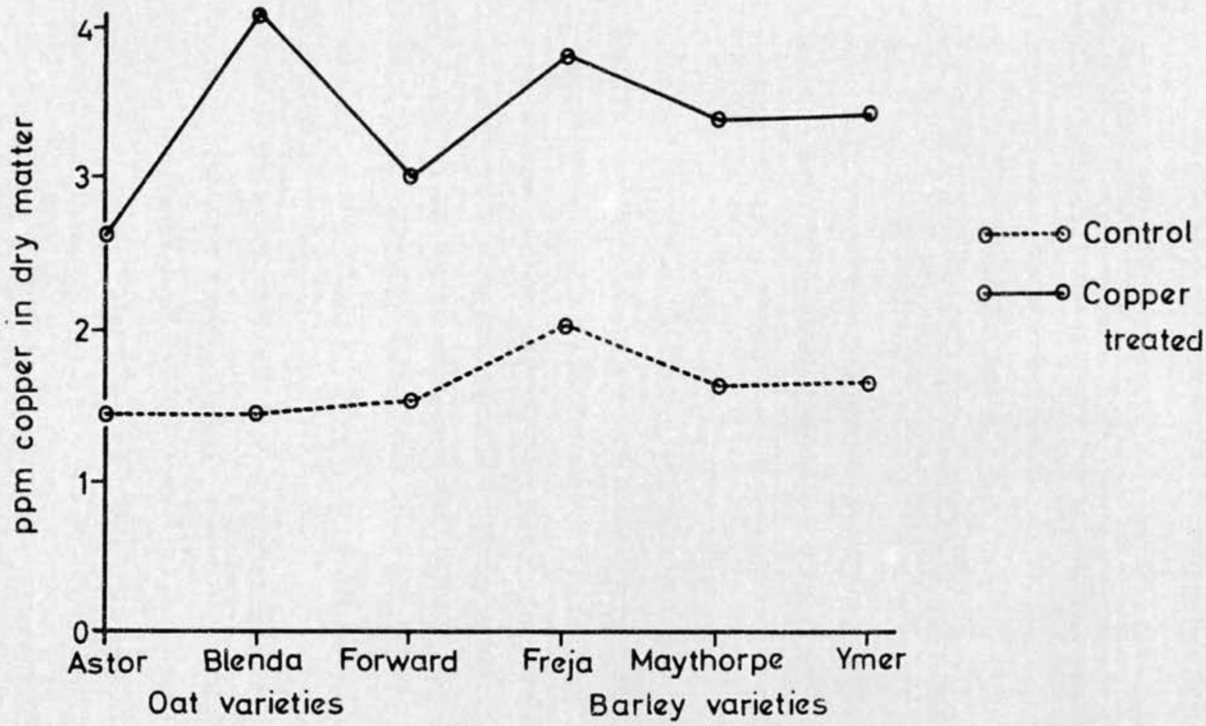
Neither the percentage moisture in the grain nor in the straw was affected by the copper treatment. With the exception of Maythorpe barley, however, the less moisture in the grain of a variety, the more moisture there was in its straw. The oats had a higher straw and lower grain moisture level than the barley. The data on bushel weights of dry grain showed no response to the copper treatment, but a bushel of barley was heavier than one of oats, Forward oats being the lightest.

The copper treatment raised the copper concentration in the straw and in the samples cut at ear emergence very significantly. The response in the grain was, however, significant at only P less than 0.05. The copper levels in straw, grain and "heading out" samples were higher in barley than in oats. Within each cereal there were also varietal differences, which are shown in figures 17 and 18. The capacity of the young Blenda oats to retain more of the applied copper than the Forward or Astor oats was particularly striking. The extractable copper level in the soil was raised more than 1 p.p.m. by the copper application, but was not influenced significantly by the varietal treatments.

(iii) Experiment 3.

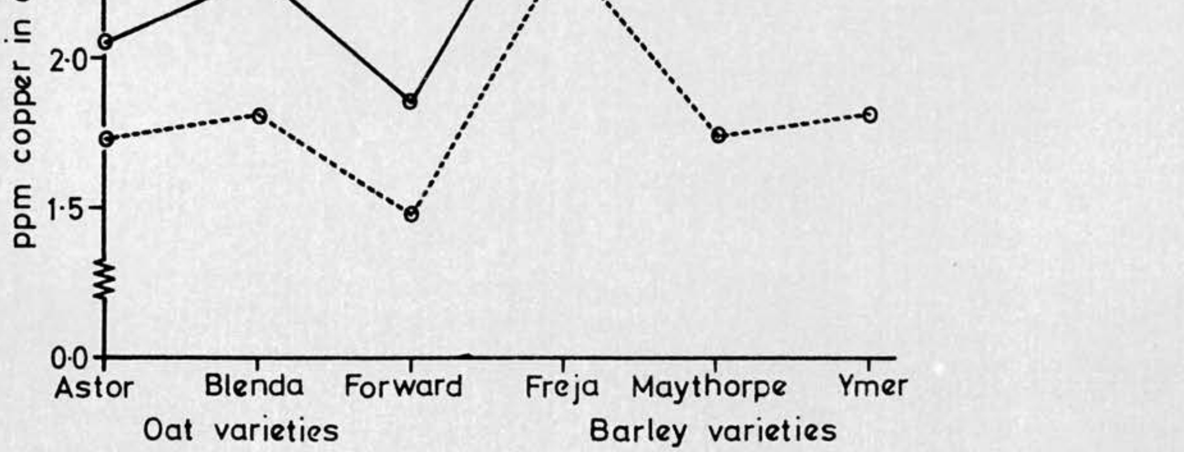
Diagram 19 shows the average copper concentration in the oat plants from the three areas at each of the sampling dates. The general trend of the curve, from a high level when the plants were young, to a lower and more constant value towards maturity, is similar to graphs produced from the results of Piper (1942 (1)), Piper and Walkley (1943) and Williams and Moore (1952), all of whom worked on Algerian oats, and of

Diagram 17. Effects of copper and variety on copper concentration in plants at ear emergence. Experiment 2 1964.



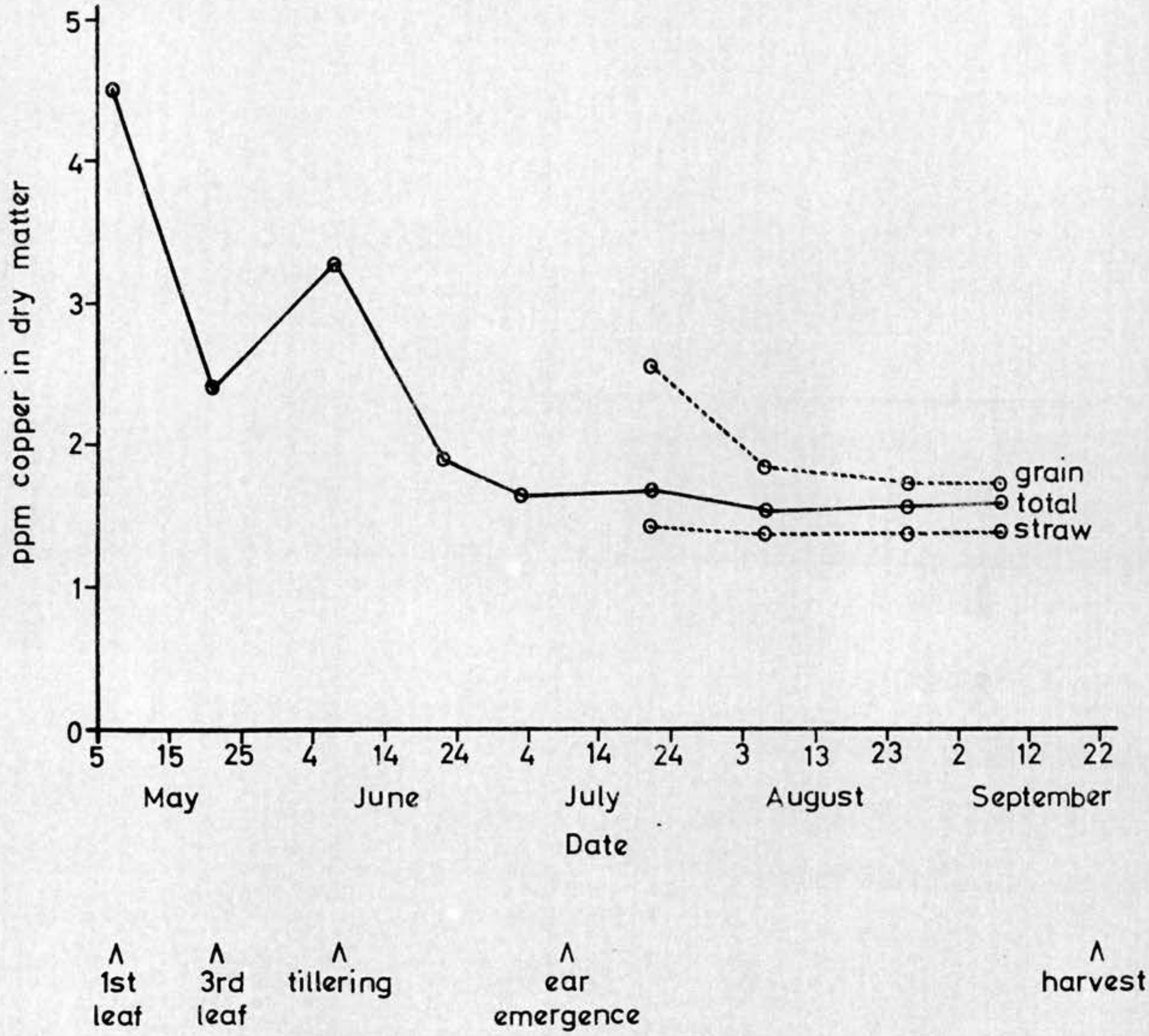
Significant differences: copper response,** oats/barley,* Blenda / Astor and Forward.*

Diagram 18. Effects of copper and variety on copper concentration in grain. Experiment 2 1964.



Significant differences: copper response,* oats / barley,** Freja / Maythorpe and Ymer,* Blenda / Forward.*

Diagram 19. Effect of development on copper concentration in Sun II oats.



Reith and Mitchell (1964) in Scotland. The rise in the curve, which occurred at tillering, has not been reported for Algerian oats. Rademacher (1940), however, has found an exactly similar effect with Rotenburger black oats and, to a lesser degree, with Victory oats. This author has linked the short period of rising copper concentration with the onset of withertip when copper is deficient. At Hexpath (Bog field) and Langtonlees, however, withertip was first observed in oats at a slightly later stage, nearer ear emergence.

(iv) Experiment 4.

The results obtained during the investigation are tabulated in appendix 7. Water was collected during most of the growing season at Hexpath, and for a full year in Edinburgh. The apparatus used did not conform to the meteorological office specifications, and so it is not surprising that the rainfall figures from nearby meteorological stations are not exactly equivalent to the volumes actually measured in this investigation. The Edinburgh collections were made in a southern suburb, which is not near any industrial plant, but is surrounded by domestic chimneys. The average of the concentrations of copper found in the rain water there, 0.022 p.p.m., corresponds closely to the 0.027 p.p.m. measured outside Bristol by Hewitt (1952), while the highest concentrations obtained were 0.063 p.p.m. in Edinburgh and 0.062 p.p.m. near Bristol. The rain containing these highest levels in Edinburgh was collected during two dry spells, one when the weather was warm and windy, and the other when it was foggy. The heaviest average daily deposition of copper occurred, however, during wet weather at the end of March.

By contrast, the average of the concentrations measured in the water collected at Hexpath was only 0.012 p.p.m. copper, with a range of 0.002 to 0.035 p.p.m. The total copper deposited at the Edinburgh site, during the period between the end of May and the beginning of September 1964, was almost double that at Hexpath (see appendix 7). The Plantation field is over a mile from any house, chimney, road or railway, but is in an exposed, windy position, and thus it seems that the only possible source of the copper found in the rain there is dust, blowing from copper treated or normal soils in the surrounding fields. The heaviest deposition of copper occurred under dry conditions, when any top soil not covered by plants would tend to be blown about.

The higher copper status of horticultural, as compared with agricultural soils, has been noted in New Zealand (Rolt 1962) and of urban, as compared with rural soils, in Scotland (Purves 1964(2)). At least some of this difference must be caused by the fallout of atmospheric pollution. It appears, therefore, that there is little likelihood of copper becoming deficient near urban areas under present conditions.

(4) Discussion of the Results of Observations and Experiments in the Field.

(a) Grain Yield.

The trials were carried out on commercial farms, where the sites were cultivated and fertilized by the farmer in the same way as the rest of the field, with the exception of any copper treatment. Thus there were many factors which varied from farm to farm, and it is not surprising that the yield responses to the 10 lb. per acre copper oxychloride foliar treatment, which are summarised in Table 23, were not constant. In no case, however, was there a diminution in yield caused by this copper dressing.

From Table 23 it is evident that, of the five sites where the E.D.T.A. extractable copper level was about 0.5 p.p.m., four gave a positive response to the copper treatment. The variable results from the fifth site, Langtonlees, are discussed above. Langtonlees was the only trial where manganese was applied at the same time as copper, and where no macro nutrient dressings were made by the farmer. There was no response to copper at any higher E.D.T.A. extractable level. Reliable yield results were not, however, obtained from either of the two sites where the extractable soil copper concentration was between 0.6 and 0.7 p.p.m. Thus, in South East Scotland, the deficiency limit for cereals appears to be above 0.5 p.p.m. extractable copper and below 1.1 p.p.m. Mitchell (1963) has given 0.8 p.p.m. as the normal limit.

Table 23

Grain yield responses to a foliar dressing of 10 lb. per acre copper oxychloride

Farm	Field	Year	Soil Series	E.D.T.A. extractable copper in seedbed	Total copper in seedbed	grain yield response	Significance of response
Hexpath	Bog	1962	Eckford	0.5 ppm	<1 ppm	+14.0 cwt/acre	* *
Hexpath	Plantation	1964	Eckford/Hexpath	0.55ppm	<1 ppm	+3.3 cwt/acre	*
Choicelee	1st Moor	1964	Hobkirk/Faw	0.5 ppm	2.4ppm	+1.6 cwt/acre	*
Choicelee	3rd Birks	1963	Hobkirk	0.5 ppm	3.1ppm	+2.6 cwt/acre	linear to coded dressings*
Oldcastles	Craigielaw	1962	Eckford	1.1 ppm	3.7ppm		none
Billie Mains	Castle Shotts	1962	Eckford	5.3ppm			none
Pirnie	Monkshott	1963	Eckford	0.6ppm	<1 ppm		none†
Langtonlees	Under Back Hill S.	1964	Hobkirk	0.5ppm	3.0ppm		none
Rosehill	No.3	1962		1.65ppm	3.9ppm	no results	
Upper Huntlywood	West Knock	1963	Eckford	0.65ppm	1.3ppm	no results	

† severe lodging occurred at Pirnie

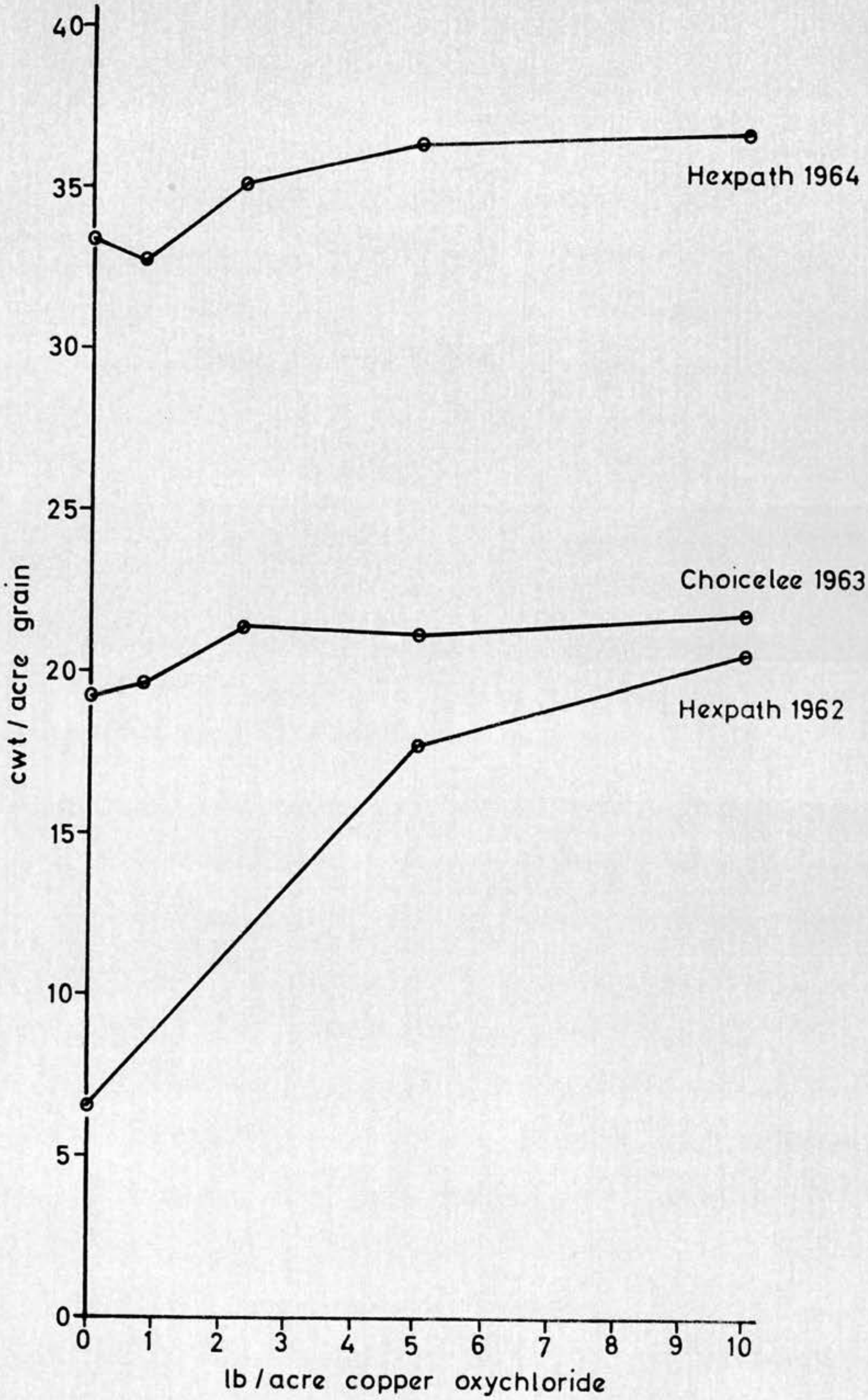
From Table 23 it can also be seen that the total copper status of the soil was a less consistent guide as to whether a yield response would be obtained from applied copper, than the E.D.T.A. extractable copper level. Where a soil contains less than 2 p.p.m. total copper, however, the depletion of this reserve by continued cropping will lead to copper deficiency in plants or stock comparatively quickly, unless the soil copper level is raised. (Purves and Ragg 1962). The observations at the ten experimental sites showed that copper deficiency can occur on Hobkirk as well as Eckford Series soils.

All the sites from which yield results were obtained were well drained (except Langtonlees) and had pH levels between 5.8 and 6.6. No conclusions could therefore be drawn about either of these factors. Although the trial at Choicelee in 1964 did not reveal any varietal differences in response to copper, the statistical analysis of yield data from that experiment was complicated so much by the position effect, that a small interaction may have been masked. The only two sites where Forward oats were used both gave a yield response.

The experiment in 1962 showed that, where reclamation disease occurred, the seedbed applications prevented the withering of the leaf tips, but only partially cured the condition of green straw and little grain. The foliar applications were more effective in raising the grain yield, and gave healthy oats with a normal moisture content. In the dry conditions of 1962, the water in the foliar sprays may have been of value to the plants, but this difference between soil and foliar treatments is similar to that obtained by Mitchell Reith and Johnston (1957(1)), who have reported that the yield response to 1 lb. copper sulphate per acre sprayed on young oats is approximately equivalent to that of 20 lb. applied to the seedbed. Davies (1962) has also found that copper applied as a foliar spray is more effective than a seedbed dressing. Younts (1964), however, has reported that the response of winter wheat to copper is equally good whether the dressing is to the soil or the leaves, provided that it is applied early. In the present work no yields of cereals were measured in later years to investigate the residual effects of soil and foliar treatments. As shown in the review of the literature, it has been found that soil dressings are more available to the succeeding year's crop than in the first year.

At three sites, foliar applications were made at more than one level of copper, and a yield response was obtained. The shapes of the response curves at these sites are shown in figure 20, where the treatment rates of copper are not coded. Steenbjerg and Boken (1950) have stated that the yield curve, drawn against available copper, is very steep over a limited range of soil copper levels. From figure 20, it appears that a point of inflection occurred at an application of between 1

Diagram 20. Effect of foliar treatments on grain yield.



and 2 lb. per acre copper oxychloride at Choicelee (3rd Birks) and Hexpath (Plantation field), and below 5 lb. per acre at Hexpath (Bog field). At these three sites, 10 lb. per acre copper oxychloride gave a yield not significantly different from that produced by 5 lb. per acre. In 1962 at Hexpath, an application of 5 lb. copper oxychloride did not produce quite such yellow straw as 10 lb., but this was the only sign at any trial that the former treatment was not sufficient for healthy development. Very approximately, the cost per acre of 5 lb. copper oxychloride, and its application as a foliar spray, equals the value of one cwt. of grain, while a treatment of 10 lb. copper oxychloride requires an increase in yield of over 1½ cwt. per acre to be economic. Although 0.8 lb. per acre copper oxychloride prevented withertip symptoms, it was not so effective at raising yield as the 5 and 10 lb. treatments. These rates may be compared with the foliar spray recommendation of 2 lb. per acre copper oxychloride by Davies (1962) and of the equivalent of 5 lb. per acre copper oxychloride by Younts (1964).

(b) Straw Yield

The straw yield was measured at only four sites, and at three of them there were no significant treatment differences. At Hexpath in 1962, however, the "C" and "D" treatments reduced the production of straw. In very acute cases of copper deficiency, cereals die before reaching maturity. In less severely deficient conditions, however, the plants fail to set grain and continue to produce tillers. (Piper 1942(1)). The straw remains green. (Mitchell Reith and Johnston 1957(1), Wallace 1961). Thus although the copper deficient plants are stunted, there is a much thicker growth of straw than is normal. Most of the minerals absorbed by the roots probably remain in the stem and leaves because there is very little, or no, grain, and because there is less leaching than usual. Loss of nutrients by leaching is most likely to occur just before plant maturity (Long Sweet and Tukey 1956). Thus the copper treatments, by curing the copper deficiency, increase the length and maturity of the straw without necessarily raising the yield. Mitchell Reith and Johnston (1957(1)), have obtained only small increases in oat straw yield after soil copper dressings, and small decreases after foliar applications.

(c) Copper Levels in Cereals

The concentration of copper in the cereal plants showed considerable variation, caused by the copper treatments, and differences in the stage of growth, variety and environment of the samples. As shown in figure 19, the copper level in the oat plants at Choicelee fell rapidly at first, and then evened out after ear emergence. This effect explains why there was little difference between the concentration of copper in the control plants in the field trials at "heading out" and at harvest. As in figure 19, there was a tendency for the level of copper in the grain to be higher than that in the straw (see Table 25).

The effect of the soil dressings on the herbage copper levels was not significant at any site in either plants at the "heading out" stage or maturity. Piper (1942(1)), Mitchell Reith and Johnston (1957(1)) and Reith and Mitchell (1964) have reported that an application of copper to the soil raises the copper level of young cereals, but that, generally, the difference between the concentration in treated and untreated plants falls as maturity is reached. Piper (1942(1)) has found that even a soil dressing of 2 cwt. per acre copper sulphate does not significantly affect the copper level in oats after ear emergence. Thus the seedbed treatments in 1962 were not heavy enough, and the herbage samples were not taken sufficiently early, for any effect on plant concentration to be apparent. Mitchell, Reith and Johnston (1957(1)) have reported that copper treatment of deficient soil can cause an increase in yield without necessarily raising the copper concentration in the grain or straw.

The foliar treatments affected the copper levels in the samples cut at ear emergence much more than those in the grain or straw. This may have been because the effects of the foliar spray decreased with the passage of time, or, as reported above for soil dressings, with the maturing of the plants. The 5 and 10 lb. per acre copper oxychloride foliar treatments significantly raised the copper level of the oat and barley plants at ear emergence at all sites except Billie Mains, where there was ample soil copper initially. The foliar treatments in 1963 and 1964 also gave rise to a very significant linear response in the copper concentration in the plants at ear emergence at all sites. At Oldcastles and Langtonlees, the increases in plant copper concentration caused by 10 lb. per acre copper oxychloride were 3.3 and 5.7 p.p.m. respectively. The high response at Oldcastles may have been caused by the spraying of the treatment at a later stage of plant development than at the other sites, although the period between spraying and sampling was about ten days longer at Oldcastles than elsewhere. The large effect at Langtonlees may have been a result of the low production of vegetative growth in Minor oats, and of the manganese application (Younts 1964). The average rise in concentration in the plants at ear emergence at all the sites, caused by the foliar application of 10 lb. per acre copper oxychloride, was 1.7 p.p.m.

The effects of the foliar treatments on the copper levels of the harvest grain and straw were much less striking. Only at Oldcastles and Langtonlees, where there was no yield response, did the grain copper concentration show very significant treatment differences. Where a yield response was obtained, on the other hand, at Hexpath (Bog and Plantation fields) the grain showed no significant differences, and at Choicelee (Third Birks and First Moor) significance was only at the 5% level. Even at Oldcastles and Langtonlees, the rises in the grain copper level, caused by the 10 lb. per acre copper oxychloride foliar spray, were only 0.5 and 0.4 p.p.m. respectively. The effect of the copper treatment was thus primarily to raise the grain yield. Where this was not possible, because of some limiting factor, (as may have been the case at Langtonlees) or because just enough copper was already available for good yields, (as probably at Oldcastles) the copper concentration in the grain rose. If plenty of copper was available, however, as at Billie Mains, the barley did not apparently respond in any way to the treatments. The effect of foliar treatments on the copper level in the straw was much less than on the plants at ear emergence, but was highly significant at all sites except Hexpath (Bog field), Billie Mains and Upper Huntlywood. An application of 10 lb. per acre copper oxychloride raised the concentration in the straw by between 0.3 and 1.4 p.p.m. at the sites where there was significance, with an average of 0.5 p.p.m. Mitchell Reith and Johnston (1957(1)), on the other hand, have obtained rises of 1 p.p.m. or more in the copper concentration of oat grain and straw by spraying the young foliage with only 1 lb. per acre copper sulphate, whether or not there was also an increase in yield. Copper, when in solution as the sulphate, may be assimilated more readily by the leaves of plants than when in suspension as the oxychloride.

The trial at Choicelee in 1964 showed that varietal differences affected the copper concentration, both at ear emergence and maturity. The copper level in the barley was higher than that in the oats. Scharrer and Schaumloffel (1960) have found that applied and not native copper is assimilated more readily by barley than oats. In the present work, however, the lines in diagrams 17 and 18, for the copper treated and untreated plants, demonstrate the lack of interaction between varietal and copper effects by being approximately parallel. The varietal differences which occurred at Choicelee were not so extreme as those found at very early stages of growth by Rademacher (1940), nor so marked as those reported in wheat grain by Greaves and

Andersen (1936). In a further investigation into varietal effects, grain was analysed from a variety experiment held in the North of Scotland in 1962 by Bain (1965). The experimental soil was not known to be copper deficient. Oats and wheat were grown on adjacent sites in the same field. Thus, although the analytical results, which are given in appendix 8, make it seem very likely that wheat grain generally contains more copper than oats, this has not been proved because of the lack of randomisation. The statistical significance of the differences between the results for each variety is shown in Table 24.

Table 24

Significance of varietal effects on copper concentration in grain from North of Scotland trial

Oat varieties	Mean copper concentration	Wheat varieties	Mean copper concentration
Vollbringer	4.24 p.p.m.	Vilmorin line G	5.31 p.p.m.
Marino	3.84 "	Cappelle	4.94 "
Sun II	3.83 "	Champlein	4.84 "
Astor	3.80 "	Vilmorin 5905	4.81 "
M.G.H. 856	3.72 "	Rallye	4.54 "
Flamingskrone	3.64 "	Prestige	4.27 "
Karin	3.62 "		
Manod	3.28 "		

All means not joined by the same line are significantly different at the 1% level.

Table 25

Mean copper concentrations from untreated plots

Variety	Site	p.p.m. E.D.T.A. extractable copper in seedbed	Plant copper concentrations (p.p.m.)		
			"heading out"	grain	straw
Freja barley	Billie Mains	5.3	3.4	3.4	2.4
" "	Choicelee (First Moor)	0.5	2.0	2.4	1.7
Maythorpe barley	Oldcastles	1.1	1.8	2.2	2.0
" "	Choicelee (First Moor)	0.5	1.6	1.7	1.6
Ymer barley	Pirnie	0.6	1.4	1.9	1.5
" "	Hexpath (Plantation)	0.55	1.9	2.4	1.9
" "	Choicelee (First Moor)	0.5	1.7	1.8	1.8
Forward oats	Hexpath (Bog)	0.5	0.7	1.3	1.2
" "	Choicelee (Third Birks)	0.5	1.9	2.3	1.9
" "	Choicelee (First Moor)	0.5	1.5	1.5	1.4
Blenda oats	Rosehill 1962	1.65	3.5	-	-
" "	Rosehill 1964	1.8	1.7	-	-
" "	Choicelee (First Moor)	0.5	1.4	1.8	1.4
Astor oats	North of Scotland trial			3.8	
" "	Choicelee (First Moor)	0.5	1.4	1.7	1.6
Sun II oats	North of Scotland trial			3.8	
" " "	Upper Huntlywood	0.65	1.4	1.9	1.5
" " "	Choicelee (First Moor)	0.5	1.6	1.7	1.4

In most of the varieties used in the copper trials, the control grain and straw copper levels were very near each other (see Table 25). In Freja barley, however, the grain had a higher copper concentration than the straw at both Choicelee in 1964 and Billie Mains in 1962. At the latter site, because of the length of time between maturity and harvest, leaching of the straw may have increased the difference already present because of variety. Piper and Walkley (1943) have reported that the concentration of copper in Algerian oat grain is double that in the straw.

The effect of the native copper status of the soil on the copper concentration in the experimental oat and barley plants is summarised in Table 25. Because of varietal differences, true comparisons can only be made between the copper levels in plants of one variety growing on different soils. Thus, despite the fact that the concentration of extractable soil copper was twice as high at Oldcastles as at Choicelee, there was virtually no difference in the herbage copper content, and the ten fold difference between the soils at Choicelee and Billie Mains corresponded to a change of only 1 p.p.m. in the grain copper level. The copper concentration in the grain from the trial in the North of Scotland was, nevertheless, over double that in the Choicelee oats, and the copper levels of all the control samples from the Bog field, Hexpath and from Langtonlees were very low compared with the normal Aberdeenshire values of 2-4 p.p.m. in oat grain and 1.5-3 p.p.m. in the straw. (Mitchell Reith and Johnston 1957(1)). Thus, although there is agreement between classifications based on the concentration of copper in flowering oats (Piper and Walkley 1943), and on trial yield results, in showing that only the site at the Bog field Hexpath was very deficient, it is evident that the analysis of plants after ear emergence gives no more than a very general assessment of the soil copper status. Reith and Mitchell (1964) have suggested that only very young cereals should be analysed to diagnose a copper deficient soil.

The results from Rosehill show that the copper concentration in the control oats in 1962 was double that in 1964 (see Table 25). The variety, stage of growth sampled, yield of dry matter per plant, extractable copper content of the control soil, and lack of response to additional copper in the soil, were similar in the two years. In 1961 the field was under grass, however, while in 1963 turnips were grown on the site. Mitchell Reith and Johnston (1957(1)) have shown that copper availability is lower after turnips than potatoes, while an increase in available copper after grass (Steenbjerg 1940) may have been augmented by the effects of the poor drainage at the site when left uncultivated (Adams and Elphick 1956).

(d) Other Effects of Copper Treatment of Cereals

The results obtained from the measurement of the bushel weights of grain were contradictory. The copper treatments at Choicelee in 1963 produced lighter grain than the control, while the plants affected by withertip at Hexpath (Bog field) andIangtonlees, very probably gave lighter grain than normal.

Kent (1963) has shown that grain from copper deficient oat plants contains a large proportion of single, empty husks, although the weight per grain is comparatively high.

The results of the nitrogen analyses of plants from Hexpath in 1962 showed that, at the "heading out" stage and in the straw, but not in the grain, the control oats had a higher concentration of nitrogen than treated plants. These results are similar to those obtained by Ennis and Brogan (1961). Lucas (1948), Wood and Womersley (1946) and Younts and Patterson (1964), on the other hand, have found that copper treatment of deficient plants reduces the nitrogen concentration in both the grain and straw. The total content of nitrogen per plant sampled at ear emergence was independent of treatment. The fact that the nitrogen concentration in the straw from the seedbed treatments was higher than in that from the foliar treatments, is another indication that foliar applications are more effective than seedbed dressings in curing copper deficiency.

There was no correlation between the occurrence of "grey speck" and the rate of copper application at Langtonlees, Upper Huntlywood or Choicelee (First Moor). At the Hexpath site in 1962, where no "grey speck" was observed, the plants at ear emergence contained an adequate concentration of manganese, the level of which was unaffected by the seedbed copper dressings, but lowered by the foliar copper applications. The total uptake of manganese per plant sampled at this stage was not influenced by the foliar treatments, and so the reduction in the manganese concentration was a dilution effect, caused by the increased yield in response to copper. A soil dressing of copper may, on the other hand, slightly increase the availability of manganese to the oat plant, so that both yield and uptake rise together, and the concentration remains comparatively constant. Although Steinberg (1950) and Steenbjerg and Boken (1950) have stated, in short reviews, that soil copper treatment can lead to manganese deficiency, Younts and Patterson (1964) have reported that copper seedbed dressings generally do not affect the manganese concentration in wheat at ear emergence.

The iron concentrations in the samples cut from the Bog field, Hexpath at ear emergence, were between the figures given by Mitchell (1954) for typical analyses of oat grain and oat straw, and were probably therefore not unusual. The levels in the Upper Huntlywood plants were only slightly lower, and were unaffected by the copper treatments, perhaps because of the variable and very low availability of manganese at that site. All the copper applications at Hexpath in 1962 lowered the concentration of iron in the plants at ear emergence. Although the effect of the soil dressings may have been simply a result of the combination of unchanged uptake and increased yield, the total iron contents of the plants given foliar treatments were very significantly lower than those of any of the other oats. Thus, apparently, the presence of a comparatively high level of copper in the young plants caused a reduction in the uptake of iron.

Copper can reduce the uptake of iron by plants (Willis and Piland 1934, Forster 1954), and can cause iron deficiency chlorosis (Sommer 1945, Willis and Piland 1936). Sometimes these effects are both found in the same plant (Forster 1954, Brown _et alia_ 1955), but, on the other hand, copper induced chlorosis can appear on leaves in which there is an extremely high level of iron (Wallace 1951). The formation of inactive copper-organic complexes in the plant, in preference to less stable iron-organic complexes concerned in chlorophyll synthesis, has been suggested as a probable explanation of copper induced chlorosis in beet (Hewitt 1963, pp.206-212). A similar conclusion can be drawn from the work of Hunter and Vergnano (1953) on oats. As it was the foliar treatments, and not the soil dressings, which caused the reduction in iron uptake at Hexpath, it seems that both effects of copper on iron take place within the plant. The method by which the antagonism is effective is not, however, clear.

(e) Copper in the Soil.

From a comparison (see Table 23) of the levels of total and E.D.T.A. extractable copper in the few samples from the trial areas, it appears that a smaller proportion of the total copper in the Hobkirk soils is extractable than in the Eckford soils. The total copper levels in these Hobkirk soils are lower, however, than those reported by Purves and Ragg (1962). The level of extractable copper, where comparison is possible, shows no sign of being affected by the previous crop. Thus 0.5 p.p.m. copper was extracted by E.D.T.A. from untreated soils containing 3 p.p.m. total copper after potatoes (Third

Birks, Choicelee) and after rape (Langtonlees). Between 0.5 and 0.6 p.p.m. was also extracted from soils of very low total copper status after turnips (Pirnie and Bog field, Hexpath) and lea grass (Plantation, Hexpath). No evidence has therefore been found from soil analyses of the reported mobilisation of copper by potatoes (Mitchell Reith and Johnston 1957(1)), or lea (Steenbjerg 1940). It is possible, however, that, at Rosehill in 1963, the turnip crop reduced the E.D.T.A. recovery of the soil copper dressings.

The effect of all the copper treatments was to raise the E.D.T.A. extractable copper status of the soil for at least two and a half years. The differences between the residual effects of treatments tended to become more significant, although not necessarily larger, with time, perhaps as any effect of unevenness in application became less marked. At the sites of the experiment laid down in 1963 and of experiment 1 in 1964, however, the effect of the coded treatments on the soil copper levels was always significantly linear. As there appeared to be no progressive fixing of the applied copper in a form unextractable by E.D.T.A., nor any marked loss through leaching, the recovery of the added copper was calculated from the average of the values found at all sampling dates at each site. The results from the sites which were only sampled once after treatment were not included in the recoveries listed in Table 26, because the effect of the sampling date was sometimes significant. From Table 26 it can be seen that the recovery from the soil copper applications was about 50 to 60%. A similar result has been obtained by Mitchell Reith and Johnston (1957(1)) by E.D.T.A. extraction of soil 8 or 20 months after soil copper dressings. The recovery of copper from the foliar treatments, on the other hand, was only about 20 to 30%, except at Rosehill, where it was not influenced by the method of application. At every site, except Rosehill, the straw and grain were removed from the trial at harvest, before very much leaching could have occurred. At Rosehill, however, there was very little grain, and the straw was left lying in the field. Thus nearly all the copper remaining in the plants was returned to the soil. The removal of copper in the harvested plants at Hexpath was, however, less than 0.01 lb. per acre in any treatment, and cannot, therefore, give the whole explanation of the difference between the recoveries from the foliar and seedbed dressings. At Hexpath (Bog field), 2.5 lb. per acre copper was applied to the crop in the "B" treatment, about 0.9 lb. was recoverable from the soil by extraction with E.D.T.A., and, by analogy with the soil application figures, about 1.2 lb. was lost by leaching or "fixed" in an unextractable form in the soil. There remains approximately 0.4 lb. per acre copper which has not been accounted for. There seems no reason for this to have been lost in drainage more easily than the copper applied to the soil, and therefore it may have been "fixed" in decomposing plant material or bacterially in the soil, or have been exuded by the oat roots at a lower depth than was sampled. This "fixation" or transportation apparently did not occur under the conditions of poor drainage and heavier soil at Rosehill. It may, however, have rendered the copper more available to clover at Hexpath, as shown by the 1964 analyses.

Table 26

Recovery of applied copper by E.D.T.A. extraction of soil

(i) 1962 experiment

Farm	Number of sampling dates	Treatments	A	B	C	D	E
		Addition of copper to soil (p.p.m.)	0	1.2	2.5	2.5	5
Hexpath	11	p.p.m. recovered	0	0.43	0.77	1.40	2.41
		% recovery	-	36	31	56	48
Rosehill	5	p.p.m. recovered	0	0.61	1.22	1.56	2.35
		% recovery	-	51	49	62	47

(ii) 1963 experiment

Farm	Number of sampling dates	Treatments	F	G	H	J	K
		Addition of copper to soil (p.p.m.)	0	0.2	0.6	1.2	2.5
Pirnie	3	p.p.m. recovered	0	0.07	0.10	0.22	0.47
		% recovery	-	35	17	18	19
Upper Huntlywood	3	p.p.m. recovered	0	0.06	0.10	0.26	0.51
		% recovery	-	30	17	22	20
Choicelee	3	p.p.m. recovered	0	0.05	0.17	0.30	0.55
		% recovery	-	25	28	25	22

It was found that the sampling date could affect the E.D.T.A. extractable copper status of a soil. This was not surprising, as the time of sampling has been shown to influence other soil measurements. (Piper and Prescott 1951, Robertson and Simpson 1954). These authors have, however, not found any correlation between the season or weather and fluctuations in the levels of soil pH, "available" K or "available" P. Steenbjerg (1940) (reported by Leeper 1952) has found that the level of copper extracted by ammonium nitrate solution from a single soil varied from week to week, being very much higher in mid-September than in June or November. It is not known, however, if this rise and fall was seasonal, or caused by the particular conditions in that soil. No comparable rise was found in the autumn at any site in the present work, where, however, there does appear to have been a seasonal effect.

The recovery of copper from the treatments at Hexpath (Bog field) and Rosehill was large in spring 1963, and the levels of E.D.T.A. extractable copper in the control plots at Pirnie, Choicelee (Third Birks) and Upper Huntlywood were also higher then, than at harvest 1963, or in the spring or autumn of 1964. This high "availability" in spring 1963 may have been linked with the abnormally long severe winter that year. During the winter, the top soil was frozen for far longer than usual, preventing gentle leaching, and the heavy snow falls left the soil wet when the thaw started. At two sites, there was also a significant increase in availability in spring 1964, and at Hexpath (Bog field) there was a tendency in 1964 for the level of extractable copper to be high in March, but to drop in May and gradually to increase again in the late autumn. The possible influences of crop changes and soil moisture contents on the results from Hexpath and Rosehill are discussed above, in the section on the 1962 experiment. No correlation was found at any site between recent rainfall and any change in the extractable copper level.

(f) Variation in the Occurrence of Copper Deficiency in Cereals

Table 27 shows the number of fields in South East Scotland in which withertip or blind ear has been seen by an experienced observer. Withertip was not reported in this area before 1954, and, since then, has occurred almost exclusively in Berwickshire, although, in East Lothian, cereal plants have exhibited a syndrome associated with deficiency of several nutrients, including copper. As shown in Table 27, the disease occurred most often in 1962 and more frequently in 1959 and 1964 than in the remaining years. On the basis of the reports which are discussed in the review of the literature, it was thought that hot dry conditions during May and June might have caused this increase in incidence. The rainfall, hours of sunshine and temperature records from the meteorological station at Marchmont House, Berwickshire, showed, however, that none of the data for March, April, May, June or July was peculiar to the years 1959, 1962 and 1964. It was found, on the other hand, (as shown in Table 28) that February was very dry in each of these three years, and that the rainfall in the four months preceding sowing was abnormally low. There was a negative correlation, significant at P less than 0.05, between the number of fields in which withertip was observed, and the total rainfall during the months of December, January, February and March in the area. Thus, although there are too few observations for any very reliable deductions, withertip appears to occur more frequently in Berwickshire after a dry winter than after a wet one.

Table 27

Number of fields in South East Scotland in which copper deficiency symptoms in cereals have been reported.[1]

Year	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964
Number of fields	3	0	0	2	3	7	2	3	11	2	6

1. Reports from the agricultural advisers of the extension staff of the Edinburgh School of Agriculture.

Table 28

Rainfall in inches at Marchmont House, Berwickshire.[2]

Year	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	
Feb.	2.18	2.60	3.30	3.23	2.36	0.55	2.31	2.02	1.02	1.85	0.98	2.18+
Total for Dec. Jan. Feb. & March	9.15	8.49	10.46	10.64	9.22	7.61	12.21	8.38	7.76	10.46	5.97	9.54+

2. Figures from the files of the Meteorological office

+ Average figures for 1916-1950

From data given by the Department of Agriculture (1957, 1959, 1960, 1962, 1964, 1965(1), 1965(2)), it has been estimated that the average annual increase in the production of oat and barley grain per acre in Berwickshire was maintained in 1959, 1962 and 1964. Thus the conditions leading to withertip in copper deficient fields did not reduce the yields of cereals generally. The estimated yields for oats and barley in the 1963 season, on the other hand, are very much lower than those for 1962 or 1964. In 1963, the small response to applied copper in the field experiment, and the lack of occurrence of withertip, may have been caused by increased availability of soil copper in the spring, as found by E.D.T.A. extraction, or by the generally poor growing conditions throughout the season, or by a combination of these factors.

5. Conclusions from Observations and Experiments in the Field

All the conclusions refer to the sandy soils of the Eckford and Hobkirk Series, under the conditions of farming practised in South East Scotland.

(a) The E.D.T.A. extractable copper concentration in a soil gives a better assessment of the likelihood of an immediate yield response to copper in cereals than does the total soil copper level. Soils containing 0.5 p.p.m. extractable copper generally produce an increased yield after copper treatment.

(b) E.D.T.A. extraction of a soil generally recovers 20-30% of foliar applications of copper and about 50% of soil dressings. There is no progressive decrease in these recoveries during a period of two and a half years after the copper treatment, and the copper from both foliar and soil dressings is readily available to clover plants.

(c) The recovery of applied copper by E.D.T.A., and, to a lesser extent, the extraction of native copper, fluctuates according to the sampling date. This variation appears to be seasonal at some sites, and may sometimes be associated with the weather.

(d) Observations suggest that withertip may occur more commonly on copper deficient soil after a very dry than a wet winter.

(e) Rain water in a suburb of Edinburgh contains about twice as much copper as that in a rural area of Berwickshire.

(f) A seedbed dressing of 10 lb. per acre copper oxychloride, or a foliar application of as little as 0.8 lb., prevents withertip in oats and barley, but larger increases of grain yield follow foliar spraying at a rate of 5 lb. per acre.

(g) The cereal grain yield responses to foliar treatments with 5 lb. and 10 lb. per acre copper oxychloride appear to be similar, and to show no varietal differences.

(h) The straw yield is not increased by copper dressing.

(i) The copper concentrations in oat and barley plants vary according to the stage of growth, the variety of the cereal and the extractable copper content of the soil. Under the same conditions, the concentration in barley is higher than that in oats.

(j) Foliar copper dressings of young cereals raise the copper concentration in the plants at ear emergence more than in the straw, and do not generally affect the concentration in the grain. Seedbed dressings of up to 20 lb. per acre copper oxychloride cause no change in the copper level of oat or barley plants after ear emergence.

(k) The rise in yield, following foliar copper applications to deficient oats, leads to a lowering of the nitrogen and manganese concentrations in the plants at ear emergence. The marked reduction in the iron concentration at this stage is probably a result of a decrease in iron uptake after foliar copper treatment.

GREENHOUSE EXPERIMENTS

(1) 1962

(a) Aims

(i) To observe closely the growth of oats on copper deficient soil.

(ii) To compare the effects of periods of drought on the development and copper uptake of oats poorly and well supplied with copper.

(b) Statistical Design

A factorial randomised block design was used, with four replicates of two copper levels and seven water regimes. The detailed layout is given in appendix 4. As growth was uneven, once a week all the pots on the window side of the bench were put next the gangway while the gangway pots were moved next the window. The pots remained in the same positions relative to the length of the greenhouse. The statistical analysis of variance on the results is shown in Table 29.

Table 29

Analysis of variance on data from greenhouse experiment 1962

Factor	Degrees of freedom
Total	55
Replicates	3
Treatments	13
Error	39
Splitting of treatment sum of squares:	
Treatments	13
Copper	1
Water	6
Copper x water	6

(c) Experimental Materials

Table 30

Copper concentrations in materials used in greenhouse experiment 1962

Material	p.p.m. copper
soil	less than 1
sand	less than 1
polythene flower pot	0.7
polythene water container	0.4
glass wool	less than 1
black paint for outside of pots	negligible
nutrients	less than 1
water	less than 0.001
seed grain	3.8

All the materials used in the experiment were analysed for copper, and the results are shown in Table 30. The soil, which was from the Eckford Series, was obtained from near the surface of a field in which withertip had been observed. As local sand was found to contain over 0.3 p.p.m. E.D.T.A. extractable copper, the recommendation of Hewitt (1952) was followed, and sand for the experiment was bought from Leighton Buzzard. Yellow polythene pots were used and were painted black on the outside, so that little light would penetrate below the soil surface. It was calculated that 0.6 μg copper was added to each pot as contamination in the combined nutrients, and this was considered negligible compared with the addition of 7.4 μg copper per pot in the seed grain. As treatments involved differing watering regimes, it was especially important that the water should not be a source of contamination. Deionised, rather than distilled, water was used because it could be produced easily in the greenhouse. It was stored in a heavy polythene container.

(d) Experimental Methods

(i) Water testing. Methods for checking the purity of water, beyond a simple conductivity test, were examined. It was preferable that the method chosen should be simple, because the greenhouse used in 1962 and 1963 was situated at Boghall, some distance from a laboratory, and it was convenient and desirable to check the purity of the water immediately before watering the plants. The dithizone tests for total metals described by Stout and Arnon (1939), Sandell (1959 page 17) and Hewitt (1952 page 185) were considered and, although all three tests gave similar results on three water samples, the latter was preferred. Hewitt's method is the simplest, as it does not require ammonia, the colour of the solvent layer is the most stable, and the results can be evaluated without constant reference to standard solutions of copper and zinc. Duplicate samples of water tested by Hewitt's method in the greenhouse were checked by the zinc dibenzyl dithiocarbamate method for copper. By the simple test, contamination was found to be less than 0.001 p.p.m. total heavy metals, and the second procedure showed that less than 0.001 p.p.m. copper was present.

(ii) Preparation of pots

Each pot was washed thoroughly and rinsed with deionised water. The drainage holes were covered with 5 g. teased glass wool. Forty-two pounds of riddled soil and 21 lb. sand were mixed, and nutrients at the rates shown in Table 31 were applied in a solution of 450 ml. from a polythene sprinkler. The resulting batch of soil was thoroughly mixed and portions of 15.4 lb. were weighed into each of four pots, which were, as far as possible, from one replicate. The nutrient status of the mixture is given in Table 32. All the copper deficient soil was filled into pots before any copper treated batch was begun. The copper dressing was calculated to raise the total copper level to 10 p.p.m., two thirds of the average level found in local soils. (Purves and Ragg 1962) Manganese was included in the added nutrients because "grey speck" had occurred in the field from which the soil was taken.

Table 31

Nutrient additions to greenhouse pots 1962

To all pots:

0.31 g. K_2SO_4, Analar, per pot	≡ 40 lb. K per acre
0.18 g. $NaH_2PO_4 2H_2O$, Analar, per pot	≡ 10 lb. P per acre
0.60 g. NH_4NO_3 Analar, per pot	≡ 60 lb. N per acre
0.64 g. $MnSO_4 4H_2O$, Analar, per pot	≡ 45 lb. Mn per acre

To half the pots:

0.14 g. "Blitox" per pot ≡ 20 lb. Cu per acre
≡ 40 lb. copper oxychloride per acre

Note: calculations on a basis of 2,000,000 lb. soil per acre

Table 32

Nutrient status of mixed soil and sand for greenhouse pots 1962

pH : 7.2

level of "available" K: very low

level of "available" P: moderate

level of E.D.T.A. extractable Cu: (a) 0.44 p.p.m. in control pots

(b) 11.2 p.p.m. in copper treated pots

All pots were brought to field capacity by being placed in a heavy polythene bath of deionised water until the soil surface became moist. They were then allowed to drain for at least 24 hours. Forty oat seeds dressed with "Ceresan" were sown in each pot and covered with 1.1 lb. dry sand so as to prevent puddling during watering.

(iii) Water regimes

The water capacity of the soil and sand mixture was ascertained by soaking 7 Kg. of it in a pot, and reweighing when no further drainage occurred. At the same time, a sample taken just before the first weighing was dried at room temperature to constant weight. Thus the total weight of water contained by a known weight of dry soil when raised to field capacity was determined. The average of four determinations was taken, and the total weight of a pot containing 16.5 lb. dry soil, after being raised to 40% or 80% water capacity, was calculated.

Table 33

Water regimes 1962

Treatment	Period at 40% water capacity	
a	none	
b	28th May to 13th August:	continuously after 3rd leaf.
c	28th May to 25th June:	3rd leaf to the beginning of ear emergence.
d	11th June to 9th July:	5th leaf to the end of flowering.
e	25th June to 23rd July:	the beginning of ear emergence to early maturity.
f	9th July to 6th August:	the end of flowering to near maturity.
g	23rd July to 13th August:	during maturation.

The pots in the greenhouse were weighed every day and watered, where necessary, to the weight corresponding to 80% water capacity, except during the periods shown in Table 33, when pots in the appropriate regimes were kept at 40% water capacity. The deionised water was applied from a polythene watering can which was protected from dust with polythene sheeting. This method did not give soils uniformly at 40% or 80% water capacity, (Hudson 1957) as all watering, after the initial soaking, was on to the surface, while water was taken up by the roots throughout the soil. Thus the soil near the surface was continuously wetter than 80 or 40% water capacity, while the soil near the base of the pot was drier than these levels. Very little evaporation took place from the polythene pots. By 23rd July, when the drier period of the "g" treatment was due to start, the transpiration rate was so low that the pots did not drop to the weight corresponding to 40% water capacity until 2nd August. Thus they were at the lower water level for only a fortnight instead of the intended month.

(iv) Sampling

The plants were harvested three times at about monthly intervals. All greenhouse sampling was done by the author who, at each harvest, took ten plants from each pot as representatively as possible with regard to height, bulk and maturity. Each plant was cut just above the soil surface with stainless steel secators which were cleaned between samples, and all the plants from a pot were placed in a clean paper bag lined with greaseproof paper. The samples were dried in these bags at 90°C to constant weight and, where possible, the grain was separated from the straw by hand. Milling and storing were carried out in the same way as for the field samples, except in the case of very small yields, which were chopped up with secators.

Initial soil samples were taken by removing three small handfuls from each batch during the mixing before pot filling. The handfuls from all the control batches were mixed together, as were the subsamples from copper treated batches, to give two composite samples. After the final harvest, soil samples were taken by augering five times to the full depth of each pot, keeping the holes as far apart as possible. The pot samples, which were kept separate, were treated in the same way as those from the field experiments.

(e) Results

Data from the experiment are detailed in appendix 9.

(i) Observations and comments.

The oats, variety Vigor, were sown on 9th May and emerged on the 15th. When they were about 3" high, they were thinned to 30 plants in each pot. By the first harvest, on 12th June, the plants were in 5th leaf. Plate 9 shows a general view of the pots on 22nd June, when the leaves had just ceased growing. The ears started to emerge on 25th June and the second harvest was made during flowering, on 6th July. The remaining ten plants per pot were harvested on 13th August, when they were ripe. No withertip was observed at any time, and the only colour difference was the comparatively bluer and darker appearance of the leaves of the oats in the "b" treatment.

On the 11th July, very heavy rain came through the greenhouse roof in places. As a result, the "b" high copper level pot in the first replicate was flooded and the plants partially uprooted, and so no reliable results were obtained from it at the third harvest. To prevent any further accidents, the greenhouse was lined with thin polythene sheeting.

From the results of the E.D.T.A. extractable copper analyses of the harvest soils, (see appendix 9) it was realised that gross contamination must have occurred in the seventh control batch of soil and sand before pot filling. Batches 6 and 8 had low copper levels, and yet an addition of about 9 mg. copper must have been made to batch 7, presumably during

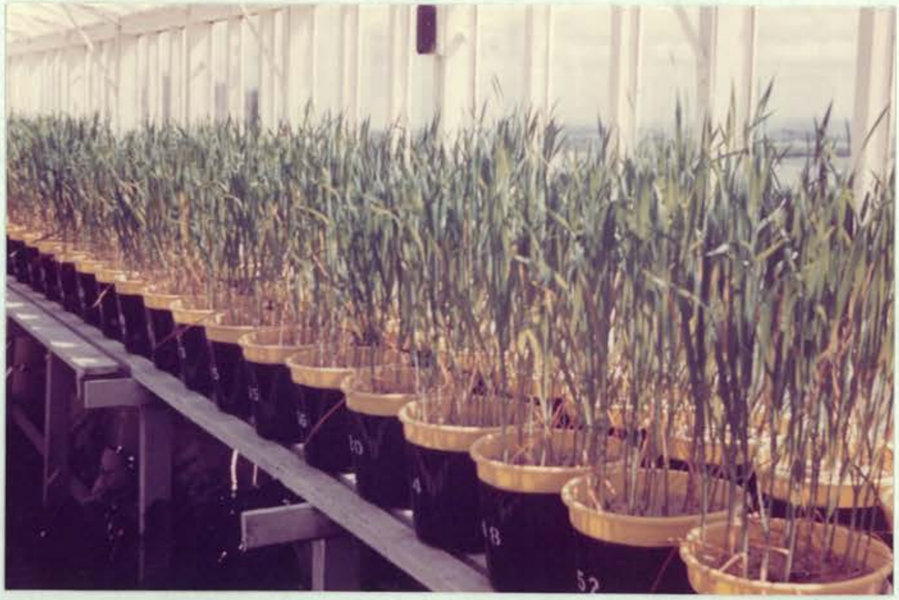


Plate 9. Greenhouse experiment June 1962.

Plate 10. Control (pot 56) and copper treated (pot 52) oats, July 1962.

mixing. The copper mesh covering the ventilator along the ridge of the greenhouse roof was a constant contamination hazard in 1962, and was removed before the beginning of the 1963 experiment. The doubling of the E.D.T.A. extractable copper level in the soil of batch 7 had no effect on the straw yield, but increased the grain yield and the numbers of ears and grains per plant to almost the same extent as the copper treatment. The seventh batch of soil was filled into four pots, all of which were in the first replicate, which also contained the flooded pot mentioned above. Statistical analysis was therefore confined to the remaining three replicates.

At the time of the first harvest, there were only two water regimes, as the periods of restricted watering had not yet begun in treatments "d", "e", "f" and "g", and the pots in the "c" regime had not yet been returned to the 80% water capacity level. Thus the results from the pots in the same replicate and the same copper treatment for water regimes "a", "d", "e", "f" and "g" were averaged, as were the figures from pots in treatments "b" and "c" (see appendix 9). At the second harvest the results from treatments "a", "f" and "g" were similarly averaged in replicates and copper treatments so that the statistical analysis would not be complicated by unequal replication.

(ii) Development.

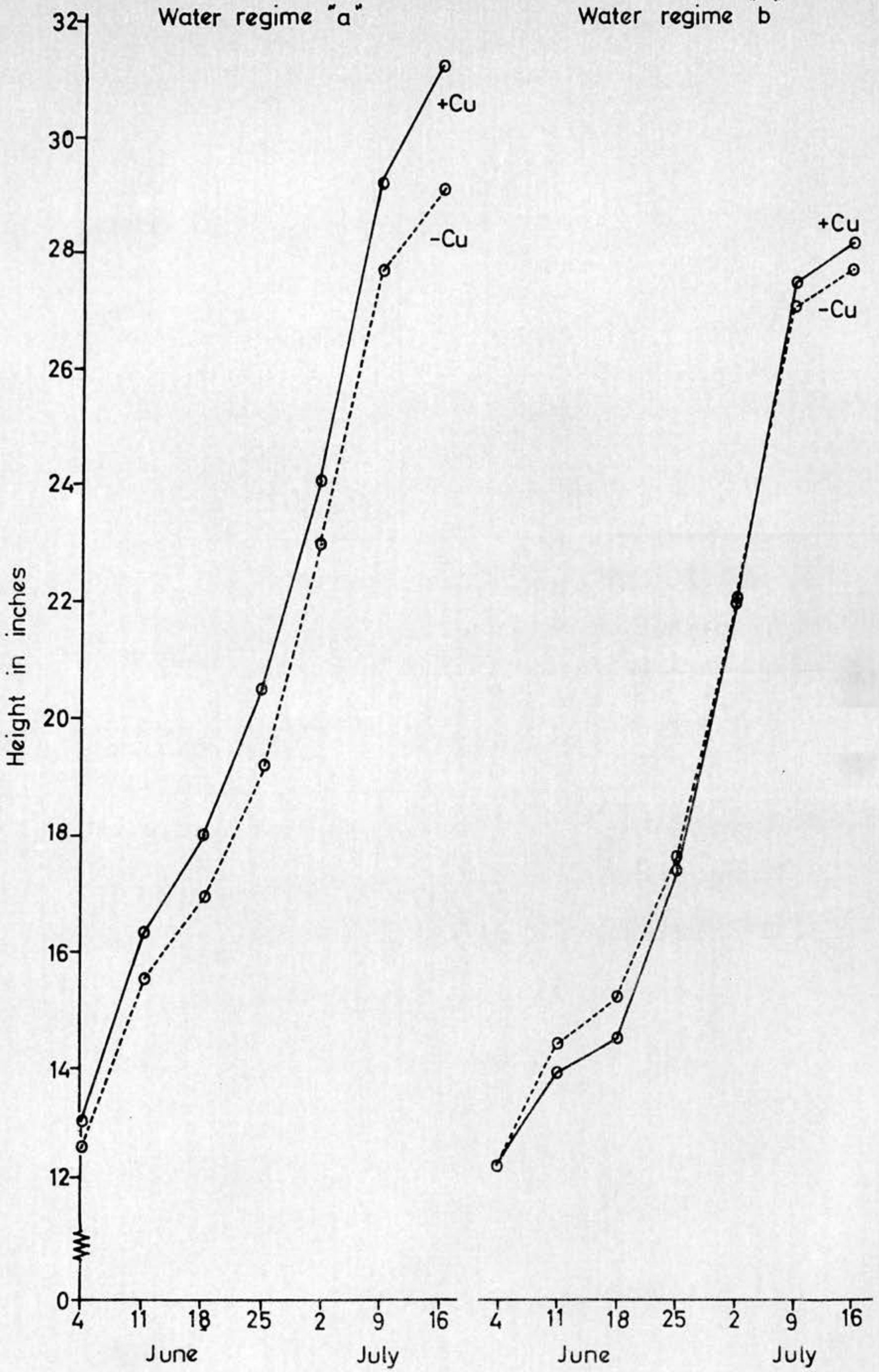
The "b" plants and the copper treated oats matured a little faster than the others. Height differences between plants at 80% and 40% water capacity and between copper treated and untreated oats were visible from the time of the first harvest. Plate 10 shows the variation on 2nd July between copper treated (pot 52) and control (pot 56) plants in the "g" water regime. Diagram 21 illustrates the growth of the oats in the "a" and "b" treatments. After the 18th June, the copper treatment increased the height of the wet "a" regime plants by an inch or more, but had, generally, no effect on the height of the dry "b" regime plants.

There was also an interaction between the effects of copper and water on the count of tillers at maturity (see appendix 9). The wettest treatments "a" and "g", led to many tillers at the low copper level but none at the high copper level, while there was no significant difference caused by the copper treatment in the other water regimes. As shown in Table 34, the copper treatment raised the number of ears and grains and lowered the weight of 1,000 grains. A lowered grain count is a mild form of the "blind ear" which is characteristic of copper deficiency.

(iii) Yield.

From Table 34 it is seen that the copper treatment raised the dry weight of the plants very significantly at every harvest. The yields at the first and second harvests and of the straw were increased by about 10%, while the grain yield was raised by 18%.

Diagram 21. Effects of copper and water supply on growth of oat plants, 1962.



Note: In diagrams 21-29, 31, 34, 35, 37: -Cu: control, +Cu: copper treatment.

Table 34

Significance of effects of copper and water supply on yield. Greenhouse 1962

Data	Copper			Water		
	Treatment	Mean	Significance of difference between means	Treatment	Mean	L.S.D.
Dry yield at first harvest (g/pot)	+	3.84	* *	wet	3.94	* *
	-	3.44		dry	3.34	
Dry yield at second harvest (g/pot)	+	11.63	* *	a,f,g	11.55	0.27 * *
	-	10.55		b	10.34	0.20 *
				c	10.16	
				d	11.72	
				e	11.68	
Yield of dry grain (g/pot)	+	7.10	* *	a	6.47	0.69 * *
	-	5.99		b	6.46	0.51 *
				c	6.36	
				d	7.23	
				e	6.38	
				f	6.24	
				g	6.67	
Yield of dry straw (g/pot)	+	9.00	* *	a	9.28	0.76 * *
	-	8.27		b	7.83	0.56 *
				c	7.79	
				d	8.92	
				e	8.65	
				f	8.64	
				g	9.32	
Number of ears per pot	+	128	* *			12 * *
	-	108				9 *
Number of grains per pot	+	219	* *			23 * *
	-	180				17 *
Weight of 1,000 grains (g)	+	32.4	*			1.3 *
	-	33.3				

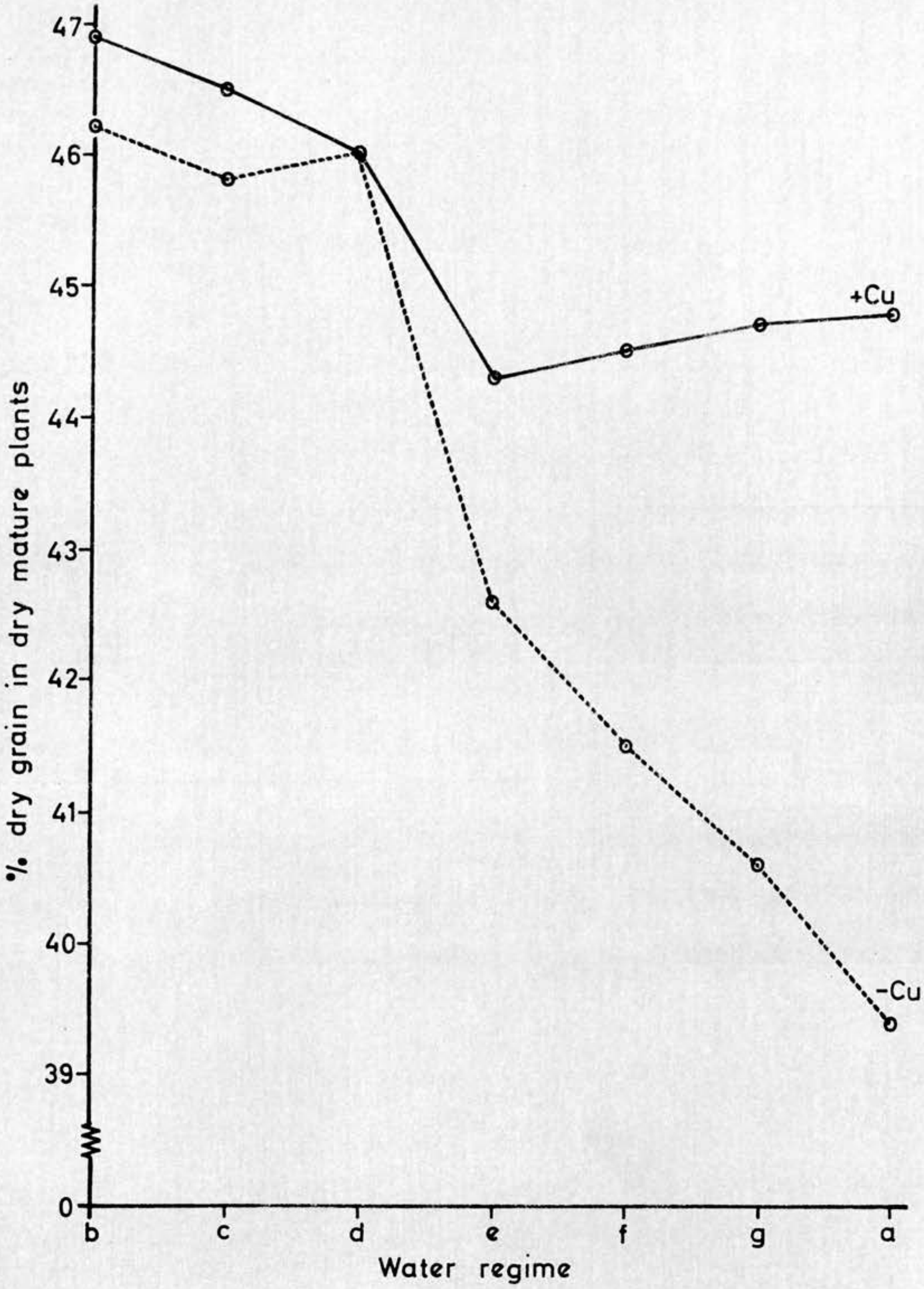
Note: There were no significant interactions between the effects of copper and water on any of the data in Table 34.

In all samples except the grain, regime "a" led to a significantly higher yield than "b". At all stages, the "c" regime plants had similar yields to those in the "b" treatment, and the "a" and "g" regime yields were also close to one another. The latter similarity is not surprising, as the "g" treatment period of restricted watering occurred so late in the development of the plants. The yield from the "d" treatment, in which the pots were at the drier water level just before and during ear emergence, was higher than from any other water treatment. Van der Paauw (1949) and Roberts (1928), on the other hand, have both reported from more extensive experiments that dry conditions during this critical period of development lead to poor grain yields. In Table 34 it is also shown that the "b" and "c" plants produced far less straw than any of the other water treatments. Van der Paauw (1949) has found that early drought causes a diminution in oat straw yield. The periods of comparative dryness occurring later, in treatments "e" and "f" also, however, lowered the straw yield. Treatment "d" was again anomalous, with a yield similar to those of regimes "a" and "g". There were no significant interactions between the effects of copper and water on yield.

There was an interaction between the effects of copper and water on the proportion of grain in the plants at maturity, (see diagram 22). In water treatments "a", "f" and "g", the differences caused by the copper treatment were highly significant. Thus the longer an ample water supply was

Diagram 22. Effects of copper and water supply on % grain in mature plants.

Greenhouse 1962.



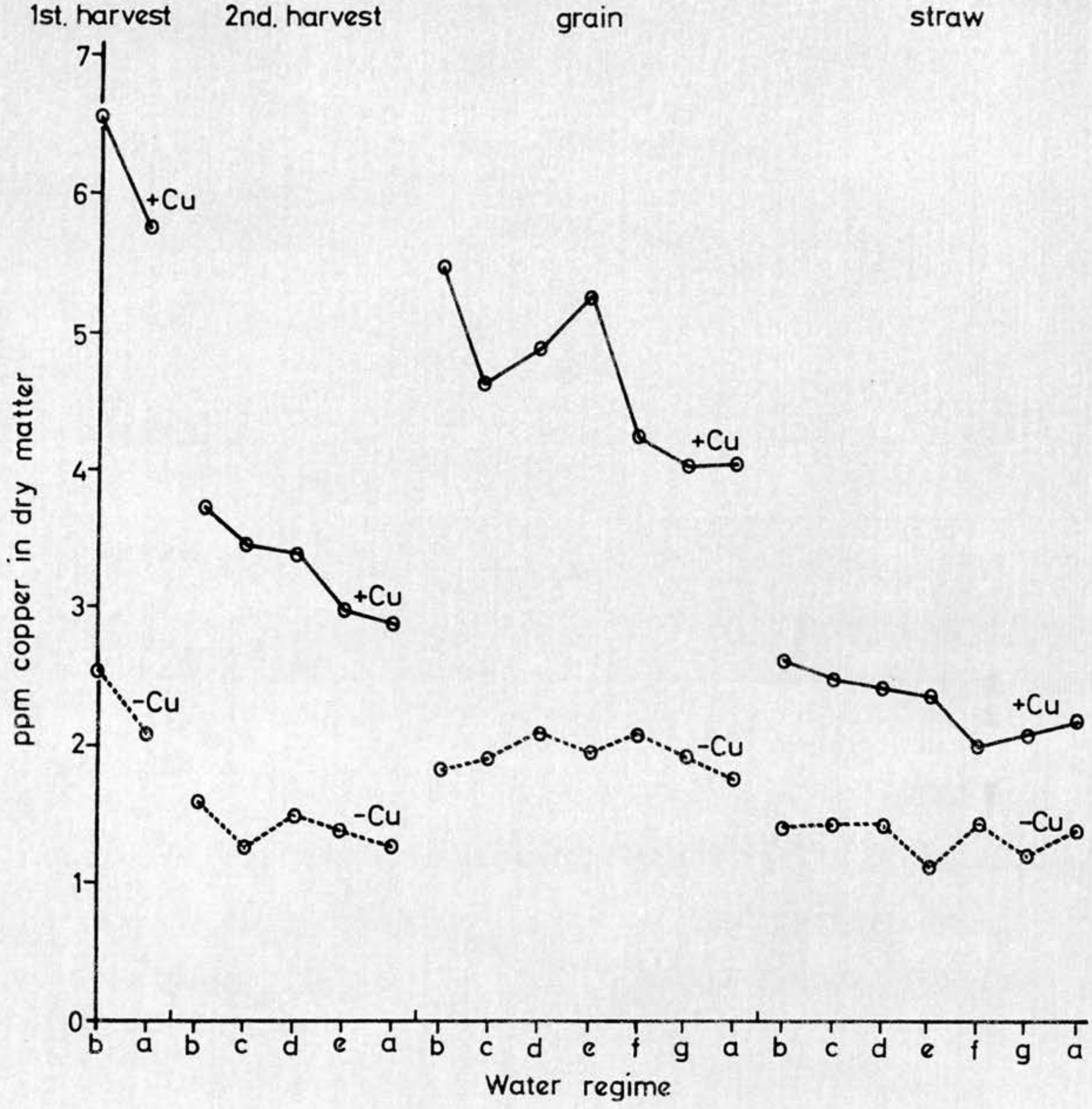
Significant interaction: Copper x water, L.S.D. = 1·9%*, 2·6%**.

maintained, the more readily the copper deficiency symptom of a low ratio of grain to straw appeared. From diagram 22 it is also evident that the effect of the water regimes "b", "c" and "d", in each of which the soils were at 40% water capacity during some time before ear emergence, was to raise the percentage of grain at the low copper level. The difference is, however, not large enough to be significant at the high copper level. Van der Paauw (1949) has found that an early drought leads to a higher ratio of grain to straw than one occurring late in the development of oat plants with full nutrition.

(iv) Copper concentration in plants.

As shown in diagram 23, interactions between the water and copper effects were significant at all harvests. In every water regime, nevertheless, the copper treatment always caused a rise in the herbage copper concentration. Although this increase was larger in the young than in the mature plants, the soil dressing of copper, unlike those in the field trials, increased the concentration in the grain by about 3 p.p.m. The water treatments caused greater differences at the high than the low soil copper level. Thus the "b" water regime led to a higher copper concentration in the copper treated plants than the "a" treatment at second harvest and maturity, while this effect was significant in the control plants only at second harvest. When ample copper was available, the grain in the three regimes ("b", "d" and "e") in which the water rate was low during ear emergence and

Diagram 23. Effects of copper and water supply on copper concentration in oat plants. Greenhouse 1962.



Significant interactions of copper x water:

1st. harvest.	L.S.D.	0·2 9ppm.*	
2nd. harvest.	L.S.D.	0·24ppm.*	0·33ppm.**
grain.	L.S.D.	0·60ppm.*	0·81 ppm.**
straw	L.S.D.	0·26 ppm.*	0·35ppm.**

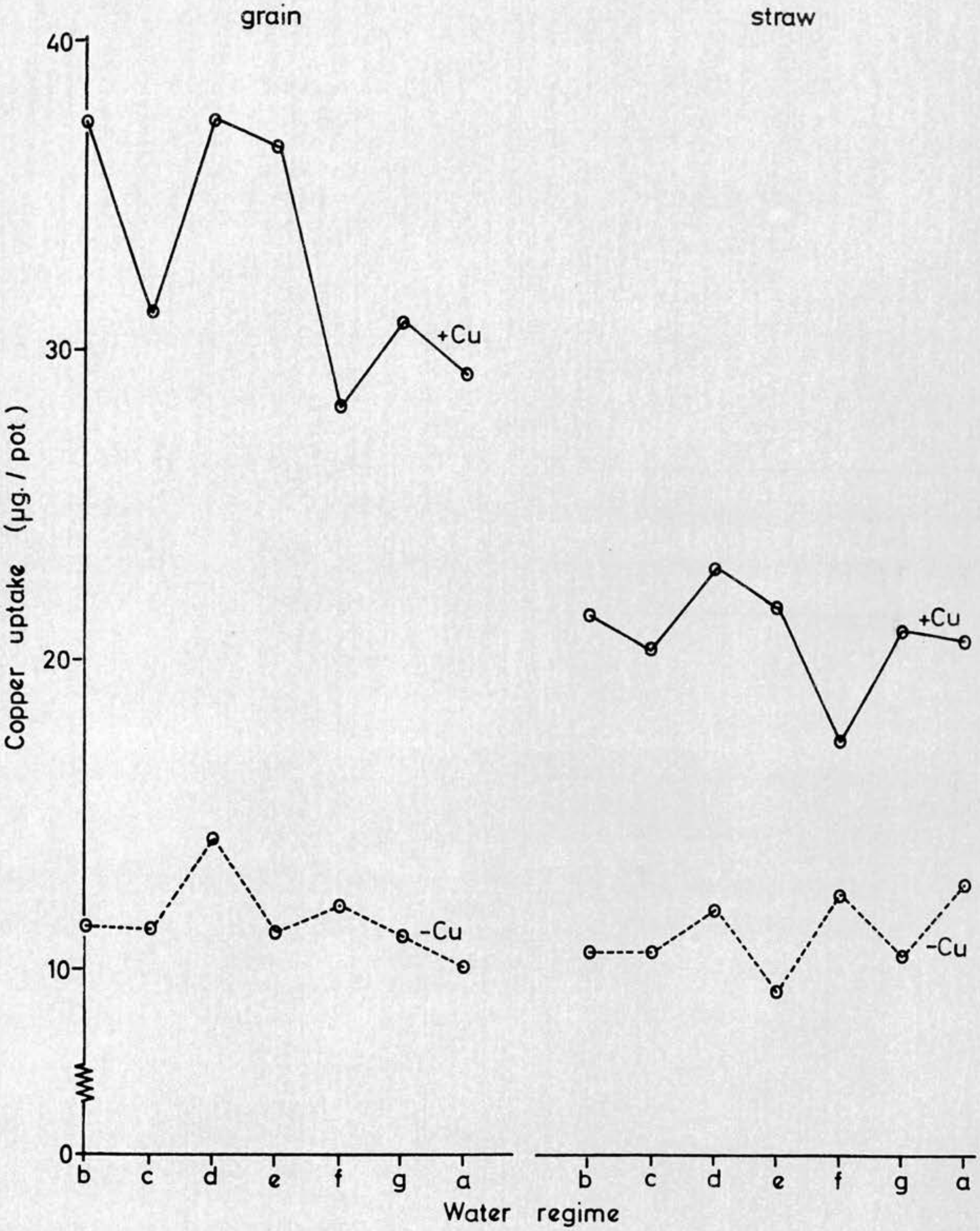
flowering, had higher concentrations than that in the other water treatments, except "c". The copper concentration in the straw from the copper treated pots, on the other hand, was highest in the water regimes "b", "c", "d" and "e", which were at the lower water rate before and during ear emergence. As in the yield data, the results from the "a" and "g" regimes were always similar. The plants in the "b" and "c" treatments, however, had different copper concentrations at the second harvest at both copper levels. The ten days between the renewal of the 80% water capacity level and the second harvest were thus enough to lower the copper concentration in the "c" plants from the "b" level. From the second harvest results of the copper treated plants, it is evident, however, that a period of ten days was not long enough at 40% water capacity to cause a rise in concentration, while three weeks were sufficient, (see diagram 23).

(v) Copper uptake.

The copper treatment raised the copper uptake of the plants very significantly at each harvest, doubling the uptake in the straw and at second harvest, and nearly tripling it in the first harvest plants and the grain. The water treatment caused no significant difference in the uptake of copper by the plants at first harvest, but, at the second harvest, the uptake by the "b" and "d" oats was significantly larger than that by the "a" and "c" plants.

There was no interaction between the effects of copper and water on the plants at the first or second harvests. The

Diagram 24. Effects of copper and water supply on total copper content of grain and straw. Greenhouse 1962.



Significant interactions of copper x water:

grain: L.S.D. 4·3 μg.* straw: L.S.D. 2·4 μg.* 3·3 μg.**

absorption results from the final harvest are illustrated in diagram 24. In the grain, there were no significant differences between the water treatments at the low copper level. At the high copper level, the "b", "d" and "e" regimes, which were at the drier water rate during ear emergence and flowering, led to a higher uptake of copper than the "a" treatment. Thus, if there is ample copper and restricted water during the formation of the grain, the total copper content in the grain is high.

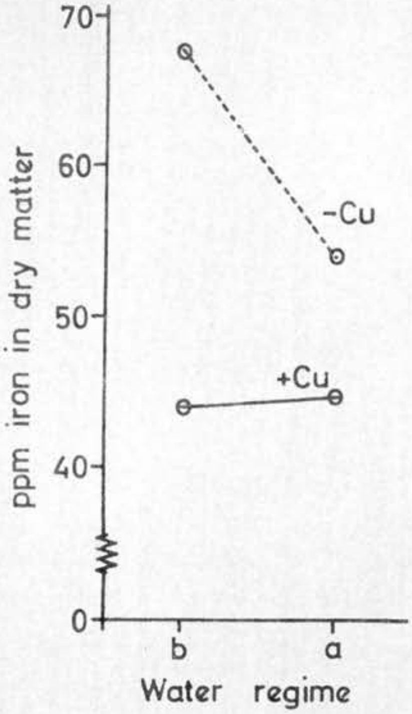
(vi) Iron and manganese levels.

Diagrams 25 and 26 show the iron concentration and uptake in the plants at first harvest. The copper treatment lowered the iron concentration in both water regimes, but reduced the iron uptake significantly at only the drier water level. The effect of the water supply was very large on the concentration of iron in the control plants, but negligible in the copper treated oats. The uptake of iron, on the other hand, was significantly affected by the water regimes at the high but not the low copper level.

There were no significant treatment differences in the results from the analysis of the plants at first harvest for manganese. This lack of response to the copper treatment may have been caused by the manganese soil dressing. The manganese concentration in the plants was very high, although fairly near the average found by Williams and Moore (1952) in oats at a similar stage of development.

Diagram 25. Effects of copper and water supply on iron concentration in oats.

First harvest, greenhouse 1962.

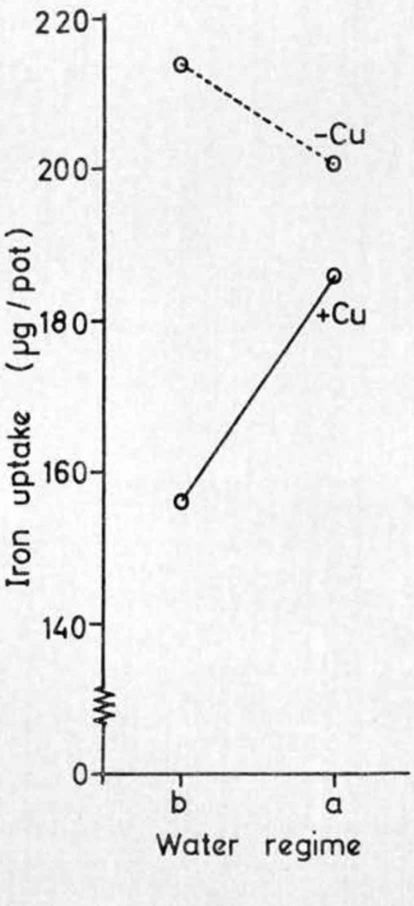


Significant interaction of copper x water:

L.S.D. 5·7ppm.*, 8·6ppm.**

Diagram 26. Effects of copper and water supply on iron absorption by oats.

First harvest, greenhouse 1962.



Significant interaction of copper x water:

L.S.D. 28µg.*, 42µg.**

(vii) Soil copper.

The copper treatment raised the concentration of E.D.T.A. extractable copper from 0.4 to 11 p.p.m. initially, and at harvest the average of the copper treated pots was 8 p.p.m. Practically no leaching occurred after the initial soaking of the pots, and so either the 3 p.p.m. was lost at this stage, or was fixed by the soil in a non-extractable form. The loss of copper in harvested plants was less than 0.1 p.p.m. and therefore negligible. The recovery of the copper at harvest was about 81%, which is higher than that from field soils after a seedbed dressing. The copper treated pots in the "a" and "c" regimes contained a very significantly lower level of extractable copper than in the "g" and "d" treatments.

(2) 1963

(a) Aims

To ascertain the effects of light intensity and rate of watering on the severity of copper deficiency in oats, on the yield response to copper treatment, and on copper uptake.

(b) Statistical Design

The experiment was factorial in design, with three intensities of illumination, two water regimes and two copper levels. There were four replicates. The layout, which is given in appendix 4, was completely randomised within the light treatments. To minimise any effects of unevenness of intensity within these light blocks, each pot was moved into a neighbouring position every second day, as shown in appendix 4. The analysis of variance is given in Table 35. As the light treatments were not replicated, a true statistical test of the significance of the differences between their effects was not possible. If, however, a difference was very obvious, it was highly probable that it was caused by the light treatments, because the oats grown in 1962 did not show any significant differences between replicates 2, 3 and 4, which occupied the same positions on the bench as the three light treatments. Any interaction between the effects of light and water or light and copper could, however, be examined statistically.

Table 35

Analysis of variance on data from greenhouse experiment 1963

Factor	Degrees of freedom
Total	47
Replicates	3
Treatments	11
Error	33
Splitting of treatment sum of squares:	
Treatments	11
Copper	1
Water	1
(Light)	2
Copper x light	2
Copper x water	1
Water x light	2
Copper x water x light	2

(c) Experimental Materials

The soil and sand mixture was that used in 1962. During the winter it had been left unwatered in the pots, which were covered with brown paper to prevent contamination. In the spring of 1963, the soil had a pH of 6.6, and a very low K and a moderate P status. The level of E.D.T.A. extractable copper was 0.39 p.p.m. in the control soil and 7.8 p.p.m. in the copper treated pots. Fresh glass wool, sand, nutrients and deionised water were from the same sources and were found to be as pure as those tested in 1962. The seed grain had, however, been collected from the harvest of the control plots in the copper trial at Hexpath in 1962, and was therefore variety Forward, containing 1.3 p.p.m. copper.

(d) Experimental Methods

The soil from the four contaminated pots was discarded. The remaining soil, after the removal of all roots and glass wool, was mixed in eight batches of six pots. Each batch, of either control or copper treated soil, was made up from the contents of one pot from each of all but one of the 1962 water treatments, and was filled into the pots of a 1963 replicate. The nutrients were applied at the same rates and in the same way as in 1962, except that no copper was added to any soil. The pots were filled, soaked and allowed to drain, and the oats were sown as in 1962, but were covered with 1.5 lb. sand.

The two copper levels were thus the control and the higher concentration resulting from the 1962 treatment. Half the pots were kept throughout the experiment at 40% water capacity and half at 80%. This was done, as in 1962, by watering to the appropriate calculated weight. There were three illumination treatments: "light", "medium" and "dark".

The control or "light" treatment pots were on part of the bench with clear windows. The sloping glass roof and the side windows along the rest of the bench were coated with green shading, and much of the glass round the "dark" treatment was covered with brown paper. The general layout of the experiment can be seen from plate 11.

Each batch of soil was sampled separately when the pots were filled, but otherwise the sampling technique was similar in 1962 and 1963.

The illumination in the greenhouse was measured by a method which involves the photo-destruction of oxalic acid in the presence of uranium ions, and was developed in 1962 from the outline given by Hewitt (1952 p.163). The detailed procedure, which is given in appendix 3, is very similar to that described by Heinicke (1963). To calibrate the method, integrator tubes were suspended at varying distances from a strong light bulb in a dark room for 67 hours. The intensity of the light falling on the tubes was measured using an L.A.P. lux meter. The results, which are given in Table 36, show that, in this low range, the destruction of oxalic acid per lux did not vary with intensity. In the greenhouse, light entered the tube from all directions, instead of from only one as in the calibration. When allowance was made for this, the average daily illumination in the "light" part of the greenhouse during the dull June in 1963 was calculated to be about 490 kilolux-hours. It has been estimated (Taylor and Smith 1961) that the mean daily

Plate 11. Greenhouse experiment June 1963.

Pot 3: "wet," "dark," +Cu.

Pot 6: "wet," "dark," -Cu.

Pot 33: "wet," "light," +Cu.

Plate 12. Effects of treatments. Greenhouse July 1963.

illumination at Boghall during an average June is 550 kilolux-hours. The integrator measures the total, cumulative illumination over a period. It has been reported, however, that plants cannot utilise light energy efficiently at intensities outside the range 5 to 1,000 foot candles (Trumble 1951). On very sunny days, therefore, the illumination measured by the integrator is not all available to the plants. In 1963, one tube was hung above the plants in each light treatment, and thus a measure of the illumination in each block was obtained. Two of the integrator tubes are just visible in position in plate 11.

Table 36

Calibration of light integrator

Light intensity in ft. candles	mg. oxalic acid destroyed in 67 hours	μg. oxalic acid destroyed per kilolux-hour.
100	0.26	5.7
200	0.48	5.2
400	0.98	5.4
600	1.49	5.4
800	2.15	5.8

(e) Results

Data from the experiment are detailed in appendix 10.

(i) Environmental measurements. The correlation between the hours of sunshine at the nearby Bush House meteorological station and the mg. oxalic acid destroyed, during the same period, in the "light" treatment integrator, was highly significant (r = + 0.90). After 1st June, conditions of illumination were kept as stable as possible, and the illumination in the "medium" block was within a range of

41-49%, averaging 44%, of that in the "light" treatment, while the "dark" block received only 12-21%, averaging 17%, of the illumination in the "light" treatment.

The temperatures in the "medium" and "light" blocks were similar, and about 2 or 3 degrees C higher than that in the "dark" treatment.

(ii) Observations and comments. The oats were sown on May 8th and emerged on May 14th. Four days later, when the plants were about 2" high, they were thinned to 30 per pot, and the illumination treatments were begun. The first harvest was made on 13th June, when the oats were in 6th leaf. The ears emerged between 24th June and 8th July, and the plants were flowering on 16th July, at the second harvest. The final harvest was made when the plants were ripe, on 19th August.

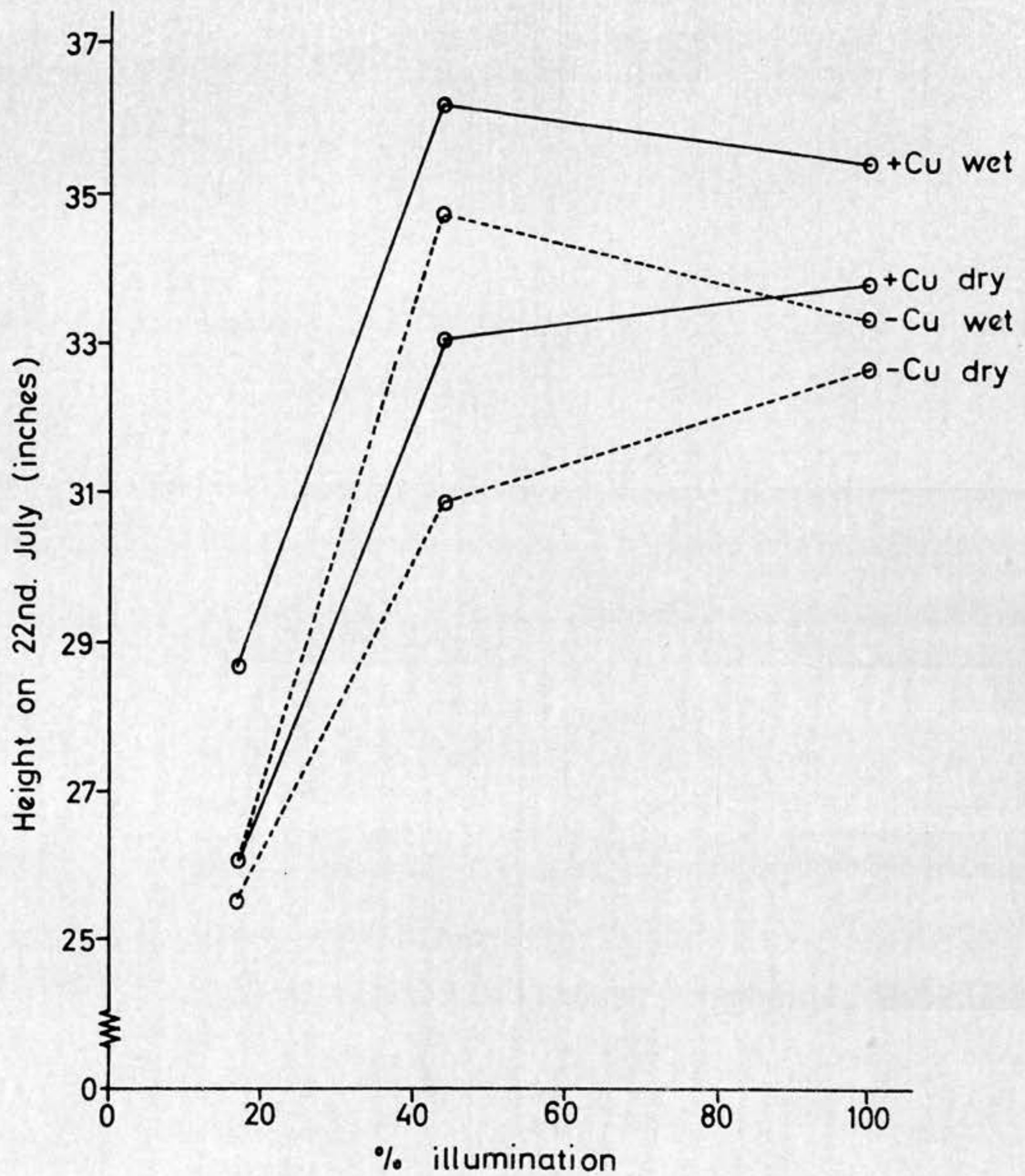
Much of the weather was cold and dull, and so transpiration was much less than in 1962, especially in the "dark" treatment. The soils were at water capacity on 14th May, and the weights of the pots did not fall to the 80% water capacity level until 27th, 29th and 31st May in the "light", "medium" and "dark" blocks respectively. The "dry" regime pots were not watered until 1st, 3rd or 8th June, when they reached 40% water capacity. From mid-June, the oats were subject to attack by aphids, and the greenhouse was fumigated thrice with nicotine. Because of lodging, canes, which had been thoroughly washed, were used to support all the plants between the second and third harvests.

(iii) Development. The "dark" treatment plants developed more slowly at every stage than the rest of the oats, and produced only six leaves, instead of seven like the other plants. Development was also retarded at the low copper level, but was accelerated in the "dry" regime. The plants in the "dark" block were weak, lodged very badly (see plate 12) and produced many adventitious roots. The latter development has been reported to be a sign of copper deficiency (Hagin 1960), but in the present work it was associated only with lack of illumination, and did not respond to the copper treatment. Other points in common, between the appearance of the "dark" block plants and the description (Hagin 1960) of withertip oats, were: stunted growth and little internode elongation, many tillers, little grain and many white "blind" ears, slow development and particularly slow maturation. The distinctive chlorosis and withering of the leaf tips caused by lack of copper did not, however, occur in any plants in the greenhouse.

After June 3rd, the plants at the high copper level were always taller than the equivalent oats at the low copper level. There was thus no difference in the response to copper between the "wet" and "dry" treatments, as was obtained in 1962. In the "medium" block, the plants receiving the "wet" treatment were taller at maturity than those in the "dry" regime, but the difference between the water regimes was not significant in the "light" block, (see diagram 27). The plants in the "dark" block grew in height at the same rate

Diagram 27. Effect of treatments on maximum height of oat plants.

Greenhouse 1963



Significant effects:

Copper response,** interaction of water x light: L.S.D.: 1·3in.*, 1·8in.**

as those in the "light" treatment until about 1st July, after which the stems of the former did not elongate normally.

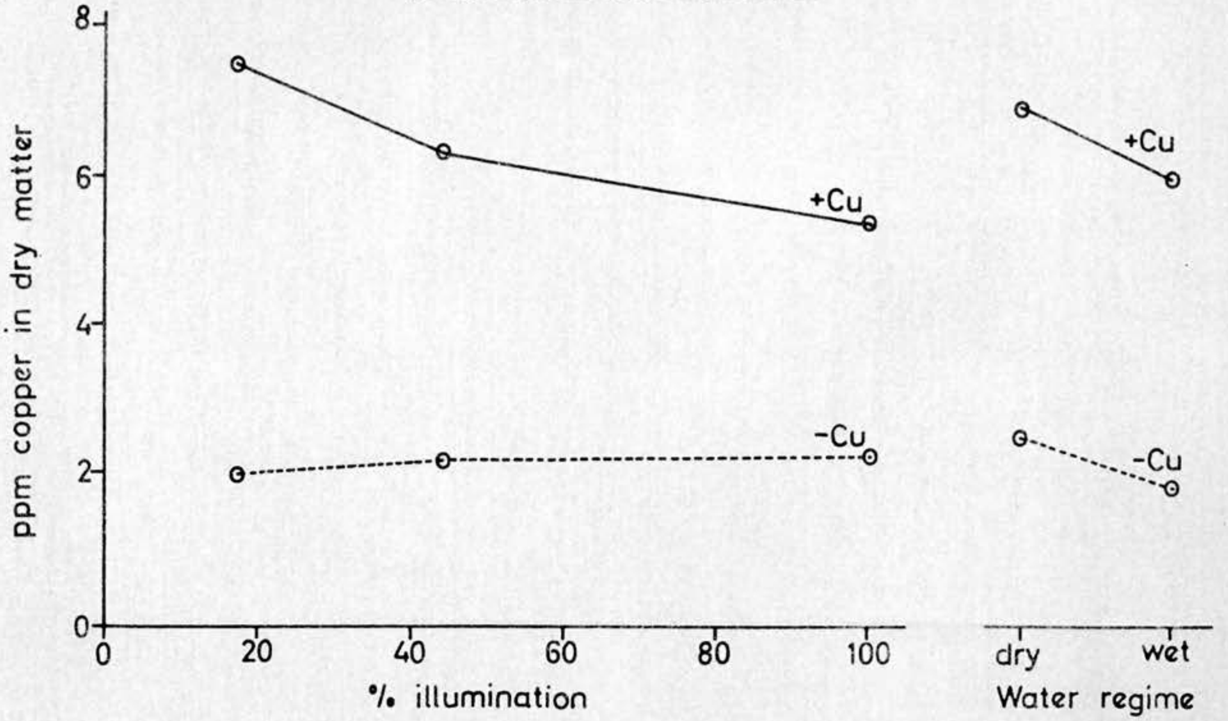
As illustrated in plate 12, tillering was stimulated by lack of both copper and illumination. There was an interaction between the effects of copper and light, in that the copper treatment lowered the rate of tillering very significantly in the "dark" block only. In the "light" and "medium" treatments very few tillers developed (see appendix 10). Lack of copper lowered the count of ears per plant but caused a drop in the number of grains which was not quite significant at P = 0.05 (see Table 37). There was a second order interaction between the copper, light and water factors on the weight of 1,000 grains, the only significant effect of copper being in the plants given the "dark" "wet" treatment, where the control grain was lighter than that at the high copper level.

(iv) Yield. The copper treatment caused a significant rise in yield of dry matter at each harvest, as shown in Table 37. Thus the plants on the control soil were copper deficient, and enough of the metal was still available from the 1962 dressing to rectify this lack. The yield of grain was larger from the "wet" than the "dry" regime, and it is highly probable that the reduction to 17% illumination diminished the yield at each stage. The only significant interaction from the yield data was between the effects of water and light on the straw. The illumination treatments caused different yields, the plants in the "light" block producing most straw and those in the "dark" block least. Only in the plants in the "medium" block, however, did the "wet" treatment cause more straw than the "dry". In contrast to the 1962 results, neither the copper nor the water treatments led to any significant differences in the percentage grain in the final harvest yield.

(v) Copper concentration in plant material. There was an accident during the drying of the second harvest samples, causing gross contamination. No analytical results were therefore obtained from the flowering stage of development in 1963.

As shown in Table 37, the rise in copper concentration as a result of the copper treatment was similar to that in 1962 in the young oats, but very much less in the grain. There was no response to copper in the straw. The "dry" treatment caused higher copper concentrations in the young plants, the grain and the straw than the "wet" regime, while the plants in the "dark" block contained a higher level of copper than those in the "light" treatment at all stages. First order interactions between the effects of the three factors on the concentration at first harvest show that adverse conditions involving low light or water levels influenced the copper concentration in the young oats more when there was an ample supply of copper than when the metal was deficient (see diagram 28). There were no significant interactions on the grain or straw concentrations.

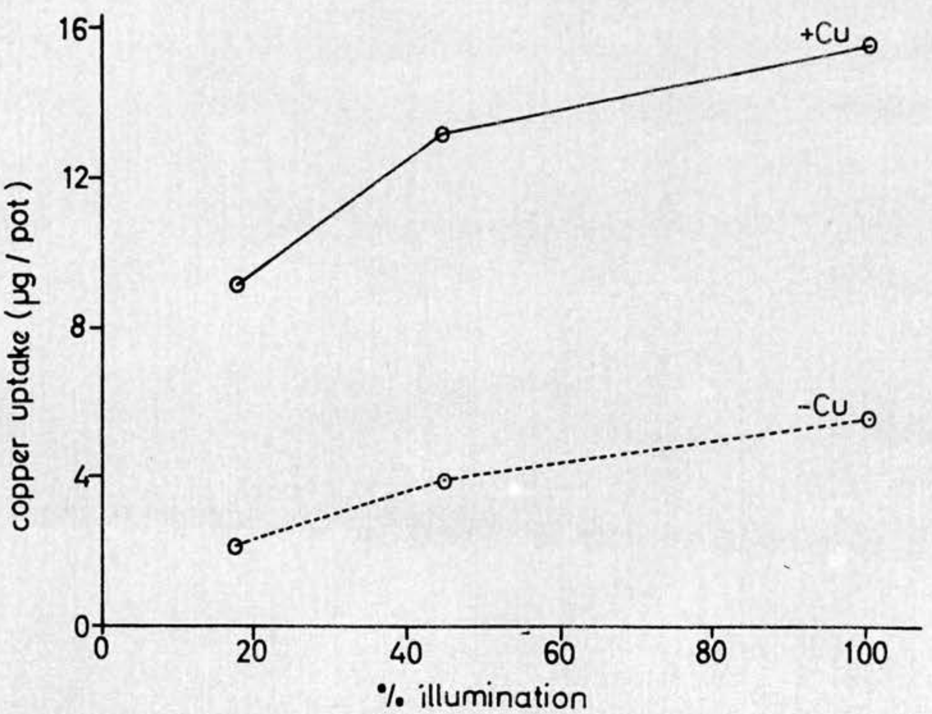
Diagram 28. Effect of treatments on copper concentration in oats at first harvest, greenhouse 1963



Significant interactions:

copper x light: L.S.D. 0·20ppm,* 0·27ppm;** copper x water: L.S.D. 0·17ppm,* 0·22ppm.**

Diagram 29. Effect of treatments on copper absorption by oats. First harvest, greenhouse 1963



Significant interaction of copper x light: L.S.D. 1·6 μg.*

Table 37

Significance of the effects of the main factors in the greenhouse experiment 1963

Data	Copper			Water			Light †	
	Treatment	Mean	Significance of difference between means	Treatment	Mean	Significance of difference between means	Treatment	Mean
Dry yield at first harvest (g/pot)	+ -	2.06 1.77	* *			none	dark medium light	1.15 1.94 2.66
Dry yield at second harvest (g/pot)	+ -	7.33 6.63	* *			none	dark medium light	4.49 7.06 9.39
Yield of dry grain (g/pot)	+ -	4.01 3.67	*	wet dry	4.03 3.65	* *	dark medium light	2.61 3.96 4.95
Yield of dry straw (g/pot)	+ -	5.02 4.66	*	wet dry	5.16 4.52	* *	dark medium light	3.92 4.83 5.77
Number of ears per plant at final harvest	+ -	6.92 6.27	* *	wet dry	6.82 6.37	*	dark medium light	4.63 7.09 8.06
Number of grains per plant at final harvest	+ -	12.37 11.53	none	wet dry	12.41 11.48	*	dark medium light	8.8 12.4 14.7
p.p.m. copper in dry plants from first harvest	+ -	6.42 2.18	* *	wet dry	3.90 4.70	* *	dark medium light	4.79 4.28 3.83

Table 37 (contd.)

Data	Copper			Water			Light †	
	Treatment	Mean	Significance of difference between means	Treatment	Mean	Significance of difference between means	Treatment	Mean
p.p.m. copper in dry grain	+ -	5.90 4.76	* *	wet dry	4.85 5.81	* *	dark medium light	6.26 5.22 4.51
p.p.m. copper in dry straw			none	wet dry	3.00 3.95	* *	dark medium light	4.61 3.08 2.72
copper uptake at first harvest (μg/pot)	+ -	12.6 3.90	* *	wet dry	7.68 8.85	*	dark medium light	5.68 8.59 10.5
copper uptake in grain (μg/pot)	+ -	22.7 16.8	* *			none	dark medium light	16.3 20.5 22.4
copper uptake in straw (μg/pot)	+ -	17.3 14.9	*	wet dry	15.0 17.2	*	dark medium light	18.0 14.7 15.6
copper uptake at final harvest (μg/pot)	+ -	40.0 31.7	* *	wet dry	34.1 37.6	* *		

† No rigorous statistical comparison of the effects of the light treatments is possible.

Note: The following interactions were significant: Water x light on the yield of straw, water x light on the count of ears, copper x light, copper x water and water x light on the concentration of copper in the plants at first harvest, and copper x light on the copper uptake at first harvest.

(vi) Copper uptake. As shown in Table 37, the copper treatment raised the copper uptake of the whole plants very much at the first harvest, and still very significantly at the final harvest. More of the extra copper available through the fertilization was assimilated by the grain than the straw, but this difference was less marked than in 1962. The "dry" regime led to a higher copper uptake in the whole plants than the "wet" treatment, both at the first and final harvests. The total copper content of the plants at maturity was unaffected by the light treatments, but the "dark" block plants took up less copper into their grain, but more into their straw, than those in the "light" block. The only significant interaction between any of the factors occurred at the first harvest, and is illustrated in diagram 29. Reduction in illumination lowered copper uptake, and this effect was larger at the high than the low copper level. Thus the response to copper was least in the "dark" block plants.

(vii) Potassium concentration in grain. The grain from the "light""wet" regime contained about 0.57% K. As the concentration in the dry matter of healthy oat grain has been reported to be 0.59 to 0.66% K (Welte 1956), the greenhouse plants were not deficient in potassium, despite the low "availability" in the soil. The copper treatment had no effect on the potassium concentration in the grain (see appendix 10).

(viii) Soil copper. The E.D.T.A. extractable copper level in the copper treated soil at harvest was about 7 p.p.m.

above that in the untreated soil. There was a significant interaction between the water and copper effects. At the high copper level, the drier soil contained more extractable copper than the wet soil, while there was no difference between the results from the water treatments at the low copper level. The recovery of the added copper, after allowance had been made for dilution with the fresh surface sand, was about 82%.

(3) 1964

(a) Aims

Two experiments were carried out in the greenhouse in 1964. The aim of experiment (1) was to study oat plants growing on soils containing four levels of copper: the control and copper treated soils used in 1962 and 1963 and two intermediate levels formed by mixing these in varying proportions. The aim of experiment (2) was to study oats growing at the residual high and low copper levels in the soil from the 1963 experiment, and under varying water regimes from germination.

(b) Statistical Design

In experiment (1), a randomised block design was used, with four replicates of the four copper treatments. As the levels of copper in the soil were not spaced in any regular way, it was not possible to calculate the shape of the response curve of any of the data. The simple analysis of variance is shown in Table 38. Experiment (2) was factorial, with two levels of copper and five water regimes. There were four replicates, and the arrangement was in randomised blocks. The analysis of variance is shown in Table 39. In experiment (1), all the pots were kept at 40% water capacity, which was one of the water regimes of experiment (2). Thus two treatments were common to both experiments. The detailed layout in the greenhouse is given in appendix 4, where it is seen that the treatments of both experiments were randomised together in replicate blocks.

Table 38

Analysis of variance on data from greenhouse experiment (1) 1964

Factor	Degrees of freedom
Total	15
Replicates	3
Copper treatments	3
Error	9

Table 39

Analysis of variance on data from greenhouse experiment (2) 1964

Factor	Degrees of freedom
Total	39
Replicates	3
Treatments	9
Error	27
Splitting of treatment sum of squares:	
Copper	1
Water	4
Copper x water	4

(c) Experimental Materials and Methods

All fresh material came from the same sources as in

1963, and was found to be as pure as that analysed in 1962. The soil and sand mixture which had been used in 1962 and 1963 was left unwatered in the pots after the 1963 harvest. In March 1964, the roots and glass wool were removed. About 55 lb. of the soil from the four pots in the 1963 "dry" "light" low copper treatment were mixed thoroughly with about 7 lb. of the soil from the "dry" "light" high copper treatment to give batch 9. Batch 10 was a mixture of the remaining "dry" "light" regime soils. All other low copper level soil from the 1963 replicate 1 was mixed and filled into the pots of the 1964 replicate 1. The other replicates were similarly filled from a mixture of the contents of the remaining pots of the corresponding 1963 replicate. The methods of fertilization, filling of pots and bringing to field capacity were similar in 1963 and 1964, but nutrients were applied at rates equivalent to 60 lb. N, 10 lb. P, 60 lb. K and 45 lb. Mn per acre. Analyses of samples taken at pot filling showed that the pH was 6.3 and the levels of "available" P and K were moderate and low respectively. The average concentrations of E.D.T.A. extractable copper in experiment (2) were 6.1 and 0.27 p.p.m. in the high and low treatments respectively, while in experiment (1) the levels were:

Treatment W: 6.1 p.p.m. (residual from 1963)
Treatment X: 4.6 p.p.m. (batch 10)
Treatment Y: 0.93 p.p.m. (batch 9)
Treatment Z: 0.27 p.p.m. (control from 1963)

The water regimes, which were established before the grain was sown, were maintained by watering the pots to calculated weights, as in previous years, and were:

Treatment P: 80% water capacity
Treatment Q: 40% water capacity
Treatment R: 20% water capacity
Treatment S: 40% water capacity until 9th June, and then 80% water capacity
Treatment T: 20% water capacity until 9th June, and then 40% water capacity

While these water levels were being reached, by drainage and evaporation from the bare soil, the pots were transferred to a new greenhouse, which had a concrete, instead of an earth floor, and far less ventilation than the former house. Thus air conditions were hotter and drier than in previous years.

On 5th May, 40 Forward oat seeds, from the control plots of the trial at Hexpath in 1962, were dressed with "Agrosan", pressed into the soil surface of each pot and covered with 1.5 lb. sand. Sampling was as in previous years, except that the fresh weight of the plants was measured in the greenhouse immediately after the oats were cut. The purity of the deionised water was checked before use by Hewitt's test.

(d) Results

Data from the experiments are detailed in appendix 11.

(i) Observations and comments. The oats in the soils at 80% and 40% water capacity emerged on 10th May and were thinned to 30 plants per pot on 19th May. There was, however, still no sign of germination in the "R" and "T" treatments at this time and so these pots were brought up to 30% water capacity. The oats in the "R" and "T" regimes emerged the next day, and the watering was gradually reduced in these two treatments until the pots were back at the 20% water capacity weight on 6th June. The first harvest was made on 8th June, when the "R" and "T" plants were in 4th leaf, and the rest were in 6th leaf. The ears began to emerge on June 17th and all plants were fully eared by June 27th. The second harvest, on 6th July, was made when the oats had just finished flowering. On 30th July the oats were ripe, and the final samples were taken. As the first harvest was cut before the change in the water supply of treatments "S" and "T", results from the "R" and "T" pots in each replicate and copper treatment were averaged for ease of statistical analysis. Similarly the means of the "Q" and "S" figures at first harvest were taken and used in the analysis of data from both experiments (1) and (2).

At the beginning of June, the leaves of the oats in the "P" treatment were pale green (see plate 13). This effect was observed first at the low copper level. On 17th June an additional 20 lb. per acre N, as a solution of 1.1% NH_4NO_3, was applied to all the soils, and the colour of all the plants darkened slightly. The temperature in the greenhouse was above 40°C on 24 days during the experiment, and, although the floor was kept damp as much as possible, many plants were attacked by mildew from about 20th June onwards. The third replicate was near the door, and, from 7th July, the plants in it were supported by strings running the length of the bench, and protected from wind by polythene sheeting on these strings.

(ii) Development. The lower the water supply, the

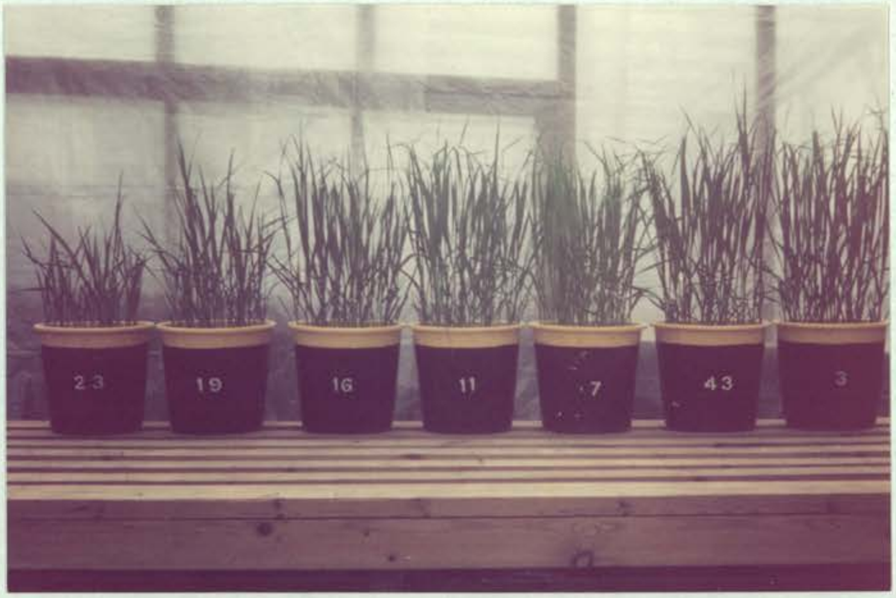


Plate 13. Effect of treatments. Greenhouse June 1964.

Pot 23: "R," -Cu.

Pot 19: "R," +Cu.

Pot 16: "Q," -Cu. ("Z")

Pot 11: "Q," +Cu. ("W")

Pot 7: "P," -Cu.

Pot 43: "Q," "X."

Pot 3: "P," +Cu.

faster the plants developed, so that stem elongation was completed first in the "Q" plants and next by both the "P" and "R" oats. The "R" plants, in spite of their later germination, were therefore at the same stage of development as the "P" oats at the time of the second harvest. The development of all the copper treated plants at all stages of growth was slightly faster than that of the corresponding oats at the lowest copper level. No withertip was, however, observed on any plant during the experiment. The plants in the "R" treatment produced small roots compared to the other oats, and in this regime, the low copper level plants had a particularly poor root system.

The copper treated oats grew about an inch taller than the untreated plants, but there was no real difference between the heights of the oats in the "W", "X" and "Y" treatments. As illustrated in plate 13, the oats grown at 80% water capacity were taller on 6th June than those at 40%, and very much taller than those at 20%. These differences continued throughout the experiment, until at maturity the average height of the "P" oats was 30", of the "Q" oats, 28", and of the "R" oats, 24". As in 1963, the response to copper was similar in all water treatments. There were no significant differences between the numbers of tillers produced at the four copper levels in experiment (1), but in experiment (2), on 20th July, there were more tillers on the control plants than on the copper treated oats. At harvest ten days later, however, the copper treatment had no significant effect on the number of the few remaining tillers.

There were no significant differences between the numbers of ears or grains, or the weight per grain from the treatments in experiment (1). Experiment (2) showed, however, (see Table 40) that the plants in the highest copper treatment, on an average over the five water regimes, produced significantly more ears and a greater number of lighter grains than the low copper level oats. There was no interaction between the copper and water effects, and so the benefit of the increased replication in the factorial design was demonstrated in revealing the significance of these small copper responses.

(iii) Yield.

Experiment (1). The yields from the first harvest are illustrated in diagram 30. All the copper treatments raised the yield above that of the control, but "X" caused a larger increase than the highest, "W", treatment. The difference between the "X" and "Y" yields was not significant. Thus it appears that, at 40% water capacity, the copper level in the copper treated soil used in experiment (2) was too high to give maximum yields, which would have been obtained at a level between "X" and "Y", or between 1 and 4 p.p.m. E.D.T.A. extractable copper. There were no significant differences caused by the copper treatments in the yields at either of the other harvests. Thus not only the slight toxic effect of the "W" treatment, but also the beneficial general copper effect was either masked by the differences between pots or was lost as the plants grew older. Piper (1942(1)) has reported that slight copper toxicity can cause a temporary depression of growth in young oats.

Table 40

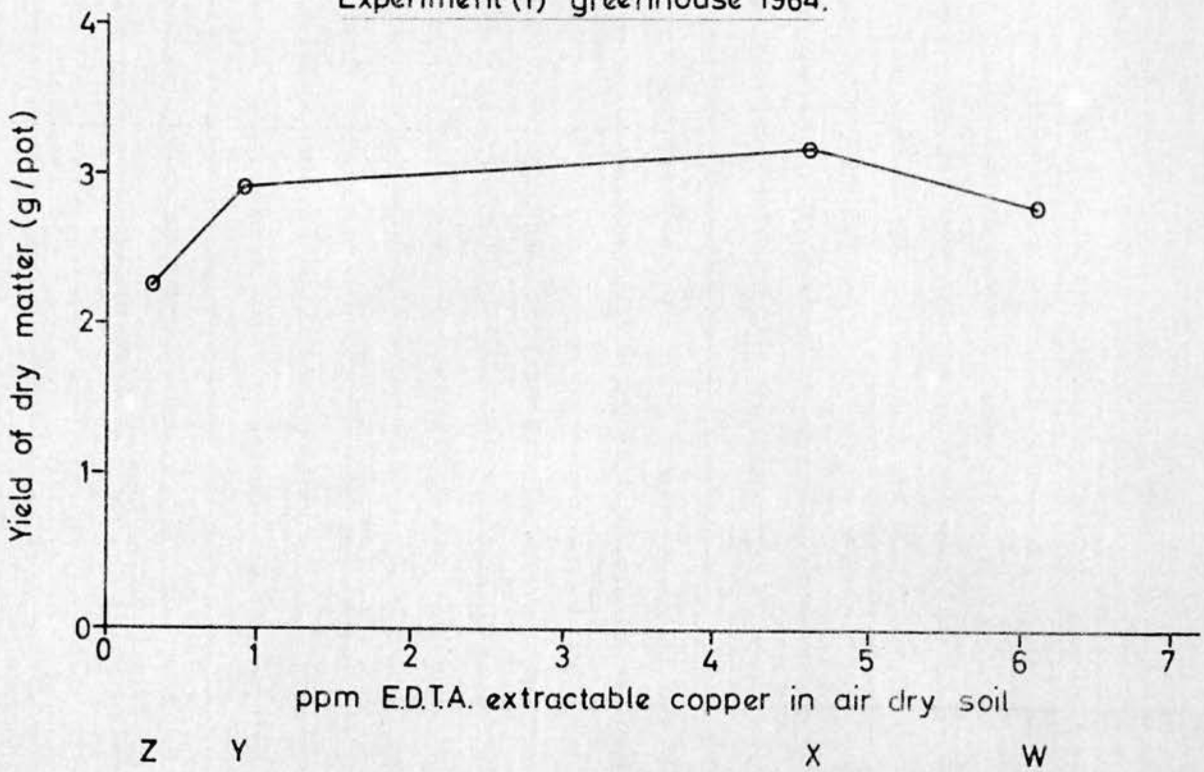
Significance of effects of copper and water supply on yield.
Greenhouse experiment (2) 1964.

Data	Copper			Water		
	Treat-ment	Mean	Significance of difference between means	Treat-ment	Mean	L.S.D.
Dry yield at first harvest (g/pot)	+	2.60	*	P	3.83	0.34 * *
	-	2.32		Q,S.	2.52	
				R,T.	1.03	
Dry yield at second harvest (g/pot)	+	11.4	*	P	13.2	1.3 * *
	-	10.7		Q	12.2	1.0 *
				R	7.3	
				S	13.2	
				T	9.4	
Yield of dry grain (g/pot)			none	P	7.4	2.3 * *
				Q	7.1	1.7 *
				R	5.2	
				S	7.5	
				T	7.1	
Yield of dry straw (g/pot)			none	P	8.09	0.75 * *
				Q	6.72	0.56 *
				R	4.80	
				S	7.82	
				T	6.74	
Number of ears per pot	+	106	* *			12 * *
	-	98				9 *
Number of grains per pot	+	202	*			23 * *
	-	190				17 *
Weight of 1,000 grains (g.)	+	34.4	* *			1.2 * *
	-	35.4				0.9 *

Note: There were no significant interactions between the effects of copper and water on any of the data in Table 40.

Diagram 30. Effect of treatments on yield of plants at first harvest.

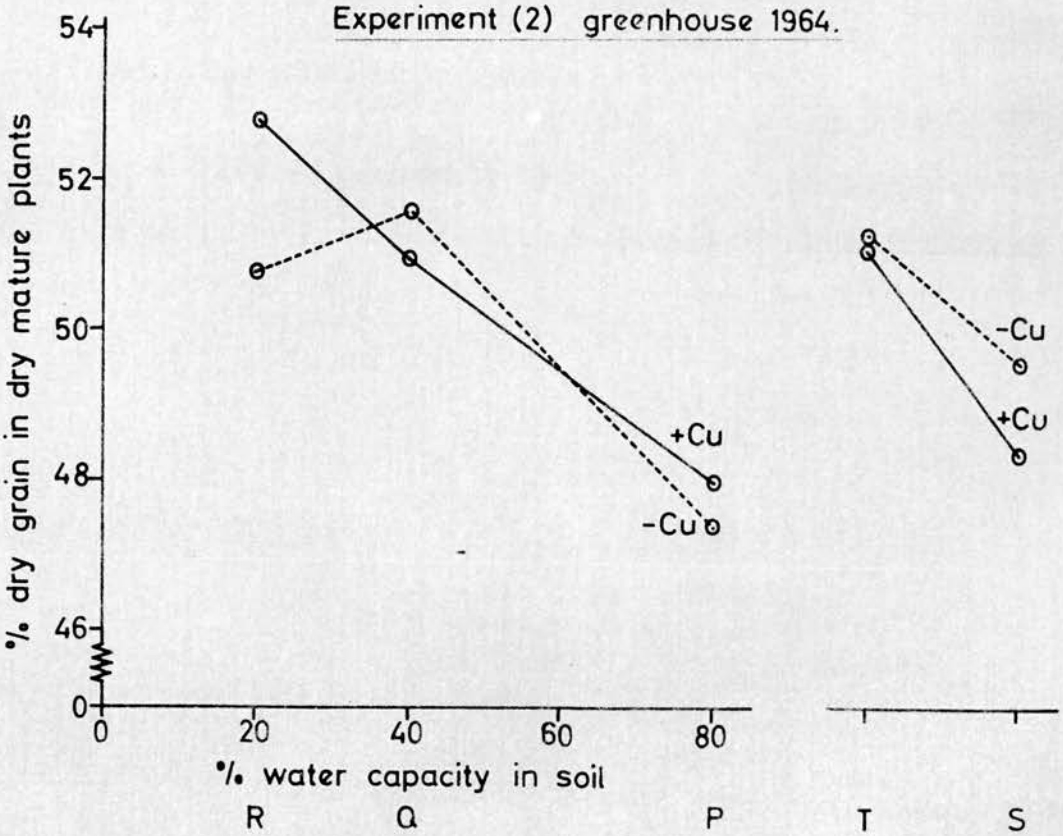
Experiment (1) greenhouse 1964.



Significant differences: Z / W, X and Y.** W / X.**

Diagram 31. Effect of copper and water supply on % grain in mature plants.

Experiment (2) greenhouse 1964.

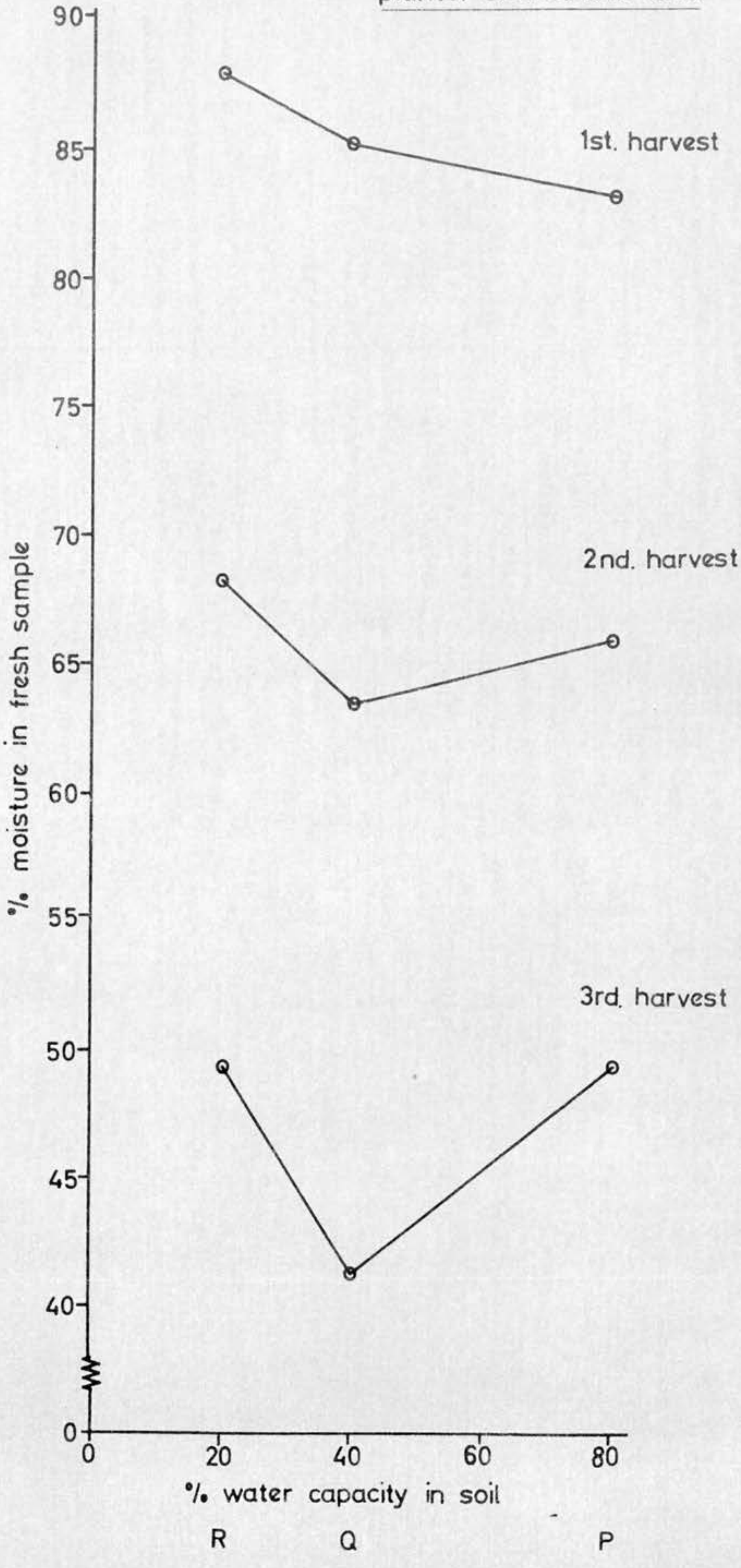


Significant interaction of copper x water: L.S.D. 1·5%,* 2·0%.**

Experiment (2). As shown in Table 40, a greater yield was obtained at the high than the low copper level in the first and second harvests but not in the grain or straw. A continuous decreased water supply reduced the yield, but the third harvest results show that an early period at a reduced water level had no lasting effect. The copper treatment caused no general significant difference in the percentage of grain in the final harvest. There was, however, a significant interaction, a larger percentage of grain at the high than the low copper level occurring only in the "R" regime (see diagram 31).

(iv) Moisture concentration in plant material. In experiment (1), the samples taken at the first and second harvests from the control, "Z", contained more moisture than those from any other treatment. At all three harvests in experiment (2), the plants at the high copper level also had a lower moisture concentration than the control. The plants in the "R" treatment always had a higher moisture concentration than those in the "Q" regime, but the results from the "P" treatment varied relative to those of the other regimes (see diagram 32). It was observed that the "R" regime plants never wilted on hot days, unlike the oats in the "P" and "Q" treatments. Plants with a high level of moisture have been reported to be hardier in conditions of drought than those with less bound water. (Vaadia, Raney and Hagan 1961). The capacity to retain a high concentration of moisture remained with the "T" treatment oats after the raising of the level of watering, for

Diagram 32. Effect of water supply on concentration of moisture in oat plants. Greenhouse 1964.



Significant differences:

1st harvest: Q / P and R**, P / R**

2nd harvest: Q / P and R**, P / R**

3rd harvest: Q / P and R**

the "T" plants contained the highest level of moisture at both the second and final harvests.

(v) Copper concentration in plant material.

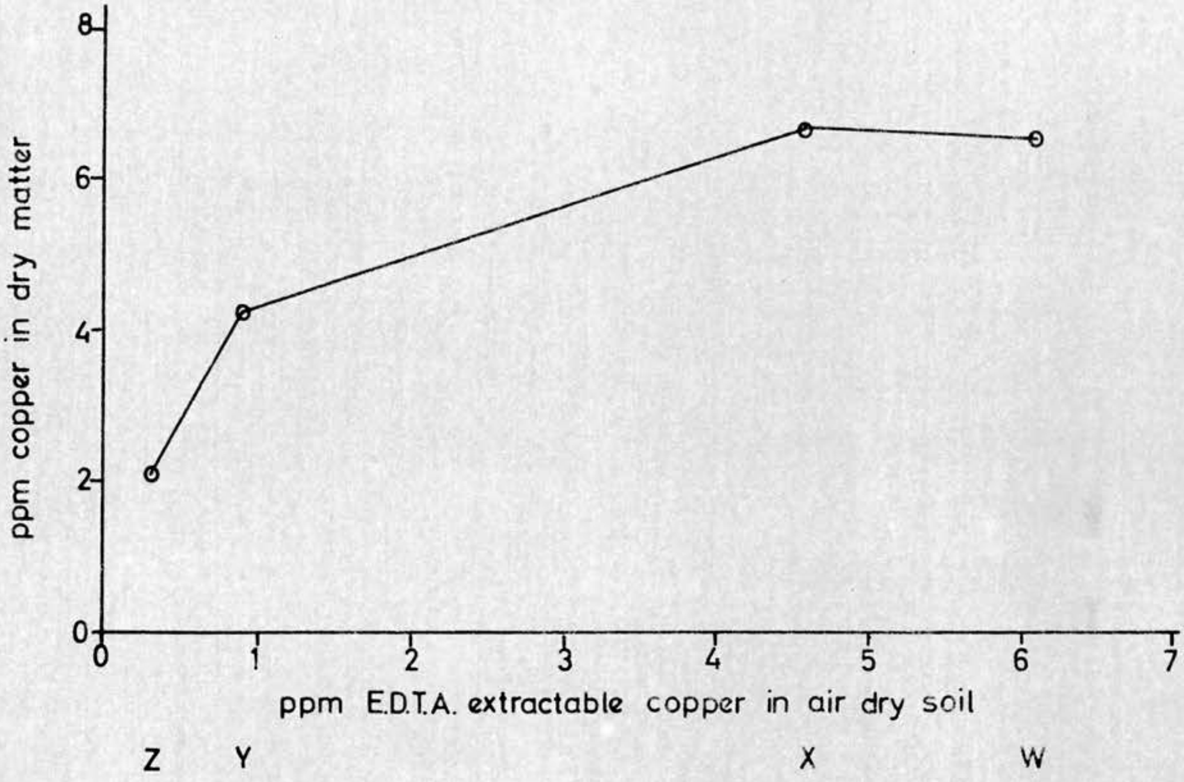
Experiment (1). The copper concentrations in the first harvest samples are shown in diagram 33. The comparatively large differences between the levels in the "X", "Y" and "Z" plants reflect the variation in the soil copper status in these treatments. The concentration in the "W" oats was, however, not higher than that in the "X" plants. The concentration in the second harvest samples and in the grain was increased to a similar extent by all the copper treatments, but there was no response in the straw.

Experiment (2). The copper treatment raised the concentration in the samples at each harvest, as shown in diagram 34. There were also always significant interactions between the effects of copper and water. The water treatments had a large effect at the high copper level, the "Q" regime results being intermediate between the high "R" concentrations and the lower "P" figures (see diagram 34). There was, however, a significant water effect at the low copper level only at the first harvest, when the "R" plants had a higher concentration than either the "P" or "Q" oats. At all harvests and at both copper levels, the concentrations in the "Q" and "T" oats and in the "P" and "S" plants, were similar.

(vi) Copper uptake.

Experiment (1). At the first harvest, the uptake in

Diagram 33. Effect of treatments on copper concentration in plants at first harvest. Experiment (1) greenhouse 1964.

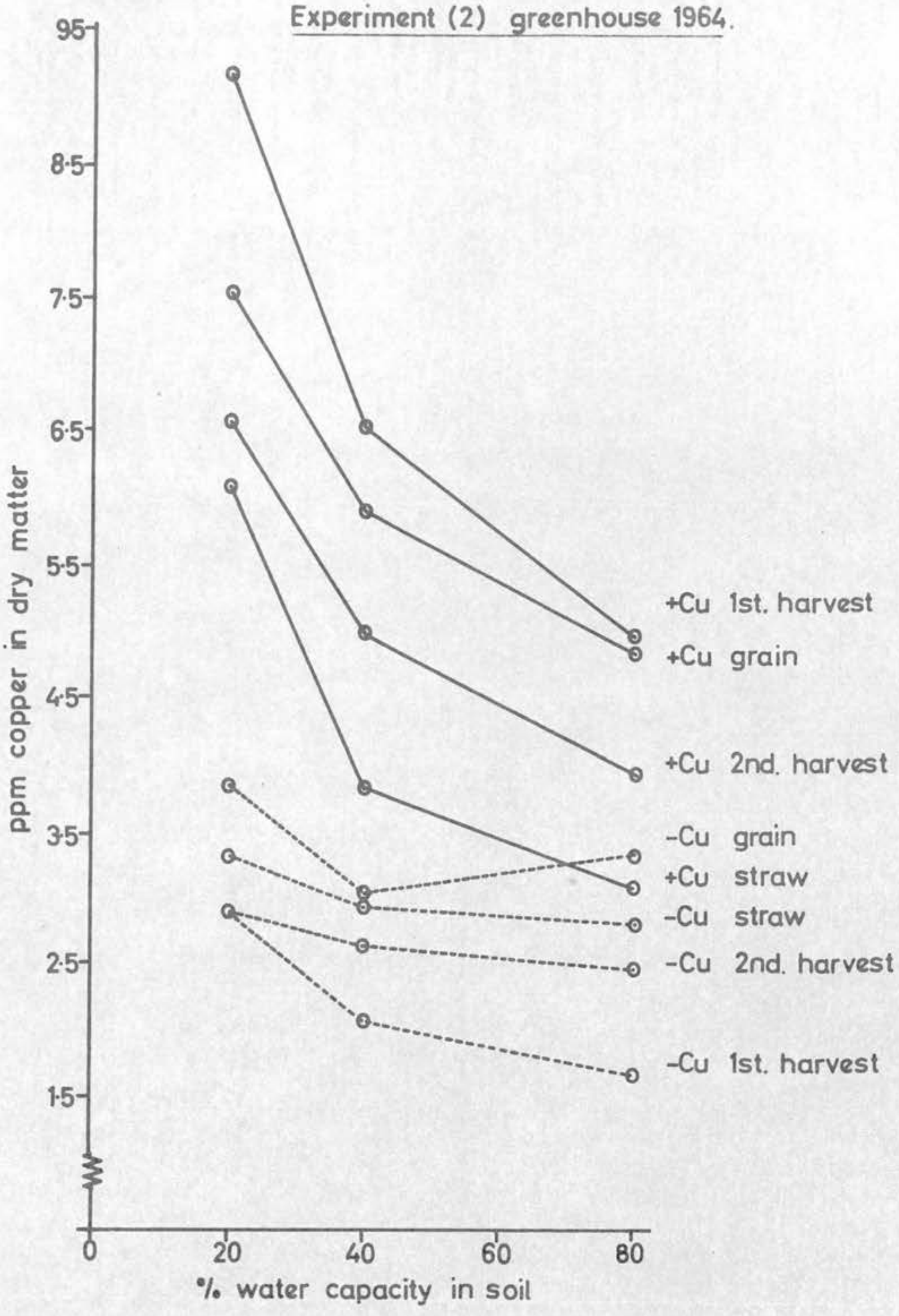


Significant differences:

Z / W, X and Y,** Y / W and X.**

Diagram 34. Effects of treatments on copper concentration in oat plants.

Experiment (2) greenhouse 1964.



Significant interactions of copper x water:

1st. harvest	L.S.D.	0·5 ppm*	0·8 ppm**
2nd. harvest	L.S.D.	0·8 ppm*	1·1 ppm**
grain	L.S.D.	0·8 ppm*	1·1 ppm**
straw	L.S.D.	0·8 ppm*	1·2 ppm**

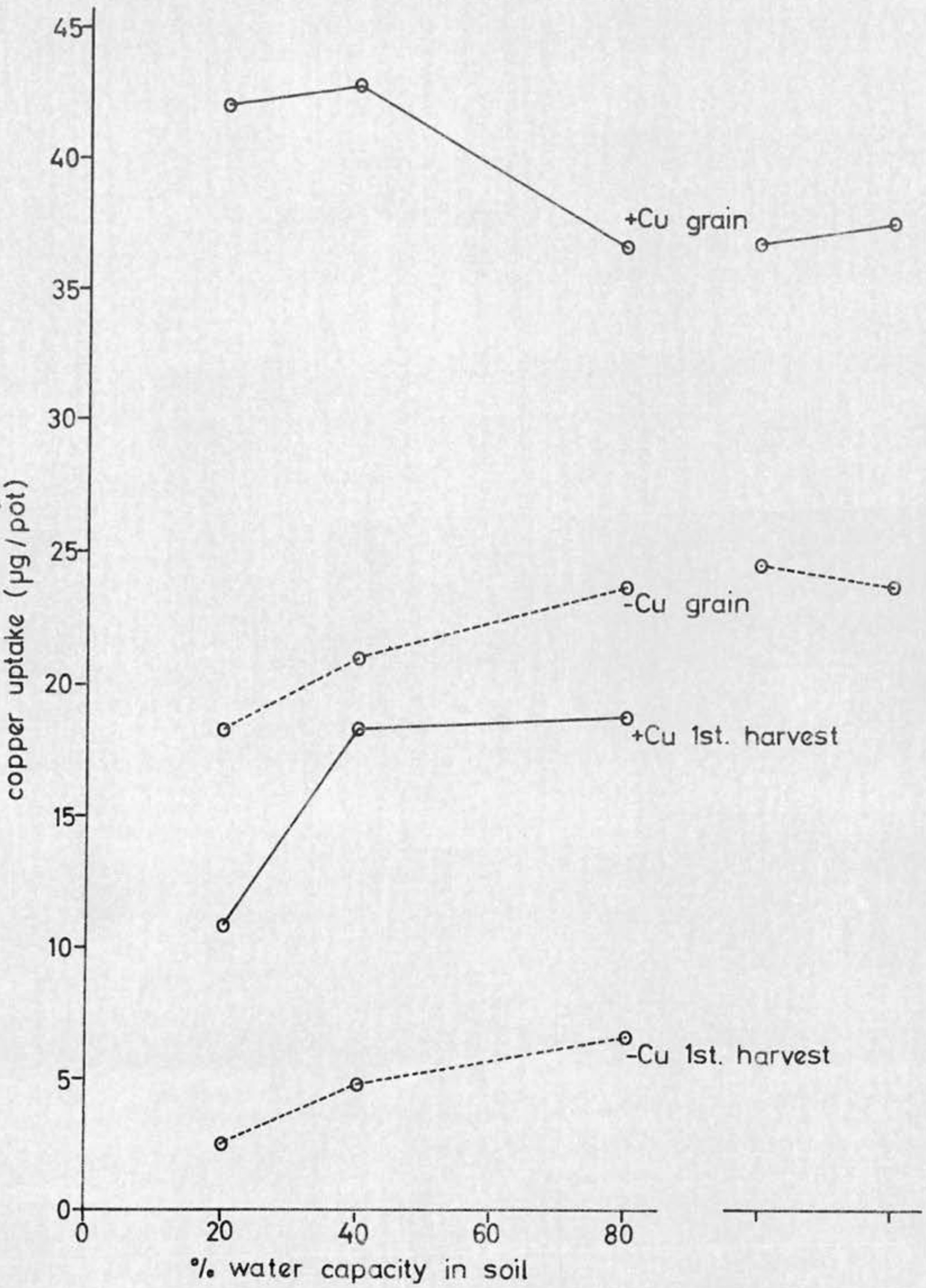
each treatment was very significantly different from that of every other treatment. The plants in the "W" treatment absorbed less than those in "X", but more than those in "Y", while the total content was least in the "Z" plants (see appendix 11). At the second and third harvests, the copper uptake of the control plants was very much lower than that of the "W", "X" or "Y" oats, but there were no significant differences between the means from these copper treatments.

Experiment (2). The plants at the high copper level contained much more copper than the controls at every harvest. The "R" regime led to a lower uptake than the "P" and "Q" treatments at the first and second harvests, but the water treatments had no significant effect on the uptake into the straw. At first harvest, the increase in uptake caused by the copper treatment was largest in the "Q" regime and smallest in the "R" treatment (see diagram 35). As in 1962, on the other hand, the response to increased availability of copper was generally greater on the uptake into the grain when the level of watering was low during ear emergence and flowering ("Q" and "R"). The total copper content of the grain from treatment "T" was, however, similar to that of "P" and "S". There were no interactions between the effects of copper and water on the uptake in either the straw or the second harvest samples.

(vii) Iron and manganese levels. The first harvest samples from experiment (1) were analysed for iron and manganese, and the concentration of iron in the control, "Z", plants was

Diagram 35. Effects of treatments on absorption of copper by oat plants.

Experiment (2) greenhouse 1964.

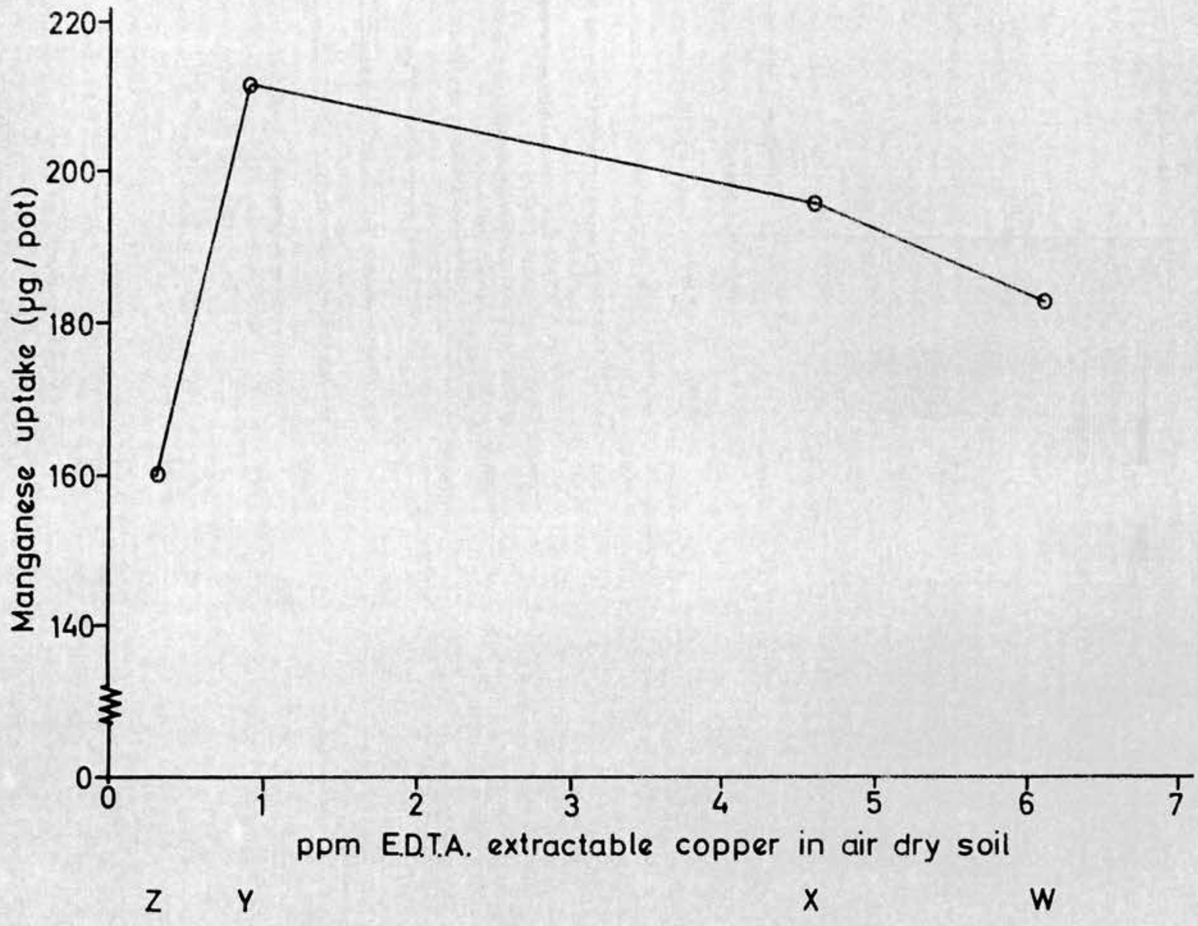


Significant interactions of copper x water:

1st. harvest: L.S.D. 1·9µg* 2·6µg** grain: L.S.D. 5·6µg* about 50% higher than that in the oats from any other treatment. There were no significant differences, however, between the results for the uptake of iron. The manganese concentration, in spite of the manganese dressings each year and the lowering of the soil pH, was very much smaller than that found in the greenhouse oats in 1962. As Williams and Moore (1952) have shown that the concentration of manganese rises as the oat plant develops, the levels in the young plants in 1964 were probably slightly higher than usual (Goodall and Gregory 1947, Mitchell 1954). The concentration of manganese was not affected by the copper treatments, but the uptake was higher in treatments "X" and "Y" than in "Z". From diagram 36 it is seen that a small rise in the soil copper content, which was just enough to cure copper deficiency in oats, caused a large increase in manganese uptake, whereas continued copper application above this critical level led to a reduction in uptake.

(viii) Soil copper. The water treatments did not have a significant effect on the E.D.T.A. extractable copper level of the soil at harvest. The concentrations of extractable copper fell very markedly in all the pots between the harvest in 1963 and the spring sampling in 1964, and the low values continued throughout the 1964 growing season. No similar drop occurred during the previous winter, when the pots were left to dry out in the same greenhouse. After allowance for the dilution of the soil with sand from the surface of the pots, the recovery of added copper by the E.D.T.A. extraction at harvest was about 74%.

Diagram 36. Effect of treatments on absorption of manganese by plants at first harvest. Experiment (1) greenhouse 1964.



Significant differences: Z / X and Y*

(4) Discussion of the Results

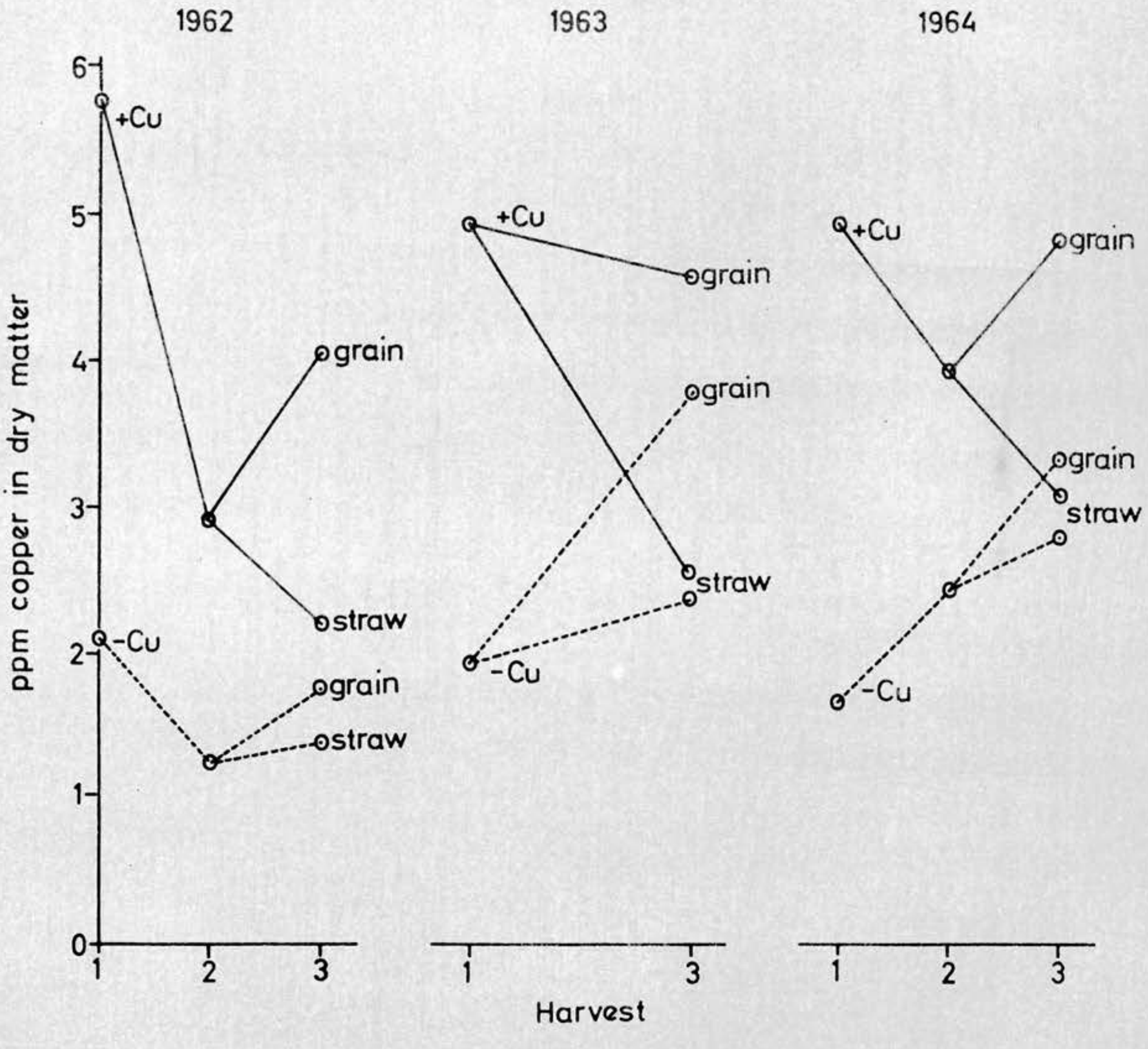
(a) Soil Copper and its Availability

The E.D.T.A. extractable copper concentration in the control soil was always very low, being less than the level in any of the field trials. The absence of withertip in the control oats in the greenhouse therefore appears to demonstrate that plants can extract copper more efficiently from soil in pots than from soil in the field. Alternative explanations are that oats require less copper for healthy growth under greenhouse conditions, or that contamination occurred, or that E.D.T.A. extraction results of field soils and dried soils in pots are not comparable. A combination of these possibilities may occur. Piper (1942(1)) has reported that the response in uptake of oats to soil copper treatment is greater in pots than in the field. Also, oats in a greenhouse in the summer mature more quickly and produce less dry matter than in the field, and so will require less copper. Contamination is always possible in work on micro nutrients. There is no reason to doubt the efficacy of E.D.T.A. as a copper extractant of the soils in 1962 and 1963, but the drop in the level of extractable copper during the winter of 1963 may invalidate exact comparison of field and pot values in 1964.

The rate of copper application was calculated so as to bring the total copper concentration in the treated soil to two thirds of the average found in local soils. There was little or no leaching, and, probably because of the very sandy texture of the soil in the pots (Scharrer and Schaumloffel 1960), little fixation occurred. The recovery of the applied copper by E.D.T.A. was, therefore, much higher than in the field experiments, averaging 77%. The extractable status of the treated soil fell from 11 to 6 p.p.m. during the three years, mainly because of the annual addition of pure sand to the surface of the pots. These levels were not extraordinarily high, for the concentration of extractable copper in arable soils in South East Scotland is normally in the range 1 - 10 p.p.m. (Purves 1964(1)) and soils on several farms in East Lothian contain between 10 and 24 p.p.m. In experiment (1) in 1964, nevertheless, there was a temporary reduction of yield in young plants at E.D.T.A. extractable concentrations of both 0.3 and 6 p.p.m. copper. These values may be slightly low because of the way in which the soil was stored, but the total copper content of the "W" soil cannot have been more than 8 p.p.m.

In 1962, the copper treatment increased the yield of grain by 18%, compared with only 8% in 1963, and no effect in 1964. This reduction in response was caused by a rise in the level of available copper in the control pots, for in 1964 the residual effect of the treatment was still strong enough to be slightly toxic, and the copper concentration in the grain in the control plants in comparable treatments in 1963 and 1964 was about double that in 1962 (see diagram 37). The change in concentration was not a varietal effect because the level in the treated grain rose only relatively little over the same period, and Forward oats in the Bog field

Diagram 37. Effects of copper and growth on copper concentration in oat plants in full light and on soil at 80% water capacity.



Hexpath contained less than 1.4 p.p.m. copper. The copper levels in the first harvest samples from the treatments at full light and 80% water capacity were similar in all three years (see diagram 37). There was the normal fall of the concentration from these levels in the copper treated plants during the growing season every year, but a rise in the concentration in the control oats occurred during 1963 and 1964. Thus it appears that there was an increase in the copper available to the control oats after the first harvest in these two years. The analyses of the control soils showed a very small rise in the E.D.T.A. extractable concentration during the 1963 growing season, but no change in 1964. The increase in copper availability cannot have been caused by gross contamination of the soil, because it was uniform throughout the control pots, and was evident well after the soils were mixed. The similarity of the results in 1963 and 1964, in all replicates in 1964, and in all water treatments, shows that contamination did not come from one of the greenhouses, nor from the canes and string used to support the older plants, nor from the water. If bringing the soil to field capacity after six months of dryness, and then keeping it moist, caused a gradual release of an increasing quantity of the native copper, the plants at first harvest in 1964 should have contained more copper than in 1963, for the soil was soaked about 70 days before the second harvest in 1963, and 70 days before the first harvest in 1964. It is of interest that Wood and Womersley (1946) have reported a marked rise in copper availability and uptake in oats in pots during ear emergence. They ascribed this increase to a rise in temperature which occurred at the time.

The concentration in the copper treated oats remained fairly constant over the three years (see diagram 37), while the level of total copper in the soil decreased because of the annual dilution with sand. These figures and the uptake results show that the availability of the applied copper to the plants did not decrease with time. Scharrer and Schaumloffel (1960) have reported that the residual and first year effects of a dressing of copper sulphate to a soil in pots were equal.

(b) Development

In the greenhouse experiments, no withertip was seen and the plants at the low copper level appeared to be healthy. Detailed observation revealed, however, that the development of the control plants was always retarded, and that they were generally stunted, with more tillers, fewer ears and a smaller number of heavier grains than the treated plants. All these symptoms have been associated with copper deficiency (Piper 1942(1), Hagin 1960, Kent 1963). The higher level of moisture in the control plants, which may have been caused by comparative immaturity, and by anatomical abnormalities similar to those described by Hagin (1960) and Ford (1940) in copper deficient oats, seems to be at variance with the reported copper deficiency symptoms of limpness and loss of turgor (Piper 1942(1)).

The height and the number of ears and grains were also reduced, and the weight per grain increased, by decreasing the water supply or illumination. Lack of water, however, accelerated development, as has been reported by Russell (1950). The appearance of plants under conditions of low illumination resembled that of copper deficient plants in some respects. The effect of the copper treatment on development was generally constant under very different conditions of watering and lighting. Many tillers were, however, produced only when low copper availability was combined with a high continuous water supply in 1962, and with low illumination in 1963. Also, in 1962, there was no response to copper in the height of the oats at the low water level.

(c) Yield

In all three years, the rise in the copper status of the soil as a result of the 1962 treatment caused an increase in yield of over 10% in the young plants and of nearly 10% in the oats at flowering. Thus, by definition, (Goodall and Gregory 1947 p.46) in spite of the absence of withertip symptoms, the young control plants were copper deficient. The copper treatment raised the grain yield by 18% in 1962. Piper (1942(1)) has reported that the first effect of slight copper deficiency is a decrease in grain production. Unlike in the field experiments, the copper treatment increased the yield of both straw and grain in 1962 and 1963. The lack of positive response to copper in the field straw yields was attributed to the immaturity of the control straw and the large growth of tillers. Under greenhouse conditions, however, comparatively few tillers were produced, and these did not generally grow very tall. The mild deficiency in the pot plants also delayed development only slightly, and so the straw of the control plants at the third harvest was apparently mature. All the effects of copper deficiency in increasing straw yield were therefore very small in the greenhouse, while the treatment still raised the height of the plants, and thus the straw yield. There are reports that oat straw yield is unaffected (Ennis and Brogan 1961, Wood and Womersley 1946) or reduced (Scharrer and Schaumloffel 1960), or sometimes increased and sometimes unaffected (Steenbjerg and Boken 1949) by copper treatment of deficient soil in pots.

In general, a moderately low water supply reduced the yield of young plants and straw more than the yield of grain. This effect, which has been reported by Russell (1950 p.362) and Van der Paauw (1949), occurred to a larger extent in the control plants than in the treated oats in 1962. Thus the longer the copper deficient oats in 1962 were maintained at a high water level after earing, the lower was the percentage grain in the total yield at maturity. There was, however, no significant interaction in any year between the effects of the copper treatment and the water or illumination regimes on the yield of oat plants. Thus, as found by Piper (1942(1)) but not by Steenbjerg and Boken (1949), the yield response to copper was unaffected by the rate of water supply.

(d) Concentration of Copper in Plant Material

The copper treatment raised the copper concentration very significantly in all samples except the straw in 1963 and the straw in experiment (1) in 1964. The response to copper was larger in the young plants than at maturity every year (see diagram 37). This effect has also been observed by Piper (1942(1)) and Rademacher (1940). The large effect on the copper concentration in the grain of a heavy dressing of copper to the soil was in marked contrast to the lack of response to smaller applications in the field. A bigger rise in the copper concentration in the grain than in the straw of oats in pots after soil copper treatment has also been reported by Rademacher (1940).

The levels of copper in all the plants at flowering were higher than 1 p.p.m, and thus were above the deficiency limit given by Piper and Walkley (1943). The average concentration in the grain from the treatments well supplied with copper, water and light in the three years was 4.5 p.p.m. Values of 5 p.p.m. copper occur in grain on soils in Aberdeenshire (Mitchell Reith and Johnston 1957(1)). At all stages of growth, the copper concentration in the plants on the copper treated soil increased with decreasing illumination and water supply. The environmental differences generally had little effect on the copper deficient plants except when they were young, but in extreme cases, as in the grain and straw in 1963, the combined effect of low water supply and low illumination raised the concentration more than the copper treatment. Thus, as shown in a review by Goodall and Gregory (1947 p.112), the conditions of water and light supply should be taken into account when the results of plant analyses are used diagnostically.

(e) Copper Uptake

The copper treatment raised the total copper content of the plants in every environmental treatment at every stage of growth in every year. The additional copper available after treatment was taken up preferentially into the grain rather than the straw. Thus, in the control plants, between 51 and 54% of the total uptake at maturity was in the grain, while in the treated oats the grain contained between 57 and 61% of the larger total uptake. Varietal differences are important, but Scharrer and Schaumloffel (1960) have stated that a mature oat plant is copper deficient if more than 50% of the absorbed copper is in the straw.

In general, there was a significantly higher uptake of copper from pots kept at 40% water capacity than from those at 80 or 20%. Thus, under conditions which limited growth through lack of water, increased uptake of copper occurred, but, when drought was severe, the absorption was no higher than that of plants given ample water. Piper (1942(1)) has found, on the other hand, that there was no difference between the total copper content in flowering oats over a range from 45 to 90% water capacity at low soil copper levels, but that after a heavy application of copper, the plants at the 45% water level took up slightly less copper than those at 60 or 75%. Results may vary for one element for, although there is agreement that absorption of potassium is usually less under dry than wet conditions, and uptake of nitrogen is generally unaffected, conflicting results have been reported for phosphate. (Russell 1950 p.364, Vaadia Raney and Hagan 1961, Wadleigh and

Richards 1951). As absorption of copper was highest at 40% water capacity in the present work, the toxic effect of the high soil copper level in 1964 may have been more marked than would have been the case in other water regimes. In the grain in 1962 and 1964, the differences caused by water supply occurred at the high copper level but not in the control treatment. From the 1962 results it was evident that the concentration in the grain was affected by changes in water supply only when these occurred during ear emergence and flowering. It has been reported that translocation of material to wheat grain is accelerated during a water deficit. (Zholkevich, Prusakova and Lizandr 1958).

In 1963, the young plants with least illumination absorbed less than those with full light. This occurred to a larger extent at the high than at the low copper level. Uptake of salts has been found to be affected by both illumination and concentration of salt in the nutrient medium. (Russell and Barber 1960). Although the total copper content per plant at maturity was unaffected by illumination, the distribution of this copper was probably altered by lack of light. The low yield of grain in the "dark" treatment contained only 45% of the total copper content of the control plants and 50% of the uptake in the oats at the high copper level.

Absorption of copper by oat plants is thus generally higher under comparatively bright dry conditions than dull wet ones. The uptake of a nutrient by a plant depends both on the availability of the nutrient in the soil and on the ability of the plant to take it up. As the level of E.D.T.A. extractable copper in this sandy soil was little affected by the water regimes, it was probably the capacity of the plants to absorb copper which was influenced by the environmental treatments.

(f) Manganese Levels in Plants

The copper treatment had no affect on the concentration of manganese in the young plants analysed, nor on the high absorption of manganese in 1962. In experiment (1) in 1964, however, the very much smaller uptake of manganese was increased about 30% by the lowest copper treatment and, to a smaller extent, by higher levels of copper in the soil. The rise in manganese absorption, but not concentration, after small soil copper dressings is similar to that obtained in the field experiment at Hexpath in 1962. If heavier copper treatments than those in the greenhouse experiment (1) in 1964 follow the trend (shown in diagram 36) of reducing the absorption of manganese from the level obtained at about 1 p.p.m. E.D.T.A. extractable copper, the reports (Steinberg 1950, Steenbjerg and Boken 1950) of "grey speck" induced by copper application would be explained. The yield results from experiment (1) in 1964 show, however, that copper toxicity would also result from heavier dressings of copper to a sandy soil.

(g) Iron Levels in Plants

At the 40% water capacity level, the iron concentration in the young control plants in 1962 and 1964 was about 50%

higher than that in the treated plants, while, when more water was supplied, the level in the control oats was only 20% higher. There were no significant differences between the uptake results from the treatments in 1964, but in 1962 the copper application reduced the iron absorption in the drier water regime. From the field experiment results, it was suggested that the concentration of copper within the plant, rather than in the soil, causes antagonism to iron. Thus it is interesting that, in the greenhouse, an application of copper to the soil caused a reduction in the absorption of iron at the drier water level, where the concentration of copper in the plant was higher.

(5) Conclusions

The conclusions refer to oat plants growing on sandy soil in pots in a greenhouse during the summer. The copper deficiency in the control soil was not severe enough to cause withertip symptoms.

(a) Mild copper deficiency generally reduces the height, number of ears and number of grains of oat plants, increases the weight per grain and, where conditions of light and moisture favour tiller formation, increases the number of tillers.

(b) Copper deficiency may reduce yield at all stages of growth, including straw, but copper toxicity affects the yield of young plants more than those at maturity.

(c) The yield response to copper treatment is not affected by various water regimes, nor by reductions in illumination. The proportion of grain in the mature plant is, however, decreased by ample water supply after ear emergence to a greater extent at low than at high soil copper levels.

(d) Copper treatment, reduced illumination, and reduced water supply all increase the concentration of copper in oat plants at all stages of growth.

(e) Absorption of copper by oat plants is generally higher from a soil at 40% water capacity than from one at 80%, but uptake into the grain is increased only at high levels of soil copper when the water supply is reduced during ear emergence and flowering. Absorption of copper by young oats may decrease as the intensity of the illumination of the plants falls.

(f) Copper deficient oat plants contain a higher concentration of moisture than normal plants.

(g) A small dressing of copper to a deficient soil may raise the manganese uptake of young oats.

(h) The iron concentration, and sometimes the iron uptake, of young copper deficient oats are reduced by copper treatment.

SUMMARY

In field trials on soils of known copper status, plots of oats and barley were given foliar and seedbed copper treatments, and in factorial pot experiments, oats under various conditions of water supply and illumination were also grown on deficient and treated soil. The following conclusions were made from the results of observations and experiments.

In fields on a few farms in South East Scotland, copper deficiency is lowering cereal yields and sometimes causing withertip. The soils in these fields are in the Eckford or Hobkirk Series, and have an E.D.T.A. extractable copper level of about 0.5 p.p.m. Withertip is seen most frequently when the preceding winter is dry, but there appears to be no correlation between its occurrence and weather conditions during the growing season. Oat plants, nevertheless, generally absorb less copper from sandy soil at about 80% water capacity than from soil at 40%, and the uptake in young plants may be decreased when illumination is reduced.

On deficient soils, similar increases in grain yield appear to be caused by foliar treatments of 5 and 10 lb. per acre copper oxychloride, although even 0.8 lb. is sufficient to cure withertip. Field seedbed copper dressings are less effective at raising grain production than foliar treatments, and cause no response in straw yield. Under greenhouse conditions, on the other hand, copper treatment of deficient

soil may increase both grain and straw yields. No evidence was found of copper toxicity in the field, but a temporary reduction in the yield of young plants in pots occurred when sandy soil contained about 8 p.p.m. applied copper.

The concentration of copper in barley plants is generally higher than that in oats, although there are varietal differences in oats, barley and wheat. The concentration is also higher in young plants than in those at maturity and is influenced by moisture and light conditions. In the field, foliar treatments and moderate applications of copper to the seedbed have little effect on the copper level in mature plants, but a heavy soil dressing in pots may raise the concentration in the grain.

Copper deficient oat plants contain a larger concentration of moisture than copper treated plants. Iron absorption may be reduced in young oat plants containing a high level of copper, and small copper dressings on a deficient soil may cause an increase in uptake of manganese.

E.D.T.A. extraction of field soils gives a guide to the availability of copper near deficiency levels, and generally recovers 20 to 30% of foliar applications and about 50% of soil dressings for up to two years. The applied copper remains readily available to plants. The sampling date, however, influences the result of an E.D.T.A. extraction of a soil, particularly after copper treatment.

The comparatively high concentration of copper in urban rain water makes copper deficiency unlikely in or near built up areas.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the advice and guidance given by Professor S.J. Watson, Dr. A.M. Smith and Dr. D. Purves. The author also thanks Dr. D.N. Lawley for advice on the statistical analysis of the results of two experiments, Mr. M. Ragg for identification of the soils at experimental sites, and members of the Soil Science, Spectrochemistry and County Advisory departments of the Edinburgh School of Agriculture, who helped select the field trial sites and assisted with field work. The co-operation and interest of the farmers on whose land experiments were carried out was much appreciated.

BIBLIOGRAPHY

ADAMS, A.F.R. and ELPHICK, B.L. (1956) Copper content of some soils and pasture species in Canterbury. N.Z.Jl Sci. Technol. 38A, 345-358.

ALSTON, A.M. (1964) A study of some factors affecting the magnesium nutrition of plants. Ph.D. thesis, Edinburgh University.

ANDREW, C.S. (1963) Copper deficiency symptoms of some tropical and temperate pasture legumes. Aust. J. agric. Res. 14, 654-659.

ANDRUS, S. (1955) Determination of copper in plant materials by means of zinc dibenzyl dithiocarbamate. Analyst, Lond. 80, 514-516.

A.O.A.C. (1960) Methods of analysis. 9th ed. Washington: Association of official agricultural chemists.

ARNON, D.I. (1950) Functional aspects of copper in plants. Pages 89-114 in "Symposium on copper metabolism" ed. McElroy and Glass. Baltimore: John Hopkins.

BAIN, R. (1965) Report on cereal variety trials carried out at Craibstone during the period 1960-1964. North of Scotland College of Agriculture, Crop Husbandry Dept. New Series Report No.1.

BAROCCIO, A. and SAPONARO, A. (1960) (The state of copper in various soils and the effects on cultivated plants. I. Serial determination of active copper in soils.) (Italian) Annali Sper. agr. 14, 385-400.

BATEY, T. (1961) Copper deficiency in farm crops. Cambridge: N.A.A.S. (Eastern Region).

BEESON, K.C. and MATRONE, G. (1950) Nutrient element content of native forages in relation to land forms and soil types in the N. Carolina coastal plain. Pages 370-398 in "Symposium on copper metabolism" ed. McElroy and Glass. Baltimore: John Hopkins.

BEESON, K.C. (1953) Report on copper and cobalt in plants. J. Ass. off. agric. Chem. 36, 405-411.

BERINGER, H. (1963) (Uptake and effect on barley of the trace element copper in ionic and chelated form.) (German). Z. PflErnahr. Dung. Bodenk. 100, 22-34. Summary in Soils Fertil., Harpenden. (1963) 26, 125.

BLEVINS, R.L. and MASSEY, H.F. (1959) Evaluation of two methods of measuring available soil copper and the effects of soil pH and extractable aluminium on copper uptake by plants. Proc. Soil Sci. Soc. Am. 23, 296-298.

BORCHARDT, L.G. and BUTLER, J.P. (1957) Determination of trace amounts of copper. Analyt. Chem. 29, 414-419.

BROWN, J.C., HOLMES, R.S., SHAPIRO, R.E. and SPECHT, A.W. (1955) Effects of phosphorus and copper salts on iron chlorosis of rice in flooded and non-flooded soil and the associated enzymatic activity. Soil Sci. 79, 363-372.

BROWN, J.C., TIFFIN, L.O. and HOLMES, R.S. (1958) Carbohydrate and organic acid metabolism with C^{14} distribution affected by copper in Thatcher wheat. Pl. Physiol., Lancaster. 33, 38-42.

BUCKPITT, W.A. and PURVES, D. (1960) Cereal crop failure due to copper deficiency in S.E. Scotland. 5th int. Congr. Nutr. Washington. Abst. p.40.

BUTLER, E.J. and NEWMAN, G.E. (1956) Urinary excretion of copper and its concentration in the blood of normal human adults. J. clin. Path. 9, 157-161.

CHENG, K.L. and BRAY, R.H. (1953) Two specific methods of determining copper in soil and plänt material. Analyt. Chem. 25, 655-659.

COCHRAN, W.G. and COX, Gertrude M. (1960) Experimental designs. 2nd ed. New York: John Wiley and Sons Inc.

COPPENET, M. and CALVEZ, J. (1955) (The origin of copper deficiencies in soils of Brittany.) (French) C. r. hebd. Seanc. Acad. Sci., Paris 241, 1068-1070.

DAVIES, D.B. (1962) Copper deficiency in cereals. N.A.A.S. "open" summer conference of advisory plant pathologists, Bristol, 1962.

DAWSON, C.R. (1950) The copper protein ascorbic acid oxidase. Pages 18-47 in "Symposium on copper metabolism" ed. McElroy and Glass. Baltimore: John Hopkins.

DAWSON, J.E. and NAIR, C.K.N. (1950) Chemical nature of the copper complexes in peat, soils and plants. Pages 315-335 in "Symposium on copper metabolism". ed. McElroy and Glass. Baltimore: John Hopkins.

DEPARTMENT of Agriculture and Fisheries for Scotland.

(1957)	Agricultural	Statistics	1955	Scotland.	Edinburgh:	H.M.S.O.
(1959)	"	"	1957	"	"	"
(1960)	"	"	1958	"	"	"
(1962)	"	"	1959 & 1960	"	"	"
(1964)	"	"	1961 & 1962	"	"	"
(1965(1))	"	"	1963	"	"	"

DEPARTMENT of Agriculture and Fisheries for Scotland (1965(2)) Personal communication.

ENNIS, Marie T. and BROGAN, J.C. (1961) The availability of copper from copper-humic acid complexes. Ir. J. agric. Res. 1, 35-42.

FARMER, V.C. (1950) Spectrographic analysis of plant ash in the carbon arc. Spectrochim. Acta 4, 224-228.

FERRES, Helen M. (1951) Influence of light and temperature on nutrient uptake and use, with particular reference to zinc. Pages 240-243 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

FISHER, R.A. and YATES, F. (1957) Statistical tables. 5th ed. Edinburgh: Oliver and Boyd.

FLEMING, G.A. and DELANEY, J. (1961) Copper and nitrogen in the nutrition of wheat on cutaway peat. Ir. J. agric. Res. 1, 81-82.

FORD, J.M. (1940) Appendix to: Experiments on copper deficient land at Dandaragan, Western Australia. J. Dep. Agric. West. Aust. 17, 161-177.

FORSTER, W.A. (1952) Some effects of metals in excess on crop plants grown in soil culture. V and VI. Long Ashton Res. Stn. Rep. 1952, 204-218.

FORSTER, W.A. (1953) Determination of copper in plant material. Analyst Lond., 78, 614-616.

FORSTER, W.A. (1954) Toxic effects of heavy metals on crop plants grown in soil culture. Ann. appl. Biol. 41, 637-651.

FRITZSCH, K. (1958) (Occurrence and significance of latent copper deficiency) (German). Z. PflKrankh. 65, 257-267.

GILBERT, F.A. (1952) Copper in nutrition. Adv. Agron. 4, 147-177.

GILBERT, F.A. (1957) Contribution to the knowledge of copper as a plant nutrient. Agrochimica 1, 136-151.

GOLDSCHMIDT, V.M. (1954) Geochemistry. Oxford: Clarendon Press.

GOODALL, D.W. and GREGORY, F.G. (1947) Chemical composition of plants as an index of their nutritional status. Imp. Bur. Hort. Plantation Crops. Tech. Commun. No.17.

GREAVES, J.E. and ANDERSEN, Ariel. (1936) Influence of soil and variety on the copper content of grains. J. Nutr. 11, 11-18.

HAGIN, M. (1960) (Disturbances in growth and development caused by copper deficiency in oats) (German). Z. PflErnahr. Dung. Bodenk. 90, 37-50.

HALLSWORTH, E.G., GREENWOOD, E.A.N. and AUDEN, Jane (1957) Some nutrient interactions affecting the growth of pasture legumes in acid soils. J. Sci. Fd Agric. 8, supplement, 60-65.

HARMER, P.M. (1945) Studies on the effect of copper sulphate applied to organic soil on the yield and quality of several crops. Proc. Soil Sci. Soc. Am. 10, 284-294.

HARRIS, H.C. (1948) Copper deficiency in relation to the nutrition of oats. Proc. Soil Sci. Soc. Am. 12, 278-281.

HEINICKE, D.R. (1963) The micro climate of fruit trees. I. Light measurements with uranyl oxalate actinometers. Can. J. Pl. Sci. 43, 561-568.

HEMINGWAY, R.G. (1962) Copper, molybdenum, manganese and iron contents of herbage as influenced by fertilizer treatments over a three year period. J. Br. Grassld Soc. 17, 182-187.

HENKENS, C.H. (1957) (The copper status of agricultural land in Holland) (Dutch) Landbouwvoorl. 14, 629-633.

HENKENS, C.H. (1961) (The copper content of the soil determined with biological and chemical methods.) (Dutch). Versl. landbouwk. onderz. No.67.10., Wageningen.

HENRIKSEN, A. and JENSEN, H.L. (1958) Chemical and microbiological determinations of copper in soil. Acta Agric. scand. 8, 441-469.

HENRIKSEN, A. (1959) (Top dressing with copper sulphate.) (Danish) Tidsskr. PlAvl 62, 871-878.

HEWITT, E.J. (1952) Sand and water culture methods used in the study of plant nutrition. Commonw. Bur. Hort. Plantation Crops. Tech. Commun. No.22.

HEWITT, E.J. (1963) Essential nutrient elements: requirements and interactions in plants. Pages 137-360 in "Plant physiology." ed. Steward. New York: Academic Press.

HOFFMANN, W. (1952) (Reclamation disease and effects of the trace element copper.) (German) Landw. Forsch. 4, 39-45.

HUDSON, J.P. (1957) Study of plant responses to soil moisture. Pages 113-128 in "Control of the plant environment" ed. Hudson, London: Butterworth.

HUNTER, J.G. and VERGNANO, O. (1953) Trace element toxicities in oat plants. Ann. appl. Biol. 40, 761-777.

IVANOV, D.N. (1950) (The distribution of copper in soils and the role of copper fertilizers in increasing yields of agricultural crops.) (Russian) Trudy pochv. Inst. 34, 143-189. Summary: Soils Fertil., Harpenden (1952) 15, 383.

JOHNSON, W.C. (1955) (ed.) Organic reagents for metals. Vol. 1. 5th ed. Chadwell Heath: Hopkin and Williams Ltd.

KENT, N.L. (1963) Defective oats commonly known as double grains. J. natn. Inst. agric. Bot. 9, 306-318.

LAWTON, K. (1955) Chemical composition of soils. Pages 53-84 in "Chemistry of the soil". ed. Bear. New York: Reinhold publishing corporation.

LEEPER, G.W. (1951) Availability of copper, manganese and zinc in soils. Pages 80-84 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

LEEPER, G.W. (1952) Factors affecting availability of inorganic nutrients in soils with special reference to micro nutrient metals. A. Rev. Pl. Physiol. 3, 1-16.

LEES, H. (1950) A note on the copper retaining power of a humic acid from peat soil. Biochem. J. 46, 450-451.

LIPMAN, C.B. and MACKINNEY, G. (1931) Proof of the essential nature of copper for higher green plants. Pl. Physiol., Lancaster. 6, 593-599.

LONG, W.G., SWEET, D.V. and TUKEY, H.B. (1956) Loss of nutrients from plant foliage by leaching as indicated by radioisotopes. Science, N.Y., 123, 1039-1040.

LUCAS, R.E. (1945) Effect of the addition of sulphates of copper, zinc and manganese on the absorption of these elements by plants grown on organic soils. Proc. Soil Sci. Soc. Am. 10, 269-274.

LUCAS, R.E. (1948) Effect of copper fertilization on carotene, ascorbic acid, protein and copper contents of plants grown on organic soils. Soil Sci. 65, 461-469.

LUNDBLAD, K., SVANBERG, O. and EKMAN, P. (1949) The availability and fixation of copper in Swedish soils. Pl. Soil 1, 277-302.

MADAN, R.C. (1958) Absorptiometric determination of traces of copper in rubber chemicals and products. Rubber Age, 84, 451-454.

MARTENS, R.I. and GITHENS, R.E. (1952) Small amounts of copper in dyes and rubber chemicals. Analyt. Chem. 24, 991-993.

MARTIN, G. and LAVOLLAY, J. (1950) (Effect of copper on the permanganate titration of humic extracts.) (French) 4th Int. Congr. Soil Sci. Amsterdam, 3, 83-88.

MITCHELL, R.L. (1954) Spectrochemical analysis of plant material. Pages 48-67 in "Plant analysis and fertilizer problems." Colloquium, 8th Int. bot. Congr. Paris. Paris: I.R.H.O.

MITCHELL, R.L. (1955) Trace elements. Pages 253-285 in "Chemistry of the soil". ed. Bear. New York: Reinhold publishing corporation.

MITCHELL, R.L. (1957) Trace element content of plants. Research, Lond., 10, 357-362.

MITCHELL, R.L., REITH, J.W.S. and JOHNSTON, Isabel M. (1957(1)) Soil copper status and plant uptake. Pages 249-261 in "Plant analysis and fertilizer problems." 2nd colloquium, 6th Int. Congr. Soil Sci., Paris 1956. Paris: I.R.H.O.

MITCHELL, R.L., REITH, J.W.S. and JOHNSTON, Isabel M. (1957(2)) Trace element uptake in relation to soil content. J. Sci. Fd Agric. 8, supplement, 51-59.

MITCHELL, R.L. (1963) Soil aspects of trace element problems in plants and animals. Jl R. agric. Soc. 124, 75-86.

MITCHELL, R.L. (1964) Spectrochemical analysis of soils, plants and related materials. Commonw. Bur. Soils. Tech. Commun. No. 44A.

MULDER, E.G. (1950) Copper and molybdenum in the nutrition of higher plants and micro organisms. Pages 41-52 in "Trace elements in plant physiology." Lotsya - A biological miscellany, Vol.3. Waltham: Chronica Botanica Co.

NEELKANTAN, V. and MEHTA, B.V. (1961) Evaluation of methods of measuring available copper in soils of Kaira district. J. Indian Soc. Soil Sci. 9, 293-297.

NEILANDS, J.B. and STUMPF, P.K. (1958) Outlines of enzyme chemistry. 2nd ed. John Wiley.

PAAUW, F. Van der (1949) Water relations of oats with special attention to the influence of periods of drought. Pl. Soil 1, 303-341.

PACK, M.R., TOTH, S.J. and BEAR, F.E. (1953) Copper status of New Jersey soils. Soil Sci. 75, 433-441.

PATERSON, D.D. (1939) Statistical technique in agricultural research. New York: McGraw-Hill book company, Inc.

PEECH, M. (1941) Availability of ions in light sandy soils as affected by soil reaction. Soil Sci. 51, 473-486.

PEROLD, I.S. (1951) Manganese and copper deficiencies in the South coastal region of South Africa. Pages 136-144 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

PIPER, C.S. (1942(1)) Investigations on copper deficiency in plants. J. agric. Sci., Camb. 32, 143-178.

PIPER, C.S. (1942(2)) Soil and plant analysis. University of Adelaide.

PIPER, C.S. and WALKLEY, A. (1943) Copper, zinc and manganese in some plants of agricultural interest. J. Coun. scient. ind. Res. Aust. 16, 217-234.

PIPER, C.S. and BECKWITH, R.S. (1951) Uptake of copper and molybdenum by plants. Pages 144-155 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

PIPER, C.S. and PRESCOTT, J.A. (1951) Factors determining the amount and availability of plant foods in soils. Pages 72-79 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

PURVES, D. and RAGG, J.M. (1962) Copper deficient soils in South East Scotland. J. Soil Sci. 13, 241-246.

PURVES, D. (1964(1)) The effect of high levels of E.D.T.A. - extractable copper in soil on growth of clover. Page 3 in "Experimental work 1964" Edinburgh School of Agriculture.

PURVES, D. (1964(2)) Contamination of Edinburgh garden soils with copper and lead. Page 6 in "Experimental work 1964" Edinburgh School of Agriculture.

RADEMACHER, B. (1940) (Variations in content, course of assimilation and uptake of copper by oat plants.) (German) Bodenk. PflErnahr. 19, 80-108.

RAGG, J.M. (1960) Soils of the country round Kelso and Lauder. Memoirs of the soil survey of Great Britain. Edinburgh: H.M.S.O.

REITH, J.W.S. and MITCHELL, R.L. (1964) Effect of soil treatment on trace element uptake by plants. Pages 241-254 in "Plant analysis and fertilizer problems." 4th int. colloquium, 1962. Paris: I.R.H.O.

RICEMAN, D.S. (1951) Plant nutrition problems from an Australian viewpoint. Pages 163-169 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

RICEMAN, D.S. and JONES, G.B. (1958) Distribution of zinc and copper in subterranean clover grown in culture solutions supplied with graduated amounts of zinc. Aust. J. agric. Res. 9, 73-122.

ROBERTS, R.A. (1928) Correlation of yield in oats with meteorological observations, Bangor 1903-1926. J. agric. Sci., Camb. 18, 297-316.

ROBERTSON, G.M. and SIMPSON, K. (1954) Variations in the chemical composition of soils as found on repeated sampling and analysis. Edinburgh School of Agriculture, Tech. Bull. No.8.

ROLT, W.F. (1962) The copper nutrient status of some soils of the North Island, New Zealand. Int. Soil Conf. N.Z., 842-846.

RUSSELL, E.J. (1950) Soil conditions and plant growth. 8th ed. London: Longmans, Green and Co.

RUSSELL, G. and HART, P.J. (1958) Determination of copper in gelatin. Analyst Lond. 83, 202-207.

RUSSELL, R.S. and BARBER, D.A. (1960) Relationship between salt uptake and the absorption of water by intact plants. A. Rev. Pl. Physiol. 11, 127-140.

SANDELL, E.B. (1959) Colorimetric determination of traces of metals. 3rd ed. New York: Interscience publishers, Inc.

SCHARRER, K. and SCHAUMLOFFEL, E. (1960) (Uptake of copper by spring cereals on copper deficient soils.) (German) Z. PflErnahr. Dung. Bodenk. 89, 1-17.

SCHUURMANS, E. and STEINER, A.A. (1958) (Colorimetric determination of traces of copper in nutrient culture solutions, plant material and substrates with zinc dibenzyl dithiocarbamate.) (Dutch) Chem. Weekbl. 54, 198-202.

SMITH, P.F. (1962) Mineral analysis of plant tissues. A. Rev. Pl. Physiol. 13, 81-108.

SOMERS, E. and GARRAWAY, J.L. (1957) Modifications in the method of determining copper with biscyclohexanone oxalyldihydrazone. Chemy Ind., p.395.

SOMMER, Anna L. (1931) Copper as an essential for plant growth. Pl. Physiol. Lancaster 6, 339-345.

SOMMER, Anna L. (1945) Copper and plant growth. Soil Sci. 60, 71-79.

SORTEBERG, A. (1962) Copper relationships with oats on adding cultivated to uncultivated peat. Soil Sci. 94, 80-86.

STEEL, R.G.D. and TORRIE, J.H. (1960) Principles and procedures of statistics with special reference to the biological sciences. New York: McGraw-Hill book company Inc.

STEENBJERG, F. (1940) (Copper in the soil with special reference to the white tip disease.) (Danish) Tidsskr. PLAvl 45, 259-368.

STEENBJERG, F. and BOKEN, Else (1949) (Copper in soil and plants III) (Danish) Tidsskr. PLAvl 52, 375-459.

STEENBJERG, F. and BOKEN, Else (1950) Copper contents and copper deficiency in Danish soil types. Pl. Soil 2, 195-221.

STEINBERG, R.A. (1950) Copper nutrition of green plants and fungi. Pages 115-140 in "Symposium on copper metabolism" ed. McElroy and Glass. Baltimore: John Hopkins.

STILES, W. (1961) Trace elements in plants. 3rd ed. Cambridge University Press.

STOUT, P.R. and ARNON, D.I. (1939) Experimental methods for the study of the role of copper, manganese and zinc in the nutrition of higher plants. Am. J. Bot. 26, 144-149.

SWAINE, D.J. (1955) Trace element content of soils. Commonw. Bur. Soil Sci. Tech. Commun. No.48.

TAYLOR, S.M. and SMITH, L.P. (1961) Estimation of averages of radiation and illumination. Met. Mag., Lond. 90, 289-294.

TEAKLE, L.J.H., TURTON, A.G. and THROSSELL, G.L. (1940) Experiments on copper deficient land at Dandaragan, Western Australia. J. Dep. Agric. West. Aust. 17, 161-177.

THOMPSON, J.H. and RAVENSCROFT, M.J. (1960) Determination of copper in lead and lead cable sheathing alloys. Analyst Lond. 85, 735-738.

TRUMBLE, H.C. (1950) Trace elements and phosphate in herbage plant nutrition. Pages 336-369 in "Symposium on copper metabolism" ed. McElroy and Glass. Baltimore: John Hopkins.

TRUMBLE, H.C. (1951) Climatic factors and the nutrition of herbage plants. Pages 1-9 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

UNICAM (1961) Determination of copper in plant materials. Cambridge: Unicam Instruments Ltd.

VAADIA, Y., RANEY, F.C. and HAGAN, R.M. (1961) Plant water deficits and physiological processes. A. Rev. Pl. Physiol. 12, 265-292.

VERMAAT, J.G. and BIE, G.J. Van der (1950) On the occurrence of copper in tropical soils. Pl. Soil 2, 257-282.

VIRO, P.J. (1955) Use of ethylene diamine tetraacetic acid in soil analysis. Soil Sci. 79, 459-465.

WADLEIGH, C.H. and RICHARDS, L.A. (1951) Soil moisture and the mineral nutrition of plants. Pages 411-450 in "Mineral nutrition of plants." ed. Truog. University of Wisconsin.

WALLACE, T. (1951) Diagnosis of mineral status of plants with special reference to deficiencies, excesses and interactions of nutrient elements. Pages 100-113 in "Plant and animal nutrition in relation to soil and climatic factors." Br. Commonw. scient. official Conf. Australia 1949.

WALLACE, T. (1961) Diagnosis of mineral deficiencies in plants by visual symptoms. 3rd ed. London: H.M.S.O.

WAYGOOD, E.R. (1950) Physiological and biochemical studies in plant metabolism. II. Respiratory enzymes in wheat. Can. J. Res. C28, 7-62.

WELLS, N. (1957) Soil studies using sweet vernal to assess element availability. III. Copper in New Zealand soil sequences. N.Z. Jl Sci. Technol. B38, 884-902.

WELTE, E. (1956) (On the potassium content of plants) (German) Pages 75-107 in "Potassium symposium, London 1956" International Potash Institute.

WESTERHOFF, H. (1955) (Copper determination in soil) (German) Landw. Forsch. 7, 190-193.

WILLIAMS, C.H. and MOORE, C.W.E. (1952) Effect of stage of growth on copper, zinc, manganese and molybdenum contents of Algerian oats grown on 13 soils. Aust. J. agric. Res. 3, 343-361.

WILLIAMS, T.R. and MORGAN, R.R.T. (1954) A rapid method for the determination of copper in plant material. Chemy Ind., p.461.

WILLIS, L.G. and PILAND, J.R. (1934) The influence of copper sulphate on iron absorption by corn plants. Soil Sci. 37, 79-83.

WILLIS, L.G. and PILAND, J.R. (1936) The function of copper in soils and its relation to the availability of iron and manganese. J. agric. Res. 52, 467-476.

WOOD, J.G. and WOMERSLEY, H.B.S. (1946) Development and metabolism of copper deficient oat plants. Aust. J. exp. Biol. med. Sci. 24, 79-94.

YOUNTS, S.E. (1964) Response of wheat to rates, dates of application, and sources of copper and to other micro nutrients. Agron. J. 56, 266-269.

YOUNTS, S.E. and PATTERSON, R.P. (1964) Copper lime interactions in field experiments with wheat. Agron. J. 56, 229-232.

ZHOLKEVICH, V.N., PRUSAKOVA, L.D. and LIZANDR, A.A. (1958) Fiziol. Rastenii Akad. Nauk S.S.S.R., 5, 333-340. Quoted by Vaadia, Raney and Hagan (1961).

(1960) Fertilisers and feeding stuffs regulations 1960. Eighth schedule. (Methods of analyses of feeding stuffs). London: H.M.S.O.

APPENDIX 1

Analysis of copper in plant material using zinc dibenzyl dithiocarbamate

Reagents: Nitric acid (analar)

Hydrogen peroxide 100 volume (analar)

Carbon tetrachloride (analar)

Cotton wool (B.P.C.)

Mixed acid (nitric acid (analar), sulphuric acid (micro-analytical reagent) and 60% perchloric acid (analar) in the proportions 14:3:2 respectively)

Reagent solution (0.03% zinc dibenzyl dithiocarbamate in carbon tetrachloride)

Standard solution (0.000,393% cupric sulphate (analar) and 0.5% sulphuric acid (micro analytical reagent) in water)

Metal free water.

2 g. oven dry plant material are weighed into a 100 ml. Pyrex kjeldahl flask and washed down with 19 ml. mixed acid. The sample is heated very gently until the vigorous reaction subsides, and then more strongly until white sulphuric acid fumes are produced. If the solution chars, 1 ml. nitric acid is added and the mixture again heated to fumes. The resulting colourless, very pale yellow, or purple solution is cooled, mixed with 1 ml. hydrogen peroxide, and boiled to white fumes. 3 ml. water are added twice and the solution is boiled to white fumes after each addition. The solution is then brought to the boil with 20 ml. water, cooled, and mixed with 25 ml. water.

When the digest is at room temperature, 10.0 ml. reagent solution are added and the mixture shaken vigorously for 1½ minutes using a polythene stopper in the kjeldahl flask.

The mixture is poured immediately after shaking into a separating funnel. The outlet of the funnel is dried and a little clean cotton wool is inserted to remove droplets of water from the solvent solution. About one ml. of the carbon tetrachloride layer is allowed to run through and rinse the cotton wool and a clean dry 2 cm. colorimetric cell. The rest of the clear yellow carbon tetrachloride solution is run into the cell and the optical density is read at 438 $m\mu$ against carbon tetrachloride. Two or three blanks are taken through the whole method every time it is used and their average optical density is subtracted from the sample reading. The result is calculated from a graph of optical density at 438 $m\mu$ against μg. copper, obtained by using the above method on aliquots of the standard solution containing 0 to 20 μg. copper.

APPENDIX 2

Analysis of E.D.T.A. extractable soil copper

Reagents: Extracting solution (0.02 M (0.744%) ethylene diamine tetra acetic acid, disodium salt, in water)

Sulphuric acid (micro analytical reagent)

Hydrogen peroxide 100 volume (analar)

Carbon tetrachloride (analar)

Cotton wool (B.P.C.)

Filter papers (Whatman No.3)

Reagent solution (0.03% zinc dibenzyl dithiocarbamate in carbon tetrachloride)

Standard solution (0.000,393% cupric sulphate (analar) and 0.5% sulphuric acid (micro analytical reagent) in water)

Metal free water.

10 g. air dry soil are weighed into a clean dry polythene bottle and 100.0 ml. extracting solution are added. The mixture is shaken for one hour, allowed to stand for one hour, and then filtered into a pyrex flask through a filter paper which has previously been washed with extracting solution, rinsed with water, and allowed to drain for at least half an hour. 20.0 ml. of the filtrate, or a suitable aliquot, are transferred to a 100 ml. pyrex kjeldahl flask and mixed with 2 ml. sulphuric acid and 2 ml. hydrogen peroxide. The solution is made up to 44 ml. and brought to the boil. It is simmered very gently for 30 to 45 minutes until it is colourless or very pale yellow, brought to a rising boil, and then cooled. The volume is made up to 50 ml. and the colorimetric determination carried out as for the

herbage digests. Two or three blanks are taken through the whole method every time it is used, and their average optical density is subtracted from the sample reading. The result is calculated from a graph of optical density at 438 mμ against μg. copper, obtained by using the above method on aliquots of the standard solution containing 0 to 20 μg. copper.

APPENDIX 3

Method of light integration

Reagents: 0.0946% oxalic acid solution

0.08% potassium permanganate solution

0.177% uranyl acetate solution

30% sulphuric acid solution.

40 ml. oxalic acid solution and 1 ml. uranyl acetate solution are mixed in a clear glass, stoppered, numbered, boiling tube, which is then kept in the dark. When a reading is required, the tube is suspended at the desired place in a wire holder. After a suitable time, usually 48 hours, it is again placed in the dark. The solution is mixed with 10 ml. 30% sulphuric acid, heated to near boiling point and titrated with the standardised potassium permanganate solution. A tube containing identical contents is kept continually in the dark before titration. The equivalent, in mg. oxalic acid, of the difference between the two titrations is calculated, using the factor found during the standardisation of the permanganate solution. This is the weight of oxalic acid destroyed by the light falling on the tube.

APPENDIX 4

Statistical layouts of experiments

(1) Field experiments 1962

Treatments: A : control

B : 5 lb./acre copper oxychloride sprayed on crop

C : 10 lb./acre " " " " "

D : 10 lb./acre " " " " seedbed

E : 20 lb./acre " " " " "

(i) Hexpath

C	A	D	B	E	B	E	A	C	D
E	A	C	D	B	E	D	B	A	C

(ii) Billie Mains

D	E	A	C	B	D	B	E	A	C
A	B	D	C	E	E	D	C	A	B

(iii) Oldcastles

C	B	D	E	A	B	A	E	D	C	
D	E	A	B	C	D	D	E	A	B	C

(iv) Rosehill

C E D A B C E B A D C B E A D A B D C E

(2) Field experiments 1963.

Treatments: F : control

G : 0.8 lb./acre copper oxychloride

H : 2.3 lb./acre " "

J : 5.0 lb./acre " "

K : 10.0 lb./acre " "

(i) Pirnie

G	F	J	K	H	K	F	G	J	H
K	J	H	F	G	G	F	K	H	J

(ii) Upper Huntlywood

G	K	F	J	H	G	K	J	F	H
J	K	H	F	G	K	H	J	G	F

(iii) Choicelee

G	K	J	F	H	J	G	K	F	H
F	K	G	H	J	H	K	J	G	F

(3) Field experiments 1964

1. Treatments as for 1963.

(i) Hexpath

F	J	H	K	G	J	K	F	H	G
H	J	K	G	F	F	H	K	J	G

(ii) Langtonlees

G	J	H	K	F	K	F	H	J	G
J	F	K	H	G	G	H	J	F	K

2. Treatments: A : Astor oats

B : Blenda oats

C : Forward oats

D : Freja barley

M : Maythorpe barley

Y : Ymer barley.

The half of each variety whole plot which was sprayed with 10 lb./ acre copper oxychloride is indicated by a + sign.

Choicelee:

+	+		+				+		+	+					+	+	+	+		+		+	+
Y		B		M		C		B		D		D		C		A		C		A		B	
	C		A		D		Y		M		A		B		M		Y		Y		D		M
		+		+	+	+		+			+	+	+	+					+		+		

(4) Greenhouse experiment 1962

Treatments: Copper: − : control

+ : soil dressing equivalent to 40 lb. copper oxychloride per acre.

Water: period at 40% water capacity, instead of 80% water capacity.

a none

b 28th May until 13th August

c 28th May until 25th June

d 11th June until 9th July

e 25th June until 23rd July

f 9th July until 6th August

g 23rd July until 13th August

Window.

	− e		+ b	+ a	− c	+ c	+ d	− b	− f	− g	− d	− a	+ f	+ e	+ g	+ b	− c	− a		− e	+ f	− b	+ e	− g	+ c	+ a	− f	+ g	+ d		− d
− d	− a	− b	+ e	+ g	+ f	− f	− g	+ d	+ a	+ b	+ c			− c	− e	+ d	+ a	− g		+ g	− d	− f	+ c	+ f		− a	− e	− b	+ e	− c	+ b

Passage.

Note: once a week all pots at the window side of the bench were moved next the passage, while those at the passage side were moved to the window.

(5) Greenhouse experiment 1963.

Treatments: Copper: + and - : residual from 1962.

Illumination: Light: control, full light

Medium : green shading

Dark : heavy green shading and brown paper shading

Water : wet : 80% field capacity

dry : 40% field capacity.

```
A                          Window

 + - - + - - + +     - + + + + - - +     - - + + - - + +
 w d w d d w w w     w w d d d d w w     w d d d d w d w
     D A R K           M E D I U M          L I G H T
 - + - - - + + +     + + - + - - - -     - - + + - + + -
 d d w w d w d d     w w d d w d w d     d w w w w d w d

B                          Window

 + - + - - - + +     - + + - + - - -     - - - + + - + +
 d d d w w d w d     d w w d d w d w     d d w w w w d w
     D A R K           M E D I U M          L I G H T
 + + - - + - - +     + - + + + + - -     + - - + + - - +
 w w d w d d w w     w w w d d d d w     w w d d d d w d

                     w = wet          d = dry
```

Note: The pots were in the positions shown in layout A at the beginning of the experiment. Every second day, each pot was moved, within a light block, one place to the right and across the bench. The pots on the right hand edge of a light treatment were moved to the left hand end of the block and across the bench. Thus after the first move the positions were as shown in layout B. After eight moves the pots were again in layout A.

(6) Greenhouse experiments 1964.

Treatments: Copper : + and - : residual from 1962.

X : E.D.T.A. extractable copper concentration in the spring soil = 4.6 p.p.m.

Y : E.D.T.A. extractable copper concentration in the spring soil = 0.9 p.p.m.

Water : P : 80% field capacity

Q : 40% field capacity

R : 20% field capacity

S : 40% field capacity until 9th June, and then 80% water capacity

T : 20% field capacity until 9th June, and then 40% water capacity

Note: treatment Q+ = treatment W

treatment Q- = treatment Z

P-	T-	Q-	QX
Q+	Q-	QY	S-
T+	QY	R-	R+
R-	R+	T-	Q+
P+	S-	P-	P+
QX	S+	S+	T+
T-	R-	Q+	T-
S-	Q+	QX	Q-
QY	P-	R+	P-
Q-	T+	S-	R-
R+	P+	P+	S+
S+	QX	T+	QY

Note: In appendices 5-11, all concentrations in plant material are on an oven dry basis unless otherwise stated, and all concentrations in soils are on an air dry basis.

APPENDIX 5

Results from field experiment laid down in 1962

Treatments: A : control

B : 5 lb./acre copper oxychloride sprayed on crop

C : 10 lb./acre " " " " "

D : 10 lb./acre " " " " seedbed

E : 20 lb./acre " " " " "

(i) Hexpath

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Yield of dry matter in g. per plant cut at ear emergence	Replicate 1	1.04	1.27	1.46	1.34	1.22
	" 2	0.99	1.38	1.23	1.21	1.38
	" 3	1.12	1.26	1.30	1.32	1.46
	" 4	1.03	1.28	1.14	1.35	1.22
	Mean	1.06	1.30	1.28	1.30	1.32
Yield of grain in cwt./acre at 18% moisture	Replicate 1	(5.8)†	16.6	(19.9)†	18.2	12.4
	" 2	4.6	16.9	19.3	12.6	14.6
	" 3	6.2	18.1	22.6	13.4	18.2
	" 4	9.3	19.8	20.3	18.3	18.3
	Mean	6.5	17.8	20.5	15.6	15.9
Yield of oven dry straw in cwt./acre	Replicate 1	27.3	19.1	21.4	17.5	28.5
	" 2	25.6	22.6	22.9	25.4	23.5
	" 3	29.4	37.8	24.0	25.2	33.0
	" 4	29.6	35.2	28.7	28.2	34.1
	Mean	28.0	28.7	24.2	24.1	29.8

† estimated figure for a missing plot

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
% moisture in grain	Replicate 1	(28.3)†	22.0	(21.7)†	22.0	22.1
	" 2	31.9	21.5	21.9	23.2	21.9
	" 3	25.6	20.7	20.6	22.2	21.2
	" 4	26.4	20.7	21.5	22.3	21.3
	Mean	28.0	21.2	21.4	22.4	21.6
% moisture in straw	Replicate 1	63.6	54.7	48.9	56.1	60.9
	" 2	64.6	52.0	53.7	59.3	58.6
	" 3	59.8	51.8	49.4	56.3	54.4
	" 4	60.6	51.2	55.0	60.5	53.6
	Mean	62.2	52.4	51.8	58.0	56.9
Weight of a bushel of fresh grain in lb.	Replicate 1	(31.1)†	36.4	(40.1)†	39.1	39.6
	" 2	28.5	38.2	38.7	35.6	37.8
	" 3	26.8	35.3	36.6	30.8	33.7
	" 4	27.5	35.5	34.3	33.5	32.5
	Mean	28.5	36.4	37.4	34.8	35.9
% by weight of fresh grain which was green	Replicate 1	(7.6)†	2.0	(1.7)†	1.7	2.2
	" 2	10.3	1.9	1.7	2.9	1.6
	" 3	5.6	0.9	0.4	2.0	1.2
	" 4	5.8	0.8	1.9	2.1	1.2
	Mean	7.3	1.4	1.4	2.2	1.6
% by weight of oven dry grain in the total harvest yield	Replicate 1	(15.0)†	41.6	(43.2)†	46.0	26.4
	" 2	12.9	38.1	40.8	28.9	33.8
	" 3	14.8	28.1	43.5	30.4	31.1
	" 4	20.4	31.5	36.6	34.7	30.5
	Mean	15.8	34.8	41.0	35.0	30.4

† estimated figure for a missing plot

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. copper in plants at ear emergence. Averages of duplicate analyses	Replicate 1	0.66	1.33	1.37	0.98	0.86
	" 2	0.55	1.03	1.41	0.80	1.04
	" 3	0.92	1.02	1.28	0.97	1.03
	" 4	0.67	1.47	1.18	0.97	0.96
	Mean	0.70	1.21	1.31	0.93	0.97
p.p.m. copper in grain. Averages of duplicate analyses	Replicate 1	(1.32)†	1.37	(1.44)†	1.41	1.43
	" 2	1.21	1.32	1.52	1.26	1.25
	" 3	1.31	1.41	1.24	1.41	1.35
	" 4	1.42	1.47	1.36	1.45	1.40
	Mean	1.32	1.39	1.39	1.38	1.36
p.p.m. copper in straw. Averages of duplicate analyses	Replicate 1	1.16	1.45	1.30	1.24	1.18
	" 2	1.14	1.36	1.30	1.28	1.38
	" 3	1.23	1.20	1.43	1.27	1.21
	" 4	1.21	1.21	1.22	1.18	1.26
	Mean	1.18	1.30	1.31	1.24	1.26
% nitrogen in plants at ear emergence	Replicate 1	1.54	1.43	1.40	1.48	1.28
	" 2	1.61	1.32	1.28	1.35	1.47
	" 3	1.51	1.26	1.35	1.40	1.32
	" 4	1.57	1.26	1.29	1.40	1.28
	Mean	1.56	1.32	1.33	1.41	1.34
% nitrogen in grain	Replicate 1	(2.09)†	1.96	(1.98)†	2.16	2.14
	" 2	1.93	1.97	1.85	1.83	1.88
	" 3	1.83	1.79	1.72	1.83	1.79
	" 4	1.86	1.96	1.74	1.93	1.89
	Mean	1.93	1.92	1.82	1.94	1.92

†estimated figure for a missing plot

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
% nitrogen in straw	Replicate 1	0.94	0.74	0.53	0.73	0.69
	" 2	0.88	0.59	0.60	0.77	0.78
	" 3	0.90	0.56	0.56	0.66	0.59
	" 4	0.72	0.53	0.55	0.63	0.57
	Mean	0.86	0.60	0.56	0.70	0.66
p.p.m. manganese in plants at ear emergence	Replicate 1	43.9	36.4	30.2	35.1	44.6
	" 2	37.9	34.5	25.8	46.0	36.9
	" 3	31.2	28.4	19.1	31.1	33.3
	" 4	32.5	24.3	21.7	38.2	26.9
	Mean	36.4	30.9	24.2	37.6	35.4
p.p.m. iron in plants at ear emergence	Replicate 1	55.4	27.5	24.8	44.5	37.4
	" 2	62.4	28.1	30.7	42.5	39.0
	" 3	41.9	23.2	24.1	31.7	28.7
	" 4	38.6	22.0	26.0	42.8	40.1
	Mean	49.6	25.2	26.4	40.4	36.3
p.p.m. chlorophyll in plants at ear emergence	Replicate 1	81.0	82.5	85.0	87.0	66.0
	" 2	75.0	74.0	70.0	71.0	76.5
	" 3	88.0	62.0	83.0	78.0	76.0
	" 4	79.5	75.5	74.0	78.0	76.0
	Mean	80.9	73.5	78.0	78.5	73.6
p.p.m. E.D.T.A. extractable copper in soil sampled 25th April 1962 (untreated seed-bed) (average of duplicate samplings)	Replicate 1	0.44	0.50	0.45	0.47	0.46
	" 2	0.54	0.48	0.44	0.56	0.49
	" 3	0.51	0.52	0.54	0.50	0.68
	" 4	0.52	0.45	0.38	0.41	0.42
	Mean	0.50	0.49	0.45	0.48	0.51

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. E.D.T.A. extractable copper in soil sampled 8th October 1962	Replicate 1	0.48	0.82	0.74	0.96	1.39
	" 2	0.43	0.59	0.96	0.99	2.41
	" 3	0.44	0.44	1.34	0.49	1.02
	" 4	0.40	0.59	0.48	0.66	2.80
	Mean	0.44	0.61	0.88	0.78	1.90
p.p.m. E.D.T.A. extractable copper in soil sampled 24th April 1963	Replicate 1	0.46	0.69	0.76	1.24	3.94
	" 2	0.50	0.84	1.40	1.58	4.48
	" 3	0.50	0.67	0.98	1.98	2.14
	" 4	0.55	0.76	0.80	1.20	2.35
	Mean	0.50	0.74	0.98	1.50	3.23
p.p.m. E.D.T.A. extractable copper in soil sampled 23rd September 1963	Replicate 1	0.45	0.64	0.92	1.01	1.92
	" 2	0.42	0.64	0.62	1.59	1.48
	" 3	0.40	0.54	1.27	0.94	2.14
	" 4	0.36	0.65	0.90	1.52	1.52
	Mean	0.41	0.62	0.93	1.26	1.76
p.p.m. E.D.T.A. extractable copper in soil sampled 23rd March 1964	Replicate 1	0.44	0.91	2.30	2.10	8.00
	" 2	0.40	0.66	1.10	0.63	1.85
	" 3	0.42	0.50	1.13	1.70	2.49
	" 4	0.32	0.56	0.84	2.02	3.20
	Mean	0.40	0.66	1.34	1.61	3.88
p.p.m. E.D.T.A. extractable copper in soil sampled 24th April 1964	Replicate 1	0.56	2.07	2.17	2.39	5.37
	" 2	0.50	1.48	1.59	2.74	3.84
	" 3	0.48	0.92	0.98	1.72	3.86
	" 4	0.44	0.91	0.82	1.41	1.90
	Mean	0.50	1.34	1.39	2.06	3.74

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. E.D.T.A. extractable copper in soil sampled 21st May 1964	Replicate 1	0.40	0.82	0.78	1.60	1.37
	" 2	0.42	0.73	0.93	1.32	1.52
	" 3	0.40	0.67	0.82	0.84	0.99
	" 4	0.37	0.72	0.66	1.93	1.27
	Mean	0.40	0.74	0.80	1.42	1.29
p.p.m. E.D.T.A. extractable copper in soil sampled 22nd June 1964	Replicate 1	0.48	0.65	0.93	0.74	2.47
	" 2	0.40	0.94	0.76	1.18	4.00
	" 3	0.56	0.80	1.12	1.20	3.08
	" 4	0.40	0.60	0.76	1.34	1.92
	Mean	0.46	0.75	0.89	1.12	2.87
p.p.m. E.D.T.A. extractable copper in soil sampled 21st July 1964	Replicate 1	0.42	1.36	1.82	3.72	2.61
	" 2	0.37	1.38	1.88	5.46	9.74
	" 3	0.52	1.80	3.50	4.97	5.97
	" 4	0.44	1.44	2.28	7.60	5.64
	Mean	0.44	1.50	2.37	5.44	5.99
p.p.m. E.D.T.A. extractable copper in soil sampled 26th August 1964	Replicate 1	0.50	0.68	0.66	1.64	2.65
	" 2	0.43	0.68	1.14	1.70	0.92
	" 3	0.44	0.75	1.04	1.26	1.36
	" 4	0.43	0.71	0.92	1.74	2.20
	Mean	0.45	0.70	0.94	1.58	1.78
p.p.m. E.D.T.A. extractable copper in soil sampled 28th September, 1964.	Replicate 1	0.48	0.98	1.70	1.92	1.80
	" 2	0.36	0.98	1.08	2.00	3.97
	" 3	0.38	0.52	0.92	1.12	1.71
	" 4	0.42	0.68	0.76	1.00	1.82
	Mean	0.41	0.79	1.12	1.51	2.32

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. E.D.T.A. extractable copper in soil sampled 26th October 1964 Average of duplicate samplings	Replicate 1	0.46	0.89	0.88	2.18	3.09
	" 2	0.42	0.94	1.06	1.33	2.84
	" 3	0.46	0.83	1.12	1.64	2.78
	" 4	0.40	0.76	1.00	1.65	1.70
	Mean	0.44	0.86	1.02	1.70	2.60
p.p.m. copper in flowering clover, cut 22nd June 1964	Replicate 1	2.10	5.30	7.72	6.40	6.88
	" 2	1.84	5.90	6.20	5.50	7.26
	" 3	3.14	7.06	8.10	8.17	8.32
	" 4	2.72	7.08	7.96	6.78	7.80
	Mean	2.45	6.34	7.50	6.71	7.56
(ii) Billie Mains						
Yield of grain in cwt/acre at 18% moisture	Replicate 1	24.2	22.4	23.9	23.2	22.6
	" 2	22.6	23.8	(23.6)†	23.8	22.9
	" 3	23.1	26.4	26.6	22.6	25.2
	" 4	25.5	23.7	21.6	23.7	23.6
	Mean	23.8	24.1	23.9	23.3	23.6
p.p.m. copper in plants at ear emergence. Averages of duplicate analyses	Replicate 1	3.04	3.40	3.62	2.98	3.04
	" 2	3.48	3.38	3.66	3.86	3.74
	" 3	3.87	3.80	4.00	3.86	3.88
	" 4	3.36	3.72	3.61	3.34	3.54
	Mean	3.44	3.58	3.72	3.51	3.55
p.p.m. copper in grain. Averages of duplicate analyses	Replicate 1	3.31	3.46	3.55	3.61	3.67
	" 2	3.38	4.10	3.49	3.76	4.16
	" 3	3.39	3.26	3.74	3.90	3.58
	" 4	3.34	3.64	3.33	3.12	3.63
	Mean	3.36	3.62	3.53	3.60	3.76

†estimated figure for a missing plot

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. copper in straw. Averages of duplicate analyses	Replicate 1	2.38	2.58	2.24	2.48	2.42
	" 2	2.50	2.53	2.45	2.47	2.48
	" 3	2.22	2.12	2.44	2.40	2.40
	" 4	2.40	2.38	2.27	2.28	2.35
	Mean	2.38	2.40	2.35	2.41	2.41
p.p.m. E.D.T.A. extractable copper in untreated seed-bed sampled 12th April 1962	Replicate 1	5.71	6.92	4.97	5.29	6.61
	" 2	5.59	5.00	6.28	6.33	5.70
	" 3	4.13	3.58	4.64	5.40	5.39
	" 4	5.04	5.26	4.14	4.85	4.63
	Mean	5.12	5.19	5.01	5.47	5.58
p.p.m. E.D.T.A. extractable copper in soil sampled 4th October 1962	Replicate 1	5.20	7.35	5.47	5.88	7.02
	" 2	5.49	5.18	6.45	7.14	6.12
	" 3	4.35	3.94	4.55	5.63	6.00
	" 4	5.30	5.55	4.27	5.40	4.96
	Mean	5.08	5.50	5.18	6.01	6.02
p.p.m. E.D.T.A. extractable copper in soil sampled 7th May 1963	Replicate 1	5.51	7.06	6.13	5.73	7.22
	" 2	6.02	5.22	6.41	6.92	6.59
	" 3	4.37	4.43	4.96	6.11	6.06
	" 4	5.73	5.35	4.17	5.79	5.24
	Mean	5.41	5.52	5.42	6.14	6.28
(iii) Oldcastles						
Yield of grain in cwt/acre at 18% moisture	Replicate 1	25.1	26.8	28.0	26.8	28.5
	" 2	30.7	28.7	33.6	29.5	30.3
	" 3	30.4	24.9	31.3	27.0	27.0
	" 4	36.6	26.0	29.0	30.3	29.3
	Mean	30.7	26.6	30.5	28.4	28.8

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
Yield of oven dry straw in cwt/acre	Replicate 1	11.2	10.6	9.1	9.6	6.4
	" 2	12.2	14.0	11.3	15.4	12.0
	" 3	9.7	10.4	13.2	10.2	7.6
	" 4	15.8	10.3	10.0	14.9	13.5
	Mean	12.2	11.3	10.9	12.5	9.9
% moisture in grain	Replicate 1	15.3	16.2	16.5	15.4	14.9
	" 2	14.9	15.1	15.5	15.6	15.0
	" 3	15.2	15.0	15.1	16.3	15.9
	" 4	15.6	15.4	15.2	15.0	15.1
	Mean	15.2	15.4	15.6	15.6	15.2
% moisture in straw	Replicate 1	57.2	56.1	61.7	59.7	64.7
	" 2	56.2	53.9	54.7	50.9	55.7
	" 3	59.5	58.7	53.7	59.6	63.2
	" 4	51.2	55.8	57.5	49.4	51.1
	Mean	56.0	56.1	56.9	54.9	58.7
Weight of a bushel of fresh grain in lb.	Replicate 1	49.8	50.5	49.5	49.1	50.1
	" 2	49.9	50.8	52.3	50.0	50.4
	" 3	50.0	50.2	50.0	50.4	50.2
	" 4	49.8	51.9	50.6	50.7	52.1
	Mean	49.9	50.8	50.6	50.0	50.7
% by weight of oven dry grain in the total harvest yield	Replicate 1	64.8	67.5	71.6	69.6	78.5
	" 2	67.4	62.7	70.9	61.1	67.4
	" 3	72.0	66.2	66.0	68.5	74.4
	" 4	65.5	67.4	70.4	62.5	64.0
	Mean	67.4	66.0	69.7	65.4	71.1

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. copper in plants at ear emergence. Averages of duplicate analyses	Replicate 1	1.96	4.64	4.48	1.81	1.81
	" 2	1.36	4.02	3.54	1.58	2.01
	" 3	2.01	3.48	7.47	1.58	1.66
	" 4	1.91	2.88	5.00	1.66	1.92
	Mean	1.81	3.76	5.12	1.66	1.85
p.p.m. copper in grain. Averages of duplicate analyses	Replicate 1	2.28	2.84	2.80	2.24	2.62
	" 2	1.98	2.66	2.56	2.11	2.28
	" 3	2.44	2.79	2.82	2.17	2.50
	" 4	2.27	2.46	2.90	2.21	2.52
	Mean	2.24	2.69	2.77	2.18	2.48
p.p.m. copper in straw. Averages of duplicate analyses	Replicate 1	2.03	2.80	3.52	2.36	2.10
	" 2	1.89	3.09	3.27	2.18	2.06
	" 3	2.12	2.95	3.74	2.15	2.16
	" 4	1.90	2.64	3.15	2.10	2.18
	Mean	1.98	2.87	3.42	2.20	2.12
p.p.m. E.D.T.A. extractable copper in soil sampled 22nd March 1962. Averages of duplicate analyses	Replicate 1	1.14	1.02	1.10	0.98	1.00
	" 2	1.14	1.08	1.16	1.14	1.16
	" 3	1.00	0.98	1.07	1.02	1.00
	" 4	1.12	1.16	1.16	1.10	1.16
	Mean	1.10	1.06	1.12	1.06	1.08
p.p.m. E.D.T.A. extractable copper in soil sampled 21st September 1962	Replicate 1	1.29	1.62	2.06	1.70	2.63
	" 2	1.38	1.39	2.10	3.93	3.53
	" 3	1.11	1.23	1.78	2.43	3.28
	" 4	1.36	1.98	1.94	1.72	1.93
	Mean	1.28	1.56	1.97	2.44	2.84

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
(iv) Rosehill						
p.p.m. copper in plants at ear emergence, 1962	Replicate 1	3.49	4.11	4.58	4.20	3.80
	" 2	3.90	4.04	4.80	3.89	3.49
	" 3	3.28	3.89	5.65	3.84	3.38
	" 4	3.27	5.46	4.47	3.78	4.16
	Mean	3.48	4.38	4.88	3.93	3.71
p.p.m. E.D.T.A. extractable copper in untreated seed-bed 25th April 1962. Average of duplicate samplings	Replicate 1	1.73	1.76	1.97	1.82	1.90
	" 2	1.74	1.62	1.68	1.79	1.73
	" 3	1.54	1.84	1.77	1.44	1.68
	" 4	1.41	1.34	1.38	1.48	1.32
	Mean	1.60	1.64	1.70	1.63	1.66
p.p.m. E.D.T.A. extractable copper in soil. Replicates 1 and 2 sampled on 8th Nov. 1962, replicates 3 and 4 on 26th Nov. 1962	Replicate 1	2.14	2.38	3.26	6.78	4.91
	" 2	2.18	2.18	3.36	2.80	3.58
	" 3	1.76	2.46	3.11	3.32	4.02
	" 4	1.61	2.06	2.18	2.95	2.22
	Mean	1.92	2.27	2.98	3.96	3.68
p.p.m. E.D.T.A. extractable copper in soil sampled 6th May 1963	Replicate 1	2.16	3.66	4.20	4.60	6.57
	" 2	2.10	3.32	3.59	6.52	5.47
	" 3	1.80	2.40	2.82	2.64	6.86
	" 4	1.51	1.88	2.32	2.64	2.57
	Mean	1.89	2.82	3.23	4.10	5.37
p.p.m. E.D.T.A. extractable copper in soil sampled 17th October 1963	Replicate 1	2.15	2.98	3.82	3.44	3.88
	" 2	1.82	2.61	3.01	2.66	3.66
	" 3	1.87	2.54	2.68	2.90	5.29
	" 4	1.46	2.38	3.07	2.98	3.59
	Mean	1.82	2.63	3.14	3.00	4.10

Data		Treatment A	Treatment B	Treatment C	Treatment D	Treatment E
p.p.m. E.D.T.A. extractable copper in soil sampled 23rd April 1964	Replicate 1	2.18	2.52	3.96	3.50	4.54
	" 2	1.90	2.28	3.45	3.43	3.46
	" 3	1.70	2.78	3.19	2.50	3.36
	" 4	1.46	1.88	2.51	2.49	2.99
	Mean	1.81	2.36	3.28	2.98	3.59
p.p.m. E.D.T.A. extractable copper in soil sampled 20th October 1964	Replicate 1	1.95	2.59	3.53	3.73	4.62
	" 2	1.86	2.55	3.43	3.18	5.18
	" 3	1.76	2.55	3.10	3.25	4.80
	" 4	1.67	1.98	2.69	2.46	3.59
	Mean	1.81	2.42	3.19	3.16	4.55
p.p.m. copper in oats at ear emergence, 1964	Replicate 1	1.78	1.92	1.88	1.82	1.86
	" 2	1.76	1.97	1.88	1.66	1.90
	" 3	1.68	1.92	1.83	1.61	1.92
	" 4	1.56	1.70	1.82	1.91	2.16
	Mean	1.70	1.88	1.85	1.75	1.96

APPENDIX 6

Results from field experiment laid down in 1963

Treatments:	F: control	∴ log (treatment +1) = 0.00
	G: 0.8 lb./acre copper oxychloride	∴ " " " = 0.26
	H: 2.3 lb./acre " "	∴ " " " = 0.52
	J: 5.0 lb./acre " "	∴ " " " = 0.78
	K: 10.0 lb./acre " "	∴ " " " = 1.04

(i) Pirnie

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
Yield of grain in cwt/acre at 18% moisture	Replicate 1	-	-	-	-	-
	" 2	30.8	26.9	28.5	29.3	30.2
	" 3	27.9	27.9	27.2	(28.0)†	26.1
	" 4	27.3	28.2	30.1	30.3	30.8
	Mean	28.7	27.7	28.6	29.2	29.0
% moisture in grain	Replicate 1	21.3	23.2	23.6	23.1	21.1
	" 2	19.7	20.6	20.4	20.1	19.4
	" 3	19.7	20.7	20.5	24.0	21.4
	" 4	20.7	19.6	19.9	19.3	19.8
	Mean	20.4	21.0	21.1	21.6	20.4
% moisture in straw	Replicate 1	50.9	47.9	38.4	46.6	49.4
	" 2	44.5	42.1	45.5	51.8	49.0
	" 3	45.3	45.0	47.7	48.0	46.4
	" 4	46.0	47.4	48.0	45.4	46.8
	Mean	46.7	45.6	44.9	48.0	47.9

† estimated figure for a missing plot

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. copper in plants at ear emergence	Replicate 1	1.39	1.79	1.93	2.02	1.99
	" 2	1.37	1.43	1.64	1.48	1.55
	" 3	1.29	1.33	1.65	1.96	1.66
	" 4	1.43	1.41	1.40	1.44	1.55
	Mean	1.37	1.49	1.66	1.72	1.69
p.p.m. copper in grain	Replicate 1	1.56	1.33	1.60	1.47	1.81
	" 2	2.08	1.56	1.87	2.10	2.39
	" 3	1.75	1.65	1.73	1.71	1.79
	" 4	2.13	1.94	1.99	2.19	1.96
	Mean	1.88	1.62	1.80	1.87	1.99
p.p.m. copper in straw	Replicate 1	1.61	1.48	1.78	1.61	1.80
	" 2	1.56	1.50	1.59	1.67	1.97
	" 3	1.23	1.49	1.76	1.94	1.94
	" 4	1.54	1.45	1.70	1.72	1.68
	Mean	1.48	1.48	1.71	1.74	1.85
p.p.m. E.D.T.A. extractable copper in untreated seed-bed. 24th April 1963.	Replicate 1	0.60	0.68	0.58	0.66	0.74
	" 2	0.61	1.14	0.91	0.56	0.50
	" 3	0.63	0.54	0.68	0.80	0.83
	" 4	0.53	0.66	0.59	0.69	0.98
	Mean	0.59	0.76	0.69	0.68	0.76
p.p.m. E.D.T.A. extractable copper in soil sampled 30th Sept. 1963	Replicate 1	0.42	0.46	0.54	0.52	0.68
	" 2	0.41	0.57	0.60	0.54	0.67
	" 3	0.48	0.46	0.46	0.53	0.58
	" 4	0.40	0.46	0.61	0.54	0.73
	Mean	0.43	0.49	0.55	0.53	0.66

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. E.D.T.A. extractable copper in soil sampled 24th April 1964	Replicate 1	0.50	0.57	0.55	0.79	0.66
	" 2	0.52	0.79	0.56	1.18	2.22
	" 3	0.55	0.71	0.80	0.91	1.31
	" 4	0.50	0.52	0.64	0.98	0.74
	Mean	0.52	0.65	0.64	0.96	1.23
p.p.m. E.D.T.A. extractable copper in soil sampled 1st Oct. 1964	Replicate 1	0.46	0.50	0.52	0.62	1.02
	" 2	0.50	0.46	0.50	0.56	0.76
	" 3	0.45	0.54	0.54	0.64	0.97
	" 4	0.54	0.53	0.66	0.58	1.04
	Mean	0.49	0.51	0.56	0.60	0.95
(ii) Upper Huntlywood						
p.p.m. copper in plants at ear emergence. Average of duplicate analyses	Replicate 1	1.34	1.52	1.71	2.18	2.20
	" 2	1.62	1.96	1.98	2.04	2.10
	" 3	1.07	1.48	1.47	1.28	1.61
	" 4	1.60	1.49	2.12	1.94	2.00
	Mean	1.41	1.61	1.82	1.86	1.98
p.p.m. copper in grain	Replicate 1	1.94	2.11	1.82	2.09	2.09
	" 2	2.06	2.61	2.15	2.29	2.32
	" 3	1.35	1.73	1.40	1.32	1.86
	" 4	2.30	1.73	2.56	1.92	1.88
	Mean	1.91	2.04	1.98	1.90	2.04
p.p.m. copper in straw	Replicate 1	1.70	1.81	1.66	2.02	1.86
	" 2	1.56	2.04	1.86	1.92	1.94
	" 3	1.08	1.36	1.22	1.14	1.40
	" 4	1.64	1.33	2.04	1.74	1.66
	Mean	1.50	1.64	1.70	1.70	1.72

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. manganese in plants at ear emergence	Replicate 1	4.1	4.8	4.7	4.6	2.8
	" 2	5.5	4.0	3.8	4.6	5.2
	" 3	10.2	8.2	7.7	12.2	9.1
	" 4	3.6	5.4	6.7	5.0	5.4
	Mean	5.8	5.6	5.7	6.6	5.6
p.p.m. iron in plants at ear emergence	Replicate 1	27.7	23.1	41.5	42.0	30.1
	" 2	22.7	24.1	24.6	25.7	25.2
	" 3	17.2	17.6	17.5	19.8	21.7
	" 4	21.0	19.4	20.9	24.0	20.8
	Mean	22.2	21.0	26.1	27.9	24.4
p.p.m. E.D.T.A. extractable copper in untreated seedbed 29th April 1963	Replicate 1	0.66	0.70	0.66	0.66	0.56
	" 2	0.68	0.64	0.64	0.65	0.70
	" 3	0.67	0.68	0.68	0.68	0.64
	" 4	0.61	0.59	0.70	0.64	0.64
	Mean	0.66	0.65	0.67	0.66	0.64
p.p.m. E.D.T.A. extractable copper in soil sampled 7th October 1963	Replicate 1	0.66	0.74	0.68	0.92	1.07
	" 2	0.71	0.65	0.68	0.80	1.08
	" 3	0.62	0.68	0.68	0.93	0.96
	" 4	0.61	0.62	0.79	0.70	0.84
	Mean	0.65	0.67	0.71	0.84	0.99
p.p.m. E.D.T.A. extractable copper in soil sampled 24th April 1964	Replicate 1	0.58	0.60	0.68	0.80	0.86
	" 2	0.62	0.59	0.68	0.78	1.11
	" 3	0.60	0.80	0.91	0.89	1.50
	" 4	0.58	0.72	0.88	0.76	1.77
	Mean	0.60	0.68	0.79	0.81	1.31

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. E.D.T.A. extractable copper in soil sampled 1st October 1964	Replicate 1	0.56	0.64	0.61	0.90	0.83
	" 2	0.58	0.68	0.68	1.14	0.94
	" 3	0.60	0.68	0.76	0.74	1.18
	" 4	0.58	0.59	0.71	1.06	1.06
	Mean	0.58	0.65	0.69	0.96	1.00
(iii) Choicelee						
Yield of grain in cwt/acre at 18% moisture	Replicate 1	19.7	(19.0)†	19.5	19.9	22.2
	" 2	19.5	19.7	22.3	21.9	23.9
	" 3	18.1	18.7	21.9	19.7	21.9
	" 4	19.3	20.9	22.1	23.2	19.1
	Mean	19.2	19.6	21.4	21.2	21.8
Yield of oven dry straw in cwt/acre	Replicate 1	15.3	16.0	13.9	12.2	16.0
	" 2	14.9	16.7	17.9	16.9	18.5
	" 3	13.4	14.9	16.1	15.1	13.7
	" 4	13.5	15.4	16.8	15.1	12.7
	Mean	14.3	15.8	16.2	14.8	15.2
% moisture in grain	Replicate 1	21.7	22.5	20.1	21.8	22.9
	" 2	19.7	20.3	22.9	21.9	22.8
	" 3	21.9	23.1	22.4	22.0	25.0
	" 4	24.5	21.8	23.5	23.8	22.5
	Mean	22.0	21.9	22.2	22.4	23.3
% moisture in straw	Replicate 1	38.0	41.9	36.9	36.7	37.1
	" 2	36.3	36.2	35.0	39.8	35.9
	" 3	41.6	40.6	43.1	40.7	42.5
	" 4	45.6	36.6	37.1	41.9	38.4
	Mean	40.4	38.8	38.0	39.8	38.5

† estimated figure for a missing plot

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
Weight of a bushel of oven dry grain in lb.	Replicate 1	32.7	32.3	31.8	32.3	31.9
	" 2	33.7	31.5	30.1	30.7	28.3
	" 3	31.9	31.6	32.0	29.2	31.3
	" 4	29.5	31.5	31.3	30.3	28.6
	Mean	32.0	31.7	31.3	30.6	30.0
% by weight of oven dry grain in the total harvest yield	Replicate 1	51.4	49.4	53.4	57.3	53.3
	" 2	51.7	49.1	50.4	51.4	51.5
	" 3	52.5	50.8	52.7	51.6	56.8
	" 4	54.0	52.6	51.9	55.7	55.2
	Mean	52.4	50.5	52.1	54.0	54.2
p.p.m. copper in plants at ear emergence	Replicate 1	1.58	2.07	2.10	2.29	3.14
	" 2	2.06	1.98	2.04	2.34	2.66
	" 3	2.00	2.02	2.15	2.38	2.61
	" 4	1.86	2.05	2.15	2.37	2.60
	Mean	1.88	2.03	2.11	2.34	2.75
p.p.m. copper in grain	Replicate 1	2.03	2.17	2.26	2.43	2.70
	" 2	2.38	2.42	1.96	2.35	2.37
	" 3	2.41	2.14	2.30	2.34	2.42
	" 4	2.25	2.01	2.02	2.14	2.27
	Mean	2.27	2.18	2.14	2.32	2.44
p.p.m. copper in straw	Replicate 1	1.66	1.85	1.86	2.01	2.21
	" 2	2.02	1.92	2.00	2.02	2.31
	" 3	1.94	1.89	1.86	1.92	2.34
	" 4	1.87	1.76	1.95	1.86	2.07
	Mean	1.87	1.86	1.92	1.95	2.23

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. E.D.T.A. extractable copper in untreated seedbed 24th April 1963	Replicate 1	0.50	0.46	0.49	0.45	0.48
	" 2	0.50	0.49	0.50	0.54	0.46
	" 3	0.49	0.50	0.44	0.52	0.48
	" 4	0.52	0.48	0.44	0.49	0.49
	Mean	0.50	0.48	0.47	0.50	0.48
p.p.m. E.D.T.A. extractable copper in soil sampled 11th October 1963	Replicate 1	0.42	0.50	0.52	0.96	1.30
	" 2	0.46	0.45	0.56	0.72	1.04
	" 3	0.45	0.44	0.64	0.68	0.76
	" 4	0.48	0.47	0.56	0.75	0.84
	Mean	0.45	0.46	0.57	0.78	0.98
p.p.m. E.D.T.A. extractable copper in soil sampled 14th April 1964	Replicate 1	0.38	0.38	0.60	0.64	1.00
	" 2	0.44	0.46	0.68	0.64	0.78
	" 3	0.39	0.46	0.56	0.72	0.98
	" 4	0.43	0.41	0.48	0.62	1.10
	Mean	0.41	0.43	0.58	0.66	0.96
p.p.m. E.D.T.A. extractable copper in soil sampled 28th Sept. 1964	Replicate 1	0.36	0.40	0.46	0.60	0.82
	" 2	0.56	0.59	0.59	0.70	0.64
	" 3	0.42	0.43	0.54	0.94	1.41
	" 4	0.42	0.54	0.62	0.82	0.82
	Mean	0.44	0.49	0.55	0.76	0.92

APPENDIX 7

Results from field experiments in 1964

Experiment 1: Treatments: F: control

G: 0.8 lb./acre copper oxychloride

H: 2.3 lb./acre " "

J: 5.0 lb./acre " "

K: 10.0 lb./acre " "

(i) Hexpath

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
Yield of grain in cwt/acre at 18% moisture	Replicate 1	36.8	34.8	37.2	36.2	36.9
	" 2	33.0	30.6	32.4	36.1	35.4
	" 3	32.7	35.8	37.3	38.5	38.2
	" 4	31.0	30.2	(33.4)†	35.0	36.4
	Mean	33.4	32.8	35.1	36.4	36.7
% moisture in grain	Replicate 1	25.6	25.2	25.2	25.3	25.4
	" 2	23.4	24.5	24.2	24.2	24.3
	" 3	25.2	24.1	25.1	24.7	24.1
	" 4	24.6	23.1	23.7	23.4	23.3
	Mean	24.7	24.2	24.6	24.4	24.3
% moisture in straw	Replicate 1	43.1	50.5	45.8	42.0	45.4
	" 2	47.1	34.7	39.3	47.4	47.1
	" 3	43.6	38.6	39.0	45.2	41.3
	" 4	42.4	36.5	37.2	35.0	41.1
	Mean	44.0	40.1	40.3	42.4	43.7

† estimated figure for a missing plot

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
Weight of a bushel of dry grain in lb.	Replicate 1	38.1	40.4	42.7	40.5	41.9
	" 2	37.2	42.5	40.6	39.4	37.4
	" 3	41.9	37.3	38.3	40.9	42.6
	" 4	36.8	(38.6)†	39.2	41.2	37.5
	Mean	38.5	39.7	40.2	40.5	39.8
p.p.m. copper in plants at ear emergence	Replicate 1	1.49	2.38	2.41	2.64	3.36
	" 2	2.24	2.53	2.62	2.78	2.58
	" 3	2.09	2.24	2.28	2.34	2.63
	" 4	1.70	2.95	1.90	2.84	3.12
	Mean	1.88	2.52	2.30	2.65	2.92
p.p.m. copper in grain	Replicate 1	2.38	2.30	2.36	2.38	2.63
	" 2	2.49	2.97	2.57	2.59	2.68
	" 3	2.66	2.18	2.55	2.20	3.03
	" 4	1.88	3.60	2.49	2.49	2.72
	Mean	2.35	2.76	2.49	2.42	2.76
p.p.m. copper in straw	Replicate 1	1.90	2.03	2.16	2.22	2.42
	" 2	1.94	2.05	2.01	2.20	2.24
	" 3	1.78	1.78	2.38	2.18	2.54
	" 4	1.96	2.24	2.06	2.26	2.50
	Mean	1.90	2.02	2.15	2.22	2.42
p.p.m. E.D.T.A. extractable copper in untreated seedbed 24th April 1964	Replicate 1	0.49	0.64	0.57	0.51	0.59
	" 2	0.58	0.62	0.55	0.58	0.54
	" 3	0.55	0.58	(0.57)†	0.61	0.54
	" 4	0.48	0.57	0.49	0.49	0.50
	Mean	0.52	0.60	0.54	0.55	0.54

† estimated figure for a missing plot

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. E.D.T.A. extractable copper in soil sampled 11th Sept. 1964	Replicate 1	0.48	1.31	0.68	1.04	1.50
	" 2	0.53	0.57	0.72	1.86	1.21
	" 3	0.58	0.62	0.64	0.90	1.05
	" 4	0.45	0.42	2.16	1.01	1.21
	Mean	0.51	0.73	1.05	1.20	1.24

Experiment 1: (ii) Langtonlees

		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
Yield of grain in cwt/acre at 18% moisture	Replicate 1	20.4	22.7	23.5	22.7	24.6
	" 2	22.4	20.7	23.0	21.0	24.0
	" 3	16.9	23.5	20.3	(21.2)†	24.8
	" 4	21.5	19.8	21.4	21.7	20.1
	Mean	20.3	21.7	22.0	21.6	23.4
Yield of oven dry straw in cwt/acre	Replicate 1	8.2	12.1	10.2	9.6	7.8
	" 2	8.8	7.2	9.6	8.1	7.5
	" 3	7.8	7.8	6.6	10.9	8.4
	" 4	8.3	6.0	8.8	8.7	6.0
	Mean	8.3	8.3	8.8	9.3	7.4
% moisture in grain	Replicate 1	23.5	24.4	24.8	23.7	22.9
	" 2	21.1	25.1	23.8	23.5	23.0
	" 3	27.4	22.9	22.2	24.8	23.5
	" 4	21.8	23.6	23.1	23.2	21.8
	Mean	23.4	24.0	23.5	23.8	22.8
% moisture in straw	Replicate 1	55.4	56.5	56.9	57.7	56.0
	" 2	55.4	58.1	56.5	56.8	53.1
	" 3	63.2	56.1	52.5	57.2	57.2
	" 4	56.6	55.3	56.9	54.2	53.4
	Mean	57.6	56.5	55.7	56.5	54.9

† estimated figure for a missing plot

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
Weight of a bushel of dry grain in lb.	Replicate 1	39.0	37.9	38.5	38.6	39.2
	" 2	38.9	38.7	39.0	39.8	38.8
	" 3	36.2	38.8	39.2	38.1	39.1
	" 4	39.8	39.3	39.3	38.3	39.6
	Mean	38.5	38.7	39.0	38.7	39.2
% by weight of fresh grain which was green	Replicate 1	7.1	5.4	5.6	3.2	4.2
	" 2	3.2	9.1	8.3	6.9	5.3
	" 3	11.0	3.8	3.9	6.5	5.3
	" 4	3.7	5.4	7.0	6.4	5.6
	Mean	6.2	5.9	6.2	5.8	5.1
% by weight of oven dry grain in the total harvest yield	Replicate 1	67.1	60.6	65.4	66.0	72.1
	" 2	67.6	70.2	66.3	68.0	72.4
	" 3	64.1	71.2	71.6	(67.9)†	70.7
	" 4	68.0	73.0	66.5	67.2	73.3
	Mean	66.7	68.8	67.5	67.3	72.2
p.p.m. copper in plants at ear emergence	Replicate 1	1.07	1.30	2.51	3.03	4.01
	" 2	1.10	1.43	2.38	5.40	10.69
	" 3	0.82	1.35	1.94	2.64	5.46
	" 4	1.07	2.94	5.34	5.54	6.78
	Mean	1.02	1.76	3.04	4.15	6.74
p.p.m. copper in grain	Replicate 1	1.04	1.20	1.16	1.32	1.78
	" 2	1.55	1.59	1.60	1.95	1.82
	" 3	0.92	1.30	1.49	1.05	1.38
	" 4	1.76	1.48	1.64	2.01	1.88
	Mean	1.32	1.39	1.47	1.58	1.72

† estimated figure for a missing plot

Data		Treatment F	Treatment G	Treatment H	Treatment J	Treatment K
p.p.m. copper in straw	Replicate 1	1.36	1.33	1.48	1.60	1.61
	" 2	1.36	1.40	1.59	1.62	1.88
	" 3	1.16	1.26	1.41	1.36	1.56
	" 4	1.48	1.36	1.41	1.62	1.74
	Mean	1.34	1.34	1.47	1.55	1.70
p.p.m. E.D.T.A. extractable copper in untreated seedbed 9th April 1964	Replicate 1	0.51	0.68	0.54	0.54	0.52
	" 2	0.52	0.49	0.44	0.48	0.58
	" 3	0.50	0.40	0.46	0.43	0.42
	" 4	0.47	0.48	0.48	0.46	0.52
	Mean	0.50	0.51	0.48	0.48	0.51
p.p.m. E.D.T.A. extractable copper in soil sampled 13th Sept. 1964	Replicate 1	0.52	0.63	0.83	1.43	0.84
	" 2	0.54	0.49	0.68	0.87	1.36
	" 3	0.48	0.51	0.78	0.82	1.18
	" 4	0.41	0.46	0.59	0.74	0.72
	Mean	0.49	0.52	0.72	0.96	1.02

Experiment 2: Choicelee

Data		Astor oats −	Astor oats +	Blenda oats −	Blenda oats +	Forward oats −	Forward oats +	Freja barley −	Freja barley +	Maythorpe barley −	Maythorpe barley +	Ymer barley −	Ymer barley +
Yield of grain in cwt/acre at 18% moisture	Replicate 1	32.5	31.1[L]	25.9[L]	32.1	29.8	24.2[L]	33.9[L]	39.0	27.9[L]	35.5	30.6	26.6[L]
	" 2	28.9[L]	32.0	30.6[L]	35.0	26.6[L]	32.5	36.6	33.5[L]	37.5	33.7[L]	37.3	38.3[L]
	" 3	29.2	28.8[L]	31.3[L]	38.4	28.8[L]	31.0	36.4[L]	35.3	38.3	36.0[L]	35.1	38.3[L]
	" 4	23.0	28.8[L]	38.3	30.7[L]	28.1	28.7[L]	29.6[L]	29.9	34.3	38.8[L]	31.2[L]	37.4
	Mean	28.4	30.2	31.5	34.0	28.3	29.1	34.1	34.4	34.5	36.0	33.6	35.2
Yield of oven dry straw in cwt/acre	Replicate 1	11.7	10.5	12.5	12.7	12.3	10.9	19.3	20.6	16.5	17.3	14.4	11.1
	" 2	10.1	12.7	13.7	14.2	12.2	15.5	20.9	19.6	18.9	16.0	17.7	17.7
% moisture in grain	Replicate 1	21.1	21.8	21.8	22.1	23.1	23.4	26.7	26.4	25.2	25.1	25.5	25.6
	" 2	21.2	20.7	21.4	22.0	22.9	23.5	26.0	25.4	25.3	24.4	25.7	25.7
	" 3	20.3	19.8	21.5	22.0	21.6	23.5	25.4	26.1	25.0	23.4	25.0	24.5
	" 4	19.7	20.0	20.9	21.8	22.4	21.3	24.9	25.1	24.1	23.0	24.2	24.2
	Mean	20.6	20.6	21.4	22.0	22.5	22.9	25.8	25.8	24.9	24.0	25.1	25.0

[L] figures for sub-plots which were lower down the slope

− : control

+ : 10 lb. per acre copper oxyhhloride

Data		Astor oats		Blenda oats		Forward oats		Freja barley		Maythorpe barley		Ymer barley	
		-	+	-	+	-	+	-	+	-	+	-	+
% moisture in straw	Replicate 1	43.0	48.6	39.2	39.3	40.3	38.0	23.8	25.6	19.4	26.3	28.8	27.6
	" 2	43.8	49.4	44.0	46.5	40.6	40.7	19.3	23.5	19.1	23.0	25.6	35.6
	" 3	42.7	40.1	41.4	39.0	36.8	42.6	24.5	23.7	25.2	21.2	25.0	30.6
	" 4	40.2	40.4	41.9	40.2	32.6	35.0	22.1	25.3	25.8	20.6	27.5	30.1
	Mean	42.4	44.6	41.6	41.2	37.6	39.1	22.4	24.5	22.4	22.8	26.7	31.0
Weight of a bushel of oven dry grain in lb.	Replicate 1	37.7	36.0	36.7	37.0	33.5	34.5	40.0	40.4	39.6	44.1	40.7	38.1
	" 2	37.2	37.0	37.4	37.9	35.4	33.2	43.2	40.5	41.7	40.9	37.2	42.8
	" 3	38.9	38.6	36.9	37.9	36.2	34.8	40.6	41.5	41.4	41.5	41.2	42.3
	" 4	38.4	37.8	38.9	38.8	34.5	36.6	38.1	41.0	38.4	41.8	39.2	41.6
	Mean	38.0	37.4	37.5	37.9	34.9	34.8	40.5	40.8	40.3	42.1	39.6	41.2
p.p.m. copper in plants at ear emergence	Replicate 1	1.42	2.28	1.54	3.49	1.50	1.98	1.74	3.89	1.49	3.38	1.53	3.24
	" 2	1.51	3.38	1.34	4.02	1.37	2.80	2.08	3.95	2.08	3.48	1.98	3.55
	" 3	1.61	2.50	1.64	4.66	1.58	4.99	2.22	4.53	1.51	3.03	1.48	3.68
	" 4	1.24	2.41	1.22	4.24	1.62	2.36	2.09	2.92	1.49	3.72	1.64	3.32
	Mean	1.44	2.64	1.44	4.10	1.52	3.03	2.03	3.82	1.64	3.40	1.66	3.45

-: control   +: 10 lb. per acre copper oxychloride

Data		Astor oats		Blenda oats		Forward oats		Freja barley		Maythorpe barley		Ymer barley	
		-	+	-	+	-	+	-	+	-	+	-	+
p.p.m. copper in grain	Replicate 1	1.74	2.03	1.55	2.40	1.45	1.50	2.07	3.12	1.45	2.18	1.88	2.16
	" 2	1.92	2.22	1.72	2.28	1.51	2.09	2.82	2.34	2.13	2.34	1.88	2.28
	" 3	1.88	2.20	1.90	1.91	1.60	1.86	1.90	2.93	1.70	2.59	1.66	2.74
	" 4	1.32	1.78	2.03	2.52	1.36	1.95	2.64	2.52	1.66	2.62	1.90	1.93
	Mean	1.72	2.06	1.80	2.28	1.48	1.85	2.36	2.73	1.74	2.43	1.83	2.28
p.p.m. copper in straw	Replicate 1	1.64	1.68	1.43	1.92	1.34	1.66	1.60	2.22	1.70	2.14	1.84	2.36
	" 2	1.61	1.84	1.36	1.80	1.40	1.94	1.61	1.86	1.59	2.03	2.08	2.10
	" 3	1.54	1.90	1.60	1.88	1.64	1.70	1.76	2.14	1.64	2.07	1.68	2.24
	" 4	1.51	1.64	1.42	2.16	1.28	2.05	1.82	1.80	1.48	2.38	1.55	2.05
	Mean	1.58	1.76	1.45	1.94	1.42	1.84	1.70	2.00	1.60	2.16	1.79	2.19
p.p.m. E.D.T.A. extractable copper in untreated seed-bed 14th April 1964	Replicate 1	0.64	0.51	0.53	0.47	0.44	0.62	0.60	0.50	0.53	0.52	0.42	0.56
	" 2	0.65	0.50	0.42	0.46	0.42	0.73	0.52	0.50	0.54	0.50	0.56	0.45
	" 3	0.48	0.44	0.54	0.52	0.40	0.55	0.51	0.52	0.51	0.54	0.57	0.50
	" 4	0.43	0.42	0.50	0.51	0.61	0.56	0.42	0.48	0.44	0.52	0.54	0.51
	Mean	0.55	0.47	0.50	0.49	0.47	0.62	0.51	0.50	0.50	0.52	0.52	0.50

-: control +:10 lb. per acre copper oxychloride

Data		Astor oats		Blenda oats		Forward oats		Freja barley		Maythorpe barley		Ymer barley	
		-	+	-	+	-	+	-	+	-	+	-	+
p.p.m. E.D.T.A. extractable copper in soil sampled 23rd Sept. 1964	Replicate 1	0.50	0.82	0.49	2.96	0.55	1.52	0.50	2.24	0.46	2.87	0.55	0.83
	" 2	0.58	2.01	0.52	1.98	0.53	1.28	0.60	1.36	0.44	1.30	0.79	1.17
	" 3	0.56	1.32	0.54	2.53	0.50	2.11	0.56	3.16	0.62	1.55	0.48	1.35
	" 4	0.50	1.26	0.55	1.02	0.66	1.40	0.50	4.33	0.47	1.39	0.61	2.98
	Mean	0.54	1.35	0.52	2.12	0.56	1.58	0.54	2.77	0.50	1.78	0.61	1.58

- :control　　　+:10 lb. per acre copper oxychloride

Experiment 3: p.p.m. copper in oats sampled from areas a, b and c.

Date sampled	7th May	21st May	7th June	22nd June	3rd July	21st July		6th August		26th August		8th September	
						grain	straw	grain	straw	grain	straw	grain	straw
Area a	5.20	2.88	3.44	1.99	1.57	2.41	1.34	1.60	1.28	1.30	1.24	1.51	1.20
Area b	4.33	2.14	3.43	1.96	1.76	2.59	1.54	2.36	1.45	2.10	1.49	1.94	1.48
Area c	3.99	2.20	2.95	1.74	1.62	2.68	1.38	1.55	1.39	1.78	1.36	1.74	1.50
Mean	4.51	2.41	3.27	1.90	1.65	2.56	1.42	1.84	1.37	1.73	1.36	1.73	1.39

Experiment 4: Investigation on rain water.

(i) Hexpath

Collection period	Rainfall in inches†	ml. collected	p.p.m. copper in water[x]	μg. copper collected	Mean daily copper deposited (g./acre)
1964					
May 21st to June 7th	1.17	about 500	0.013	6.5	0.41
June 7th to June 22nd	1.20	670	0.007	4.7	0.34
June 22nd to July 21st	0.61	270	0.015	4.0	0.15
July 21st to Aug. 6th	0.36	170	0.035	6.0	0.41
Aug. 6th to Aug. 26th	4.12	1,600	0.002	3.2	0.17
Aug. 26th to Sept. 8th	0.76	270	0.003	0.8	0.07
May 21st to Sept. 8th	8.22	3,480	0.012[o] 0.007[z]	25.2	0.25

† figures from the meteorological station at Marchmont House, Berwickshire

x average of duplicate determinations

o average of concentrations measured

z average concentration calculated from the total μg. copper and ml. collected.

(ii) Edinburgh

Collection period	Rainfall in inches†	ml. collected	p.p.m. copper in water[x]	µg. copper collected	Mean daily copper deposited (g./acre)
1964					
May 22 - June 7	0.87	about 500	0.021	10.5	0.72
June 7 - June 23	1.24	720	0.005	3.6	0.24
June 23 - July 21	0.37	250	0.062	15.5	0.60
July 21 - Aug.6	0.18	90	0.026	2.3	0.15
Aug.6 - Aug.26	2.83	1,480	0.006	8.9	0.48
Aug.26 - Sep.8	0.63	340	0.010	3.4	0.28
May 22 - Sep.8	6.12	3,380	0.022[o] 0.013[z]	44.2	0.45
Sep.8 - Oct.1	1.50	1,050	0.004	4.2	0.20
Oct.1 - Oct.22	2.33	1,450	0.005	7.2	0.37
Oct.22 - Nov.12	0.16	135	0.063	8.5	0.44
Nov.12 - Dec.3	0.89	680	0.011	7.5	0.39
Dec.3 - Dec.22	1.96	1,270	0.007	8.9	0.51
Dec.22 - Jan.14	1.35	1,020	0.011	11.2	0.53
1965					
Jan.14 - Feb.4	1.14	620	0.014	8.7	0.45
Feb.4 - Feb.25	0.26	220	0.045	9.9	0.51
Feb.25 - Mar.19	1.38	680	0.041	27.9	1.38
Mar.19 - Apr.15	2.54	1,750	0.026	45.5	1.84
Apr.15 - May 6	1.44	920	0.025	23.0	1.20
May 6 - May 27	2.11	1,290	0.012	15.5	0.81
May 22nd 64 - May 27th 65.	23.18	14,465	0.022[o] 0.015[z]	222.2	0.65

† figures from the meteorological station at Blackford Hill Observatory, Edinburgh

x average of duplicate determinations

o average of concentrations measured

z average concentration calculated from the total µg. copper and ml. collected.

APPENDIX 8

p.p.m. copper in grain from a field experiment conducted by R. Bain† in 1962

(i) Oats

Variety	Replicate 1	2	3	4	Mean
Manod	3.20	3.43	3.06	3.42	3.28
M.G.H. 856	3.81	3.84	3.87	3.37	3.72
Marino	3.84	3.78	3.75	3.98	3.84
M.G.H. 462 (Karin)	3.71	3.48	3.59	3.71	3.62
Sun II	3.38	3.95	3.89	4.10	3.83
Vollbringer	3.90	4.46	4.21	4.38	4.24
Flamingskrone	3.50	3.59	3.49	3.98	3.64
Astor	3.66	3.92	3.97	3.64	3.80

(ii) Winter wheat

Variety	Replicate 1	2	3	4	Mean
Champlein	5.18	4.87	4.69	4.64	4.84
Vil. 5905	5.05	4.26	5.04	4.88	4.81
C6 (Rallye)	4.80	4.62	4.45	4.29	4.54
Prestige	4.47	3.65	4.37	4.59	4.27
Cappelle	4.92	5.02	4.86	4.97	4.94
Vil. line G	5.60	5.18	5.26	5.21	5.31

† Crop Husbandry Dept., North of Scotland College of Agriculture. (reference: Bain 1965)

APPENDIX 9

Results from greenhouse experiment 1962

Treatments: copper: - control
+ soil dressing equivalent to 20 lb./acre copper

water: period at 40% water capacity instead of 80%

- a none
- b 28th May until 13th Aug.
- c 28th May until 25th June
- d 11th June until 9th July
- e 25th June until 23rd July
- f 9th July until 6th Aug.
- g 23rd July until 13th Aug.

Data	Water treatment	Copper treatment	Replicate 1	2	3	4	Mean†
Oven dry yield in g. per 10 plants at first harvest	Average a,d,e,f,g	+	4.09	4.44	3.92	4.10	4.15
	Average a,d,e,f,g	-	3.80	3.67	3.88	3.66	3.74
	Average b,c	+	3.98	3.62	3.40	3.56	3.53
	Average b,c	-	3.50	3.22	3.18	3.06	3.15
Oven dry yield in g. per 10 plants at second harvest	Average a,f,g	+	11.93	12.23	11.38	12.62	12.08
	Average a,f,g	-	10.82	10.72	11.59	10.78	11.03
	b	+	10.95	11.18	10.98	10.44	10.87
	b	-	10.51	9.48	9.85	10.08	9.81
	c	+	10.54	10.49	10.72	10.56	10.59
	c	-	10.41	10.67	9.15	9.34	9.72
	d	+	11.13	12.48	11.93	12.01	12.14
	d	-	12.12	10.97	11.83	11.12	11.31
	e	+	11.30	12.12	12.84	12.43	12.46
	e	-	11.00	11.75	10.64	10.29	10.89

† mean of replicates 2, 3 and 4.

Data	Water treat-ment	Copper treat-ment	Replicate 1	2	3	4	Mean†
Yield in g. of oven dry grain per 10 plants	a	+	6.21	7.01	7.16	7.48	7.22
	a	-	7.20	6.06	5.30	5.80	5.72
	b	+	-	7.35	6.30	6.65	6.77
	b	-	6.66	5.92	6.19	6.33	6.15
	c	+	7.40	6.80	7.06	6.43	6.76
	c	-	6.78	6.49	5.74	5.66	5.96
	d	+	6.97	7.58	7.90	7.58	7.69
	d	-	6.97	6.44	6.97	6.91	6.77
	e	+	6.91	6.93	7.08	6.89	6.97
	e	-	6.62	6.16	5.68	5.51	5.78
	f	+	7.85	6.36	6.40	7.16	6.64
	f	-	6.58	5.04	6.27	6.22	5.84
	g	+	6.41	8.21	6.95	7.72	7.63
	g	-	6.20	5.36	6.28	5.50	5.71
Yield in g. of oven dry straw per 10 plants	a	+	9.19	9.18	9.24	9.65	9.37
	a	-	9.22	8.96	10.00	8.64	9.20
	b	+	-	8.40	8.23	7.82	8.15
	b	-	7.53	7.29	7.55	7.68	7.51
	c	+	8.04	8.16	8.36	8.02	8.18
	c	-	7.88	7.93	7.47	6.82	7.41
	d	+	8.08	9.52	9.58	9.38	9.49
	d	-	8.22	8.16	8.40	8.47	8.34
	e	+	8.14	9.26	9.42	8.82	9.17
	e	-	8.29	8.07	8.57	7.75	8.13
	f	+	9.44	8.47	8.49	9.05	8.67
	f	-	9.14	7.81	9.05	8.94	8.60
	g	+	8.08	10.61	9.02	10.23	9.95
	g	-	8.71	8.10	9.49	8.44	8.68
% by weight of grain in the final harvest	a	+		44.4	45.0	44.9	44.8
	a	-		41.4	35.6	41.2	39.4
	b	+		48.0	45.4	47.3	46.9
	b	-		46.1	46.2	46.4	46.2

† mean of replicates 2, 3 and 4

Data	Water treat-ment	Copper treat-ment	Replicate 1	2	3	4	Mean†
% by weight of grain in the final harvest (contd.)	c	+		46.5	47.1	45.9	46.5
	c	-		46.4	44.6	46.6	45.8
	d	+		45.5	46.4	46.1	46.0
	d	-		45.3	46.4	46.3	46.0
	e	+		43.9	44.1	44.9	44.3
	e	-		44.3	40.8	42.8	42.6
	f	+		44.0	44.2	45.2	44.5
	f	-		40.3	42.0	42.3	41.5
	g	+		44.9	44.8	44.4	44.7
	g	-		40.8	40.5	40.6	40.6
Number of ears per plant at final harvest	a	+	12.4	12.8	12.8	13.3	13.0
	a	-	13.1	11.6	11.2	10.5	11.1
	b	+	-	12.9	12.0	11.8	12.2
	b	-	11.9	10.2	10.5	10.8	10.5
	c	+	12.8	11.5	11.8	11.0	11.4
	c	-	11.4	11.1	9.6	9.1	9.9
	d	+	12.4	13.9	14.7	12.9	13.8
	d	-	12.4	10.6	11.9	11.8	11.4
	e	+	12.5	12.4	13.1	12.7	12.7
	e	-	12.3	11.0	10.8	10.4	10.7
	f	+	15.0	12.2	12.0	12.6	12.3
	f	-	12.3	9.3	12.1	10.9	10.8
	g	+	12.2	15.2	13.3	14.0	14.2
	g	-	12.3	10.3	12.4	10.3	11.0
Number of grains per plant at final harvest	a	+	19.9	22.0	21.9	22.7	22.2
	a	-	22.1	18.6	18.1	17.8	18.2
	b	+	-	22.3	20.5	20.5	21.1
	b	-	20.4	16.3	17.9	19.1	17.8
	c	+	22.6	20.0	20.9	18.7	19.9
	c	-	19.7	19.3	16.6	15.8	17.2

† mean of replicates 2, 3 and 4

Data	Water treatment	Copper treatment	Replicate 1	2	3	4	Mean†
Number of grains per plant at final harvest (contd.)	d	+	21.6	23.9	26.0	22.5	24.1
	d	-	21.8	18.8	20.9	20.9	20.2
	e	+	22.3	20.8	22.0	22.3	21.7
	e	-	20.6	18.0	17.3	16.6	17.3
	f	+	26.2	19.5	18.7	22.3	20.2
	f	-	20.7	15.3	20.0	18.1	17.8
	g	+	20.5	25.8	22.9	24.3	24.3
	g	-	19.4	16.5	19.5	16.6	17.5
Number of tillers per 10 plants at final harvest	a	+	3	0	0	0	0
	a	-	0	2	9	3	4.7
	b	+	-	1	4	0	1.7
	b	-	0	0	1	0	0.3
	c	+	0	2	1	1	1.3
	c	-	0	3	0	0	1.0
	d	+	0	0	0	0	0
	d	-	0	0	0	0	0
	e	+	0	3	0	0	1.0
	e	-	0	0	3	3	2.0
	f	+	0	3	0	0	1.0
	f	-	1	2	2	1	1.7
	g	+	0	0	0	0	0
	g	-	1	4	3	4	3.7
p.p.m. copper in plants at first harvest	Average a,d,e,f,g	+	5.37	5.70	5.57	5.98	5.75
	Average a,d,e,f,g	-	2.24	2.04	2.03	2.19	2.09
	Average b,c	+	6.43	6.42	6.60	6.68	6.57
	Average b,c	-	2.90	2.64	2.42	2.57	2.54

† mean of replicates 2,3 and 4

Data	Water treat-ment	Copper treat-ment	Replicate 1	2	3	4	Mean†
p.p.m. copper in plants at second harvest	Average a,f,g	+	2.62	2.77	2.85	3.09	2.90
	Average a,f,g	-	1.41	1.19	1.29	1.26	1.25
	b	+	3.60	3.63	3.81	3.76	3.73
	b	-	1.92	1.58	1.76	1.48	1.61
	c	+	3.36	3.34	3.45	3.54	3.44
	c	-	1.47	1.32	0.93	1.48	1.24
	d	+	3.14	3.44	3.26	3.50	3.40
	d	-	2.02	1.41	1.34	1.66	1.47
	e	+	2.74	2.88	3.09	2.95	2.97
	e	-	1.40	1.20	1.30	1.61	1.37
p.p.m. copper in grain	a	+	3.66	3.92	4.25	3.99	4.05
	a	-	2.13	1.62	2.02	1.64	1.76
	b	+	-	6.00	5.18	5.29	5.49
	b	-	2.34	1.94	1.93	1.70	1.86
	c	+	4.92	4.62	4.10	5.16	4.63
	c	-	1.85	1.73	1.88	2.11	1.91
	d	+	4.65	5.18	4.53	4.91	4.87
	d	-	2.64	2.18	1.86	2.26	2.10
	e	+	4.38	4.99	4.74	6.02	5.25
	e	-	2.13	1.88	2.05	1.91	1.95
	f	+	3.57	4.16	4.80	3.78	4.25
	f	-	2.36	2.45	1.60	2.20	2.08
	g	+	3.60	3.92	3.78	4.41	4.04
	g	-	2.00	1.92	1.93	1.93	1.93
p.p.m. copper in straw	a	+	2.60	2.19	2.25	2.12	2.19
	a	-	1.52	1.27	1.22	1.64	1.38
	b	+	-	2.50	2.76	2.64	2.63
	b	-	1.92	1.40	1.37	1.42	1.40
	c	+	2.53	2.53	2.40	2.53	2.49
	c	-	1.51	1.45	1.25	1.57	1.42

† mean of replicates 2,3 and 4

Data	Water treatment	Copper treatment	Replicate 1	2	3	4	Mean†
p.p.m. copper in straw (contd.)	d	+	2.29	2.39	2.37	2.48	2.41
	d	−	1.61	1.32	1.28	1.63	1.41
	e	+	2.10	2.44	2.30	2.34	2.36
	e	−	1.37	1.22	1.14	1.03	1.13
	f	+	2.10	2.13	2.19	1.70	2.01
	f	−	1.50	1.46	1.67	1.18	1.44
	g	+	2.33	2.12	2.00	2.14	2.09
	g	−	1.36	1.14	(1.19)ǂ	1.28	1.20
Copper uptake in μg. per 10 plants at first harvest	Average a,d,e,f,g	+		25.3	21.8	24.4	23.8
	Average a,d,e,f,g	−		7.5	7.8	8.0	7.8
	Average b,c	+		23.3	22.4	23.8	23.2
	Average b,c	−		8.5	7.6	8.0	8.0
Copper uptake in μg. per 10 plants at second harvest	Average a,f,g	+		33.9	32.5	38.9	35.1
	Average a,f,g	−		12.8	15.0	13.6	13.8
	b	+		40.6	41.8	39.2	40.5
	b	−		15.0	17.3	14.9	15.7
	c	+		35.0	37.0	37.4	36.5
	c	−		14.1	8.5	13.8	12.1
	d	+		42.9	38.9	42.0	41.3
	d	−		15.5	15.8	18.5	16.6
	e	+		34.9	39.7	36.7	37.1
	e	−		14.1	13.8	16.6	14.8
Copper uptake in μg. per 10 plants in grain	a	+		27.5	30.4	29.8	29.2
	a	−		9.8	10.7	9.5	10.0
	b	+		44.1	32.6	35.2	37.3
	b	−		11.5	11.9	10.8	11.4

† mean of replicates 2, 3 and 4

ǂ estimated figure for a missing pot

Data	Water treat-ment	Copper treat-ment	Replicate 1	2	3	4	Mean†
Copper uptake in μg. per 10 plants in grain (contd.)	c	+		31.4	28.9	33.2	31.2
	c	−		11.2	10.8	11.9	11.3
	d	+		39.3	35.8	37.2	37.4
	d	−		14.0	13.0	15.6	14.2
	e	+		34.6	33.6	41.5	36.6
	e	−		11.6	11.6	10.5	11.2
	f	+		26.5	30.7	27.1	28.1
	f	−		12.3	10.0	13.7	12.0
	g	+		32.2	26.3	34.0	30.8
	g	−		10.3	12.1	10.6	11.0
Copper uptake in μg. per 10 plants in straw	a	+		20.1	20.8	20.5	20.5
	a	−		11.4	12.2	14.2	12.6
	b	+		21.0	22.7	20.6	21.4
	b	−		10.2	10.3	10.9	10.5
	c	+		20.6	20.1	20.3	20.3
	c	−		11.5	9.3	10.7	10.5
	d	+		22.8	22.7	23.3	22.9
	d	−		10.8	10.8	13.8	11.8
	e	+		22.6	21.7	20.6	21.6
	e	−		9.8	9.8	8.0	9.2
	f	+		18.0	18.6	15.4	17.3
	f	−		11.4	15.1	10.5	12.3
	g	+		22.5	18.0	21.9	20.8
	g	−		9.2	(11.3)‡	10.8	10.4
p.p.m. iron in plants at first harvest	Average a,d,e,f,g	+		49.3	43.7	41.3	44.8
	Average a,d,e,f,g	−		54.0	53.4	54.5	54.0
	Average b,c	+		46.1	43.5	42.7	44.1
	Average b,c	−		73.0	61.8	68.4	67.7

† mean of replicates 2, 3 and 4

‡ estimated figure for a missing pot

Data	Water treatment	Copper treatment	Replicate 1	2	3	4	Mean†
Iron uptake in μg. per 10 plants at first harvest	Average a,d,e,f,g	+		219	171	169	186
	Average a,d,e,f,g	-		198	207	199	201
	Average b,c	+		167	148	152	156
	Average b,c	-		235	197	209	214
p.p.m. manganese in plants at first harvest	Average a,d,e,f,g	+		226	206	204	212
	Average a,d,e,f,g	-		214	153	221	196
	Average b,c	+		175	228	193	199
	Average b,c	-		178	211	216	202
Manganese uptake in μg. per 10 plants at first harvest	Average a,d,e,f,g	+		1003	808	836	882
	Average a,d,e,f,g	-		785	594	809	729
	Average b,c	+		634	775	687	699
	Average b,c	-		573	671	661	635
p.p.m. E.D.T.A. extractable copper in soil at final harvest	a	+	7.00	7.24	7.31	7.45	7.33
	a	-	0.70x	0.46	0.35	0.37	0.39
	b	+	-	8.62	7.31	7.94	7.96
	b	-	0.74x	0.41	0.54	0.39	0.45
	c	+	7.80	7.26	6.19	6.78	6.74
	c	-	0.37	0.43	0.37	0.37	0.39

† mean of replicates 2, 3 and 4

x soils mixed in batch 7 before pot filling

Data	Water treat-ment	Copper treat-ment	Replicate 1	2	3	4	Mean†
p.p.m. E.D.T.A. extract-able copper in soil at final harvest (contd.)	d	+	7.08	9.36	8.43	7.42	8.40
	d	-	0.61ˣ	0.40	0.41	0.38	0.40
	e	+	7.26	7.86	8.43	7.80	8.03
	e	-	0.40	0.35	0.34	0.37	0.35
	f	+	9.02	8.38	7.74	8.16	8.09
	f	-	0.76ˣ	0.39	0.42	0.32	0.38
	g	+	7.52	9.56	7.84	8.41	8.60
	g	-	0.34	0.48	0.46	0.42	0.45

† mean of replicates 2, 3 and 4

ˣ soils mixed in batch 7 before pot filling

APPENDIX 10

Results from greenhouse experiment 1963

Treatments: Copper: + and -: residual from 1962

Light: L: control, full light

M: green shading

D: heavy green shading and brown paper shading

Water: wet: 80% field capacity

dry: 40% field capacity

Data	Treatment			Replicate				Mean
	Light	Water	Copper	1	2	3	4	
Oven dry yield in g. per 10 plants at first harvest	D	wet	+	1.28	1.04	1.31	1.39	1.26
	D	wet	-	1.10	0.95	1.06	1.23	1.08
	D	dry	+	1.10	1.15	1.22	1.26	1.18
	D	dry	-	1.15	1.02	1.12	1.00	1.07
	M	wet	+	1.93	2.20	2.00	2.34	2.12
	M	wet	-	1.81	1.90	2.06	1.55	1.83
	M	dry	+	2.01	2.36	2.00	1.78	2.04
	M	dry	-	2.13	1.71	1.61	1.66	1.78
	L	wet	+	3.39	1.98	3.06	3.51	2.98
	L	wet	-	2.51	2.46	2.40	2.59	2.49
	L	dry	+	2.99	3.10	2.58	2.47	2.78
	L	dry	-	2.32	2.26	2.12	2.75	2.36
Oven dry yield in g. per 10 plants at second harvest	D	wet	+	4.64	4.81	4.74	5.69	4.97
	D	wet	-	3.76	4.53	3.86	4.56	4.18
	D	dry	+	3.95	4.78	4.74	4.34	4.45
	D	dry	-	4.63	3.94	4.57	4.50	4.41
	M	wet	+	7.29	8.54	7.69	8.18	7.92
	M	wet	-	7.82	6.79	7.07	7.26	7.24
	M	dry	+	6.10	7.47	7.68	6.58	6.96
	M	dry	-	6.77	5.66	6.56	5.23	6.06

Data	Treatment			Replicate				
	Light	Water	Copper	1	2	3	4	Mean
Oven dry yield in g. per 10 plants at second harvest (contd.)	L	wet	+	10.82	7.98	10.20	10.48	9.87
	L	wet	-	9.43	8.37	8.69	8.78	8.82
	L	dry	+	10.47	10.67	9.17	8.86	9.79
	L	dry	-	9.06	8.98	8.05	9.93	9.00
Yield in g. of oven dry grain per 10 plants	D	wet	+	2.41	3.04	3.49	3.08	3.00
	D	wet	-	2.50	2.40	2.22	2.66	2.44
	D	dry	+	1.99	2.63	2.79	2.46	2.47
	D	dry	-	2.79	2.31	2.50	2.45	2.51
	M	wet	+	4.19	5.08	4.30	4.81	4.60
	M	wet	-	4.24	4.05	3.73	4.25	4.07
	M	dry	+	3.43	4.28	3.88	3.54	3.78
	M	dry	-	4.03	3.39	3.65	2.57	3.41
	L	wet	+	6.14	4.29	5.36	5.50	5.32
	L	wet	-	4.71	4.88	4.84	4.51	4.74
	L	dry	+	5.50	5.18	4.66	4.28	4.90
	L	dry	-	4.59	5.39	3.99	5.32	4.82
Yield in g. of oven dry straw per 10 plants	D	wet	+	3.76	4.05	3.82	4.87	4.12
	D	wet	-	4.07	4.40	3.90	3.87	4.06
	D	dry	+	4.01	2.99	4.21	4.06	3.82
	D	d ry	-	3.70	4.03	3.30	3.73	3.69
	M	wet	+	5.28	6.20	5.50	5.83	5.70
	M	wet	-	5.20	4.75	4.97	5.62	5.14
	M	dry	+	4.10	4.96	4.72	4.27	4.51
	M	dry	-	4.42	3.89	4.06	3.53	3.98
	L	wet	+	7.23	5.05	6.40	6.39	6.27
	L	wet	-	5.79	5.65	5.59	5.62	5.66
	L	dry	+	6.16	6.02	5.31	5.32	5.70
	L	dry	-	5.24	5.63	4.81	6.04	5.43

Data	Treatment			Replicate				Mean
	Light	Water	Copper	1	2	3	4	
% by weight of grain in the final harvest	D	wet	+	39.1	42.9	47.7	38.7	42.1
	D	wet	-	38.0	35.3	36.3	40.7	37.6
	D	dry	+	33.2	46.8	39.9	37.7	39.4
	D	dry	-	43.0	36.4	43.1	39.6	40.5
	M	wet	+	44.2	45.0	43.9	45.2	44.6
	M	wet	-	44.9	46.0	42.9	43.1	44.2
	M	dry	+	45.6	46.3	45.1	45.3	45.6
	M	dry	-	47.7	46.6	47.3	42.1	45.9
	L	wet	+	45.9	45.9	45.6	46.3	45.9
	L	wet	-	44.9	46.3	46.4	44.5	45.5
	L	dry	+	47.2	46.2	46.7	44.6	46.2
	L	dry	-	46.7	48.9	45.3	46.8	46.9
Number of ears per plant at final harvest	D	wet	+	4.7	5.2	5.5	5.2	5.2
	D	wet	-	4.1	4.3	3.8	4.7	4.2
	D	dry	+	4.5	4.6	5.0	4.8	4.7
	D	dry	-	4.5	4.0	4.5	4.7	4.4
	M	wet	+	7.4	8.9	7.9	8.8	8.2
	M	wet	-	7.4	7.0	6.8	7.7	7.2
	M	dry	+	6.2	7.8	7.0	6.3	6.8
	M	dry	-	6.9	6.3	6.3	4.8	6.1
	L	wet	+	9.5	6.7	8.8	8.7	8.4
	L	wet	-	7.8	7.8	7.6	7.4	7.6
	L	dry	+	9.5	8.7	7.4	7.0	8.2
	L	dry	-	7.7	8.9	6.4	9.0	8.0
Number of grains per plant at final harvest	D	wet	+	8.0	9.6	10.6	9.7	9.5
	D	wet	-	8.2	9.9	8.2	9.3	8.9
	D	dry	+	8.5	8.4	8.6	8.4	8.5
	D	dry	-	8.7	8.4	8.1	8.3	8.4
	M	wet	+	13.0	15.2	13.2	15.4	14.2
	M	wet	-	13.3	12.3	11.3	14.1	12.8
	M	dry	+	10.5	13.7	11.8	10.9	11.7
	M	dry	-	12.3	10.7	11.0	8.9	10.7

Data	Light	Water	Copper	1	2	3	4	Mean
	Treatment			Replicate				
Number of grains per plant at final harvest (contd.)	L	wet	+	18.0	11.8	15.9	16.5	15.6
	L	wet	-	13.1	14.2	13.6	13.4	13.6
	L	dry	+	17.3	15.5	13.5	12.8	14.8
	L	dry	-	14.2	16.5	11.8	16.8	14.8
Number of tillers per 10 plants at final harvest	D	wet	+	5	3	2	7	4.2
	D	wet	-	18	19	12	7	14.0
	D	dry	+	13	2	10	12	9.2
	D	dry	-	17	13	11	13	13.5
	M	wet	+	2	0	0	0	0.5
	M	wet	-	0	0	2	2	1.0
	M	dry	+	1	0	0	0	0.2
	M	dry	-	0	3	1	4	2.0
	L	wet	+	0	0	0	0	0.0
	L	wet	-	0	1	0	3	1.0
	L	dry	+	0	1	0	2	0.8
	L	dry	-	1	0	3	1	1.2
p.p.m. copper in plants at first harvest	D	wet	+	7.08	7.42	7.16	7.22	7.22
	D	wet	-	1.69	1.70	1.92	1.94	1.81
	D	dry	+	7.70	8.28	7.91	7.53	7.86
	D	dry	-	2.13	2.15	2.25	2.50	2.26
	M	wet	+	5.63	5.99	(5.78)†	5.50	5.72
	M	wet	-	1.69	1.62	1.87	1.96	1.78
	M	dry	+	6.65	6.97	7.34	6.92	6.97
	M	dry	-	2.60	2.41	2.83	2.73	2.64
	L	wet	+	5.18	4.82	4.97	4.79	4.94
	L	wet	-	2.00	1.73	1.99	1.95	1.92
	L	dry	+	6.00	5.98	5.64	5.64	5.82
	L	dry	-	2.65	2.70	2.58	2.68	2.65
p.p.m. copper in grain	D	wet	+	6.14	6.34	6.19	6.56	6.31
	D	wet	-	4.53	6.72	4.75	4.35	5.09
	D	dry	+	8.17	7.05	7.48	7.76	7.62
	D	dry	-	6.07	5.90	6.22	5.98	6.04

† estimated figure for a missing pot

Data	Treatment			Replicate				Mean
	Light	Water	Copper	1	2	3	4	
p.p.m. copper in grain (contd.)	M	wet	+	5.52	4.92	5.03	5.12	5.15
	M	wet	-	3.82	3.79	4.82	4.39	4.20
	M	dry	+	5.83	6.24	6.38	6.42	6.22
	M	dry	-	5.05	5.11	5.42	5.62	5.30
	L	wet	+	4.30	4.39	4.74	4.91	4.58
	L	wet	-	3.55	4.68	3.29	3.51	3.78
	L	dry	+	5.44	5.60	5.40	5.63	5.52
	L	dry	-	4.06	4.00	4.52	4.18	4.19
p.p.m. copper in straw	D	wet	+	4.79	3.92	3.30	3.73	3.94
	D	wet	-	4.59	3.40	4.36	2.66	3.75
	D	dry	+	6.09	4.65	5.87	6.10	5.68
	D	dry	-	6.85	4.11	5.60	3.70	5.06
	M	wet	+	2.90	2.53	3.13	2.99	2.89
	M	wet	-	1.83	2.45	2.31	3.46	2.51
	M	dry	+	3.32	3.27	3.33	3.72	3.41
	M	dry	-	3.73	4.34	3.17	2.88	3.53
	L	wet	+	2.40	2.47	2.87	2.40	2.54
	L	wet	-	2.05	3.07	2.49	1.85	2.36
	L	dry	+	2.90	2.88	3.24	3.70	3.18
	L	dry	-	3.21	2.26	2.97	2.82	2.82
Copper uptake in μg. per 10 plants at first harvest	D	wet	+	9.06	7.72	9.38	10.04	9.05
	D	wet	-	1.86	1.62	2.04	2.39	1.98
	D	dry	+	8.47	9.52	9.65	9.49	9.28
	D	dry	-	2.45	2.19	2.52	2.50	2.42
	M	wet	+	10.87	13.18	(11.98)†	12.87	12.22
	M	wet	-	3.06	3.08	3.85	3.04	3.26
	M	dry	+	13.37	16.45	14.68	12.32	14.20
	M	dry	-	5.54	4.12	4.56	4.53	4.69
	L	wet	+	17.56	9.54	15.21	16.81	14.78
	L	wet	-	5.02	4.26	4.78	5.05	4.78
	L	dry	+	17.94	18.54	14.55	13.93	16.24
	L	dry	-	6.15	6.10	5.47	7.37	6.27

† estimated figure for a missing pot

Data	Treatment Light	Water	Copper	Replicate 1	2	3	4	Mean
Copper uptake in μg. per 10 plants in grain	D	wet	+	14.8	19.3	21.6	20.2	19.0
	D	wet	-	11.3	16.1	10.5	11.6	12.4
	D	dry	+	16.3	18.5	20.9	19.1	18.7
	D	dry	-	16.9	13.6	15.6	14.6	15.2
	M	wet	+	23.1	25.0	21.6	24.6	23.6
	M	wet	-	16.2	15.4	18.0	18.7	17.0
	M	dry	+	20.0	26.7	24.8	22.7	23.6
	M	dry	-	20.4	17.3	19.8	14.4	18.0
	L	wet	+	26.4	18.8	25.4	27.0	24.4
	L	wet	-	16.7	22.8	15.9	15.8	17.8
	L	dry	+	29.9	29.0	25.2	24.1	27.0
	L	dry	-	18.6	21.6	18.0	22.2	20.1
Copper uptake in μg. per 10 plants in straw	D	wet	+	18.0	15.9	12.6	18.2	16.2
	D	wet	-	18.7	15.0	17.0	10.3	15.2
	D	dry	+	24.4	13.9	24.7	24.8	22.0
	D	dry	-	25.3	16.6	18.5	13.8	18.5
	M	wet	+	15.3	15.7	17.2	17.4	16.4
	M	wet	-	9.5	11.6	11.5	19.4	13.0
	M	dry	+	13.6	16.2	15.7	15.9	15.4
	M	dry	-	16.5	16.9	12.9	10.2	14.1
	L	wet	+	17.4	12.5	18.4	15.3	15.9
	L	wet	-	11.9	17.4	13.9	10.4	13.4
	L	dry	+	17.9	17.3	17.2	19.7	18.0
	L	dry	-	16.8	12.7	14.3	17.0	15.2
% potassium in grain	L	wet	+	0.55	0.59	0.55	0.59	0.57
	L	wet	-	0.55	0.59	0.56	0.59	0.57
p.p.m. E.D.T.A. extract-able copper in the soil at final harvest	D	wet	+	6.75	7.76	6.81	7.47	7.20
	D	wet	-	0.40	0.44	0.41	0.46	0.43
	D	dry	+	6.55	7.37	8.18	7.90	7.50
	D	dry	-	0.40	0.46	0.43	0.44	0.43

Data	Treatment			Replicate				Mean
	Light	Water	Copper	1	2	3	4	
p.p.m. E.D.T.A. extract-able copper in the soil at final harvest (contd.)	M	wet	+	7.12	7.49	7.45	7.22	7.32
	M	wet	-	0.41	0.46	0.45	0.43	0.44
	M	dry	+	7.30	7.73	8.24	7.51	7.70
	M	dry	-	0.50	0.47	0.48	0.50	0.49
	L	wet	+	6.94	7.55	7.16	7.10	7.19
	L	wet	-	0.48	0.47	0.41	0.44	0.45
	L	dry	+	7.43	7.69	8.00	7.47	7.65
	L	dry	-	0.46	0.40	0.43	0.42	0.43

Environmental data.

Period of measurement	Maximum Temperature °C		Light integration: mg. oxalic acid destroyed			hours sunshine †
	Treatment		Treatment			
	Dark	Light	Dark	Medium	Light	
20 May	17	18.5	0.08	2.21	4.98	0.0
21 May	23	26	0.08	3.95	9.16	3.2
22 May	24.5	27.5	0.16	5.29	12.32	7.6
23 May	28.5	32	0.16	5.37	13.75	10.0
24 May	14	15.5	0.08	1.26	2.92	0.1
25,26 May	33.5	35	0.79	8.61	18.72	3.7
27,28 May	32	35.5	1.11	11.77	28.12	21.2
29,30 May	29.5	35	1.34	14.14	33.50	23.2
31 May, 1 June	36	40	5.45	13.11	28.91	19.2
2,3 June	32	34	5.06	14.06	31.05	22.0
4,5 June	27	30	2.53	8.14	18.64	7.1
6,7 June	27	31	2.05	6.64	16.35	6.0
8,9 June	37	37.5	5.29	11.61	26.70	16.3
10,11 June	39	40.5	4.42	10.51	22.67	14.2
12,13 June	27	30	1.81	3.71	8.65	1.3
14,15 June	31	34	3.78	7.47	18.25	8.8
16,17 June	33.5	35	2.75	6.45	14.86	5.7
18,19 June	30	32	3.93	7.79	18.72	9.0
20,21 June	31	34	3.38	7.39	17.15	8.7
22,23 June	33	36.5	5.58	11.88	28.08	10.0
24,25 June	28	30.5	4.33	8.89	20.69	5.5
26,27 June	30	32	2.20	4.80	11.33	4.1
28,29 June	11.5	12	0.39	1.02	2.36	0.0
30 June 1,2,3 July	20.5	23	2.04	4.80	10.15	0.5
4,5,6,7 July	32	30	3.46	8.34	18.56	1.9
8,9 July	27	30	3.30	7.47	16.91	4.6
10,11 July	21.5	24	1.89	4.17	9.83	0.5
12,13 July	37	-	2.44	6.45	13.45	7.8
14,15 July	28	32	2.92	7.27	17.00	4.5
16,17 July	26	30	3.00	8.14	19.37	8.6
18,19 July	35	36.5	2.77	8.30	17.15	9.8
20,21 July	40	42	5.22	13.99	32.02	18.7
22,23 July	41	43	3.08	9.25	20.63	9.8
24,25 July	31	32.5	4.19	11.46	24.74	17.6
26,27 July	35	36.5	4.43	12.33	27.04	20.9
28,29 July	40	44	4.74	13.36	31.07	24.0
30,31 July	42	44	3.64	10.99	25.14	17.6
1,2 August	35	39	2.45	8.22	19.76	10.5
3,4 August	21	23	0.95	2.92	6.64	0.0
5,6 August	27.5	29.5	1.34	4.27	9.17	1.1
7,8 August	31	34	2.69	8.70	18.97	11.0
9,10 August	30	32.5	2.37	8.38	18.26	9.4
11,12 August	26.5	28	1.90	6.01	13.83	2.3
13,14 August	27.5	30	1.74	6.01	13.52	5.3
15,16 August	29.5	30.5	1.66	5.68	12.47	8.4
17,18 August	29.5	30	0.95	3.71	7.50	

† results from Bush meteorological station

APPENDIX 11

Results from greenhouse experiments 1964

Experiment (1) Treatments:

W: E.D.T.A. extractable copper concentration in the spring soil = 6.1 p.p.m.

X: E.D.T.A. extractable copper concentration in the spring soil = 4.6 p.p.m.

Y: E.D.T.A. extractable copper concentration in the spring soil = 0.9 p.p.m.

Z: E.D.T.A. extractable copper concentration in the spring soil = 0.3 p.p.m.

Experiment (2) Treatments:

Copper: + and - residual from 1962

Water: P: 80% field capacity

Q: 40% field capacity

R: 20% field capacity

S: 40% field capacity until 9th June, and then 80% field capacity

T: 20% field capacity until 9th June, and then 40% field capacity

Note: W ≡ Q + and Z ≡ Q -

Data	Treatment		Replicate				Mean
	Water	Copper	1	2	3	4	
Oven dry yield in g. per 10 plants at first harvest	P	+	3.98	3.87	3.61	3.77	3.81
	P	-	3.55	3.99	3.36	4.50	3.85
	Average Q,S	+(W)	2.68	2.86	2.67	2.93	2.78
	Q	X	3.53	3.03	3.10	3.03	3.17
	Q	Y	2.84	2.79	3.04	2.98	2.91
	Average Q,S	-(Z)	2.36	2.08	2.26	2.34	2.26
	Average R,T	+	0.89	1.08	1.32	1.48	1.19
	Average R,T	-	0.84	0.86	0.85	0.90	0.86

Data	Treatment		Replicate				Mean
	Water	Copper	1	2	3	4	
Oven dry yield in g. per 10 plants at second harvest	P	+	13.5	14.0	13.1	14.1	13.7
	P	-	11.7	14.0	11.3	13.7	12.7
	Q	+(W)	13.7	12.3	12.3	13.0	12.8
	Q	X	12.2	12.9	12.6	12.4	12.5
	Q	Y	12.3	14.3	12.3	12.6	12.9
	Q	-(Z)	12.2	13.2	10.4	10.7	11.6
	R	+	7.3	7.1	9.2	8.7	8.1
	R	-	6.2	6.4	6.6	7.0	6.6
	S	+	11.9	14.0	12.8	11.6	12.6
	S	-	15.0	13.4	13.3	13.4	13.8
	T	+	9.3	8.9	10.7	10.3	9.8
	T	-	9.2	8.9	9.1	8.9	9.0
Yield in g. of oven dry grain per 10 plants	P	+	7.51	8.64	7.10	7.24	7.62
	P	-	6.72	7.61	6.48	7.76	7.14
	Q	+(W)	7.30	7.00	6.81	7.95	7.26
	Q	X	6.53	7.55	7.16	6.28	6.88
	Q	Y	7.51	7.03	6.99	7.28	7.20
	Q	-(Z)	6.76	7.26	6.75	6.85	6.90
	R	+	5.17	5.45	5.48	6.12	5.56
	R	-	5.20	4.74	5.00	4.18	4.78
	S	+	7.43	8.20	7.00	7.00	7.41
	S	-	8.92	7.03	7.21	7.35	7.63
	T	+	7.13	7.07	6.62	6.90	6.93
	T	-	6.73	7.64	6.97	7.49	7.21
Yield in g. of oven dry straw per 10 plants	P	+	8.10	9.28	7.61	8.11	8.28
	P	-	7.58	8.56	7.07	8.44	7.91
	Q	+(W)	7.28	6.65	6.63	7.35	6.98
	Q	X	6.46	7.01	6.76	6.49	6.68
	Q	Y	7.21	6.37	6.54	6.94	6.76
	Q	-(Z)	6.68	6.64	6.35	6.21	6.47
	R	+	5.12	4.54	5.00	5.18	4.96
	R	-	4.87	4.53	5.10	4.05	4.64

Data	Treatment: Water	Treatment: Copper	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
Yield in g. of oven dry straw per 10 plants (contd.)	S	+	7.59	8.69	8.00	7.26	7.88
	S	-	9.25	7.46	7.20	7.15	7.76
	T	+	7.27	6.40	6.53	6.35	6.64
	T	-	6.93	6.78	6.71	6.91	6.83
% by weight of grain in the final harvest	P	+	48.1	48.2	48.3	47.2	48.0
	P	-	47.0	47.1	47.8	47.9	47.4
	Q	+(W)	50.1	51.3	50.7	52.0	51.0
	Q	X	50.3	51.9	51.4	49.2	50.7
	Q	Y	51.0	52.5	51.7	51.2	51.6
	Q	-(Z)	50.3	52.2	51.5	52.5	51.6
	R	+	50.2	54.6	52.3	54.2	52.8
	R	-	51.6	51.1	49.5	50.8	50.8
	S	+	49.5	48.5	46.7	49.1	48.4
	S	-	49.1	48.5	50.0	50.7	49.6
	T	+	49.5	52.5	50.3	52.1	51.1
	T	-	49.3	53.0	51.0	52.0	51.3
% moisture in plants at first harvest	P	+	84.0	83.2	83.0	81.7	83.0
	P	-	84.7	83.6	82.9	82.4	83.4
	Average Q,S	+(W)	85.0	85.7	84.3	83.9	84.7
	Q	X	84.8	85.7	85.5	84.0	85.0
	Q	Y	85.8	85.7	84.2	84.0	84.9
	Average Q,S	-(Z)	86.4	86.2	85.8	85.0	85.8
	Average R,T	+	89.0	88.4	87.2	86.7	87.8
	Average R,T	-	88.6	89.0	88.1	86.8	88.1
% moisture in plants at second harvest	P	+	66.3	65.3	65.8	65.1	65.6
	P	-	67.6	65.7	66.2	65.8	66.3
	Q	+(W)	63.2	62.4	63.1	63.1	63.0
	Q	X	63.9	62.8	63.8	62.5	63.2
	Q	Y	64.1	63.4	63.4	61.9	63.2
	Q	-(Z)	64.9	63.3	64.4	63.5	64.0

Data	Treatment		Replicate				Mean
	Water	Copper	1	2	3	4	
% moisture in plants at second harvest (contd.)	R	+	69.8	67.6	66.4	64.9	67.2
	R	-	71.2	68.8	70.5	67.0	69.4
	S	+	66.5	66.0	65.5	64.2	65.6
	S	-	67.2	66.3	66.2	65.0	66.2
	T	+	70.8	68.4	68.5	67.3	68.8
	T	-	72.4	71.2	70.8	70.1	71.1
% moisture in plants at final harvest	P	+	47.6	47.0	50.8	50.3	48.9
	P	-	51.5	46.5	50.7	49.7	49.6
	Q	+(W)	38.5	36.5	43.3	42.7	40.2
	Q	X	42.3	41.8	42.7	43.2	42.5
	Q	Y	44.7	37.1	41.9	42.0	41.4
	Q	-(Z)	44.9	39.6	45.4	39.5	42.4
	R	+	52.4	45.1	46.0	43.2	46.7
	R	-	54.4	49.9	56.8	47.2	52.1
	S	+	50.4	49.6	53.0	48.3	50.3
	S	-	51.3	49.0	50.1	48.0	49.6
	T	+	54.7	47.4	51.1	48.4	50.4
	T	-	57.6	51.4	54.6	54.3	54.5
Number of tillers per 10 plants on 20th July	P	+	0	0	1	1	0.5
	P	-	0	0	2	1	0.8
	Q	+	1	1	1	2	1.2
	Q	-	0	3	2	1	1.5
	R	+	1	0	0	0	0.2
	R	-	6	1	7	2	4.0
	S	+	0	3	2	0	1.2
	S	-	2	4	2	0	2.0
	T	+	1	1	0	1	0.8
	T	-	2	0	3	0	1.2

Data	Treatment Water	Treatment Copper	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
Number of ears per plant at final harvest	P	+	12.6	13.8	10.9	11.0	12.1
	P	-	10.3	12.4	10.0	12.2	11.2
	Q	+(W)	11.2	10.9	9.8	11.0	10.7
	Q	X	9.9	11.2	10.2	10.0	10.3
	Q	Y	11.2	10.5	10.2	10.7	10.6
	Q	-(Z)	9.9	10.9	9.6	10.0	10.1
	R	+	8.3	8.8	9.3	9.6	9.0
	R	-	7.7	8.0	7.9	6.0	7.4
	S	+	10.3	11.7	10.8	9.8	10.6
	S	-	12.2	9.8	9.6	9.7	10.3
	T	+	10.7	11.5	10.6	9.5	10.6
	T	-	9.2	10.4	9.6	10.1	9.8
Number of grains per plant at final harvest	P	+	22.9	26.3	20.4	20.8	22.6
	P	-	19.0	22.8	18.9	22.4	20.8
	Q	+(W)	21.3	19.9	19.3	21.8	20.6
	Q	X	19.0	21.0	19.4	18.3	19.4
	Q	Y	21.1	20.1	19.5	20.7	20.4
	Q	-(Z)	19.0	21.0	18.2	19.4	19.4
	R	+	15.9	16.9	16.7	18.4	17.0
	R	-	14.7	14.4	14.3	12.1	13.9
	S	+	20.1	22.5	19.1	19.0	20.2
	S	-	24.8	18.1	18.8	19.1	20.2
	T	+	21.2	21.8	20.2	20.1	20.8
	T	-	19.9	21.8	19.6	21.9	20.8
p.p.m. copper in plants at first harvest	P	+	5.00	5.07	4.93	4.76	4.94
	P	-	1.51	1.69	1.57	1.82	1.65
	Average Q,S	+(W)	5.97	7.15	6.57	6.41	6.52
	Q	X	6.18	7.22	6.62	6.54	6.64
	Q	Y	4.53	4.26	4.13	4.01	4.23
	Average Q,S	-(Z)	2.12	1.98	2.14	2.10	2.08
	Average R,T	+	9.93	9.45	8.98	8.35	9.18
	Average R,T	-	2.82	2.84	3.20	2.67	2.88

Data	Treatment		Replicate				Mean
	Water	Copper	1	2	3	4	
p.p.m. copper in plants at second harvest	P	+	3.70	3.94	2.94	5.14	3.93
	P	-	2.26	3.09	2.45	2.00	2.45
	Q	+(W)	4.76	5.84	4.66	4.72	5.00
	Q	X	4.59	4.88	4.15	4.05	4.42
	Q	Y	4.61	3.61	6.18	4.10	4.62
	Q	-(Z)	2.95	3.13	2.04	2.34	2.62
	R	+	7.45	7.36	5.78	5.70	6.57
	R	-	3.22	3.96	2.24	2.12	2.88
	S	+	4.94	5.18	3.95	4.56	4.66
	S	-	1.92	2.93	1.56	3.50	2.48
	T	+	5.14	5.32	5.24	4.43	5.03
	T	-	2.53	3.14	2.66	2.72	2.76
p.p.m. copper in grain	P	+	4.24	4.34	4.87	5.84	4.82
	P	-	4.30	2.82	2.56	3.61	3.32
	Q	+(W)	5.54	6.49	5.80	5.74	5.89
	Q	X	5.27	6.12	5.40	5.66	5.61
	Q	Y	6.19	4.68	4.55	4.74	5.04
	Q	-(Z)	4.13	2.72	2.66	2.61	3.03
	R	+	8.02	7.02	7.07	8.06	7.54
	R	-	3.10	4.49	4.18	3.57	3.84
	S	+	4.63	5.01	5.40	4.78	4.96
	S	-	3.14	3.07	3.01	3.64	3.22
	T	+	5.16	5.22	5.20	6.13	5.43
	T	-	3.12	3.18	3.02	3.84	3.29
p.p.m. copper in straw	P	+	3.09	3.42	3.30	2.52	3.08
	P	-	3.64	2.70	2.65	2.22	2.80
	Q	+(W)	4.06	3.78	4.24	3.18	3.82
	Q	X	5.46	3.59	3.53	3.84	4.10
	Q	Y	4.40	3.61	4.42	2.94	3.84
	Q	-(Z)	2.67	3.70	2.62	2.74	2.93
	R	+	6.32	5.24	7.68	5.20	6.11
	R	-	3.26	3.15	3.41	3.38	3.30

Data	Treatment		Replicate				Mean
	Water	Copper	1	2	3	4	
p.p.m. copper in straw (contd.)	S	+	3.51	3.06	3.16	2.57	3.08
	S	-	3.14	1.96	2.40	2.58	2.52
	T	+	6.08	3.93	5.47	4.36	4.96
	T	-	2.86	3.39	2.24	3.20	2.92
Copper uptake in μg. per 10 plants at first harvest	P	+	19.9	19.6	17.8	17.9	18.8
	P	-	5.4	6.7	5.3	8.2	6.4
	Average Q,S	+(W)	16.0	20.4	17.5	18.8	18.2
	Q	X	21.8	21.9	20.5	19.8	21.0
	Q	Y	12.9	11.9	12.6	11.9	12.3
	Average Q,S	-(Z)	5.0	4.1	4.8	4.9	4.7
	Average R,T	+	8.8	10.2	11.9	12.4	10.8
	Average R,T	-	2.4	2.4	2.7	2.4	2.5
Copper uptake in μg. per 10 plants at second harvest	P	+	50.0	55.2	38.5	72.5	54.0
	P	-	26.4	43.4	27.7	27.4	31.2
	Q	+(W)	65.2	71.8	57.3	61.4	63.9
	Q	X	56.0	63.0	52.3	50.2	55.4
	Q	Y	56.7	51.6	76.0	51.7	59.0
	Q	-(Z)	36.0	41.3	21.2	25.0	30.9
	R	+	54.4	52.3	53.2	49.6	52.4
	R	-	20.0	25.3	14.8	14.8	18.7
	S	+	58.8	72.5	50.6	52.9	58.7
	S	-	28.8	39.3	20.7	46.9	33.9
	T	+	47.8	47.3	56.1	45.6	49.2
	T	-	23.3	27.9	24.2	24.2	24.9
Copper uptake in μg. per 10 plants in grain	P	+	31.8	37.5	34.6	42.3	36.6
	P	-	28.9	21.5	16.6	28.0	23.8
	Q	+(W)	40.4	45.4	39.5	45.6	42.7
	Q	X	34.4	46.2	38.7	35.5	38.7

Data	Treatment Water	Treatment Copper	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
Copper uptake in μg. per 10 plants in grain (contd.)	Q	Y	46.5	32.9	31.8	34.5	36.4
	Q	-(Z)	27.9	19.7	18.0	17.9	20.9
	R	+	41.5	38.3	38.7	49.3	42.0
	R	-	16.1	21.3	20.9	14.9	18.3
	S	+	34.4	41.1	37.8	33.5	36.7
	S	-	28.0	21.6	21.7	26.8	24.5
	T	+	36.8	36.9	34.4	42.3	37.6
	T	-	21.0	24.3	21.0	28.8	23.8
Copper uptake in μg. per 10 plants in straw	P	+	25.0	31.7	25.1	20.4	25.6
	P	-	27.6	23.1	18.7	18.7	22.0
	Q	+(W)	29.6	25.1	28.1	23.4	26.6
	Q	X	35.3	25.2	23.9	24.9	27.3
	Q	Y	31.7	23.0	28.9	20.4	26.0
	Q	-(Z)	17.8	24.6	16.6	17.0	19.0
	R	+	32.4	23.8	38.4	26.9	30.4
	R	-	15.9	14.3	17.4	13.7	15.3
	S	+	26.6	26.6	25.3	18.7	24.3
	S	-	29.0	14.6	17.3	18.4	19.8
	T	+	44.2	25.2	35.7	27.7	33.2
	T	-	19.8	23.0	15.0	22.1	20.0
p.p.m. iron in plants at first harvest	Q	+(W)	34.0	32.7	33.9	31.5	33.0
	Q	X	25.5	32.3	33.0	30.9	30.4
	Q	Y	35.7	29.1	30.5	27.8	30.8
	Q	-(Z)	51.4	42.9	43.3	42.9	45.1
Iron uptake in μg. per 10 plants at first harvest	Q	+(W)	91.1	93.5	90.5	92.3	91.8
	Q	X	90.0	97.9	102.3	93.6	96.0
	Q	Y	101.4	81.2	92.7	82.8	89.5
	Q	-(Z)	121.3	89.2	97.9	100.4	102.2

Data	Treatment Water	Treatment Copper	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean
p.p.m. Manganese in plants at first harvest	Q	+(W)	63.3	69.2	56.2	72.8	65.4
	Q	X	59.3	60.4	66.0	61.8	61.9
	Q	Y	82.1	76.3	64.2	69.1	72.9
	Q	-(Z)	76.9	68.1	67.7	69.8	70.6
Manganese uptake in μg. per 10 plants at first harvest	Q	+(W)	170	198	150	213	183
	Q	X	209	183	205	187	196
	Q	Y	233	213	195	206	212
	Q	-(Z)	181	142	153	163	160
p.p.m. E.D.T.A. extractable copper in the soil at final harvest	P	+	5.90	6.32	6.38	6.18	6.20
	P	-	0.32	0.30	0.30	0.27	0.30
	Q	+(W)	5.50	6.02	6.08	5.06	5.66
	Q	X	3.86	3.59	3.79	3.83	3.77
	Q	Y	0.70	0.76	0.87	0.84	0.79
	Q	-(Z)	0.31	0.28	0.25	0.30	0.28
	R	+	6.58	6.88	5.86	6.46	6.44
	R	-	0.32	0.30	0.26	0.26	0.28
	S	+	4.68	5.02	6.49	5.62	5.45
	S	-	0.28	0.22	0.26	0.26	0.26
	T	+	6.42	5.75	6.32	5.62	6.03
	T	-	0.28	0.30	0.34	0.36	0.32