How to Unravel and Solve Soil Fertility Problems

Colin Asher, Noel Grundon and Neal Menzies

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Canberra 2002

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Asher, C., Grundon, N. and Menzies, N. 2002. How to unravel and solve soil fertility problems. ACIAR Monograph No. 83, 139p.

ISBN 1 86329 321 4 (print)
ISBN 1 86320 322 2 (electronic)

Editing by Arawang Editorial
Design and layout by Clarus Design

Foreword

Farmers in developing countries regularly harvest crops yielding far below the biological potential of their chosen variety. Although pests and diseases take their toll, and water shortages are widespread, nutritional disorders are probably the most pervasive constraint to crop yields in the tropics. Most soils used for crop production supply inadequate amounts of essential nutrients or contain toxic levels of other elements. This is the result of either an inherently low soil fertility, such as that associated with intense weathering in the humid tropics, or of nutrient depletion and organic matter decline caused by repeated cropping without replacing what has been taken from the soil. In many developing countries, such exploitation of the soil through agricultural intensification of this type is being accelerated by population growth and poverty.

Soil fertility problems can be resolved and yields increased by the judicious use of fertilizers, crop residues and/or organic manures. However, such remedies will have a fuzzy element of *muck and magic* about them unless the location-specific nutrient requirements of the crop are pinpointed. In the worst case, farmers may invest in N, P or K fertilizers only to find that they exacerbate the problem by inducing deficiencies of secondary nutrients or micronutrients. Fortunately, since the German scientist Justus von Liebig expounded the law of the minimum in 1840, scientific knowledge of plant nutrition and soil fertility has progressed enormously. While a lot of producers in developed countries such as Australia have benefited greatly from that knowledge, the benefits have bypassed many farmers in developing countries.

This monograph provides a vehicle for developing country farmers to benefit from scientific knowledge on plant nutrition and soil fertility. The book bridges the gap between the outputs of basic research and the results of applied research on soil and crop management. It is intended for use by agricultural scientists and extension staff in developing countries, and I feel sure that they will welcome so systematic and practical a guide.

In many ways this monograph encapsulates the ACIAR model. Much of the knowledge base on which the book

rests was developed in a series of bilateral ACIAR projects, in which Australian scientists from the University of Queensland married their expertise with scientists from Asia and the Pacific. The synergies created through these partnerships are reflected in the innovative approaches developed and expounded in the manuscript. ACIAR is publishing the monograph to its usual high standards, and it and the authors and editors deserve our sincere thanks for their efforts.

Eric T. Craswell
Centre for Development Research (ZEF)
Bonn, Germany
October 2001

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About the authors

Emeritus Professor Colin Asher has over 40 years' experience in solving soil fertility and plant nutrition problems, has published over 250 papers, and has been a member of the International Council on Plant Nutrition since 1993. He has worked and travelled widely in tropical developing countries including those of Central and South America, Asia, and the Pacific. He has a special interest in the diagnosis and correction of mineral nutrient problems affecting productivity of the land.

Dr Noel Grundon is an Honorary Research Fellow with CSIRO Land and Water, located at the Tropical Forest Research Centre at Atherton in North Queensland, Australia. He has published over 150 papers and has worked and travelled widely in tropical and subtropical Australia, and the developing countries of Southeast Asia. His special interest is in solving soil fertility problems, an area in which he has over 30 years' experience.

Associate Professor Neal Menzies is a Reader in Soil Science in the School of Land and Food Sciences at the University of Queensland, Australia. He has extensive experience of solving soil fertility problems in Australia, Africa, and Southeast Asia. He has a particular interest in identifying factors inhibiting plant growth on disturbed and degraded lands and in restoring the fertility of these lands.

Preface

The maintenance and improvement of the fertility of our soils has never been more important than it is today. Recent United Nations' estimates put the annual growth of the world population in the region of 100 million. Meeting the needs of this rapidly growing population for food, fibre, timber, and fuel is going to require a very large increase in agricultural and forestry production in the decades ahead.

Improving the productivity of existing farmlands by ecologically sustainable methods is essential, not only to meet the needs of a growing world population, but also to alleviate pressures to clear and cultivate remaining areas of natural vegetation, with their rich and irreplaceable flora and fauna.

In the Keynote Address to the 15th World Congress of Soil Science, Nobel Prize winning scientist Norman Borlaug commented[1]:

> We believe without doubt that the single-most important factor limiting crop yields in developing nations worldwide — and especially among resource-poor farmers — is soil fertility.
>
> Soil fertility can be restored effectively by applying the right amounts of the right kinds of fertiliser — either chemical or organic, or preferably, a combination of the two — according to the requirements of different crops, soil types, and environments.

On fragile and nutrient-depleted lands, appropriate inputs of nutrients may play an important role in protecting the soil surface from erosion by increasing plant cover, and along with the return of crop residues, allowing a rebuilding of soil organic matter reserves.

Whether dealing with lands that are now highly productive, or with degraded lands, nutrient management needs to be guided by a sound knowledge of which chemical elements are limiting without our intervention. Simple pot experiments, which are discussed in some detail in the following pages, are one of the more reliable tools by which we can gain such knowledge.

[1] Borlaug, N.E. and Dowswell, C.R. 1994. Feeding a human population that increasingly crowds a fragile planet. Keynote Lecture, 15th World Congress of Soil Science, Acapulco,10–15 July 1994. Supplement to Transactions, 15p.

This book is intended to help the reader proceed in a logical step-wise manner from recognising the existence of soil fertility problems, to characterising each problem and finding a practical and economic solution to it.

Colin Asher
Noel Grundon
Neal Menzies

September, 2001

1

Soil Fertility Problems and How to Recognise Them

The successful correction of soil fertility problems will usually involve three steps:

(a) recognising that we have soil fertility problems at a particular site;

(b) defining the precise nature of these problems; and

(c) finding a cost-effective and culturally acceptable solution to these problems.

The present section deals mainly with step (a), while Sections 2 and 3 of the book deal mainly with step (b), and Sections 4 and 5 with step (c). In Section 6, we return to a further consideration of (b).

1.1 How do we know if we have a fertility problem?

Many factors can contribute to slow or unhealthy plant growth and, ultimately, to reduced yields. These include: unfavourable weather leading to drought or waterlogging; acid rain from active volcanoes; competition by weeds; attacks by insect pests; infection by disease organisms; and shortages or excesses of particular chemical elements in the soil surrounding the roots. This book is about how to recognise and deal with shortages or excesses of chemical elements. However, we need to keep these other possibilities in

mind whenever we step into a farmer's field, and we need to learn to read the telltale signs of each factor that has the potential to reduce yields.

1.2 Which chemical elements do plants need?

For healthy plant growth, soils must provide adequate amounts of at least 13 chemical elements. These are divided into two main groups: the *macronutrients* (or major elements), which plants need in relatively large amounts; and the *micronutrients* (or trace elements), which are needed in much smaller amounts (Table 1.1).

In addition to the elements listed in Table 1.1, there are a few elements which have been found to be needed by some plants or under some circumstances (see also Section 3.2). These are called *beneficial* elements. Beneficial elements include cobalt (which is needed for biological nitrogen fixation), nickel, sodium, and silicon. Asher (1991) provides more information on these elements.

Table 1.1 List of macronutrient and micronutrient elements required for the healthy growth of all plant species.

Macronutrient	Symbol	Micronutrient	Symbol
Nitrogen	N	Chlorine	Cl
Phosphorus	P	Iron	Fe
Potassium	K	Boron	B
Calcium	Ca	Manganese	Mn
Magnesium	Mg	Zinc	Zn
Sulfur	S	Copper	Cu
		Molybdenum	Mo

1.3 How much of each nutrient will a particular crop need?

The amount of each nutrient needed for healthy growth (the *crop demand* for each nutrient) depends on two main factors: the concentration of each nutrient element needed in the plant tissues for healthy growth and development, and the amount of plant material that is going to be produced. Table 1.2 gives some estimates of the quantities of nutrients needed to grow an average and a high-yielding sweet potato crop.

Clearly, the amount of each nutrient needed for a crop yielding 50 t/ha is much greater than for a crop yielding only 12 t/ha.

For a given crop in a given place, many factors can reduce plant growth and hence reduce the demand for nutrients. Among these are poor establishment, drought, waterlogging, competition from weeds, and damage by pests and diseases.

Table 1.2 Amounts of nutrients present in tubers or tubers plus vines of a 12 t/ha or 50 t/ha crop of sweet potato (kg/ha). Based on data of O'Sullivan et al. (1997).

	Nutrients present in a crop with a tuber yield of:			
	12 t/ha		50 t/ha	
Nutrient	Tubers only	Tubers plus vines	Tubers only	Tubers plus vines
Nitrogen	26	52	110	215
Phosphorus	6	9	25	38
Potassium	60	90	250	376
Calcium	3.6	16	15	65
Magnesium	3	6.5	12.5	27
Sulfur	1.8	4.3	7.5	18
Chlorine[a]	–	–	–	–
Iron	0.060	0.160	0.250	0.670
Boron	0.024	0.074	0.100	0.310
Manganese	0.024	0.175	0.100	0.730
Zinc	0.036	0.062	0.150	0.260
Copper	0.018	0.037	0.075	0.155
Molybdenum	0.004	0.006	0.015	0.023

[a] Insufficient information available to estimate amounts of Cl present in tubers and vines.

1.4 How can we tell if plants are getting the right amount of each element?

There are three important tools that we can use to answer that question:

(a) visible symptoms of nutrient deficiency or excess;

(b) plant analysis; and

(c) soil analysis.

Each of these tools has its strengths and weaknesses. Hence, they are often best used in combination.

1.4.1 Visible symptoms

When plants are unable to extract enough of a particular element from the soil for healthy growth, they will often display a characteristic set of *symptoms of deficiency* on the leaves or other plant parts. For example, when cassava plants are deficient in P (and some other nutrients) the depth of the canopy is reduced from top to bottom by the repeated shedding of the lower (older) leaves (Figure 1.1a). Close examination of the lower leaves still attached to a P-deficient plant shows that the leaves are soft and hang limply from their petioles (Figure 1.1b). They lose their green colour from the tips and margins (Figure 1.1c) until the whole leaf is a light yellow colour, except perhaps for a small area close to where the leaf joins the petiole. Learning to read symptoms of nutrient deficiency (and also *nutrient toxicity*) is an important skill which every agronomist and extension worker should seek to develop.

Fortunately, there are useful colour-illustrated publications that can help us with this task. Some of these publications deal with all the symptoms likely to be encountered on a particular crop such as cassava (Asher et al. 1980) or sweet potato (O'Sullivan et al. 1997), or those encountered in particular groups of plant species (Weir and Cresswell 1993a, b, 1995, 1997).

The great advantage of using visible symptoms is that no special equipment or facilities are needed — only a keen eye, and the training needed to understand what we see. The main disadvantage of the method is that we tend to pick up only the more severe problems. Thus, mild deficiencies may reduce plant yields without producing clearly recognisable symptoms. This situation is sometimes called *hidden hunger*. Similarly, where a soil contains insufficient amounts of more than one nutrient element in plant-available form, the chances are that we will detect only the most severe nutrient limitation at any particular site.

Often, the symptoms are quite clear-cut, and we can be confident about our diagnosis. However, in other cases we may be unable to decide between two nutritional disorders that sometimes produce similar symptoms, e.g. between zinc deficiency and manganese deficiency.

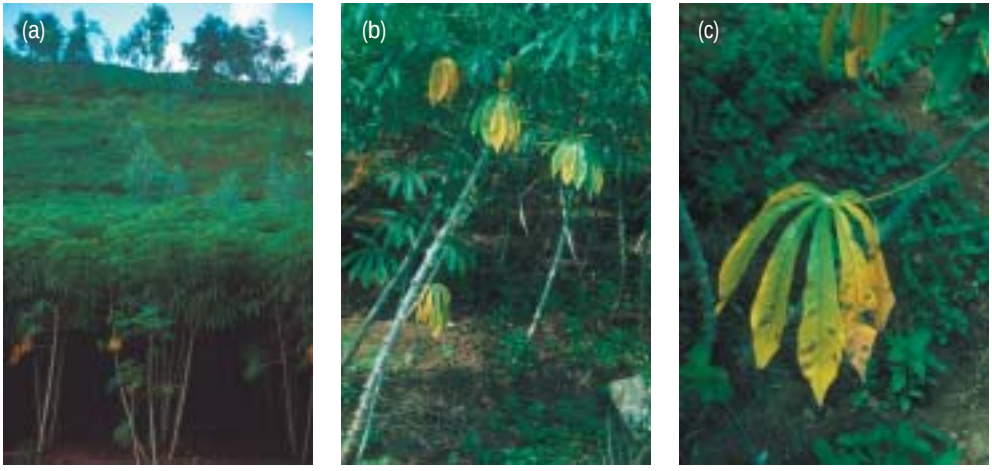

Figure 1.1 Example of visible symptoms of nutrient deficiency in a cassava crop in northern Vietnam: (a) reduction in the depth of the canopy due to shedding of P-deficient older leaves; (b) closer view of P-deficient plant showing the soft, drooping lower leaves; (c) view of individual leaf showing the progressive loss of green colour due to P deficiency (dark lesions on the leaf are due to angular leaf spot, a fungus to which P-deficient cassava plants seem particularly susceptible).

Hence, we need additional information. As discussed below, chemical analysis of leaf samples can readily provide this information if we have access to a suitably equipped laboratory. However, in developing countries this is often not the case, and we need to use other methods.

In the case of micronutrients, confirmation of the identity of a deficiency can often be obtained by a technique called *leaf painting.* In this technique, a portion of a leaf displaying the symptom under investigation is painted with a dilute solution containing the element suspected of being deficient. Greening-up (and sometimes stimulated growth) of the painted area indicates a correct diagnosis. Figure 1.2 shows some examples of leaf painting responses in yams. (More information on leaf painting can be found in Appendix 1.)

In the case of macronutrients, leaf painting does not work as well as with micronutrients. This is, in part, because the amounts that need to be absorbed by the leaf for regreening are much larger, and application of a correspondingly more concentrated solution to supply these larger amounts is likely to cause damage to the leaf. However, there are some simple *tissue tests* that can be applied in the field for at least some of the macronutrient elements (see Appendix 2). Sometimes,

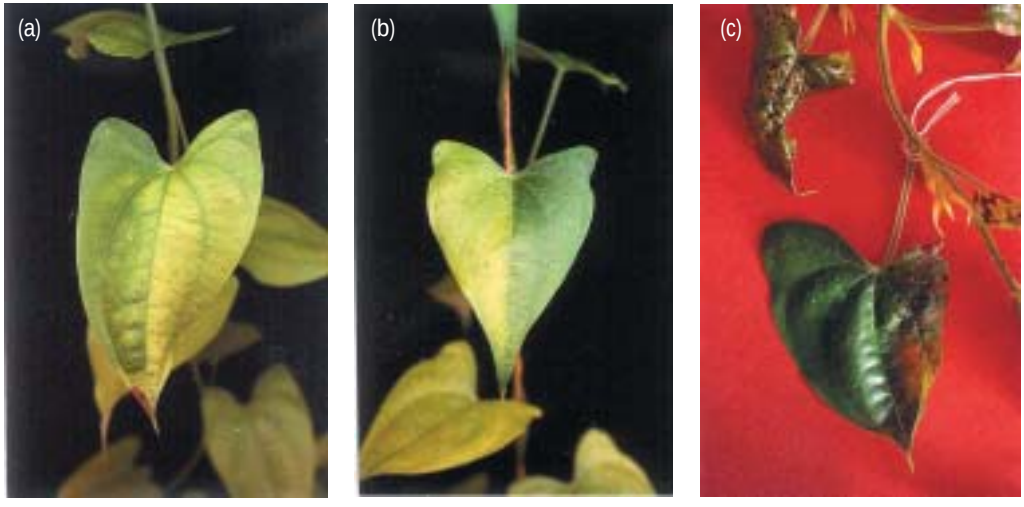

Figure 1.2 Leaf painting responses in micronutrient-deficient yam plants: (a) iron deficiency, left half of leaf painted; (b) zinc deficiency, right half of leaf painted; and (c) manganese deficiency, left half of leaf painted (note increased leaf expansion and fewer necrotic spots on painted half).

it is possible to confirm a diagnosis by applying a fertiliser containing the suspected element to selected plants or to a narrow strip across the field (*test strip*). If the treated plants improve in appearance compared with the adjacent untreated ones, our diagnosis will be confirmed (Figure 1.3).

In crops in which nitrogen deficiency is hard to distinguish from sulfur deficiency, a diagnosis may be made by laying down adjacent test strips treated with urea fertiliser (containing only nitrogen), or with sulfate of ammonia (containing both nitrogen and sulfur). If sulfur is deficient, only the sulfate of ammonia strip will green up, whereas if nitrogen is deficient, both strips should green up.

Figure 1.3 Successful diagnosis of copper deficiency in a wheat crop in Queensland, Australia. The dark green strip of crop was sprayed with a solution containing 10 g/L of $CuSO_4.5H_2O$, approximately three weeks before the photograph was taken, while the remainder of the crop was left unsprayed.

1.4.2 Plant analysis

Chemical analysis of suitable plant parts can tell us a great deal about the nutrient status of the plants from which they were taken. In most developed countries, farmers and their advisors can send plant samples to either government-run or private chemical laboratories. However, in many developing countries, these facilities are not yet readily available, and so we must use other methods.

Interpretation of the results of plant analysis tests involves comparing the concentration of each element in the sample we had analysed with published values for the same species. These published values are often given in ranges of concentrations corresponding with deficiency, adequacy, or toxicity. The regions of deficiency, adequacy, and toxicity are separated from each other by *critical concentrations*, often defined as the tissue concentrations corresponding with 90% of maximum yield (Figure 1.4). Since there is often a degree of uncertainty about the exact value of a critical concentration, some researchers prefer to indicate marginal ranges, rather than a single value. In Figure 1.4 these correspond to the ranges of concentration between 'deficiency' and 'adequacy' (marginal range for deficiency), and between 'adequacy' and 'toxicity' (marginal range for toxicity).

It is important to note that, for reliable results, we must take the same plant part at the same stage of development as was done when the test was calibrated.

Reuter and Robinson (1997) give a comprehensive account of sampling procedures, nutrient ranges, and critical values for many crop, pasture, ornamental, and forestry species.

Tissue tests are qualitative chemical tests that we can perform in the field. Although they are not as accurate as those conducted in a chemical laboratory, they can still be quite useful (see Appendix 2).

1.4.3. Soil analysis

Most of the nutrient elements exist in the soil in a number of chemical forms that differ greatly in their solubility and in the ease with which plant roots can extract them. In soil analysis, we extract the soil with a solution that experience has shown will remove amounts of a particular nutrient that are positively correlated with the amounts a plant root system might extract. The solutions range from water (boron analysis) through neutral salts (potassium, calcium, and magnesium analysis) to strongly acidic or alkaline extractants (some phosphate analyses). Peverill et al. (1999) give more information on soil analysis tests and their interpretation.

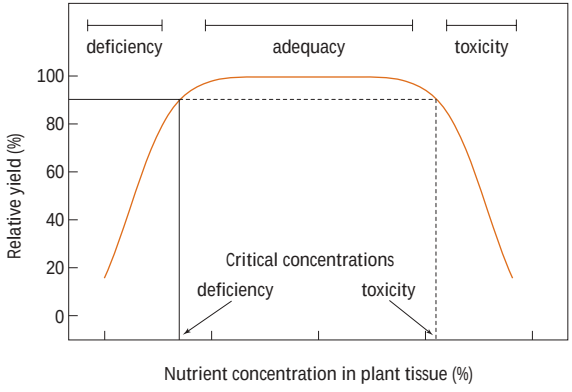

Figure 1.4 Schematic relationship between relative yield and concentration of a nutrient in plant tissue.

The big advantage of soil analysis tests over other tests is that they can give us valuable information *before* we plant the crop — we do not have to wait for the crop to start growing and for problems to start emerging, but can take early preventative action. Farmers in most developed countries have ready access to soil chemistry laboratories which can undertake soil analysis tests for them, but access is not so readily gained in many developing countries.

Soil analysis tests generally work best when used on the particular group of soils for which they were originally developed, e.g. soils of the mid-western USA, or on soils similar to those for which the tests were developed. When the same tests are applied to soils with a very different geological history, and a vastly different mineral composition, they may not work as well; indeed, they may not work at all. Hence, whenever it is planned to rely on soil analysis tests in a new situation, we need to first check by actual experiment that the test really does work on the particular soils in which we are interested. (We will return to the question of soil testing in Section 6.)

1.5 Correction of fertility problems — which nutrients and how much of each?

The amount of a nutrient we need to add to the soil to ensure healthy crop growth depends on two things — the *crop demand* for each nutrient (see Section 1.3), and the ability of the soil to supply that amount of the nutrient. In most situations, most of the dozen or so nutrient elements needed by plants (see Sections 1.2 and 1.3) can be supplied in adequate quantities by the soil, and we do not have to take any action to improve their supply. Only when the supply of a particular nutrient from the soil is likely to be insufficient to meet the crop demand do we need to consider ways of increasing the supply of that nutrient.

Common ways of increasing the supply of a nutrient are:

(a) biological nitrogen fixation (legumes, *Casuarina* spp., grass rhizosphere nitrogen fixers);

(b) use of deep-rooted trees to bring up subsoil nutrients (agroforestry and alley cropping systems);

(c) applying animal manure, or other organic nutrient sources from processes such as sewage treatment, sugar refining, or palm oil extraction; and

(d) applying chemical fertilisers.

The most appropriate way of increasing the supply of a particular nutrient will depend on local circumstances, including the farmer's financial position, and the availability and price of fertilisers and other inputs.

As we saw previously, if the soil is capable of meeting all the nutrient demands of the crop, then the requirement for additional nutrient inputs is zero. If, however, the supply of one or more nutrients from the soil is less than the demand, yields will be reduced unless we take action to increase the supply of these deficient elements. The amount of each deficient nutrient element that we need to supply will depend on two factors:

(i) the difference between the amount of nutrient needed by the crop and the amount available from the soil; and

(ii) the extent to which any additional nutrients we add to the system are 'fixed' in forms that are unavailable to the plant roots (see Sections 3.2 and 4.2.2) or are lost from the soil–plant system by leaching or other processes (see Section 5.4.2).

In summary, the amount of a nutrient that needs to be added is given by the formula :

Amount of added nutrient = Crop demand
– Amount available from soil
+ Amount of added nutrient lost due to fixation, leaching etc.

In the field, the nutrient demand of a crop and the extent of losses of added nutrients may vary substantially, depending on a range of seasonal, soil, and management factors. Hence, *field trials* conducted under conditions that are closely similar to practical farming conditions *are essential if requirements for added nutrients are to be determined accurately* (see Section 5).

1.6 Some notes on the relative importance of soil type and site history

The nutrient supply provided by a soil, and the type of problems we are likely to encounter, often differ greatly, depending on the parent material from which the soil has been derived. Hence, we would expect rather different sets of problems on soils developing on an uplifted coral reef, compared with those developed on volcanic ash, or a basaltic lava flow. However, site history is important too, the fertility usually being much higher on freshly cleared land than on land that has been under cultivation for some time.

Under bush fallow (or long-term grass fallow), the organic matter content of the soil builds up, and mineral nutrients build up also. Some of these nutrients are brought in on the wind from the ocean and are captured by the vegetation. In Tonga, for example, it has been estimated that sulfur of marine origin is deposited on the land at a rate of about 5 kg/ha/year. In addition, nitrogen from the atmosphere is converted into plant-available forms by microorganisms living in the roots of legumes and of some non-legumes like the *Casuarina* tree. Microorganisms living on root surfaces of grasses are also believed to contribute to this nitrogen fixation. Plant roots may also bring up nutrients from deep in the subsoil, and the manure of seabirds probably contributes additional nitrogen, phosphorus and other nutrients.

When the land is cultivated, the rate of breakdown (*mineralisation*) of the soil organic matter is increased, and any vegetation turned in starts to rot, adding the nutrients it contains to those that have built up in the soil during the fallow period. Each crop grown on the land will remove some nutrients (see Table 1.2, for example). Hence, in the absence of nutrient inputs via fertiliser, animal manure, or green manure crops, the chemical fertility of the soil will decline during the cropping phase. The more crops taken, the greater will be this decline in natural fertility. As cultivation hastens the breakdown of the soil organic matter, the organic matter content declines also with increasing length of time since the last fallow. The organic matter content of the soil is important for three main reasons:

(a) it holds the soil 'crumbs' together, giving the soil a good physical structure;

(b) it contains most of the soil nitrogen and large amounts of the phosphorus, sulfur, and other nutrients; and

(c) it traps on its surface substantial amounts of plant-available potassium and other nutrients.

Hence, the run-down in soil organic matter is a further reason why the fertility of a soil declines with increasing time since the last fallow.

In areas where the soils have a similar geological history, the chemical fertility of the soil may be more related to the management history of a particular site (the number and type of crops taken, fallow vegetation type, and length of time since the last fallow) than to differences in soil type.

1.7 Locating sites with soil fertility problems

In developing country agriculture, a good way to find out if there are likely to be important soil fertility problems in a particular geographical area is to travel through the area, inspecting crops, and noting any symptoms that would indicate nutrient deficiencies or excesses. To do this effectively, we do not have to be familiar with all the symptoms that could occur on all the crops in the area. It is enough to concentrate on one or two species that are commonly grown throughout the area, and for which we have good descriptions of the symptoms; e.g. maize, cassava, sweet potato, or citrus species. As we move about the area, we need to talk to farmers and other people who know the local situation well to learn all we can about their farming system. The things we would want to know will include the following:

(a) Are the symptoms we are seeing a common occurrence?

(b) Are they present right from when the land is first cultivated or do they tend to show up only towards the end of the cropping cycle when we would expect nutrient levels in the soil to have been reduced?

(c) Do they occur on some soils but not others?

(d) Are there any cultural practices such as including legumes in the system, or applying animal manure, that seem to reduce their intensity or get rid of them altogether?

The symptoms we see will often tell us what is the most severe nutrient limitation at a site, or at least narrow down its identity to a few possibilities, which might be further resolved by leaf analysis, tissue tests, leaf painting, or soil analysis, depending on which of these options are available to us. However, as noted in Section 1.4.1, other less severe limitations may be present that do not show up as visible symptoms.

Experience has shown that *pot experiments* provide a very valuable tool for uncovering the potential nutrient limitations in soils and for ranking the severity of these limitations. Pot experiments will be discussed in some detail in Sections 2 and 3 of this book.

When collecting soil for pot experiments (see Section 2.2), we should try to take the soil from sites that would

be suitable for a subsequent field experiment (see Section 5). In this regard, some desirable characteristics are that:

(a) the site is representative of a substantial area of farmland in the district or region;

(b) the site has within it sufficient area for a field trial;

(c) the owner of the land is willing to have a field trial on their land, and is keen to cooperate in the running of any such trial; and

(d) the site is secure against theft of produce, and damage by livestock.

In relation to (d), adequate fencing can keep animals out, but security against theft often depends on strong community support for the work, and the involvement of village leaders.

2

Getting Ready to Run Pot Experiments

When we first start to use pot experiments as a tool for investigating soil fertility problems, there are certain things we need to do by way of preparation, and there are some facilities and equipment items that we are likely to need. The more important issues are discussed below.

2.1 What kind of pots should we use?

For most purposes, pots holding between 1 and 2 kg of air-dry soil are convenient. Two-litre (half-gallon) polyethylene buckets are ideal, as are moulded plastic flowerpots, with or without drain holes in the bottom. If these are not available, empty ice-cream containers or metal cans may be used. If you use metal cans or flowerpots with drain holes in the bottom, you will need to line each container with a polyethylene bag. In the case of metal cans, this is to prevent contamination of the soil with any micronutrients that may be present in the can. In the case of flowerpots, the liner is needed because we will be watering on a weight basis instead of relying on natural drainage (see later) as we do not want any soluble nutrients to be lost from the soil by leaching. In practice, we use cheap, disposable plastic liners in all our pots as they not only reduce the risk of contamination of the soil, but also make the cleaning of pots easier between experiments.

2.2 How much soil do we need, and how should we collect it?

The quantity of soil needed depends on the size and number of pots to be filled. For each *nutrient omission trial* (see Section 3), we will need to have enough soil for 60 pots. At the same time, additional soil should be collected for *nutrient rate trials* (see Section 4). Usually, a rate trial will involve 24 pots, and we will conduct one rate trial for each element found to be in short supply in the omission trial. While we cannot be sure how many elements will fall into this category, experience shows that we rarely need to conduct more than five rate trials per soil. Hence, enough soil for an additional 120 pots (5×24) should be sufficient. Thus *for each 1 kg of soil held by a pot we will need to collect about 180 kg of air-dry soil* (60 + 120), e.g. if our pot holds 1.5 kg of dry soil, we will need to collect $1.5 \times 180 = 270$ kg.

Soil is usually collected from the 0–15 cm horizon of each field site (see Section 1.7 for characteristics of a good site). To obtain a representative sample of soil, subsamples are collected from 5–10 locations from within the field. The subsampling points are chosen at random over the field site, taking care to avoid patches where grass or rubbish have been burned within the last year or so, or where subsoil has been brought to the surface by the roots of a falling tree. If two distinct types of soil occur in the field, a separate omission trial should be conducted on a sample collected from each type. If this is not practical, soil collection should be limited to the soil occupying the larger area. A subsample pit 50 cm long $\times$ 50 cm wide $\times$ 15 cm deep should yield the equivalent of about 40 kg of air-dry soil.

At each subsample location, the surface litter and any growing plants should be carefully removed to expose the soil surface. Soil to the required depth is then removed with a spade or other suitable digging tool, and placed in a strong, clean bag (Figure 2.1(a)). Woven polypropylene bags with a plastic film liner are ideal as they give the soil sample some protection against waterborne contamination — an important consideration when samples must be transported between islands by boat (Figure 2.1(b)). Care should be taken to keep the sides of the sampling hole vertical so that the subsample does not contain more soil from the top 5 cm of the hole than from lower depths.

Each soil should be dried by spreading it in a shallow layer on a plastic sheet in the sun, preferably in a greenhouse (see Section 2.3). Subsamples of the same soil do not need to be kept separate at this stage. Rather, the aim should be to have the total sample thoroughly mixed by the time it has been dried and sieved (see below). The drying soil layer should be turned once or twice per day until the soil is completely air-dry. Rocks, clods, and macro-organic matter are then removed by passing the soil through a stainless steel or plastic screen with approximately 5 mm openings. Brass or galvanised wire screens should not be used because of the risk of contaminating the soil

with micronutrients. Clods may be broken up with a wooden mallet and put back through the screen.

Figure 2.1 Collecting and transporting bulk soil samples for pot experiments in Vanuatu: (a) collecting soil from an area of bush fallow on Malo Island; (b) unloading soil samples from Malo Island (visible in the background) for road transport to the research station.

The importance of thorough drying and mixing of the soil cannot be over-emphasised. Incomplete air-drying of the soil may result in the soil becoming too wet when usual volumes of stock solution are added to it (see Section 3.5.4(c)). Again, during the drying process, a gradient in soil moisture is established, with the driest soil on the top of the drying soil mass. In the absence of good mixing, some pots will receive more of this drier soil than others. Since all pots are filled to a constant air-dry weight, the combination of incomplete drying and incomplete mixing means that some pots will contain more oven-dry soil than others. One result of such differences is that when pots are watered up to the same after-planting target weight (see Section 3.5.2) the pots containing less oven-dry soil may be too wet for healthy plant growth. Again, during drying and sieving, some soils tend to separate out into finer and coarser fractions with greater and lesser water-holding capacities than the average for the well-mixed soil. Such fractionation of the soil needs to be reversed by thorough mixing if watering problems in subsequent pot experiments are to be avoided.

When dried, screened, and mixed, the soil may be returned to the original bags and stored in a cool, dry place. A subsample of about 1 kg should be set aside for pH testing (Section 2.11.3), determination of water-holding characteristics (Section 2.11.2), and possibly other laboratory tests.

2.3 Protection from rain and wind

The use of undrained pots (see Section 2.1) means that pots exposed to rain are likely to become waterlogged, thus ruining the experiment. Hence, except at all but the very driest locations, it will be necessary to provide protection from the rain. Protection from the wind may also be required, as pots are easily blown over in the wind, particularly if the test plants are allowed to grow tall. In addition, exposure to strong wind retards the growth of some plants.

If pot experiments are seen as a 'one-off' activity, as is sometimes the case in land development projects, a temporary structure of bamboo or bush poles, roofed with inexpensive plastic film, may be all that is required. However, if pot experiments are likely to be an ongoing activity, it will be worthwhile to build a relatively permanent structure to protect plants from rain and wind.

The glasshouse is a familiar feature of agricultural research stations in the temperate regions. While these certainly provide protection from wind and rain, they are designed primarily to trap heat in the cold winter months, and may not be very suitable for use in the tropics and subtropics where high temperatures can be a problem. In these regions, leaving the sides and ends completely open, or enclosed only with insect mesh, will often provide a favourable environment for pot experiments (Figure 2.2). Construction is greatly simplified by the use of clear-plastic corrugated sheeting instead of glass. Although several plastic materials are available for this purpose, polycarbonate has the best light transmission characteristics and is the most durable. In attaching the polycarbonate sheeting, care needs to be taken to make sure that the ultraviolet protective layer is on the top of the sheet, otherwise the life of the roof sheeting will be reduced.

Figure 2.2 An open-sided greenhouse built for pot experiments in Tonga, which has a subtropical climate. Note the clear polycarbonate sheeting on the roof.

2.4 Control of greenhouse air temperatures

In the tropics, where the inputs of solar energy are often much higher than in temperate regions, greenhouse air temperatures can be unacceptably high unless care is taken with the design to minimise heat build-up. As

noted in Section 2.3, it is a good idea to leave the sides and ends open as far as is possible while still giving some protection from wind. A gentle breeze blowing through the greenhouse will usually mean that the air temperatures inside are not much higher than those outside. In addition, providing some form of weatherproof ventilation along the roof ridge will allow hot air trapped under the roof to escape, and making the glasshouse roof ridge as high as possible will make cooling by convection more efficient.

Limiting the entry of solar radiation by shading is not recommended, except when working with shade-tolerant plants. Rather, the object should be to have light intensities inside the greenhouse as similar as possible to those which the plants would be exposed to in the field. Also, experience shows that running water over the roof is usually not very effective in lowering air temperatures in the greenhouse (Nualsri et al. 1993).

2.5 Avoiding excessive soil temperatures in pots

In general, plants are more sensitive to high soil temperatures than to high air temperatures. Evidence from experiments conducted in Thailand (Nualsri et al. 1993) indicates that direct solar radiation striking the sides of pots, and secondary radiation from hot greenhouse floors are major causes of overheating of soil in pots. The first problem is easily and cheaply prevented by covering the sides of the pots with reflective material, such as aluminium foil. These *radiation shields* can also be made from waterproof builders' paper with a reflective outer surface. Secondary radiation from the greenhouse floor can be reduced by hosing down the floor once or twice during the hottest part of the day.

As dry soil is easier to heat up than moist soil, careful attention to watering during hot weather will also help to keep soil temperatures down (Nualsri et al. 1993).

2.6 Need for good quality water

Reverse-osmosis, deionised, or distilled water should be used for all soil fertility work done in pots. Well water, river water, and town water supplies almost always contain amounts of essential plant nutrients that are too high for the water to be used in pot experiments. Even rainwater is not completely pure, and may contain windblown salts, plus dust and other contaminants off the collecting roof and guttering.

Water purity is usually measured in terms of electrical conductivity, EC (low EC = high purity), and the aim should be to produce water with an EC ≤ 1 µS/cm. Use of rainwater as feed water for your deioniser will greatly reduce the frequency with which the equipment needs regeneration or replacement of disposable resin cartridges, and will usually also cause a slight improvement in water quality. Similarly, if you are purifying water by distillation, the use of rainwater will

greatly reduce the frequency with which scaly deposits need to be removed from the steam generator of your still. As pyrex glass is rich in boron, your still should have a soda glass condenser rather than one made of pyrex.

Keeping your pure water pure requires close attention to laboratory and greenhouse hygiene. Containers of polyethylene, polypropylene, or fibreglass will usually be found satisfactory. New containers should be thoroughly cleaned to remove dust and any surface deposits on the plastic, then kept covered so that dust cannot enter the container while in use.

2.7 Good quality nutrient salts

If we are to detect micronutrient deficiencies in the soil, it is important that we do not make accidental additions of these elements to the soil by using impure nutrient salts. Which salts to use will be discussed later. But the point here is that only good quality salts should be used in the preliminary stages of soil testing. Once we know which elements we are dealing with, we may then be able to revert to using some cheaper, less pure materials. For omission trials (see Section 3), we recommend the use of analytical reagent (AR) grade nutrient salts wherever possible. If unavailable, it may be possible to purify the salts yourself, at least with respect to some of the micronutrients (see Appendix 3).

2.8 Weighing equipment

You will need to be able to weigh out nutrient salts either for direct addition to the pots, or for the preparation of nutrient stock solutions. For this, a balance capable of weighing down to 0.01 g or 0.001 g is required. Modern digital laboratory balances are ideal for this purpose, but top pan and other types of beam balance can also be used.

You will also need a balance for weighing soil into your pots and for weighing the pots during routine watering operations. For this purpose, the balance needs to be accurate only to about 1 or 2 g. Although digital laboratory balances are excellent for this purpose, they are relatively expensive and require a source of 110 V or 240 V power in the greenhouse. Good quality, battery-operated, digital kitchen scales are sufficiently accurate, are much cheaper than laboratory balances, and do not require an external power source.

2.9 Access to a drying oven

You will need access to an oven for drying soil samples at 105°C and for drying plant samples at 75–80°C. For the latter purpose, forced-draught ovens are better than convection ovens. As fresh plant samples are rather bulky, an oven with an internal volume of at least 0.75 m^3 is desirable.

2.10 Some other useful items

2.10.1 Greenhouse benches

If you have a smooth concrete floor in your greenhouse that can be kept clean, it is possible to do good pot experiments with the pots sitting on the floor, as in Figure 2.2. However, it saves a lot of bending if you place your pots on benches that are about waist high. Getting the pots up off the floor also makes it easier to prevent contamination with dust. Benches may be made of a wide range of materials. Having a slatted top allows for free movement of air about the pots. Placing the benches on casters allows them to be moved easily about the greenhouse, even when loaded with pots. Being able to move the benches can be an advantage if you are short of greenhouse space, since permanent working space does not then have to be left around each bench.

2.10.2 Portable AC power source

If you intend to use a digital laboratory balance for weighing pots and you do not have electricity connected to your greenhouse, or if the supply is unreliable, you may need a portable power source. Power inverters which convert DC power from a car battery to 110 V or 240 V AC power are cheap and convenient portable power sources, and some people prefer to use a car battery and inverter all the time to avoid having potentially dangerous power cords in the greenhouse.

2.10.3 Movable weighing table

If you plan to do a lot of pot experiments, you may find it worthwhile to make yourself a portable weighing table so that you can always have your balance close to the group of pots you are weighing. A good weighing table will have a stand for your deionised water bottle (Figure 2.3).

Figure 2.3 View of a portable weighing table showing deionised water bottle (top right), digital balance and pot (centre), and 240 V AC power inverter connected to a 12 V car battery (bottom).

2.11 Preliminary characterisation of each soil

2.11.1 Finding the weight of soil needed to fill a pot

The weight of air-dry soil needed to fill the pot to 3 or 4 cm below the top, is determined as follows:

(a) Weigh three empty pots.

(b) Fill each pot to about 1 cm from the top with air-dry soil, and settle the soil in the pot by gently dumping (dropping) the pot on the bench four times from a height of about 5 cm.

(c) Add or remove soil until the top of the soil is the required distance below the top of the pot.

(d) Weigh the full pots, and calculate the mean weight of air-dry soil needed to fill a pot by subtracting the weight of the empty pot.

(e) Calculate the mean (average) amount of air-dry soil needed to fill a pot to the required depth.

2.11.2 Measuring the water-holding characteristics of the soil

All pots will be watered on a weight basis. As a minimum, we need to know the moisture content of our air-dry soil and the soil at field capacity (see below for explanation of terms). It is useful also to know the moisture content at wilting point.

(a) Moisture content of air-dry soil

Three samples (about 50 g) of air-dry soil are weighed accurately, then dried to a constant weight at 105°C. This will usually take about 48 hours. Record the oven-dry weights and calculate the air-dry moisture content:

$$\text{Air-dry moisture (\%)} = \frac{100\left(\text{Weight air-dry soil} - \text{Weight oven-dry soil}\right)}{\text{Weight oven-dry soil}}$$

The mean of the three values is taken as our best estimate of the air-dry moisture percentage (Table 2.1).

Table 2.1 Estimation of air-dry moisture percentage for a lowland soil from the Soc Son District, Vietnam.

Replicate	Weight of moist soil (g)	Weight of oven-dry soil (g)	Weight of moisture (g)	Air-dry moisture (%)
1	47.32	42.88	4.44	10.4
2	46.80	42.87	3.93	9.2
3	42.99	38.75	4.24	10.9
			Mean	10.2

(b) Field capacity by the column method

Field capacity is the moisture content of a soil that has been drained at a suction of 10 kPa (0.1 bar or pF 2 in the older terminology). It corresponds approximately with the amount of water a soil will retain after it has

been thoroughly wet and allowed to drain. In pot experiments, we usually aim to keep the moisture content of the soil as close as possible to field capacity.

An estimate of field capacity can be obtained quite simply by the *column method*. The procedure is as follows:

Air-dry soil is placed in a glass jar (approximately 15–20 cm high × 8 cm diameter) or a measuring cylinder. The soil is added to the jar in four roughly-equal portions and is settled after each soil addition by gently dumping the container two or three times on the bench top. The aim should be to have the soil surface about 1 cm below the top of the container after the final addition. Another two glass containers are filled in a similar manner. Water is added slowly to the soil in each container with a wide bore pipette until the soil is wet to approximately half its depth. Evaporation from the soil surface is prevented by covering the jar with Parafilm®, plastic film or, if a screw-topped jar is used, by lightly screwing on the lid. The containers are left standing for at least 24 hours. The top 3 cm of the wet soil is then scooped out and discarded. A teaspoon may be used for scooping out the soil. The bottom 3 cm of wet soil immediately above the wetting front is discarded also. The remaining wet soil is our sample of soil at field capacity. This is placed in a drying tin or an aluminium foil 'boat'. The weight of the wet soil is recorded, and the wet soil is then dried to a constant weight at 105°C, and the dry weight recorded. The moisture content of soil is then calculated:

$$\text{Field capacity moisture (\%)} = \frac{100\left(\text{Weight wet soil} - \text{Weight oven-dry soil}\right)}{\text{Weight oven-dry soil}}$$

Once again, the mean of the three values is taken as our best estimate of the field capacity moisture percentage (Table 2.2).

Table 2.2 Estimation of field capacity for a lowland soil from the Soc Son District, Vietnam.

Replicate	Weight of moist soil (g)	Weight of oven-dry soil (g)	Weight of moisture (g)	Field capacity moisture (%)
1	34.26	28.25	6.01	21.1
2	31.65	26.18	5.47	20.9
3	36.93	30.20	6.73	22.3
			Mean	21.4

(c) Field capacity and permanent wilting point by the pressure plate method

If you have access to a pressure plate apparatus, you can determine both the field capacity and *wilting point* (moisture content when the soil is drained at a suction of 1500 kPa (= 15 bar or pF 4.2). The procedure for making these measurements is as follows:

Three samples of soil are equilibrated to 10 kPa and a further three to 1500 kPa on the pressure plate. The weight of the moist soil in each sample is determined accurately. The soil is then dried to a constant weight at 105°C, and the dry weight recorded. The moisture content at each of the two suction values is then calculated:

$$\text{Field capacity moisture (\%)} = \frac{100\left(\text{Weight wet soil at 10 kPa} - \text{Weight oven-dry soil}\right)}{\text{Weight oven-dry soil}}$$

$$\text{Wilting point moisture (\%)} = \frac{100\left(\text{Weight wet soil at 1500 kPa} - \text{Weight oven-dry soil}\right)}{\text{Weight oven-dry soil}}$$

The *available water* content of the soil is the difference in moisture content between the two suction values:

$$\text{Available water (\%)} = \text{Field capacity moisture (\%)} - \text{Wilting point moisture (\%)}$$

Again, means of the three values are taken as our best estimates of field capacity, permanent wilting point, and available water range.

2.11.3 Measuring soil pH

The pH of the soil is a very important chemical characteristic, determining the solubility and availability to plants of many elements. An accurate measure of soil pH can be obtained in the laboratory using a *pH meter*. This should be done on a representative soil sample e.g. a portion of the 1 kg subsample taken after a bulk soil sample has been dried, sieved and mixed (see Section 2.2). As the measured value of soil pH can be markedly affected by the method of measurement, it is important that soil pH is measured in a standardised way (see Appendix 4).

In the field, a reasonably good estimate of soil pH can be obtained using a battery–operated portable pH meter (Figure 2.4(a)). To obtain a representative value, readings should be taken on soil samples collected from several places in the field, and the mean value calculated. If a portable pH meter is not available, a rough indication of the soil pH can be determined in the field by moistening a sample of the soil with a solution that changes colour according to the pH (universal indicator solution), and matching the colour against a chart provided with the solution. So that the colour observed is not influenced by the natural colour of the soil, it is usual to sprinkle a white powder (barium sulfate) over the surface of the moistened soil sample and read the colour against this white background. The pH measured in this way is called the *field pH* (Figure 2.4(b)).

As the pH of a soil increases, the solubilities of aluminium and the micronutrient metals iron, manganese, zinc, and copper decrease, while the solubility of molybdenum increases. At low soil pH values, plant growth may be restricted by the presence

Figure 2.4 Measurement of soil pH in the field using either (a) a portable pH meter, or (b) a colorimetric soil pH test kit.

in the soil solution of toxic concentrations of aluminium or manganese, or by deficiencies of molybdenum or other elements (see Table 6.1 in Section 6). At high pH values, plant growth may be reduced by deficiencies of iron or other micronutrient metals. Differences in soil mineralogy and plant adaptation mean that there is no single pH value that is best for plant growth. However, in many soils, most plant species can be expected to grow well at pH values between 5.5 and 6.5.

Yield reductions due to manganese toxicity may occur if the pH measured in water is less than 5.5, and yield reductions caused by this or other soil acidity factors are very likely if the pH is less than 5.2. With such soils, we will want to test in our pot experiments the effects of overcoming this acidity with lime. However, before we can do this, we need to establish the relationship between the amount of lime added and the resulting soil pH. Details of how to establish this *lime requirement* are given in Appendix 5.

3

Understanding and Running Omission Trials

3.1 First a little theory...

About the middle of the nineteenth century, Justus von Leibig discovered a very important principle that came to be called *the law of the minimum.* In modern language, it may be stated as follows:

> If several nutrient elements are present in the soil in amounts that would be insufficient for maximum plant yield, the yield will be determined solely by the supply of that element present in smallest amount relative to the plant requirement, variation in the supply of other elements having no effect on yield.

Understanding the law of the minimum is helped by the well-known analogy of water in a wooden barrel with staves of unequal length (Figure 3.1). In this analogy, the height of individual barrel staves represents the level of supply, relative to plant needs, of individual factors that are capable of influencing yield, while the level of water in the barrel represents the actual yield.

In the example illustrated in Figure 3.1 we see that, as long as phosphorus supply is limiting plant yield, there will be no benefit in supplying increased amounts of any other element. However, once the supply of phosphorus has been improved, another element, in

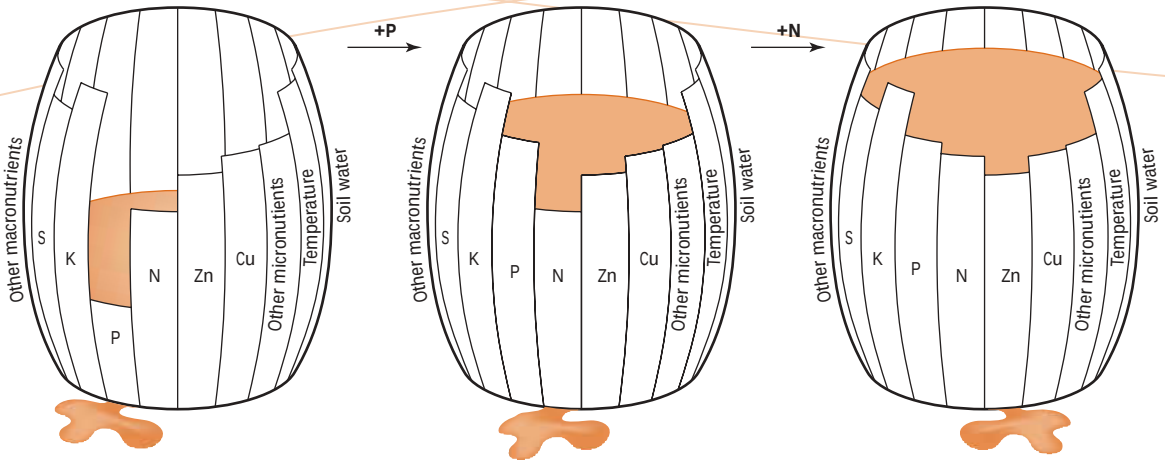

Figure 3.1 The 'water barrel' analogy of relationships between crop yield and the supply of individual nutrients.

this case nitrogen, would be expected to become the limiting element. Similarly, when additional nitrogen has been added, a further limitation is likely to be imposed by the supply of a third element, in this case, the micronutrient zinc. It is clear from this illustration that, although the soil is rather low in the three elements phosphorus, nitrogen, and zinc, there will be no value in adding nitrogen fertiliser until the phosphorus deficiency has been corrected. In the same way, there will be no benefit in adding zinc fertiliser until both phosphorus and nitrogen have been added to the system.

The law of the minimum has important implications for the design of experiments intended to identify nutrient elements likely to limit plant yields. For example, if we were to test the effect of adding each element singly to a soil, from the law of the minimum we would expect to detect a deficiency only of the element present in least supply relative to plant needs. The experiment would not give us any information about other elements also present in amounts insufficient for maximum yield. Hence, we need to study the effects of applying nutrient elements in various combinations if we are to discover all of the elements that are potentially limiting for plant growth in a particular soil.

There are two basic experimental designs that can be used — factorial designs, and omission or 'missing element' designs (Andrew and Fergus 1964). In skilled

hands, either design is capable of yielding the desired information. However, the omission designs are simpler in concept, and probably work rather better than factorial designs on soils with severe, multiple nutrient limitations.

In nutrient omission trials, we take as our reference point the yield of plants growing in a soil to which all nutrient elements have been added. We then compare the yield of plants in this 'all nutrients' treatment with those in a series of treatments in which each of the nutrient elements has been left out in turn. Thus, we would have as our treatments: all nutrients, all nutrients minus nitrogen, all minus phosphorus, and so on.

3.2 Deciding on the 'all nutrients' treatment — which salts and how much of each ?

We use mostly chloride salts of the nutrient cations and sodium salts of the nutrient anions in the 'all' treatment. This means that we cannot test for chlorine deficiency but, except in coconuts, chlorine deficiency is rarely encountered as a field problem. Similarly, we cannot test for sodium deficiency. While it has been demonstrated that sodium is essential for some species having the C_4 photosynthetic pathway, deficiencies have not been reported from the field. The ability of some plants to partially substitute sodium for potassium may result in mild potassium deficiency being missed in omission trials.

With legumes and other species that usually rely on symbiotic nitrogen fixation, we do not include nitrogen in the 'all' treatment, but we do include cobalt, because cobalt is essential for biological nitrogen fixation. Cobalt is not thought to be required for the growth of plants supplied with mineral nitrogen. To date, nickel has been demonstrated to be essential only for barley, but it seems likely that further research will extend essentiality to a wider range of species. Hence, we recommend including nickel in the 'all' treatment.

As explained in Section 1.5, optimal levels of nutrient addition depend in part on plant requirements, and in part on the extent to which the added nutrients react with soil components to produce compounds of very low solubility and hence low availability to plants. Soils rich in oxides of iron or aluminium may have a considerable capacity to 'fix' added phosphorus into forms less available to plants. Hence, the amount of phosphorus that needs to be added to obtain unrestricted plant growth may vary widely from soil to soil (see Section 4.2.2). Some soils also have the ability to 'fix' potassium in relatively unavailable forms. Thus, a knowledge of the chemical properties of the soils we are studying, and particularly their phosphorus-fixing capacity, can be very useful in choosing nutrient levels for the 'all' treatment.

There are two steps in optimising the 'all' treatment:

(a) making a 'best guess' for the rate to apply each element, based on soil chemical data (if any), our

own experience, or the published experience of others working with similar soils; and

(b) conducting a preliminary experiment in which the 'best guess' mixture of nutrients is supplied at a range of levels (see Section 3.5.1), and the effects on the yield of a test plant are measured. Usually, we would select as our 'all' treatment the lowest level of addition of the mixture that gave maximum yield. If the next-lowest treatment was very close to the maximum, we might choose a level intermediate between these two treatments.

It is worth taking some trouble to optimise the 'all' treatment for each soil, since omission trials have their greatest sensitivity when the 'all' treatment ensures an adequate but not excessive supply of each of the elements essential for plant growth. If the optimisation is imperfect, the trial may still yield useful results, but some information may be lost. For example, suppose if, after adding the 'all' mixture, the supply of one of the essential elements was sufficient to allow the test plants to achieve only 60% of maximum growth. In such a case, it follows from the law of the minimum, that we will be able to detect only those deficiencies of other elements which would cause a yield reduction greater than 40%.

3.2.1 Some examples of 'all' treatments used with various soils

As a starting point, it is useful to know the rates of nutrient addition that have been used successfully by other researchers on a diverse range of soils. As an example, the rates used for five contrasting soils are listed in Table 3.1.

Note that the phosphorus rates used vary from the equivalent of 30 kg/ha for the sandy soil which had very little capacity to 'fix' phosphate in forms unavailable to plants, to 600 kg/ha for the strongly phosphorus-fixing volcanic ash soil from Tonga. The rates of application of other elements generally vary a lot less than those for phosphorus. For boron and the other micronutrients, the rates needed will usually increase as we move from sandy textures to clay textures.

3.2.2 Preparing nutrient stock solutions

Apart from lime, which, if needed, is added as a dry powder, all chemicals in omission trials are usually added to the soil as solutions. A separate *stock solution* is needed for each element to be added. For most omission experiments, this will mean adding 13 solutions to the soil in each 'all nutrients' pot and 12 solutions to each of the other pots. It will usually be found convenient to apply each of the chemicals in a volume of 5 mL. However, if the addition of 60 or 65 mL (5×12 or 13) would make the soil too wet, the volume of each solution applied can be decreased and the concentrations increased correspondingly. The problem of excessive wetness of the soil occurs most commonly if the soil has not been properly air-dried before being placed in the pots.

Table 3.1 Rates of nutrient addition used in the 'all' treatment in pot experiments with five contrasting soils (kg element/ha).

Element	Sand, Dimbulah, Australia[a]	Loam, Solomon Islands[b]	Clay, Papua New Guinea[c]	Solodic soil, Australia[d]	Volcanic ash soil, Tonga[e]
N	100	Nil[f]	100	Nil[f]	200
P	30	50	80	280	600
K	80	100	80	168	450
Ca	35	Nil	35	162	105
Mg	30	Nil	30	34	90
S	25	40	25	404	75
Fe	6.7	Nil	5	Nil	15
B	2	1	2	5.6	6
Mn	5	7	5	18	15
Zn	4	4	4	5.6	12
Cu	3	4	3	6.7	9
Mo	0.4	0.15	0.4	0.37	1.2
Ni	Nil	Nil	0.1	Nil	0.3

[a] N.J. Grundon, unpublished data
[b] Watson and Whitman (1981)
[c] Dowling et al. (1994)
[d] Jones and Crack (1970)
[e] Halavatau (1998)
[f] The test plant was a legume reliant on symbiotic nitrogen fixation. Usually, cobalt would be added in the 'all' nutrient mixture, as this element is required for nitrogen fixation. A typical rate of application would be 0.1 kg/ha. However, cobalt was not supplied in either of these studies.

The method of calculating the weights of each salt needed to make up the stock solutions is given in Appendix 6.

Table 3.2 shows the composition of a typical set of stock solutions, and results of some of the underlying calculations, as described in Appendix 6.

Table 3.2 Examples of computation of stock solution concentrations required for a nutrient omission trial using pots with an exposed soil area of 133 or 182 cm^2 (13.0 or 15.2 cm diam.) and a volume of 5 mL for each addition of stock solution to the soil.

Element	Rate of application of element (kg/ha)	Compound	Conversion factor: weight of element to weight of compound	Rate of application of compound			Concentration of compound in stock solution (g/L)	
				(kg/ha)	(mg/pot)			
					133 cm^2	182 cm^2	133 cm^2	182 cm^2
N	100	NH_4NO_3	2.86	286	380	521	76.1	104.2
P	30	$NaH_2PO_4.2H_2O$	5.75	173	229	314	49.8	62.8
K	80	KCl	2.01	161	214	293	42.8	58.6
Ca	35	$CaCl_2$	2.79	98	131	179	26.1	35.7
Mg	30	$MgCl_2.6H_2O$	8.35	250	332	455	66.4	91.0
S	25	Na_2SO_4	4.42	111	147	202	29.5	40.4
Fe	5	FeNaEDTA[a]	6.57	32.9	43.7	59.8	8.76	12.0
B	2	H_3BO_3	5.72	11.4	15.0	20.7	3.02	4.14
Mn	5	$MnCl_2.4H_2O$	3.27	16.35	21.8	29.8	4.35	5.96
Zn	4	$ZnCl_2$	2.08	8.34	11.0	15.1	2.20	3.02
Cu	3	$CuCl_2.2H_2O$	2.68	8.04	10.7	14.6	2.13	2.92
Mo	0.4	$[NH_4]_6Mo_7O_{24}.4H_2O$	12.88	5.15	6.84	9.37	1.37	1.87
Ni	0.1	$NiCl_2.6H_2O$	4.05	0.405	0.50	0.74	0.11	0.15
Co	0.1	$CoCl_2.6H_2O$	4.04	0.404	0.50	0.73	0.11	0.15

[a] Sometimes, ferric EDTA contains appreciable amounts of sulfate. If this problem is encountered, you may need to prepare your own sulfur-free EDTA as described in Appendix 7. EDTA = ethylenediaminetetraacetic acid

3.3 Choosing the test plant

Choice of test plant is a matter of individual preference. You may wish to use a species that is important in the agricultural system with which you are working. However, sometimes this will not be convenient because of the size, growth rate, or some other characteristic of such species. Hence, we often find it convenient to use another plant species as our test plant.

The ideal test plant will be fast growing, have relatively small reserves of mineral nutrients in the seed (coconuts definitely not suitable!), and exhibit little plant-to-plant variation when grown under uniform conditions. It is helpful also, if the species is tolerant or immune to seedling diseases such as damping-off, and has seeds that are easy to obtain and easy to germinate. Among the legumes, phasey bean (*Macroptilium lathyroides*) has proved to be a good test plant, as has maize (*Zea mays*) among the non-legumes.

3.4 Note on the need for replication in pot experiments

In pot experiments, we usually put a quite a lot of effort into providing uniform growing conditions for our test plants. Thus, we provide each pot with the same weight of soil (Section 3.5.1) and accurately measured amounts of nutrients (Section 3.5.4), and we select seeds of uniform weight for planting (Section 3.6.1), cover them with measured amounts of soil (Section 3.6.3), and so on. However, despite all these precautions, when we harvest our experiment, we will find that plants we have subjected to the same treatment will vary slightly in yield.

If there is some variation even when all pots have received the same treatment, we need to have some means of distinguishing between these naturally occurring variations in yield, and those variations caused by the particular nutrient treatments that we have applied in our experiment. Fortunately, statistical techniques have been developed to help us with this problem. These techniques involve comparisons between the amount of variation in yield among pots that have been treated the same, and those that have had different treatments imposed on them. Hence, for most kinds of experiments, we need more than one pot receiving each treatment. A set of pots containing a complete single set of treatments is called a *replicate*. When all the pots in a replicate are grouped together in the greenhouse, e.g. placed on the same bench, it is often called a *block*.

We recommend the use of four replications for preliminary experiments, unless you are very short of soil or greenhouse space. We recommend four replications also for nutrient rate trials in pots (Section 4) and for the omission treatments in nutrient omission trials. However, as indicated in Section 3.10.1, we recommend increasing statistical precision in omission trials by replicating the 'all' treatment eight times instead of four.

3.5 Setting up a preliminary trial

3.5.1 Weighing the soil into the pots

If plastic pot liners are being used, it is advisable to line the pots with them about two days before weighing out the soil, and leave the lined pots exposed to the sun so that any toxic volatile substances they contain will be released (Figure 3.2).

For the preliminary trial, we recommend using 12 pots of each soil. This will allow testing the best guess 'all' treatment at the originally intended level ('best guess' × 1), and at five other levels (× 0, × 0.5, × 2, × 3, and × 4), with two replications.

In Section 2.11.1, we determined the weight of soil required per pot. We could now weigh out into each pot that full amount of soil. However, the planting operation will be made easier, and in some soils the seedling emergence made more uniform, if we keep separate enough soil to cover the seeds to the required depth after planting. The exact amount will depend on pot size and planting depth but it will usually be in the range 150–250 g. This weight of soil will need to be deducted from that weighed into each pot, and should be weighed out into a separate plastic bag for each pot (Figure 3.3).

Figure 3.2 Pots and plastic liners placed in the greenhouse to allow any toxic volatile substances to be released before the pots are filled with soil.

3.5.2 Calculating *before-planting* and *after-planting target weights* for pots

As indicated in Section 2.11.2, our aim during the experiment will be to keep the water content of the soil as close as possible to field capacity. Since we will be watering on a weight basis, we will need to know what the pot of soil will weigh when correctly watered to field capacity; i.e. we need a *target weight* to aim for. To do this, some simple calculations are needed.

Figure 3.3 (left to right) Empty pot with liner, pot containing correct weight of air-dry soil, and plastic bag containing the air-dry soil that will be used to cover the seeds after planting.

First, we calculate the oven-dry weight of the air-dry soil to fill the whole pot, using the mean air-dry moisture percentage for the soil determined in Section 2.11.2. Later, the 150–250 g air-dry soil used to cover the seeds will be taken into account.

$$\textit{Weight of oven-dry soil} = \frac{\textit{Weight of air-dry soil} \times 100}{100 + \textit{Air-dry moisture \%}}$$

For the Vietnamese lowland soil mentioned in Section 2.11.2, a pot with a diameter of 12.5 cm and a depth of 12.0 cm was found to contain 1420 g air-dry soil when filled to 1 cm from the top. The weight of oven-dry soil was thus $1420 \times 100/(100 + 10.2) = 1289$ g (see Section 2.11.2a for the calculation of the air-dry moisture percentage).

Second, we calculate the weight of soil plus water at field capacity.

$$\textit{Weight of soil + water at field capacity} = \frac{\left(100 + \textit{Field capacity moisture \%}\right) \times \textit{Weight of oven-dry soil}}{100}$$

Using our lowland soil example, this would be $(100 + 21.5) \times 1289/100 = 1566$ g (see Section 2.11.2b for the calculation of the field capacity moisture percentage).

Next, we need to calculate the amount of water that would be present in each pot when watered to field capacity, using the field capacity moisture percentage calculated in Section 2.11.2b. To do this, the weight of oven-dry soil is subtracted from the weight of soil plus water at field capacity.

$$\textit{Weight of water} = \textit{Weight of soil at field capacity} - \textit{Weight of oven-dry soil}$$

In our example, this would simply be 1566 – 1289 = 277 g. Further, if the weight of the pot plus liner was 57 g, the total weight of pot, liner, oven-dry soil and water to field capacity would be 57 + 1289 + 277 = 1623 g. As this is the weight we would be aiming for each time we watered the pots, it is sometimes called the *after-planting target weight*.

As explained in Section 3.5.1, some air-dry soil is kept separate in a plastic bag to make the planting operation easier and improve seedling emergence. Therefore, a *before-planting target weight* needs to be calculated which reflects the lower weight of oven-dry soil in the pot before planting.

Importantly, the soil is not watered to field capacity because (i) not all the soil is yet in the pot, and (ii) waterlogging of soil is detrimental to seed germination. Often, 85% of the water to field capacity is added (Section 3.5.4(g)). If 185 g (approximately 2 cm depth) of air-dry soil was kept separate to cover the seeds in our lowland soil example, the *before-planting target weight* would be based on the following calculations:

Weight of pot and liner = 57 g

Weight of air-dry soil = 1420 – 185 = 1235 g

Weight of oven-dry soil =
$100 \times 1235/(100 + 10.2) = 1121$ g

Weight of water to 85 % of field capacity =
$0.85 \times 277 = 235$ g

Thus, the *before-planting target weight* is 57 + 1121 + 235 = 1413 g, which is 210 g (i.e. 1623 – 1413) less than the *after-planting target weight*.

3.5.3 Labelling the pots

Next, smooth down the plastic pot liner over the sides of the pot and write on it the soil name, treatment, and replication number, using a waterproof marking pen. A dark-coloured (e.g. black or dark blue), good quality pen should be used, as some of the inks in cheaper pens may fade excessively in the sunlight. If in doubt, do a fading test on a spare plastic bag about 3 weeks before the start of the experiment. We do not write these details on the *radiation shield* (see Section 2.5), for two reasons: (a) the radiation shields are likely to be reused in subsequent experiments, and will soon be covered in a confusing array of information; and (b) when watering, these shields are usually removed, and it is easy to make a mistake about which shield was on which pot.

3.5.4 Adding nutrients and soil amendments

(a) Empty the soil from each pot in turn onto a clean plastic sheet about 80 cm square and spread the soil out to a depth of 2 to 3 cm with a plastic ruler.

(b) If the pH of the soil is to be adjusted, the $Ca(OH)_2$ or $CaCO_3$ powder is now sprinkled as uniformly as possible over the surface of the soil, and mixed in by taking two diagonally opposite corners of the sheet and rolling the soil backwards and forwards. Repeat with the other two corners. The amended soil should then be spread out again with the plastic ruler for the addition of the nutrients.

(c) The nutrients are now added one at a time. This can be done using glass pipettes to accurately measure out and apply the 5 mL of each stock solution to the soil from each pot, a separate pipette being used for each solution to prevent cross-contamination. However, glass pipettes are relatively expensive (several dollars each) and are easily broken in the greenhouse. A cheap alternative is to use disposable plastic syringes costing only a few cents each. We have found that, with a little practice, these can be used to dispense the required volume of solution with an acceptable level of accuracy. To apply each solution, the syringe or pipette is passed backwards and forwards above the soil surface in such a way that the stock solution is spread as evenly as possible (Figure 3.4). For most soils, the rate of application can be varied by varying the volumes of the solutions applied. Thus, if the decision has been made to apply 5 mL of each solution in the 'best guess' treatment, we would apply 4×5 mL = 20 mL in the 'best guess $\times$ 4' treatment. With 13 solutions to apply, this would add 13×20 mL = 260 mL per

Figure 3.4. Using a plastic syringe to distribute 5 mL of a nutrient stock solution evenly over a pot of soil that has been spread out thinly on a sheet of clean plastic.

pot. For most soils this will not be sufficient to cause the soil to become excessively moist. However, note that in the example of the lowland soil from Vietnam discussed in Section 3.5.2, the addition of 260 mL of solution would result in the before-planting target weight being exceeded. This problem was caused by incomplete air-drying of the soil and was overcome by using smaller volumes of more concentrated stock solutions for the 'best guess × 3' and 'best guess × 4' treatments.

(d) When all the nutrients have been added, the soil is mixed thoroughly as described in (b) above.

(e) The soil is then poured back into the pot, and settled by dumping the pot on the bench four times from a height of approximately 5 cm.

(f) If you will not be ready to plant for some time, simply cover the pots to keep out dust until you are nearly ready to plant.

(g) If you will be ready to plant in the next few days, you should calculate the *before-planting target weight* for your pots (see Section 3.5.2). For the purposes of this calculation, we suggest that you water up to 85% of the total water that will be present in the pot at field capacity *after planting*, that is, after the extra layer of soil has been added. Depending on the relative weights of soil above and below the seed, this may represent a slight overwatering of the before-planting soil, but the excess water should sink to the bottom of the pot. After the layer of air-dry soil is placed on top of the seed, any excess water in the lower layer will be redistributed upwards into this 'new' layer of soil.

(h) Now level the soil surface in the pots and apply sufficient deionised or distilled water to bring the pots up to the *before-planting target weight*. Be careful not to slosh the water on, making your planting surface uneven and bringing fine material to the surface. A good way of bringing the soil up to the required water content is to first place the pot on a

balance of suitable capacity, and then slowly apply the water through a soft plastic tube of about 5 mm internal diameter connected to your deionised water reservoir and closed off at the lower end with a sliding clamp that can be operated with one hand. By passing the gentle stream of water backwards and forwards and from side to side over the soil surface it should be possible to bring the pot of soil up to the required weight without flooding or greatly disturbing the surface.

Note that the soil will already contain some residual water from air-drying, and some water from the nutrient solutions already added. Hence, the amount of water needed to bring the pots up to target weight will be correspondingly less than the amount used in your target weight calculations.

If signs of excessive wetness appear when you first start watering-up your pots, stop immediately, and check your calculations concerning the *before-planting target weight*. If no error can be found, it is likely that you have encountered a problem of incomplete soil mixing, as discussed in Section 2.2, resulting in some pots containing less-well-dried soil, or soil with less-than-average amounts of the finer fractions of the soil. At this stage, the best course of action is to reduce the before-planting target weight until no free water remains on the top of the soil. (A corresponding adjustment should be made also to the after-planting target weight to ensure that no pots are flooded when the pots are first watered after emergence (Section 3.6.3)). Any pots already over-watered will have to be replaced with freshly prepared pots of the same treatment.

(i) Unless you are able to plant the pots on the same day as they were watered up to the before-planting target weight, gently draw the plastic pot liner up above the pot and close off with a rubber band or wire bag-tie to prevent evaporative loss of soil water until you are ready to plant. Be sure to cover the pots with newspaper to prevent overheating of the moist soil.

3.6 Germinating and planting the seed

Extra effort at this stage can yield rich rewards in improved experimental precision.

3.6.1 Stratifying our batch of seed

Commercial seed samples commonly contain a considerable range of seed sizes, resulting in substantial variation in the growth rate of individual seedlings. Variability can be reduced by restricting the range of seed sizes actually used in the experiment.

With seeds that are smooth and approximately spherical, stratification of a seed lot is easily achieved by passing the sample through a series of laboratory sieves (coarsest on the top, finest on the bottom). For your experiment, use only seed of the same class size.

Seeds that are not easily sorted by sieving can be sorted on an individual weight basis. Although this may seem to be a big task, if you have access to a well-damped laboratory balance, a great many seeds can be sorted in 2 or 3 hours. We suggest starting by weighing 100 individual seeds taken at random from the seed lot, and determining the frequency distribution of seed weight. You will then be in a position to strike a balance between conserving your seed supply and reducing variation in seed weight. Often, the mean seed weight ±10% is a satisfactory compromise.

In recent experiments in Vietnam, hybrid maize seed was found to vary from about 160 mg/seed for the smallest seeds, to about 300 mg/seed for the largest seeds. The average seed weight was about 240 mg/seed and 58% of seeds were within ±10% of this seed weight, i.e between 220 and 260 mg/seed (Figure 3.5(a)). Hence, a very considerable reduction in variability could be achieved while still retaining for use more than half of the original seed sample.

However, even when the range of seed sizes is restricted, there may still be substantial variation in the speed of germination (Figure 3.5(b)). Hence, plant-to-plant variation can be further reduced by selecting for planting only those seeds at the same stage of germination. In the example from Vietnam, after 42 hours of germination only about half of the seeds in the 220–260 mg weight range (24% of the original seed sample) were judged to be at the correct stage for planting (radicle about 4 mm long). Although variation in seed weight and in speed of germination can be expected to vary from one seed lot to another, these results show that when commencing an experiment we may need to imbibe about twice as much seed as we intend to plant (see Section3.6.2).

Figure 3.5 Variation in seed size and speed of germination in hybrid maize: (a) (left to right) seeds < 220 mg/seed, seeds between 220 and 260 mg/seed, and seeds > 260 mg/seed; (b) appearance of seeds between 220 and 260 mg/seed, 42 hours after commencement of the germination process.

3.6.2 Surface-sterilising and imbibing the seeds

Seeds often carry on their surface, spores of fungi that can overrun the germination trays, and may inhibit early growth processes. If the seed you plan to use has

been treated with a fungicidal dust, no further protection should be required. If it has not, surface sterilisation with 0.5% w/v NaOCl for 5 minutes will usually solve the problem. Seeds should be washed in several changes of distilled or deionised water after the surface sterilisation procedure.

Germination of many species is hastened and made more uniform by imbibing the seeds in a well-aerated, dilute solution of a calcium salt. $CaSO_4 2H_2O$ at 200 mM is often used. However, since the oxygen requirement of germinating seeds is high, and the seeds may be damaged if starved of oxygen, this step should be carried out only if the solution can be aerated continuously. An aquarium bubbler can be used for this purpose. The optimal time for imbibition appears to vary substantially with species, but large-seeded legumes (e.g. soybean) should not be imbibed for more than about 2 hours. By contrast, maize can be allowed to imbibe overnight. If the imbibing solution becomes discoloured, it should be drained off and replaced with fresh solution.

After imbibition, the seeds should be spread out in shallow trays lined with blotting paper or paper towels moistened with a dilute calcium salt, and covered with a loose-fitting lid to allow some gas exchange but restrict the rate of water loss. With large seeds, e.g. maize or soybean, which tend to have a relatively small area of contact with the moistened paper relative to their volume, placing a second sheet of moistened blotting paper over the top of the seeds will be advantageous. The trays should be kept out of sun and wind and checked daily, moistening the papers if necessary. When the radicle is emerging in most of the seeds is a good time to plant.

Always imbibe plenty of seeds so that you can select, for planting, seeds that are all at the same stage of germination.

3.6.3 Planting

With each pot, open the plastic pot liner and smooth it down the outside of the pot where it will not be in the way. Place the required number of germinating seeds at roughly equal spacings on the moist surface of the soil (Figure 3.6) and cover with the air-dry soil previously weighed and set aside for the purpose. With legumes, pipette 1 mL of a suspension of the appropriate strain of *Rhizobium* or *Bradyrhizobium* over each seed before covering with soil. Under hot sunny conditions, it is advantageous to cover the pots with clean newspaper for a day or two until the first seedlings emerge.

Always plant more seeds per pot than you intend to keep, so that you can conduct a thinning harvest a few days after seedling emergence, thus further improving uniformity in the experiment. For example, if your pots are of such a size that you plan to grow three maize plants in them, plant five germinated seeds and remove two after emergence. In deciding which seedlings to

Figure 3.6 Placing germinated maize seeds, that have been selected for uniform length of radicle, on the moist soil surface before covering with a layer of dry soil.

remove, pay particular attention to any that are damaged or are smaller or larger than the average seedling.

With most soils, it will not be necessary to water until after seedling emergence, there being sufficient capillary rise around the imbibed and sprouted seeds to ensure excellent establishment. Withholding additional water at planting will be particularly helpful to seedling emergence in soils that tend to disperse on wetting then form a crust.

Once the seedlings have emerged, the pots can be weighed, and additional water added, as necessary, to bring them up to field capacity, i.e. to bring them up to the *after-planting target weight*.

At this first watering, approach the after-planting target weight cautiously, being on the lookout for any pots which, due to inadequate soil mixing (see Sections 2.2 and 3.5.4(h)), may have a lower water-holding capacity than the average. If free water remains on the soil surface in a pot, after the applied water has completely entered the soil in the other pots, this free water should be carefully poured off, and the pot allowed to dry down until the appearance of the soil surface matches that of a correctly watered pot. The pot should then be weighed, the weight written clearly on the pot liner, and this new weight used as the target weight for the remainder of the experiment.

3.7 Maintaining the experiment

The experiment should be checked daily to make sure that the plants have adequate water. When the seedlings are small, it should not be necessary to water every day, but it is a wise precaution to test-weigh two or three pots just to be sure. Later, when the plants are well grown, it will be necessary to bring the pots up to the correct weight each day, and towards harvest time, twice daily watering may be needed. Application of a mulch of the polyethylene or polypropylene beads used in the manufacture of plastic buckets and other moulded products, will reduce evaporation from the soil surface and hence one source of water loss. The beads should be weighed (typically about 100 g per pot) and the weight of beads taken into account when calculating the *target weight* for watering.

When the plants become large, their weight becomes a source of error in our water management, causing us to under-water our plants, particularly those which are growing the best. This error can be overcome by correspondingly increasing the *target weights* for those treatments in which the plants have become large. In maize, the height of the tallest leaf (Figure 3.7(a)) is a non-destructive measurement that is closely related to plant fresh weight and can be used to adjust the target weight as an experiment progresses (Figure 3.7(b)).

In hot climates, potted soils can become much hotter than their counterparts in the field because of absorption of solar radiation through the sides of the pot. This may lead to damaging root temperatures. As indicated in Section 2.5, the problem can be partly solved by sitting the pots inside radiation shields made of the aluminised paper often used as a heat barrier in building construction. Watering the floor in the hottest part of the day helps also, by reducing the amount of heat radiated from the floor (Nualsri et al. 1993).

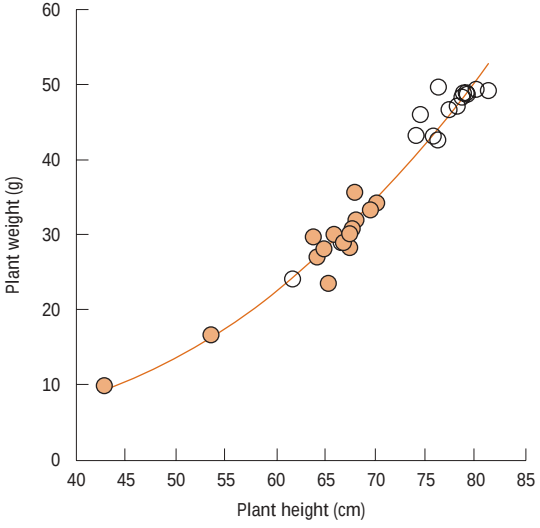

Figure 3.7 (a) Measuring height of the tallest leaf in hybrid maize in a pot experiment at the National Institute of Soils and Fertilisers near Hanoi, Vietnam. (b) Relationship between height to the tip of the tallest leaf in hybrid maize and plant fresh weight in a pot experiment with two soils (open and closed circles) and two plants per pot.

During the daily observation of the pots, careful note should be taken of any deficiency or toxicity symptoms that may appear, and we should look out for infestations of damaging insects, which should be dealt with promptly.

3.8 Harvesting

Depending on the test plant chosen, well-defined growth differences usually will be evident after 3–5 weeks and the experiment should be harvested. We have found with maize in the tropics and subtropics that treatment effects usually becoming evident after 2 weeks, but harvest is best delayed until 3 weeks to allow these treatment effects to develop fully. Only when there is a severe shortage of time, e.g. in a training course, should the plants be harvested at 2 weeks from planting.

Before harvest, any pots that have suffered unexpected damage during the experiment should be identified and discarded. Sources of damage we have observed over the years have included plants being dug up by rats, watering errors that have left one or more pots seriously waterlogged, and pots dropped on the floor during watering, with consequent severe disturbance to the root system. Usually, such problems will have been recorded on the laboratory notebook at the time the incident occurred. However, it is a good idea to 'derandomise' the experiment before harvest, locate any such pots, and remove them before the harvesting operation commences.

In most cases, harvesting will involve cutting off the plant tops about 1 cm above the soil surface. The tops should then be oven-dried at 75–80°C. Do not dry at higher temperatures, as this is likely to induce charring of the plant material. Drying time will depend on the species and the efficiency of the oven, but in most cases 48 hours in a forced-draught oven will be sufficient. When thoroughly dry, the dry weights should be recorded. For this, we recommend using a data sheet with 7 columns and 12 rows (Table 3.3). The first row is for treatment names, the next two (i.e. rows 2 and 3) for the measured dry weight of tops, the next (row 4) for the mean weights, and the fifth for the weights relative to the highest-yielding treatment. The remaining rows are for statistical calculations you will perform to test the significance of differences between the highest-yielding treatment and other treatments. Details of these calculations and a worked example are given in Appendix 8.

3.9 Results of the preliminary trial and their interpretation

Results obtained in preliminary experiments with three soils from the Asia–Pacific region (Table 3.4) may be regarded as typical.

Table 3.3 Suggested data sheet for preliminary trials.

Treatment	No fertiliser	Best guess × 0.5	Best guess	Best guess × 2	Best guess × 3	Best guess × 4
Rep 1						
Rep 2						
$\bar{x}$						
Rel. $\bar{x}$ (%)						

With the Australian and Malaysian soils, the original best guess for the 'all' treatment gave the best yield. In these soils, lower rates of application of the nutrient mixture resulted in an inadequate supply of at least one essential element, and higher rates of application resulted in an excessive supply of at least one component of the mixture. However, in the soil from Thailand, the original best guess probably underestimated the amount required of at least one essential element, although the difference between the 'best guess × 1' and the 'best guess × 2' treatments was not statistically significant.

If the plants grow vigorously in the highest-yielding treatment, and are free of any visible symptoms of nutrient deficiency or toxicity, we would be justified in accepting that treatment as the 'best guess' treatment for our omission trials. However, if growth was poor, or the plants showed symptoms of a nutrient deficiency or toxicity in the highest-yielding treatment, the problem will need to be sorted out before moving on to set up an omission trial with that particular soil.

Questions that might be asked include: Did the plants receive adequate light? (This is often a problem with old

greenhouses where growth of moulds or deterioration of the roofing material reduces light transmission.) Were soil temperatures too high? Was water management effective? Were the plants damaged by pests or diseases? Was the balance of nutrients in the 'best guess' treatment appropriate?

Where visible symptoms are present, these may give an indication of the cause of the problem, as may multi-element plant analyses if you have access to a plant chemistry laboratory. However, whatever the cause of poor growth, it is very important that such problems are corrected before proceeding any further.

Table 3.4 Relative dry matter yields (% of maximum) of maize tops in preliminary experiments conducted with soils from Dimbulah (Australia), Chembong (Malaysia), and Songkla (Thailand). (Values in the same column followed by an asterisk differ significantly at P = 0.05 from the highest yielding treatment.)

Treatment	Dimbulah, Australia[a]	Chembong, Malaysia[b]	Songkla, Thailand[c]
No fertiliser	16*	23*	3*
Best guess ×0.5	61*	54*	67*
Best guess ×1	100	100	92
Best guess ×2	55*	88*	100
Best guess ×3	0	78*	69*
Best guess ×4	1*	45*	4*

[a] N.J. Grundon, unpublished data
[b] H.A.H. Sharifuddin, pers. comm.
[c] Nilnond (1993)

3.10 What about the mineral nutrition of paddy crops?

When soils are flooded, many important chemical changes occur that can alter the supply of mineral nutrients. For example, nitrogen present in the soil as nitrate is reduced to gaseous nitrogen and lost from the soil, a process called *denitrification*. Again, insoluble oxides of iron and manganese are converted to more soluble forms, sometimes releasing large amounts of these elements in plant-available form, and the pH of acid soils rises (Forno et al. 1975) and that of alkaline soils tends to fall. In some lime-rich soils of high organic matter, flooding causes soil microorganisms to produce sufficient amounts of bicarbonate, and possibly organic acids such as acetic and butyric acid, to temporarily inhibit root function. These effects have been implicated in zinc deficiency of rice in the field (Forno et al. 1975).

From the foregoing, it is clear that studying the ability of a soil maintained at field capacity to release nutrients to a test plant such as maize, may not give a satisfactory indication of the ability of the same soil, when flooded, to release nutrients to a paddy crop. While further research on nutrient omission trials for paddy crops is needed, we suggest that the following procedures should be effective in most cases.

3.10.1 Choice of test plant

Although a number of crops are grown under paddy conditions, rice is by far the most widely grown, and has also proved to be an excellent test-plant.

3.10.2 Water management

If, in the district in which you are working, rice is normally planted into moist soil, and the field flooded after emergence, you may proceed as described up to and including Section 3.6.3 except that, after thinning, the pots should be flooded to a depth of 1–2 cm with distilled or deionised water instead of being brought up to field capacity.

If it is more usual to puddle the fields and transplant seedlings into them, we suggest flooding and puddling the soil immediately after the nutrients have been added (Section 3.5.4) and allowing the pots to stand for several days before transplanting, so that at least the more rapid chemical and biological changes have time to occur before the seedlings are introduced. In these circumstances, you will not need to determine the water-holding characteristics of the soil (Section 2.11.2) or calculate the before-planting and after-planting target weights (Section 3.5.2)

3.10.3 Nitrogen source

As denitrification results in nitrate being an ineffective nitrogen source in flooded soils, we recommend changing the nitrogen source from ammonium nitrate (Table 3.2) to urea.

3.10.4 Harvesting and interpreting the results

The procedures described in Sections 3.8 and 3.9 should be followed.

3.11 Setting up and running the omission trial

3.11.1 Treatments and experimental design

As recommended by Andrew and Fergus (1964), the 'all' treatment should be replicated eight times, and the individual omission treatments four times. For convenience, you may wish to label half your 'all' pots 'A' and half 'B' (see Table 3.6). All 60 pots of the experiment will usually be placed on the same bench, the position of the replicates and the pots within replicates being randomised. Re-randomisation at weekly intervals is desirable to reduce any effects of position on the bench; e.g. pots at the edges of the bench might be getting more sun than those in the middle. Also, if the benches are on castors, the position of benches in the greenhouse may be rotated weekly to reduce any effects of environmental gradients in the greenhouse.

For a non-leguminous test plant, and a soil in which the pH measured in water (Appendix 4) is above 5.5, we recommend the following treatments:

All[2]	All nutrients added
–N	All nutrients except N added
–P	All nutrients except P added
–K	All nutrients except K added
–Ca	All nutrients except Ca added
–Mg	All nutrients except Mg added
–S	All nutrients except S added
–Fe	All nutrients except Fe added
–B	All nutrients except B added
–Mn	All nutrients except Mn added
–Zn	All nutrients except Zn added
–Cu	All nutrients except Cu added
–Mo	All nutrients except Mo added
–Ni	All nutrients except Ni added

With a leguminous test plant, we would usually modify the 'all' treatment by including cobalt and omitting nitrogen. We would also add a –Co treatment, and delete the –N treatment. If we wished to check the effectiveness of symbiotic nitrogen fixation, we could include an additional 'all + N' treatment.

For a strongly acidic soil in which the pH in water was below 5.2, we would include in the 'all' treatment sufficient lime to raise the pH to 6.0, and we would add an 'all – lime' treatment in place of the –Ca treatment.

For soils of intermediate pH, i.e. between 5.2 and 5.5 inclusive, we would not include lime in the 'all' treatment, we would retain the –Ca treatment, and we would add an 'all + lime' treatment.

3.11.2 Treatment application

(a) If the soil has a pH of less than 5.2 in water, apply lime as described in Section 3.5.4(b).

(b) Next, apply the nutrient solutions as set out in Table 3.5, starting with the –Co treatment, if a legume, or –Ni if a non-legume, and working down the table. By proceeding in this way, from micronutrients to macronutrients, the risk of cross-contamination is reduced. Use the procedure set out in Section 3.5.4(c) to apply the nutrient solutions.

(c) Then proceed as set out in the remaining steps of Section 3.5.4.

[2] Selected on the basis of results of the preliminary experiment.

3.11.3 Planting, maintaining the experiment, and harvesting

For upland crops such as corn, proceed as in Sections 3.6, 3.7, and 3.8, except that any photographs documenting leaf symptoms and the magnitude of plant growth responses to the treatments (Figure 3.8) should be taken before harvesting (Figure 3.9). For paddy crops such as rice, water management should be as described in Section 3.10.2. For ease of recording the dry weight of plant tops, we recommend making up a data sheet with 16 columns (or 17 if there is an 'all+lime' treatment)and 18 rows (Table 3.6). The first row will contain your list of treatments, and the next four rows the yield data from the individual replicates. The remaining 13 rows will be needed for your statistical calculations (see Appendix 9).

Figure 3.8 Photographic record of plant growth responses in a soil from the Soc Son district in Vietnam that was found to be deficient in nitrogen and phosphorus.

Figure 3.9 Harvesting a pot experiment at the National Institute of Soils and Fertilisers, near Hanoi, Vietnam.

3.12 Interpreting the results of the omission trial

Since the experimental design is unbalanced, having eight replications for the 'all' treatment and only four for each of the other treatments, the simplest method of analysis is to compare each treatment in turn with the 'all' treatment. Each sub-experiment is then analysed separately, using Student's *t* test (for details of the calculations and a worked example, see Appendix 9).

Figure 3.10 shows the results of omission trials with two contrasting soils.

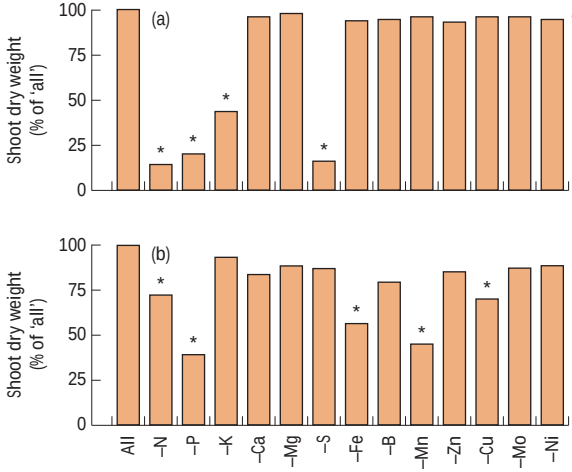

Figure 3.10 Results of nutrient omission pot trials (Halavatau et al. 1998) on (a) a volcanic ash soil from Fahefa, Tonga, using maize as the test plant, and (b) a calcareous soil from Madang, Papua New Guinea, using sweet potato as the test plant. Treatments marked with an asterisk yielded significantly less than the 'all' treatment ($P < 0.05$).

In soil (a) in Figure 3.10, we see that the natural supply of calcium, magnesium, iron, boron, manganese, zinc, copper, molybdenum, and nickel was adequate for maximum plant growth, the addition of more of these elements in the 'all' treatment having no effect on the dry weight of the shoots. However, the soil was severely deficient in nitrogen, phosphorus, and sulfur, plants in treatments in which one of these elements was omitted having shoot weights less than 25% of those in the 'all' treatment. In this soil, potassium was quite deficient also, with yields in the zero potassium treatment less than 50% of those in the 'all' treatment. However, from the law of the minimum (see Section 3.1) we would expect no response to potassium fertiliser until nitrogen, phosphorus, and sulfur had been added to the soil in amounts sufficient to lift the yield to 50% or more of the maximum (Figure 3.10(a)).

In soil (b) in Figure 3.10, moderately severe deficiencies of phosphorus, iron, and manganese were identified, along with less severe deficiencies of nitrogen and copper. Once again, no response to nitrogen or copper addition would be expected until the more severe deficiencies of phosphorus, iron, and manganese had been corrected.

Occasionally, it will be found that omission of an element causes a significant increase in yield *above* that in the 'all' treatment. When this occurs, the most likely explanation is that the level of the particular element that we have chosen to include in the 'all' treatment was too high — so high as to be toxic. Such an error will have caused some loss of sensitivity in the experiment, and it is suggested that, if the difference between the 'all' and this omission treatment is greater than 30%, the trial should be run again with a new 'all' treatment supplying less of the element concerned.

Table 3.5 Scheme for adding nutrient stock solutions to soil in an omission trial. Solutions are identified by the nutrient element they are supplying. Volume of each solution is either 0 or 5 mL per pot.

Treatment	Stock solution													
	N[a]	P	K	Ca[b]	Mg	S	Fe	B	Mn	Zn	Cu	Mo	Ni	Co[a]
– Co[a]	0	5	5	5	5	5	5	5	5	5	5	5	5	0
– Ni	5	5	5	5	5	5	5	5	5	5	5	5	0	5
– Mo	5	5	5	5	5	5	5	5	5	5	5	0	5	5
– Cu	5	5	5	5	5	5	5	5	5	5	0	5	5	5
– Zn	5	5	5	5	5	5	5	5	5	0	5	5	5	5
– Mn	5	5	5	5	5	5	5	5	0	5	5	5	5	5
– B	5	5	5	5	5	5	5	0	5	5	5	5	5	5
– Fe	5	5	5	5	5	5	0	5	5	5	5	5	5	5
– S	5	5	5	5	5	0	5	5	5	5	5	5	5	5
– Mg	5	5	5	5	0	5	5	5	5	5	5	5	5	5
– Ca[b]	5	5	5	0	5	5	5	5	5	5	5	5	5	5
– K	5	5	0	5	5	5	5	5	5	5	5	5	5	5
– P	5	0	5	5	5	5	5	5	5	5	5	5	5	5
– Na	0	5	5	5	5	5	5	5	5	5	5	5	5	5
All	5	5	5	5	5	5	5	5	5	5	5	5	5	5

[a] Note comments in Section 3.10.1 about N and Co in trials with legumes and non-legumes.
[b] Note comments about Ca in Section 3.10.1 in trials with soils with pH < 5.2 or between 5.2 and 5.5.

Table 3.6 Outline of a data sheet for recording yield data from a nutrient omission trial.

Soil name..... Test plant.....

Planting date.....Harvest date.....Units of measurement.....

Treatment	All A	All B	–N	–P	–K	. . . . .	–Ni
Rep1							
Rep2							
Rep3							
Rep 4							
.							
.							
.							
.							
.							
.							
.							

4

The Next Step — Rate Trials in Pots

4.1 Why do rate trials in pots ?

Having established which elements are likely to be limiting for plant growth, the next step is to determine, for each soil, the optimal level of supply of each of the deficient elements. We could do this directly by applying various rates and combinations of appropriate fertilisers in a field experiment and studying their effects on yield. However, there are two good reasons for conducting some nutrient rate trials in pots before starting our field work. These are:

(a) to confirm the results obtained from the omission trials; and

(b) to obtain preliminary information on the relationship between plant growth and the amount of nutrient added.

4.1.1 Need for confirmation of omission trial results

In omission trials, the 'all' treatment involves adding to the soil a large number of nutrient elements, many of which may not be deficient in any particular soil. Thus, in the examples given in Section 3.12, in one soil (Figure 3.2(b)), 8 of the 13 elements added to the soil were already present in amounts adequate for maximum plant growth, whereas in the other soil (Figure 3.2(a)), 9 of 13 were already adequate. Usually, these 'unnecessary' nutrient additions have no effect on the

outcome of the experiment. However, we do know that some nutrient elements interact quite strongly with each other, so that the addition of one may induce a deficiency of another. For example, adding potassium to the soil tends to make it harder for the roots to absorb magnesium, and *vice versa*. Indeed, there are well-documented cases of magnesium deficiency being induced in crops by heavy or repeated applications of potassium fertiliser. Similarly, zinc and copper inhibit the uptake of each other by the plant roots. Hence, when we add one nutrient element to the soil, there is always a slight risk that we will create a deficiency of another element which otherwise would not have occurred. Nutrient rate trials give us a convenient, low-cost means of eliminating any such 'false positive' results before we progress to the more expensive field experiments that will be described in Section 5. In the nutrient rate trials, the risk of these 'false positive' results is largely eliminated by adding to the soil only those nutrients already found to be deficient in the omission trials.

Again, our confidence in the results of an omission trial will be boosted if, in addition to confirming which elements were in short supply, the rate trials confirmed that the rates of application of each deficient nutrient in the 'all' treatment had been optimal for plant growth. If a large discrepancy is found between the rate of application of a nutrient giving maximum yield in a rate trial, and that previously used in the 'all' treatment of an omission trial, the omission trial should be re-run with a suitably adjusted rate of application of that element in the 'all' treatment.

4.1.2 Need for preliminary information on the form of the nutrient response

Field experiments can be quite costly in terms of physical resources and time, and hence need to be designed with care. The task of devising cost-effective fertiliser experiments can be simplified, not only by eliminating any 'false positive' results (Section 4.1.1), but also by establishing approximate relationships between the amount of each element added to the soil and the growth of the test plant. Such nutrient rate trials can be a very useful means of selecting treatments for inclusion in subsequent field trials and in reducing the size of field trials, and therefore their cost.

4.2 Some comparisons between predicted and actual responses in the field

In the greenhouse, we take precautions to prevent nutrient losses by leaching, and we try to prevent growth (and hence demand for nutrients) being reduced by drought, pests and diseases, and so on. In the field, we have less control over the situation, but on average the plants have a greater volume of soil from which to draw nutrients. Hence, there are factors operating which could cause pot experiments to overestimate or underestimate the required nutrient input in the field. In practice, it often turns out that pot experiments

slightly overestimate the amount of nutrient needed for near-maximum growth in the field (see Section 4.2.1 for an example). This tendency to overestimate nutrient requirements needs to be taken into account when designing field experiments (Section 5).

There are special problems to be considered when dealing with strongly phosphorus-fixing soils (see Section 4.2.2 for an example), or with field situations where there is a large supply of nutrients from the subsoil (see Section 4.2.3).

4.2.1 Plant responses to nitrogen

Figure 4.1 shows an example of a soil on which the response of sweet potato to nitrogen fertiliser in the field was similar to the response that had earlier been obtained in a greenhouse rate trial using maize as the test plant. The results show that the two response curves were similar in shape, but that the greenhouse trial somewhat overestimated the rate of nitrogen application needed for maximum or near-maximum yield of sweet potato in the field.

In the glasshouse trial, losses of nitrogen by leaching were prevented by using undrained pots that were watered on a weight basis, whereas in the field, the nitrogen fertiliser was split into four applications to reduce leaching losses (see Section 5.4.2). Since the apparent nitrogen requirement in the field was less than in the greenhouse, the differences between the two experiments could not have been the result of greater leaching losses in the field. On the other hand, the test plants in the greenhouse experiment were watered daily to ensure that growth, and hence nutrient demand, was

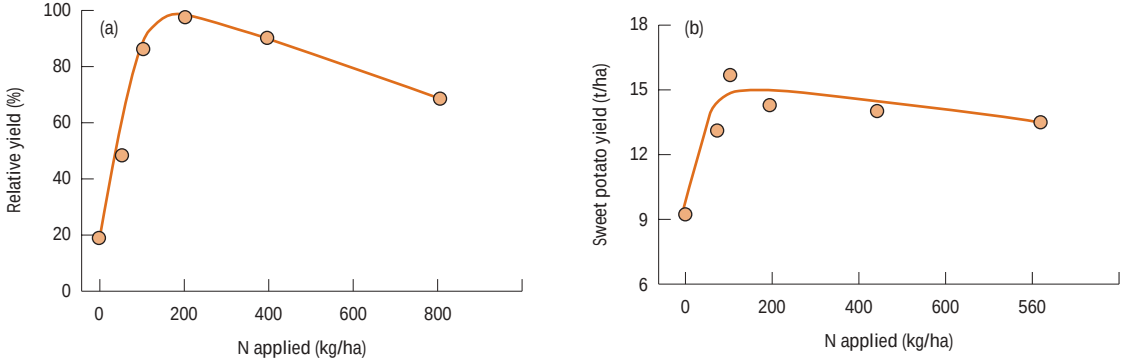

Figure 4.1 Plant responses to nitrogen fertiliser on Fahefa soil, in Tonga (Halavatau 1998): (a) response of maize grown in a greenhouse pot experiment for 4 weeks; (b) response of sweet potato grown to commercial harvest in the field (tonnes tubers/ha).

not limited by water stress. By contrast, the sweet potato plots in the field experiment were rain-fed and at times were subject to moderate levels of water stress. While it is not possible to be sure about the reasons underlying the quantitative difference between the predicted and actual nitrogen requirement of the crop in the field, the results are consistent with what is commonly observed, which is that pot experiments often slightly overestimate the nutrient requirements of field-grown plants.

4.2.2 Responses to phosphorus on strongly phosphorus-fixing soils

On strongly phosphorus-fixing soils, pot experiments in which the phosphorus is thoroughly mixed with the soil may *underestimate* the amount of phosphorus that would be needed in the field if the fertiliser were broadcast and cultivated into the soil (Figure 4.2). The amounts of a nutrient such as phosphate that are lost owing to fixation depend on the degree of contact between the fertiliser and the soil, and the length of time that they are in contact. Hence, the underestimation of phosphorus requirement on the basis of the pot trial results may be a reflection of the widely differing contact times between soil and fertiliser, e.g. 3 or 4 weeks compared with several months, and hence the greater fixation losses in the field.

Under practical farming conditions, losses of phosphorus caused by fixation can be greatly reduced by limiting the volume of soil with which the fertiliser comes into contact. With machine-planted row crops, this can be done by placing the fertiliser in a narrow band below, and preferably a little to one side of, the seed (*band placement*). With subsistence crops that are

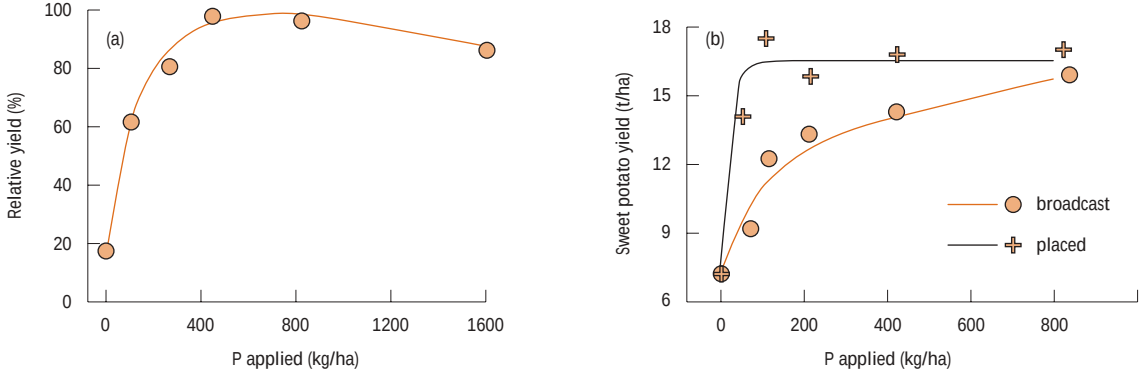

Figure 4.2 Plant responses to phosphorus fertiliser on Fahefa soil, in Tonga (Halavatau 1998): (a) response of maize in a greenhouse pot experiment; (b) response of sweet potato in a field trial.

planted in hills, the same effect may be obtained by mixing the fertiliser with a small volume of soil in the base of the hill. This is sometimes referred to as *spot placement* of the fertiliser (Figure 4.3).

Figure 4.3 Spot placement of phosphorus fertiliser in a field experiment on the mineral nutrition of sweet potato in Tonga. Future positions of planting hills are marked by wooden stakes, and measured amounts of phosphorus fertiliser (white patches) are placed near the stakes. Later, this fertiliser will be incorporated into a small volume of soil. Next, unfertilised surrounding soil will be raked up over it to from a hill into which sweet potato cuttings will be planted.

Another form of spot placement would be to put an appropriate amount of the fertiliser in the planting hole, and cover with a little soil before planting, to prevent direct contact between the mass of fertiliser and the seed or seedling. In experiments with sweet potato and taro in Tonga (Halavatau 1998), it was found that the amount of spot-placed phosphorus fertiliser needed for maximum yield on strongly phosphorus-fixing soils was only 6 to 25% of that needed if the fertiliser was broadcast over the site before planting, and mixed into the soil with disc harrows. Figure 4.2(b) demonstrates this effect for one of the sites studied in Tonga.

Whereas the pot trial results underestimated the phosphorus requirement in the field, when the fertiliser was broadcast and cultivated in, they overestimated the requirement when the fertiliser was spot placed (Figure 4.2). Possibly, the match between pot trial results and those with spot-placed fertiliser in the field could be improved if, in the pot experiments, the phosphorus was mixed with only a portion of the soil. Effects of banding on the response of sweet corn to phosphorus in pots is shown in an experiment from Vanuatu (Figure 4.4). Note how banding the phosphorus has reduced the amount of it needed to produce a given level of yield. However, at the time of writing no information was available on crop responses to banded phosphorus on that soil, so we do not know if the banded results agreed better with field behaviour than when the fertiliser was mixed through the whole soil mass. Hence, we need to be cautious about how we interpret the results of phosphorus rate trials in pots, when dealing with strongly phosphorus-fixing soils.

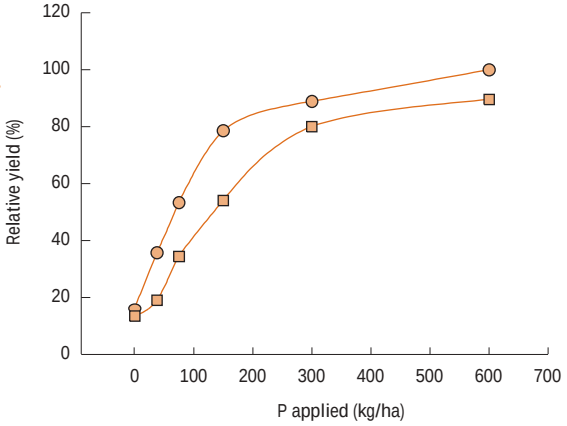

Figure 4.4 Effects of fertiliser placement on the response of sweet corn to finely ground triple superphosphate (TSP), in a pot experiment with a strongly phosphorus-fixing soil from Vanuatu (M. Melteras, unpublished data). Circles are values for TSP mixed through a 2 cm thick band of soil starting 2 cm below the seed; squares are for TSP mixed thoroughly throughout the whole soil mass.

4.2.3 Subsoil nutrients — an occasional cause of 'false positive' results

In most field situations, the highest concentrations of nutrients are in the topsoil, and it is from the topsoil that plants draw most of their nutrients. Hence, it is the supply of topsoil nutrients that we usually try to estimate in soil tests or in pot experiments. However, the topsoil is occasionally not the richest source of a particular nutrient, and the results of a pot trial with topsoil may indicate a deficiency, yet in the field, the plants grow satisfactorily without addition of the 'deficient' nutrient. A good example of this comes from recent experiments on the main island in Tonga (Halavatau 1998).

In the omission trials, plants in the 'all – sulfur' treatment showed symptoms of sulfur deficiency, and had significantly reduced yields at harvest (Table 4.1). In the subsequent rate trials in pots, sulfur deficiency symptoms were again seen at the lower rates of sulfur application, and relative yields in the zero sulfur treatments were strongly depressed (24–56% of maximum). Hence, there seemed to be clear evidence of sulfur deficiency at these sites.

When rate trials with sulfur were planted in the field, the leaves of taro (Vaini soil) or sweet potato (other soils) in the zero-sulfur plots were initially paler than in the sulfur-fertilised plots, but this colour difference gradually disappeared as the season progressed. At final harvest, there was a trend towards reduced yields in the lower sulfur treatments, with yields in the zero sulfur treatments ranging from 86 to 93% of maximum (Table 4.1). However, none of these apparent yield reductions was statistically significant.

In an attempt to find the cause of this surprising result, samples of topsoil and subsoil were collected and analysed for $Ca(H_2PO_4)_2$-extractable sulfur. The results (Table 4.1) showed that the topsoils from four sites had extractable sulfur levels that would be of

marginal or just-adequate sulfur status for soils in the USA, or in the low end of the adequate range for Australian soils (Lewis 1999), no local calibration being available for Tongan soils. By contrast, the subsoils were all well-supplied with sulfur (Table 4.1).

'False positive' results such as those described above are not very common, but they do serve to show the importance of conducting field trials before giving advice to farmers.

4.3 Recommended approach

Rate trials can be conducted separately for each deficient element *(single-element rate trials)*, or with simultaneous variation of two or more of the deficient elements *(factorial trials)*. In single-element rate trials, the effects of other deficient elements on yield are removed by applying them as a basal[3] application to all pots. However, it is not possible to study interactions amongst elements. With factorial experiments, interactions can be studied, but since the number of pots per replication increases exponentially with the number of deficient elements to be studied, factorial experiments can become very large and complex. This can be illustrated by the example of the volcanic ash soil mentioned in Section 3.12 (Figure 3.2(a)). This soil was found to be deficient in four elements: nitrogen, phosphorus, potassium, and sulfur.

Let's consider the number of pots that would be required for four single-element rate trials, compared with a single factorial experiment involving the same number of levels of each element:

Single-element rate trials :

> 6 N rates × 4 replicates (basal P, K, and S applied) = 24 pots

[3] The term 'basal nutrients' (or 'basal fertiliser application') is used to denote nutrients (or fertilisers) that are applied to all pots in a pot experiment (or all plots in a field experiment).

Table 4.1 Data on the sulfur status of four soils in the main island of Tonga.

Soil	Omission trial, relative yield, –S (%)	Rate trials, relative yield, –S (% max.)		Topsoil S (0–30cm) (mg/kg)	Subsoil S (30–60 cm) (mg/kg)
		Greenhouse	Field		
Fahefa	16	24	86	10	38
Lapaha	21	24	91	14	42
Nuku'alofa	33	37	93	10	28
Vaini	54	56	93	12	35

6 P rates × 4 replicates (basal N, K, and S applied) = 24 pots

6 K rates × 4 replicates (basal N, P, and S applied) = 24 pots

6 S rates × 4 replicates (basal N, P, and K applied) = 24 pots

Total = 96 pots

Factorial trial:

6 N rates × 6 P rates × 6 K rates × 6 S rates × 1 rep = 1296 pots

Both experimental approaches will provide estimates of the rates of application of each nutrient needed for maximum yield, and how the yield varies with rate of application as the maximum is approached. In addition to this, the factorial experiment would provide information on the *interactions* between nutrients, i.e. how plant response to one nutrient is affected by the level at which the other nutrients are supplied. However, this additional information may come at a high price — in the case of our example, an experiment that is more than 10 times larger than would be the case if single-element rate trials had been employed.

As there is a lot of internal replication within large factorial experiments, it is possible to delete a number of the treatment combinations so that we are left with a 0.5 replicate or a 0.25 replicate. In the above example, this would reduce the pot numbers to 648 or 324. However, the design and analysis of such *fractionally replicated factorials* are more complex than those of simple rate trials, and may still result in an experiment containing many more pots than if the rate trial approach had been used.

Hence, *for ease and simplicity, we recommend the use of single-element rate trials as the first step in optimising nutrient application rates*. Once the approximate optimal application rate of each deficient element is known, suitable rates and combinations can be identified for later testing in the field (see Section 5).

4.4 Choosing the sources and rates of addition of nutrient elements

In the omission trials, we use mostly chloride salts for the nutrient cations and sodium salts for the nutrient anions. We also use relatively pure nutrient salts to avoid the risk of micronutrient contamination. However, in single-element rate trials, we suggest that, wherever possible, you use a commercial fertiliser material *for the element under test*, thus moving one step closer to the practical farming situation (but see the note below about the basal nutrients). This is particularly important where the preferred fertiliser is a material with limited solubility, e.g. rock phosphate. Again, as a further step towards the practical farming situation, *the basal nutrient application made to all pots in a*

trial should be restricted to only those elements shown to be deficient in the preceding omission trial.

The number of rates of addition of the test element should be sufficient to establish a complete response curve. In most cases, six levels will be sufficient, and we recommend that the number of rates be no fewer than five. If we did a good job in optimising the composition of the 'all nutrients' treatment in the omission trial, the 'all' rate of application of each of the deficient elements we now wish to test should not be too far from the rate needed for maximum yield. Hence, we suggest using that rate plus two higher rates (e.g. the 'all' rate × 2 and ×4) and three lower rates (e.g. 0, the 'all' rate × 0.25, and 'all' rate × 0.5). This assumes that the fertiliser materials we plan to use are at least moderately soluble in water, as were all the nutrient salts used in the omission trials. If relatively insoluble nutrient sources are to be used, e.g. rock phosphate as a source of phosphorus or dolomite as a source of magnesium, these rates will need to be adjusted upwards.

When the rates of addition of the test nutrient have been selected, we suggest giving descriptive codes to the treatments indicating the chosen rates. For example, in a rates-of-potassium experiment, with rates equivalent to 0, 50, and 100 kg/ha, we suggest calling the treatments K_0, K_{50}, and K_{100}.

For those basal nutrients that are to be applied, we recommend using the same rates as employed previously in the 'all' treatment of the corresponding omission trial, unless we have some reason to believe that these had been too low or too high. We recommend also that the same relatively pure salts again be used, especially when the test element is a micronutrient, and the basal elements include macronutrients, since some commercial fertilisers such as single superphosphate may contain substantial amounts of zinc or other micronutrients (Figure 4.5)

Figure 4.5 Response of wheat on a zinc-deficient soil in Western Australia to zinc present as a contaminant in single superphosphate. The central strip of very poor growth, due to zinc deficiency, was fertilised with pure calcium phosphate, which supplied no zinc to the crop. The healthy crop each side of the strip received the same amount of phosphorus as single superphosphate manufactured from a source of rock phosphate rich in zinc. (Photo courtesy of Western Australian Department of Agriculture.)

4.5 Choosing the test plant

Often, it is convenient to stick with the same test plant that we used in the omission trials. However, if this was of a different species to the one we will be growing in the field, we may wish to move a step closer to the field situation by switching to the species we plan to grow in the field. For example, if we are working with a slow-growing woody species that may require 2–3 months to develop substantial responses to our nutrient treatments, it would be quite logical to use a fast-growing test plant at the omission trial stage, to save time (see Section 3.3). However, once we know which elements are likely to be deficient, and we turn our attention to how much of each would be needed to correct the deficiency, we may decide to use the plant species of interest as our test plant. Such a change would recognise the fact that quantitative differences in response are likely between species that differ markedly in their physiology and growth rate.

4.6 Running the trial

Apart from any changes that may be necessitated by a change of test plant (see Section 4.5), the procedures for setting up the trial, maintaining it, and harvesting will be essentially the same as described previously for preliminary trials and omission trials (see Sections 2 and 3). A data sheet with 7 columns and 13 rows will be found convenient (Table 4.2). Once again, a Student's t test can be used to show which treatments differ significantly from that giving the highest yield (see Appendix 10).

4.7 Results and their interpretation — a case study from Papua New Guinea

During a field trip in the Aiyura Valley in the Eastern Highlands of Papua New Guinea, one of the authors (CA) noticed that maize growing on hill slopes showed all-over yellowing, a symptom consistent with sulfur deficiency. No other symptoms were seen. Later, bulk samples of soil were collected from nearby north- and south-facing slopes and taken to The Papua New Guinea University of Technology, near Lae, for greenhouse pot tests (Dowling et al. 1994). The tests indicated that both soil samples were deficient in sulfur and phosphorus (Table 4.3), but apparently contained adequate amounts of all other nutrients. As the pH of the soils lay between 5.0 and 5.5, an 'all + lime' treatment was included (see Section 3.10.1), but the yield was not improved significantly by liming.

The results suggest that, for the south-facing slope, sulfur deficiency was a more severe limitation than phosphorus deficiency, which may explain why sulfur deficiency symptoms were the only ones observed (see Section 1.4.1). On the north-facing slope, both deficiencies were much less severe and of about equal intensity (Table 4.3).

Table 4.2 Example of a data sheet for a rate trial with six levels of nitrogen and four replications.

Treatment	N_0	N_{25}	N_{50}	N_{100}	N_{200}	N_{400}
Rep 1						
Rep 2						
Rep3						
Rep 4						
$\bar{x}$						
Rel. $\bar{x}$ (%)						

Next, single element rate trials were run for sulfur (with basal phosphorus), and for phosphorus (with basal sulfur) on the soil from the north-facing slope only (Dowling et al. 1995). As there was some concern that the rates of sulfur and phosphorus application in the 'all' treatment of the omission trials may have been too low (25 and 80 kg/ha, respectively), the basal rates of both nutrients were increased to 160 kg/ha for the rate trials. Again, as the crop of main interest was sweet potato, it was decided to try using sweet potato as the test plant for the rate trials.

The deficiencies of sulfur and nitrogen found in the omission trial using maize as the test plant were confirmed in the nutrient rate trials with sweet potato as the test plant (Figure 4.6). As maximum yield was reached at 80 kg S/ha and 160 kg P/ha, the suspicion that the rates used in the omission trial had been too low was confirmed. The larger responses to addition of these two elements to the north-facing soil in the rate trial than in the omission trial, again are consistent with the idea that there may have been some loss of sensitivity in the omission trial as a result of lower-than-

desirable levels of sulfur and phosphorus in the 'all' treatment (see comments in Section 3.2). However, because different test plants were used in the two types of experiment, we cannot be certain on the point of whether or not the levels of these elements used in the omission trial had been too low.

Table 4.3 Main results from nutrient omission pot trials with soils from sloping land in the Aiyura Valley, Papua New Guinea, using maize as the test plant (Dowling et al. 1994).

Treatment	Relative dry matter yield (%)	
	North-facing slope	South-facing slope
All	100	100
All – S	85	57
All – P	81	74

4.8 The next steps

Having satisfied ourselves that we know which nutrient elements are likely to be deficient for healthy plant growth, and obtained some preliminary information on relationships between the supply of these elements and plant growth, we are well-placed to undertake the important task of planning the field experiments needed to allow us to give sound advice to farmers. Let us remember that greenhouse experiments are an aid to conducting good field experiments, not a substitute for field experiments. Section 5 offers some guidance on the design and execution of field experiments.

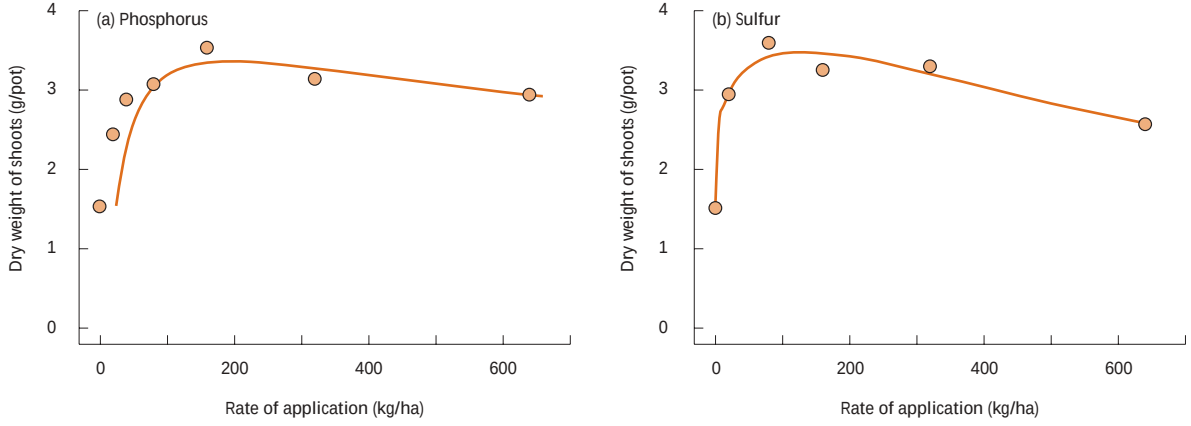

Figure 4.6 Effects of (a) phosphorus supply and (b) sulfur supply on the growth of sweet potato cv. 'Wanmun' cuttings in pot experiments with and Ultisol from Aiyura, Papua New Guinea (data points are means of three replications).

5

Field Experiments — at Last!

Well-planned and conducted field experiments are almost always necessary before we can give sound advice to farmers about soil fertility problems. Not surprisingly, then, many researchers and farm advisers are anxious to get started in the field as soon as possible. However, our experience suggests that a delay of a few weeks, while we use pot experiments to explore the nature and severity of the problems that may be present at a particular field site, can save us from many mistakes and false starts as we attempt to find solutions to soil fertility problems in farmers' fields.

5.1 Purpose and location of field trials

Field trials can be used for a variety of purposes, some of which are:

(a) to demonstrate to farmers the advantage of correcting one or more soil fertility problems that we know exist in their fields;

(b) to check the suitability of an existing fertiliser recommendation;

(c) to develop and test strategies for improving crop yields on land which is believed to have soil fertility problems; and

(d) to check the reliability or the calibration of a soil test.

Some field trials are best conducted under the controlled conditions that are possible only on a research station. However, many soil fertility problems that occur in farmers' fields cannot be reproduced on the research station, and even if they could be, farmers are usually less easily convinced by 'research station results' than they would be by results obtained in their own fields or a neighbour's field. Hence, experiments in farmers' fields are often the best way of showing them how to overcome soil fertility problems.

5.2 Some human factors that need to be considered

5.2.1 Clearly defining the problem to be investigated

It is essential that everyone associated with a field trial understands the purposes of the trial, and that the questions to be answered by the trial are stated very clearly. This is so whether the trial is to be conducted on a research station or in a farmer's field. However, when working in farmers' fields, there is a particular need to be very clear about the questions that the trial is intended to answer.

Wherever possible, farmers should be involved in this important process of problem definition. Unless a farmer agrees that the question a trial is intended to answer is a question for which they really want an answer, they are unlikely to have any real commitment to seeing the trial through to a successful completion. They are also less likely to adopt any new technology arising from the trial.

Extension officers need to settle with their farmers what questions need to be answered before giving consideration to possible experimental designs.

5.2.2 Reaching agreement about who does what

Even simple trials in farmers' fields can be quite expensive in terms of labour, and sometimes of materials also. Occasionally, such trials fail because of drought or other causes beyond human control. However, in our experience, most failures are the result of misunderstandings about what is expected of each participant. Causes of failure of field trials include: failure to stick to the agreed plan (e.g. putting fertiliser on unhealthy-looking control plots); failure to control weeds; failure to control insect pests; failure to keep animals off the plots; unauthorised removal of produce from plots before final harvest; and failure to record the yields of produce on the individual plots at harvest time. Such failures represent not only a huge waste of time and money, but also lost opportunities to learn how to make farming more efficient and more profitable.

It is essential that before a trial is commenced, all participants reach agreement about such things as:

(a) how and where the trial is to be conducted;

(b) who is to be responsible for supplying the necessary labour for planting, for weeding, for controlling pests and diseases, and for harvesting and weighing the produce, and who will pay for that labour;

(c) who is responsible for buying the seed, the fertilisers, and the pesticides;

(d) what records will be kept and who will be responsible for keeping them;

(e) who owns the produce after it is harvested; and

(f) any compensation to be paid for yield losses due to treatments in which the crop does not grow well, e.g. zero fertiliser treatments.

What has been agreed to needs to be written down, and everyone associated with the trial should be given a copy. This written statement should also include the field plan (see Section 5.7), and all experimental details relevant to the trial on the farmer's property. Where similar trials are to be conducted on a number of properties, the written statements for each trial may be very similar, but it is essential that each farmer individually agrees to what is going to be done on their property.

5.3 Some practical considerations that will apply to most field experiments

5.3.1 Choosing the site

We gave some consideration to potential sites for field experiments in Section 1.7, where it was stated that the following site characteristics are important:

(a) the site is representative of a substantial area of farmland in the district or region;

(b) the site is big enough for a field trial;

(c) the owner of the land is willing to have a field trial on their land, and is keen to cooperate in the running of any such trial; and

(d) the site is secure against theft of produce and damage by livestock.

We now need to review our earlier assessment about the suitability of each of the sites from which we have gathered bulk soil samples for pot experiments, as circumstances may have changed since that time. Also, when we gathered our soil samples, we did not know what soil fertility problems our pot experimentation would uncover, and hence we did not have a clear idea about how much land we might need for subsequent field experiments. Again, it could be that several sites from within a district, or a region, gave closely similar results in the pot trials, and we have to decide whether to restrict our activity to a representative site or to

spread the field trial activity over several sites (see also Section 5.3.2).

In selecting on which of the possible sites we should establish a field trial, we would tend to give preference to a site with land that appeared to be relatively uniform (e.g. in slope, depth of topsoil, previous use, and degree of shading by coconuts). We would also give preference to sites with a known history (e.g. number of crops since first clearing or since the last fallow period, types of crops grown, and details of any additions of fertiliser or animal manure).

5.3.2 Some points about treatment number and replication

In general, the more plots in an experiment, the greater will be the power of the statistical techniques in distinguishing between variations due to chance (*error variation*) and variations due to the treatments we have imposed (*treatment variation*). *The number of plots in an experiment is equal to the number of treatments multiplied by the number of replicates.* Hence, we can increase the number of plots either by increasing the number of treatments, or by increasing the number of replicates. However, the more plots we have, the more land we will need, the more work will be involved in planting, maintaining, and harvesting the trial, and the greater will be the cost of the trial in materials and labour. Hence, we need to reach a compromise between experimental precision and the cost and effort involved in trying to answer a particular question. In practice, we do not gain very much by increasing the plot number beyond about 20 to 25 in simple replicated field trials. In large factorial trials, there may be substantial internal replication, so that a single replicate, or even a fraction of a replicate, may well suffice.

With trials in farmers' fields we may use one of the following strategies:

(a) have the entire experiment located at one site;

(b) have one replicate of the experiment at each of a number of sites; or

(c) have more than one replicate of the experiment at each of a number of sites.

Where we think the cropping and fertiliser history of a site is the main factor likely to affect the fertiliser response in a farmer's field, we should use strategy (a). Again, with trials on research stations, we would usually have all the replications at the same site. In these cases, we will have to accept that the results apply only to that site or to other sites with closely similar soil properties and site history. We are thus moving towards a situation where the fertiliser recommendations within a district may vary from site to site depending on the particular soil conditions at each site.

Where we think, on the basis of our pot trials, that the same fertiliser recommendation could be appropriate for all sites on the same soil type/same island/whole

country, we may employ strategy (b) in an attempt to develop a generalised recommendation. Or we might prefer to use strategy (c) which would allow for some testing of treatment differences within an individual site.

5.4 Some strategies for reducing losses of added nutrients

We saw, in Section 1.5, that one of the factors determining the amount of a nutrient we would need to add to correct a deficiency is the amount of the added nutrient that is 'fixed' or in some way lost from the system. Put another way, this means that the response of our crop to a given nutrient addition will depend on how large these losses are. Since these losses are not fixed, but depend a good deal on how and when a fertiliser is applied, we need to give some thought, at the planning stage, to the application methods we believe to be most appropriate for a particular fertiliser in the particular farming system with which we are dealing.

5.4.1 Fertiliser placement to reduce losses due to 'fixation'

This is mainly a problem with phosphorus on soils high in oxides of iron and aluminium, but there are some soils in which potassium is 'fixed' in the inter-layer spaces of the clay minerals. As we saw in Section 4.2.2, *spot placement* of the fertiliser, or *band placement* near the seed, can greatly increase the effectiveness of a nutrient subject to fixation losses, by limiting the volume of soil that comes into contact with the fertiliser.

Usually, we will want to employ in our field trials the method of fertiliser application that we believe will give the best result in terms of cost-effective increases in crop yield. However, there may be circumstances where we wish to demonstrate the importance of method of application. If so, we might include in our field trial a comparison of different methods of fertiliser application. In either case, decisions have to be taken about the method(s) of application of any nutrients likely to be subject to fixation losses.

5.4.2 Split applications to reduce leaching losses

Under high rainfall conditions, large amounts of soluble nutrients may be washed beyond the root zone, and hence lost to the crop. This process is called *leaching*. Leaching losses of nitrogen fertiliser may be particularly large if the fertiliser is applied before a network of feeding roots has had time to develop (e.g. all or most applied at planting). Leaching losses can often be reduced dramatically by dividing up the total amount of fertiliser to be used into a number of doses that are applied at intervals during the growing season. The effects of these *split applications* on the relationship between the amount of fertiliser applied and the yields of sweet potato and taro are shown in Figure 5.1. Note that, for the same total amount of fertiliser applied, yields tended to increase with an increase in the number of split applications, because of decreases in the amounts of nitrogen being lost by leaching. Also note that there was no advantage in nitrogen fertiliser on taro if it was all applied as a single dressing at planting time.

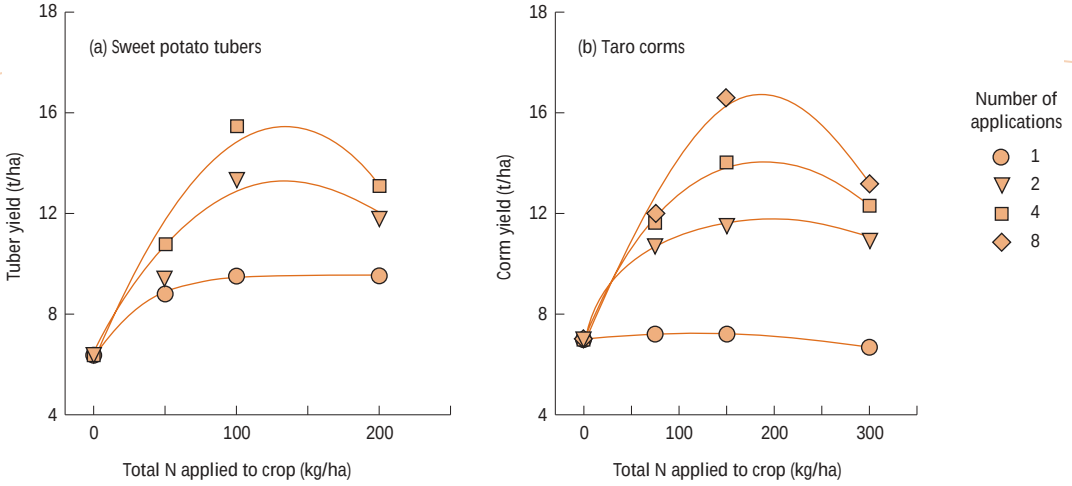

Figure 5.1 Effects of total nitrogen fertiliser applied, and number of applications into which the total nitrogen applied was split, on the yield of (a) sweet potato and (b) taro (data of Halavatau 1998).

With irrigated crops, split applications of nitrogen are easily made by adding a soluble nitrogen fertiliser, such as urea or ammonium nitrate, to the irrigation water at suitable stages during crop development. However, in rain-fed agriculture, side-dressings of fertiliser along the planting rows or around individual plants/hills may be needed.

When side-dressings of urea are applied, large losses of nitrogen may occur if the fertiliser is allowed to sit on a moist soil surface. Under these conditions, the urea is converted to ammonia gas which is lost to the atmosphere — a process called *volatilisation*. Losses due to volatilisation can be prevented or reduced by digging the fertiliser into the soil, so that as the ammonia is released it can be trapped in the soil moisture.

Once again it is necessary, at the stage of designing our field trials, to make decisions about when and how soluble fertilisers such as nitrogen are going to be applied.

5.5 Note on fertiliser composition — mineral nutrient versus oxide basis

The concentrations of most mineral nutrients in fertilisers are given as the weight of the nutrient,

expressed as a percentage of the weight of fertiliser. For example, sulfate of ammonia fertiliser (SOA) usually contains about 20% nitrogen and about 24% sulfur. In other words, 100 kg of the fertiliser will contain 20 kg of nitrogen and 24 kg of sulfur. In some countries, including Australia, this simple system is applied to all nutrient elements. However, in other countries, the concentrations of two very important elements, phosphorus and potassium, are expressed instead as the weight of the corresponding oxide, as a percentage of the weight of fertiliser. The oxide basis is sometimes used also for calcium and magnesium.

The use of the old-fashioned oxide basis makes fertilisers appear to contain more of the nutrient than they really do. Thus, single superphosphate (SSP), which contains 9.6% phosphorus, would be labelled as containing 22% phosphorus on the oxide (P_2O_5) basis, and muriate of potash (MOP), which contains 50% potassium, would be labelled as containing 60% potassium on the oxide (K_2O) basis.

These differences in ways of expressing the composition of fertilisers can be a source of confusion, particularly in places where fertilisers are imported from countries where the oxide basis is common (e.g. New Zealand, and some European countries) and where it is no longer used (e.g. Australia). The unfortunate practice of using 'P' and 'K' as abbreviations for P_2O_5 and K_2O, respectively, is a further source of confusion and should be strongly discouraged. Clearly, when discussing fertiliser application rates, or when conducting fertiliser trials, it is essential to be absolutely clear about the basis on which rates of phosphorus and potassium are being expressed.

The following conversion factors may be useful:

To convert % P to % P_2O_5, multiply by 2.3

To convert % P_2O_5 to % P, multiply by 0.44

To convert % K to % K_2O, multiply by 1.2

To convert % K_2O to % K, multiply by 0.83

5.6 Deciding on the experimental approach

The best approach to adopt will depend on the purposes of the trial (see Section 5.1). Hence, at this point, there are several ways of proceeding. *In each case, it is essential that we are very clear in our own minds about what it is that we are trying to find out.*

Let us assume that we believe, on the basis of pot experiments (Sections 3 and 4) or other evidence, that there is a deficiency of one or more nutrient elements at a particular experimental site. Usually, we will want to establish a full response curve from deficiency to adequacy for each deficient element, so that we are in a position to give good advice to farmers about the cheapest and most effective way of using the available nutrient sources to raise yields.

Let us consider how best to proceed if we had 1, 2 or more than 3 deficient nutrients at a particular site.

5.6.1 One deficient nutrient

The simplest case to consider is where we have only one deficient nutrient, and have available to us only one practical source of that nutrient. Here, we suggest a simple, single-element rate trial similar to that considered in Section 4, but this time conducted in the field, e.g.

6 levels of nutrient × 4 replications = 24 plots

As only one element is believed to be deficient, there will be no basal nutrients to apply. If rate trials with the same element are conducted at a number of sites, they can be used to check the effectiveness and calibration of the corresponding soil test(s) (see Section 6).

If there is more than one nutrient source available (or more than one method of application), we may wish to include a comparison of the effectiveness of these sources (or methods) in the experiment. As the statistical precision depends mainly on the number of plots, but does not increase greatly above about 25 plots, we can reduce the number of replications as we increase the number of nutrient sources, e.g.

6 levels of nutrient × 2 sources × 3 replications = 36 plots, or

6 levels of nutrient × 3 sources × 2 replications = 36 plots.

When considering different nutrient sources, we need to take account of their cost per unit of the deficient element as well as their effectiveness in raising the yield.

5.6.2 Two deficient nutrients

Again, let us start by considering the simple situation where there is only one practical source of each nutrient, and each of these sources provides only one of the deficient nutrients. In this case, we suggest a factorial design with five levels of each nutrient, e.g.

5 levels of nutrient A × 5 levels of nutrient B × 2 replications = 50 plots

If there is more than one nutrient source (or method of application) to consider, we suggest reaching a decision about these before running the factorial trial, as including comparisons between them within the trial is likely to make the trial too large. Some preliminary experimentation may be needed to assist in this decision, such as a single element rate trial comparing the different sources, as described in Section 5.6.1. However, in this case it would be advisable to apply the second deficient element to all the plots in the single-element trial. As the optimal rate is not yet known, apply a 'best guess' rate based on the results of the rate trials conducted in pots (Section 4).

Again, if the same fertiliser contains both deficient elements, e.g sulfate of ammonia (nitrogen and sulfur), sulfate of potash (potassium and sulfur), or single superphosphate (phosphorus, calcium, and sulfur), we may consider an experiment which focuses on the element thought to be needed in greater amount. The experiment could take the form of a simple rate trial designed with pot trial results for the 'main' element in mind (as in Section 5.6.1). However, with this approach, we cannot be certain about which element is mainly responsible for the yield increase at a particular level of fertiliser application, or whether the need for both has been fully satisfied. An alternative approach would be to use a factorial design in which adequacy of the 'lesser' nutrient were checked by adding it from another source:

6 levels of fertiliser × 2 levels (+ or –) 'lesser' nutrient × 3 replications = 36 plots

5.6.3 Three or more deficient nutrients

Before a recommendation to farmers can be made, we will need to run a factorial experiment with rates and combinations of the deficient elements. However, to run a factorial experiment with even five levels of each, as in Section 5.6.2, would result in an experiment that was very large and probably unmanageably so. Thus, if three elements were deficient, we would have $5 \times 5 \times 5 \times 1$ replicate = 125 plots, whereas with four deficient elements, we would have $5 \times 5 \times 5 \times 5 \times 1$ replicate = 625 plots.

Although there are some sophisticated experimental designs that would allow us to gain most of the information we require with fewer plots, we believe that the best way to proceed is to break the task into the following steps:

(a) For each deficient element, run a rate trial with a basal application of the other deficient elements applied at levels judged to be sufficient to overcome their deficiency, e.g. for a site that was deficient in nitrogen, phosphorus, and sulfur we would have

6 N levels (plus basal P and S) × 4 replicates = 24 plots

6 P levels (plus basal N and S) × 4 replicates = 24 plots

6 S levels (plus basal N and P) × 4 replicates = 24 plots.

(b) From the results of (a), check that basal levels used were sufficient for maximum or near-maximum yield. If not, repeat step (a), using more suitable rates of basal nutrients. If adequate, proceed to step (c).

(c) Choose a reduced number of levels of each deficient element to include in a factorial experiment. In making this decision, some economic and social factors need to be considered as well as the biological information obtained from (a).

Important among these factors are:

(i) *The relative cost of each fertiliser material* — in general, the more expensive a proposed input, the more carefully we will want to establish the relationship between rate of application and yield. Hence, we may choose to have more levels of an expensive nutrient than of a cheap nutrient.

(ii) *The farmer's economic circumstances and attitude to risk* — in commercial agriculture, we are often seeking a set of inputs that will give us maximum or near-maximum yield, and the risk of losing the value of those inputs in the event of crop failure may be acceptable, even if unwelcome. However, for a cash-poor subsistence or semi-subsistence farmer, a modest increase in yield that could be obtained with minimal financial risk may be the desired outcome.

Clearly, in the former case, we will want to choose our levels of input of the various deficient elements so that some combinations give yields close to the maximum, and others lie sufficiently above and below the maximum to allow us to estimate the most cost-effective rates and combinations of nutrients.

However, in the case of a cash-poor, risk-averse farmer, we will be much less interested in the inputs that might be needed for maximum yield. Rather, we will be interested in gaining the maximum yield advantage from each unit of cash that the farmer can afford to spend on fertiliser. Hence, we will want to have more of our treatment combinations corresponding with the lower range of yields. Further, we may choose to exclude from our trial the investigation of any mildly deficient nutrient elements which would be unlikely to become limiting because of only partial correction of the more severely deficient nutrients (see Section 3.1).

Clearly, given the differing circumstances in which we may be designing a factorial experiment, there is no one design that can be recommended. However, for the simple case of a commercial crop, and three deficient nutrients which do not differ greatly in cost, the following would be appropriate:

$4 \times 4 \times 4 \times 1$ replication = 64 plots

For each nutrient, we would suggest the four levels be selected as follows: zero; an intermediate level; a level to just give maximum yield (from rate trial results); and a higher level expected to also give maximum or near-maximum yield.

With more than three deficient nutrients, we are likely to have to employ fewer than four levels of at least some of them to keep the experiment down to a manageable size. Here are some possibilities:

$4 \times 4 \times 2 \times 2 \times 1$ replication = 64 plots;

$4 \times 3 \times 3 \times 2 \times 1$ replication = 72 plots; and

$3 \times 3 \times 3 \times 3 \times 1$ replication = 81 plots.

5.7 Preparing the field plan

Once a decision has been made about the experimental design, we must turn attention to the practical details of how this is to be laid out in the field. Here, a number of factors need to be considered, including the desired size of each plot, and the area and shape of the land available.

5.7.1 Plot size

The plot size we need will vary a good deal with the crop we will be growing. For densely planted crops, and crops with little plant-to-plant variation in yield, the plots can be smaller than for crops grown at wide plant spacings, or where plant-to-plant variation is large. Here, we can often be guided by the past experience of others.

With sweet potato, experience has shown that, for a good estimate of yield, we need to harvest a minimum of nine two-plant hills per plot (Halavatau 1998). These plants are referred to as *datum plants*. Usually, we will want the harvested area to be completely surrounded by a row of *border plants*. Hence, the minimum number of hills per plot becomes 9 + 16 = 25 hills, or 50 plants. If the hill spacing were 1 m in each direction, the minimum plot size in that case would be 5×5 m = 25 m^2, remembering that the plot boundary lies $0.5 \times$ the hill spacing (0.5 m in this example) outside the border rows.

By comparison, with taro (Halavatau 1998), which is grown as spaced single plants, we need a minimum of 16

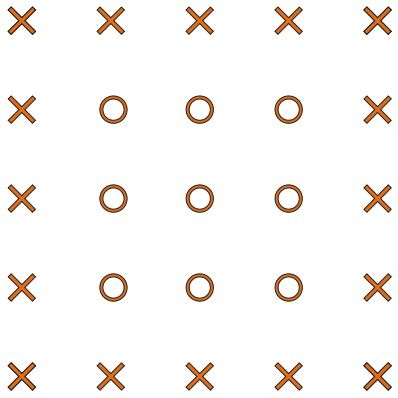

Figure 5.2 Layout of a 5 m × 5 m sweet potato plot with 1 m × 1 m hill spacing and containing 9 two-plant hills for harvest (○), and 16 border hills (×).

datum plants (+ 20 border plants = 36 plants/plot), whereas with pumpkins, for a good estimate of the yield from each plot, we need to harvest the fruit from about 30–32 plants, i.e. from about 15 or 16 two-plant hills.

5.7.2 Deciding on the plot layout in the field

The absolute minimum area of land needed for a trial is given by the area of each plot multiplied by the total number of plots. However, in practice, we will usually need more land than this as it may be necessary to avoid rocky outcrops, stumps or other obstructions in the field. Also, in some situations, we may wish to leave some space between the blocks so that we can gain access to our plots without having to cross over other plots. These spaces are sometimes called *headlands*.

In laying out our trial in the field, it is important that we position our blocks so that any variability in soil properties between plots in the same replicate is as small as possible, i.e. we want to minimise within-block variation in soil properties. For example, if the depth or chemical fertility of the topsoil increases as we move down a slope, we would place our blocks across the slope, i.e. at right angles to the gradient in topsoil depth or fertility (Figure 5.3a). (Variation in soil properties between blocks is less important than within-block variation, because allowance can be made for the former when the data are subject to statistical analysis.) Also, if there are tree stumps, patches of ash, or patches of exposed subsoil from land-clearing operations, we would adjust the position of the blocks so that these atypical areas were avoided (Figure 5.3b).

5.7.3 Randomising the treatments within blocks

For simple trials with only a few treatments, the easiest way to randomly assign the treatments to plots is to write the treatment names (codes) on small squares of paper, fold them up and place them in a wide-mouthed jar. Take out the field plan and, after mixing up the pieces of paper in the jar, withdraw one piece of paper and write down the treatment code it contains on the

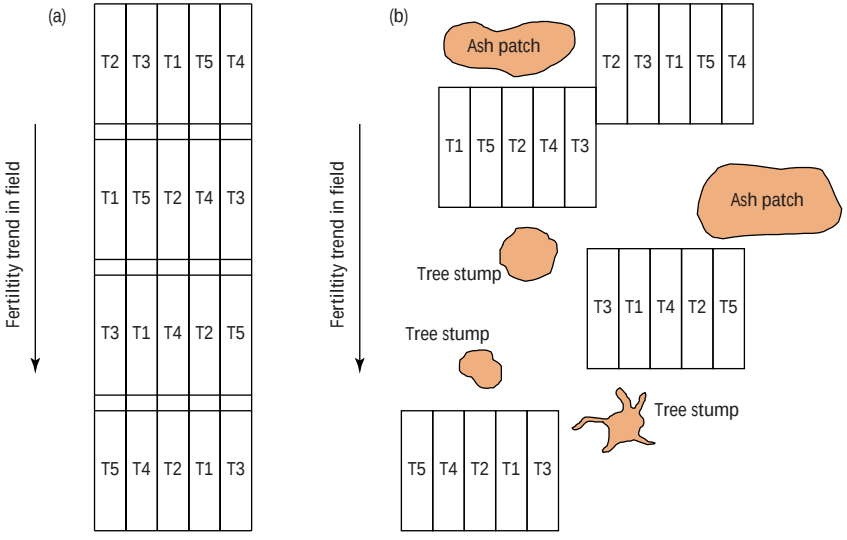

Figure 5.3 (a) Layout of blocks in a field experiment on a site with a gradient in soil fertility. (b) Layout of the same experiment where it is necessary to avoid ash patches and stumps.

first plot of Block I. Then take out the next square of paper and write its code on the next plot of the same block, until all the plots in Block I are labelled. Next, return all the paper squares to the jar, mix, and repeat for the next block. Repeat the process until treatments have been assigned to all plots in all blocks.

Using simple descriptive codes makes it easy for everyone to know what the treatments are. For example, if we had a trial with just two treatments — one receiving NPK fertiliser and the other NP fertiliser — we could call the first 'NPK' and the second 'NP', or simpler still, we could call the first '+K' and the second '–K', (as in Figure 5.4) since the purpose of such a trial would be to test whether or not potassium was needed at the trial site. Similarly, if we had a trial with five rates of phosphorus application from 0 to 280 kg P/ha, we could call the treatments 'P_0, P_{35},…P_{280}', where the subscripts indicate the rate of phosphorus application. Such simple descriptive codes for treatments (and for plots) can prevent many unfortunate mistakes resulting from the misreading of field plans or incorrectly identifying samples because less obviously descriptive codes (such as the T1, T2 etc. in Figure 5.3) have been used. Use of Roman numerals (e.g. I, II, and III) for blocks is a convenient convention also. The combination of block number and treatment code (e.g. II P_{35}) provides a simple and unambiguous method of labelling every plot in a trial.

5.7.4 Drawing the field plan

Once the plot size has been decided and the potential trial site carefully inspected, we need to prepare a field plan showing where the blocks and their individual plots will be located. The plan needs to show dimensions from fixed objects such as fences, buildings, or trees that are unlikely to be removed, so that the plots can always be located, even if surrounded by crop plants that do not form part of the trial. Permanent and accurate recording of plot locations is particularly important if it is planned to reuse the same plots for a later experiment, e.g. to measure the value to a subsequent crop of any fertiliser remaining in the soil after the forthcoming experiment.

Wooden or metal stakes may not suitable permanent markers because of the ease with which they can be 'borrowed' for other purposes. On the other hand, coconut palms marked with white or brightly-coloured house paint provide very convenient permanent markers. Figure 5.4 shows an example of a plan of a field trial set in a coconut plantation.

Where no coconut palms are available to serve as reference points, the location of one or more corners of a trial can be fixed by its distance from a pair of conveniently located fixed points (e.g. two fence posts), a method called *triangulation*.

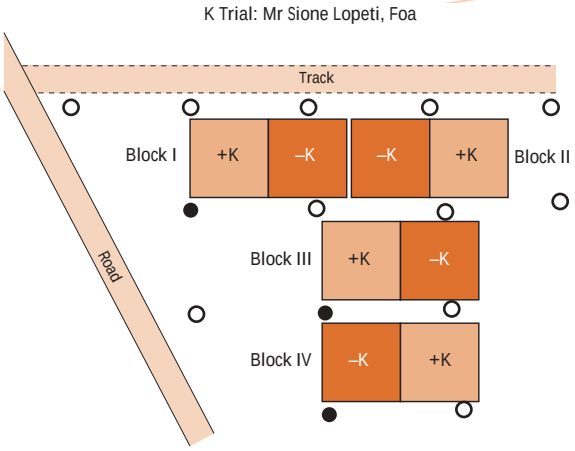

Figure 5.4 Example of a field plan for a simple field trial with two potassium treatments and four replicates (circles denote coconut palms, solid circles denote palms identified with white paint on the trunk and used as permanent markers.

5.8 Listing and assembling the materials needed for the trial

As soon as the experimental design is finalised, we should prepare a detailed list of all the materials that will be needed to establish and run the trial successfully. An early start on this work is essential as some of the materials needed may have to be imported, e.g. 'straight' fertilisers required for experimental purposes, but not commonly used in commercial crop production. The list should include the quantities of each item needed.

Although the items on the list will vary according to the purposes of the experiment, the list will usually contain many of the following items:

Equipment for laying out the plots

- Measuring tape
- String
- Paint for identifying fixed marker points, e.g. coconut palms
- Roofing nails to mark reference points on fixed markers
- Measuring rod equal to plant spacing along the row, marked at the mid-point with a saw cut
- Knotted cord for making a right angle
- Knotted cords for measuring plot length
- Stakes
- Plot labels

Equipment for taking soil samples

- Soil auger or spade
- Plastic bags
- Felt-tip pens (waterproof)
- Fertilisers

Equipment for measuring out and applying fertilisers

- Accurate balance
- Plastic bags
- Felt-tip pens (waterproof)
- Cups or cut-off soft drink cans
- Hoe (for digging in any surface-applied urea)
- Digging fork (for incorporating phosphorus fertiliser under planting hills on P-fixing soils)

Seed or other planting material

Equipment and materials for disease and pest control

- Knapsack spray(s) (if herbicide is to be used, have a separate one kept especially for this purpose)
- Snail bait
- Insecticides
- Fungicides

Equipment for recording observations and results

- Clipboard
- Data sheets

Equipment for harvesting and weighing produce in the field

- Tripod
- Spring balance
- Canvas sheet to support produce during weighing
- Clipboard
- Data sheets
- Field bins or other containers for saleable produce

5.9 Pegging out, soil sampling, applying fertiliser, and planting

5.9.1 Pegging out

The actual plot dimensions will vary depending on the crop being grown and on the size and shape of the piece of land to be used. When working with root crops grown under coconuts (a common practice in the Pacific region) and a 1 m × 1 m spacing, a plot 5 or 6 rows wide will occupy most of the useable land between 2 rows of coconuts, and the blocks will typically be only one plot wide. Although not actually in a coconut plantation, Figure 5.5 shows such an experiment in Tonga, consisting of narrow, one-plot-wide blocks. In this case, the blocks were positioned end-to-end, but often they would be side-by-side, separated by rows of coconut palms.

Figure 5.5 Appearance of a fertiliser experiment with sweet potato laid out as a sequence of narrow blocks, each one plot wide, and placed end-to-end.

The following steps would be followed if laying out a field experiment in a coconut plantation.

(a) Locating and squaring one end of each block

From the field plan, locate the first marker palm, and if it has not yet been painted, paint a strip 10–15 cm wide around the trunk at about waist height so that the palm can be easily identified when viewed from any direction. Next, on the side of the palm facing into Block I of the experiment, drive in a roofing nail into the centre of the trunk and about 40 cm above the ground. The head of this nail provides a permanent reference point from which measurements can be made to locate plots in the block, even if all the marker stakes are subsequently removed.

Next (see Figure 5.6(a)), run a string line past the marker palm, parallel to the line of coconuts, and just far enough away from them to be clear of any grass or fallen fronds that may have been stacked along the palm row. Draw the string taught and attach to stakes #1 and #2 firmly driven into the ground. Next, drive stake #3 into the ground directly opposite the head of the nail in the marker palm, and just touching the string line. A second string line is now run at right angles to the first one to mark one end of the block.

Measuring the right angle is most easily done with three people, using a piece of cord approximately 13 m long, with knots tied in it at 0.5, 3.5, 7.5, and 12.5 m from one end (knots 3, 4 and 5 m apart), so that a 3,4,5 triangle can be constructed with the first string line as its base. The procedure is as follows: hold the knot at 3.5m against stake #3, whilst a second person draws the long end of the cord tight alongside the existing string line. Drive stake #4 into the ground touching the two string lines and the 7.5 m knot. A third person now gathers up the two free ends of the cord and pulls them tight with the 0.5 m and 12.5 m knots in contact. Stake #5 is driven into the ground at the point where the two knots touch (Figure 5.6(a)). The knotted cord is now removed, as are stakes #1 and #4. A string line is now attached to stake #3 and run right across the space between the rows of coconuts so that it is just touching stake #5. This string is attached to stake #6, and stake #5 is removed.

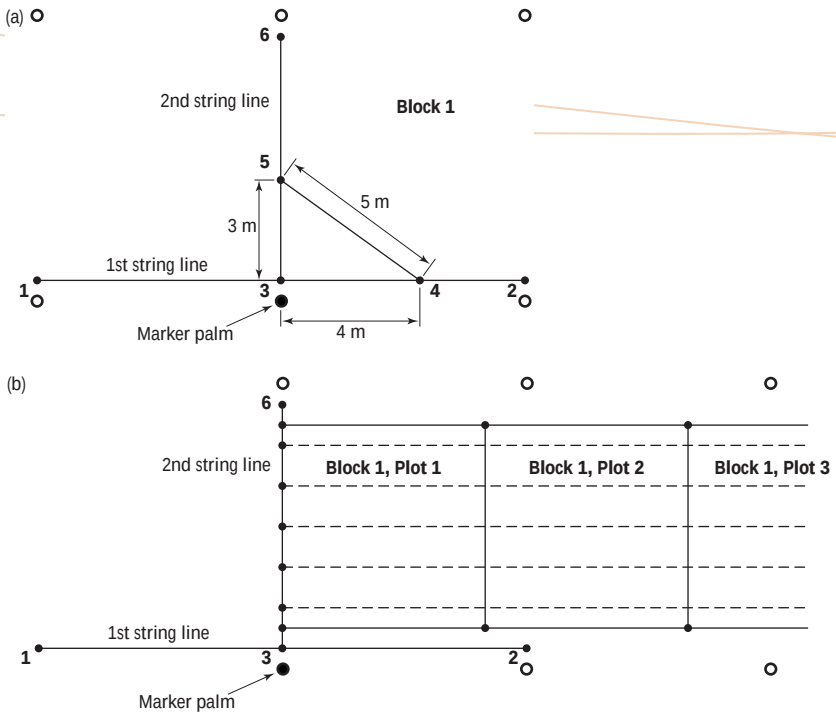

Figure 5.6 Pegging out a field experiment using a labelled palm tree (solid circle) as a permanent marker (open circles represent other palm trees): (a) locating and squaring one end of a block (for sequence of operations see text); (b) marking off the plant boundaries and planting rows.

If working in a field which is free of coconuts, it may be possible to lay out the plots at right angles to a known fertility gradient, or to the slope in the field (i.e. on the contour). When using triangulation to permanently establish the position of the corner of a block, the greatest accuracy is achieved if the distance between the two fixed objects is about the same as the distance from each of them to the corner of the block.

The above procedures can now be used to mark out one end of each of the other blocks in the trial.

(b) Marking off the plot boundaries

If fertiliser is to be broadcast over the whole area of a plot, both plot width and length need to be accurately marked out. Continuing to use the arrangement in Figure 5.6(a) as our example, now mark out the block

(plot) width and the position of planting rows along the second string line with a tape measure, using wooden or bamboo stakes. Depending on the width of the strip of land, one plot boundary may coincide with the first string line or it may run parallel to this line (Figure 5.6(b)). Next, measure the length of the plots along the block boundaries, marking the ends of the plots with stakes. When you get to the end of the block, use additional stakes to mark the planting row positions. Strings can now be run the length of the block to mark the positions of the planting rows and across the block to mark the boundaries between plots. If you position the strings close to the soil surface, you will have less trouble with people tripping over them as they work on the plots.

(c) Labelling the plots

Clear labelling of the pots is well worth the effort so that everyone will know which plot is which, even if they do not happen to have a copy of the field plan with them. Plot labels should be waterproof and sufficiently durable to still be clearly legible at the end of the growing season when the plots are harvested. The same descriptive codes used on the field plan (e.g. 'I +K' or 'III P_{70}') are recommended for the plot labels. The codes can be painted on wooden plot labels (excellent if you have the resources) or written with waterproof ink on plastic labels tied to stakes, or scratched into aluminium labels tied to stakes. The labels should be placed inside the plot boundaries in about the same position in each plot, e.g. in the centre and about 1 m in from the end of the plot. It is best to have them all facing in the same direction, so that they can all be read as you stand at one end of a block, or walk along the length of a block.

5.9.2 Taking soil samples

Where facilities exist for soil chemical analysis, it is usually a good idea to take representative soil samples from each trial site (see Section 6). These samples should be taken before any fertiliser is applied at the trial site, and great care is needed to make sure that the samples are not contaminated with fertiliser dust before, during, or after collection.

For some detailed studies, it may be necessary to take separate soil samples from each plot. However, usually, a composite sample representative of each block will be all that is needed.

Samples may be taken quite quickly with a soil auger (Figure 5.7), but if one is not available, a spade may be used. The procedure is as follows: walk a zigzag path down the length of Block I stopping every few metres to take a soil core down to the depth of cultivation (approximately 15 cm). If you are using a spade, dig a hole 15 cm deep, and trim one side of it to be straight. Then cut a slice of soil about 2 cm thick off the straight side of the hole and place this sample in a clean plastic bucket. The straight side does not have to be vertical, as long as it goes right to the bottom of the 15 cm deep hole. Indeed, it is easier to recover the slice of soil off the spade if the straight side slopes down into the hole at about 45°.

Figure 5.7 Using a soil auger to collect a soil sample in Soc Son district, Vietnam.

Place all the soil samples from the block into the same clean plastic bucket as you collect them. When you get to the end of the block, mix the samples together, and take out a representative sample of about 500 g and place it into a clean plastic bag. Label the bag with the name of the site, the name of the experiment, the block number ('I' in this case), and the date. Repeat for the remaining blocks. The samples should be sent to the research station as soon as possible, where they will be air-dried in the shade and then stored for later chemical analysis. If samples are to be analysed for nitrate nitrogen, keep them in a cool, shady place (a foam icebox is ideal) as soon as you get them. They must not be left lying around in the sun.

5.9.3 Applying the pre-plant fertiliser

The correct fertiliser for each plot should be weighed out and placed in a clean plastic bag, labelled with the block number and the treatment code. (Use of plastic bags is important in case there is a shower of rain before the fertiliser can be applied.) In trials with several treatments, it will be convenient to place all bags for the same block in a larger bag to keep them together until they are put out on the plots. After the bags of fertiliser have been placed onto the plots, and before any fertiliser is applied, the person in charge of the trial should visit each plot and check that both the plot label and the label on the bag of fertiliser correspond exactly with what is on the field plan to make sure that no errors have been made up to this point.

If fertiliser is to be spread along planting furrows, it is suggested that separate bags of fertiliser be weighed out for each planting furrow of each plot. In this case, approximately half the contents of each bag should be spread as evenly as possible over the full length of the plot, and the other half spread as evenly as possible again over the full length of the plot while walking back along the same planting furrow. If any fertiliser is left over at the end of this second pass, the remainder should be spread as evenly as possible along the rip line, not dumped at the end of the plot! Experience has shown that this gives a much more even distribution of fertiliser than if an attempt is made to spread the all fertiliser along the furrow in one operation. The fertiliser is then usually covered with a chipping hoe before planting.

A disadvantage of this method is that in crops that are transplanted, seedling deaths often occur as a result of the young roots coming into contact with the fertiliser, unless there has been enough rain to dissolve the fertiliser and move it into the surrounding soil.

Where the fertiliser is to be spot-placed under the planting hills, the fertiliser for each row of hills is again weighed out separately, but before being applied, it is divided as evenly as possible between separate containers for each hill (cut-down soft drink cans are suitable containers). The contents of each container are spread in a circular patch perhaps 30 cm in diameter, then incorporated with a chipping hoe or digging fork. Unfertilised inter-mound soil is then raked up to form a mound above the fertilised soil (see also Section 4.2.2).

5.9.4 Planting and crop establishment

For best results, planting should be delayed for a few days after the pre-plant fertiliser application, and preferably until after rain, so that the soluble fertilisers will have had a chance to dissolve, thereby reducing the risk of seedling injury.

Uneven crop establishment can be a major source of non-treatment variation in fertiliser trials, and so it is worth going to some trouble to ensure uniform establishment. In the case of transplanted crops, planting three seedlings per hill and thinning back to two of them is a help in ensuring a good, even crop establishment. However, it is a good idea also to plant some extra seeds near the trial area in case it is necessary to transplant some seedlings to fill in any gaps in seedling establishment. If dry conditions follow planting, it is better to hand-water the hills than to lose the trial.

5.10 Side-dressing with fertiliser

Applying some (or most) of the nitrogen during the growing season helps to limit leaching losses of nitrogen and so improve the effectiveness of the nitrogen fertiliser. As the amount of soluble nitrogen fertiliser applied pre-planting is reduced by applying some of the nitrogen later, the risk of fertiliser injury to the seedlings is reduced also. Side-dressing with soluble potassium fertiliser also reduces the risk of seedling injury, and leaching losses. Side-dressing with phosphorus is not a good idea on phosphorus-fixing soils, better results being likely if all the phosphorus is applied to a limited volume of soil (see Section 4.2.2) as a pre-plant application.

Urea is very soluble in water, and if rain falls on surface-applied urea, it will usually be dissolved and taken into the soil with the water. However, when solid urea is in contact with moist soil, the urease enzyme present in the soil will convert the urea to ammonium which may be lost as ammonia gas. Ammonia is also very soluble in water, and if the urea has been properly buried after application, most of the nitrogen it contains will be retained in the moist soil (see also Section 5.4.2).

However, if solid urea is left on the soil surface, very large losses of nitrogen to the atmosphere can occur as gaseous ammonia, and much of the money spent on buying and spreading the fertiliser will have been wasted. Hence chipping in the urea is essential unless there is a very strong chance of rain on the day the application is made.

5.11 Managing the trial and keeping records

5.11.1 Management

Success of a fertiliser trial depends very much on timely and effective management during the growing season. Thus, snails, weeds, insects, and fungal diseases must be controlled, side-dressings of fertiliser correctly applied at the right time, and virus-infected plants removed. Plants weakened by pests and diseases or struggling to compete with weeds will not respond properly to the fertiliser treatments we have imposed, and the results obtained will have little value. It makes good sense to protect the time and money invested in planning and establishing the trial by taking good care of it throughout the growing season. Regular inspection of the trial and noting of any emerging problems that may require action should become a habit.

5.11.2 Record keeping

Accurate records need to be kept of the dates of pre-plant fertiliser application, of planting, and of all field operations during the season. Details need to be recorded also of all pesticide applications (type, amount, date of use), and their effectiveness or otherwise noted. Rainfall records are needed also to aid in the interpretation of the trial results. A record should be kept of any symptoms of nutritional disorders that may occur, of when they occurred, and on which plots.

If an economic analysis of the results is planned, records should be kept of the costs of all the inputs for each treatment, including labour. Similarly, the value of produce resulting from each treatment should be calculated using the prices current in the market at about harvest time.

Part-way through the season, it is a good idea to measure and record plant height (or vine length) to gain an indication of the effects of the treatments on vegetative growth, and observations should be made and recorded on the effects of the treatments on flowering and seed set or fruit set. Such records assist in the interpretation of the yield results, and in the event that a trial is lost due to late-season drought or some other unfortunate occurrence, they may still permit some assessment of the effects of the fertiliser treatments on the growth of the crop.

5.12 Harvesting, sorting the produce, and measuring yield

The measurements needed here will vary with the crop concerned. When size or other quality factors are involved, it is a good idea to make separate piles of marketable or desirable tubers, fruits etc. and non-marketable or less desirable ones. The two kinds of produce from each plot can then be counted or weighed separately.

It is important to check the identity of each plot carefully against the field plan and the plot label before recording any data. A mistake at this stage, e.g. recording a yield against the wrong plot, can ruin the whole experiment. The person responsible for the trial should satisfy themself that the plot data have been accurately recorded before leaving the site at the end of the day. If there has been a mix-up, it may still be possible to correct it at this stage, whereas later on it will be much harder to sort out a mistake if one has occurred.

5.13 Understanding the results

The exact way to proceed will vary from trial to trial depending on the detail of the experimental design, e.g. was the trial testing a simple 'yes' or 'no' question at a single site or did it involve several rates of a fertiliser and perhaps several sites? However, some steps towards understanding the results will be common to most experiments.

The first step is to organise the results into a table which brings together data from all the plots that have had the same treatment, so that the average effects of the treatments can be seen. This should be done as the data become available so that they can be checked and any inconsistencies followed up while the process of gathering the data is still fresh in everyone's mind. (A data recording sheet that has the treatment codes across the page and separate rows for each replicate will help keep the data organised as they are collected.)

Next, look for differences between the averages for the various treatments. For example, was the average yield of all the +K plots higher, lower, or about the same as the average yield of all the –K plots? Later, the data can be analysed statistically to distinguish between proven differences and those that might have occurred because of the chance variations referred to in Section 3.4. However, for the present, we are trying to get some 'feel' for the outcome of the trial, and trying to identify effects that might be worth further testing by statistical means. Look also for trends in the data. For example, did the average yields of marketable tubers tend to increase with an increasing rate of phosphorus application up to a certain point, then not increase any more? Try drawing a graph with average yield per plot on the vertical axis and rate of application of the nutrient (phosphorus in the case of this example) on the horizontal axis. What does the curve look like? If different placement methods were compared, do average yields at each level of phosphorus supply tend to

be higher with one placement method than another, so that, if you plot them, you get two curves instead of one?

Having become familiar with the results you have obtained, the next step is to analyse them statistically. If you have had some training in this area, you may be able to do this yourself. If not, you will need to seek assistance with the task. The results of statistical analyses are expressed as probabilities that the difference we are testing was caused by the treatments we imposed. Thus, 'significant at $P < 0.01$' means that there is less than one chance in 100 that the difference was an accidental result due to normal place-to-place (i.e. block-to-block) variation in yield across the site. Similarly, 'significant at $P < 0.05$' means that there was less than one chance in 20 that the result was accidental. On the other hand, 'not significant' means that any apparent differences between the average values obtained for our treatments were too small to distinguish from the background variation, and hence these differences cannot be accepted as 'real' or proven differences.

Finally, convert your yields from kg/plot into tonnes/hectare and compare them with the commercial yields obtained elsewhere on the same farm or on other farms in the district. On this basis, do some of your experimental treatments look as though they might give higher yields than existing methods now in use on commercial farms or perhaps similar yields with a lower fertiliser cost? On this season's costs and prices, what would be the likely financial outcome for a farmer who abandoned his existing fertiliser practices for a new set of practices based on the results of this trial?

5.14 Reporting the results

It is important that, once analysed, the results are reported so that they become available to those who may benefit from their use. Trial results that do not get beyond the experimenter's field notebook or that remain locked away in research station files are of no use at all to the farming community. Again, everyone who has participated in a trial has a right to know what the outcome was, and researchers who do not take the trouble to share this information will soon run out of people who are willing to collaborate with them in future trials.

Reporting may take a variety of forms, including talks at farmers' meetings, radio and television interviews, newspaper articles, discussions at meetings of agronomists and extension officers, new or revised extension pamphlets, and scientific journal papers. The most appropriate form or forms of reporting will vary with the nature of the information gained from a trial or series of trials, and on the main target audience. The latter may include farmers, extension officers, fertiliser importers, government policy makers, and agronomists. All have part to play in making agriculture more efficient, and all should be kept in mind when deciding how best to report trial results.

5.15 Planning future action, based on the results

Sometimes, trial results are so clear-cut that farmers are prepared to make an immediate change to some aspect of their farming practices. However, more often, experience over several seasons is required before a convincing case for change can be made. However, it is important that discussions take place annually to identify still-existing gaps in knowledge and to plan the next season's field trials. It is not a sensible use of scarce resources to simply repeat last season's experimental program. Rather, conscious decisions have to be made concerning trials that are worth running again in their existing form, trials that should be run again in a modified form, and completely new trials arising from what was discovered in the previous season. Involving farmers in these consultations will assist in directing the program towards practical ends, and should sustain a level of farmer interest that will lead to early adoption of improved practices arising from the trials.

6

Soil Testing — Where Does That Fit In?

Much of the experimental work we have been discussing so far would become unnecessary if we had truly reliable soil chemical tests for each of the nutrient elements required by plants. However, the fact remains that there seem to be very few universal soil tests that will work well on all or even most soils. Table 6.1 shows some results from a study which measured the ability of some well-known soil tests to predict plant behaviour in pot experiments.

In the case of the widely-used Bray 2 test for phosphorus, the test had a 100% success rate, correctly predicting that all 15 soils would be deficient for the growth of maize in a series of omission trials ('all – P' treatment). The omission trials also showed that four soils contained adequate potassium and 11 were deficient (Table 6.1). The soil test for potassium correctly predicted potassium deficiency in 9 of the 11 deficient soils, but predicted an adequate supply of potassium in only 1 of the 4 soils containing adequate potassium. Hence, the success rate here was only 10 out of 15. However, with the copper test, the success rate was only 5 out of 15 (Table 6.1), a very poor result considering that all the soils were derived from similar parent material.

Although in the study in Thailand referred to above, the Bray 2 extractant (NH_4F + HCl) provided a good indication of phosphorus availability on a particular group of soils, there is no single soil test for phosphorus that can be trusted to give reliable results on all soils. Indeed, in recent research on 25 heavily fertilised

Table 6.1 Numbers of correct and incorrect predictions on the growth of maize plants in nutrient omission trials conducted using published soil tests and their critical values on 15 granitic soils in southern Thailand (Nilnond 1993).

		Plants deficient		Plants healthy	
Element	Test	Correct	Incorrect	Correct	Incorrect
P	Bray No 2	15	0	0	0
Mg	Exchangeable	9	0	3	3
K	"	9	2	1	3
S	$Ca(H_2PO_4)_2$	5	1	5	4
Zn	DTPA	4	0	4	7
Cu	DTPA[a]	1	10	4	0

[a] DPTA = Diethylenetriaminepentaacetic acid

Australian soils, the Bray 2 test incorrectly identified 8 phosphorus-deficient soils as containing enough phosphorus for plant growth (Kusumo 2000).

Such results serve to illustrate the continuing need for work to improve soil tests, and why we should always satisfy ourselves that a particular soil test actually works on our soils before putting the test into routine use.

6.1 Attributes of a good soil test

A good soil test will have four main attributes: close correlation with plant response; accurate calibration; wide applicability; and ease of measurement. Let us consider each of these in turn.

6.1.1 Correlation with plant response

A soil test may not extract the same amount of a nutrient element from a given amount of soil as would the roots of a crop, but for the test to be successful, these two quantities must be closely and positively correlated; i.e. as the soil test value increases, plant uptake of the element will increase also.

In practice, we usually do not measure uptake of the element by the plant, but rather plant growth. Growth will increase with increasing uptake over the range of nutrient deficiency, become constant in the region of adequacy, and then decrease if further increase in uptake results in a toxicity of the nutrient concerned (Figure 6.1).

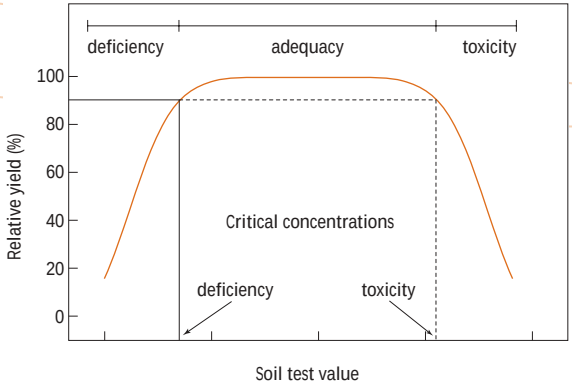

Figure 6.1 Schematic relationship between relative yield and soil test value.

6.1.2 Soil test calibration

While the form of the relationship between soil test value and plant growth may remain similar when we compare a range of soils, the quantitative relationships may differ from soil to soil. In other words, the critical concentrations for deficiency may vary from soil to soil. A good example of this comes from studies on the boron nutrition of avocado trees (T.E. Smith, unpublished data). In this case, it was found that the concentration of boron in the spring flush of leaves was well correlated with the amounts of boron extracted with hot $CaCl_2$ solution from soils of similar texture. However, in sandy soils, the increase in leaf boron concentration with increase in soil test value was much more rapid than on soils with higher clay contents. Hence, the tendency was for the critical value for the soil boron test to increase with the clay content of the soil; i.e. the calibration of the test changed with clay content.

Often we do not know why the same soil test will yield different critical values when applied to different soils. In these circumstances, we cannot predict, from knowledge of other soil properties, how the critical values will change. Hence, there is a need to check published values by actual experiment with the soils of interest to us.

6.1.3 Applicability

The ideal soil test would be well correlated with plant performance on all soils, and would have the same critical values for deficiency and toxicity on all soils, regardless of their texture and mineral composition. However, as indicated in Section 6.1.2, this wide applicability cannot be taken for granted. Hence, we need to show by experiment that a particular soil test and its published critical values apply to 'our' soils before we can interpret the results of the test with confidence.

6.1.4 Ease of measurement

Clearly, if two or more soil tests are equally accurate predictors of plant response to nutrient applications on the soils with which we are concerned, we will tend to select for routine use the test that is easier to perform, quicker, or less expensive.

6.2 Use of published information

The published experience of others with various soil tests is a logical starting point if we are considering using soil tests. Table 6.2 lists some commonly-used soil tests, along with notes on their interpretation. From Table 6.2 it is clear that, in some cases, quite wide ranges of soil test values have been found to correspond with the threshold for nutrient deficiency; i.e. there is no generally accepted critical value that can be applied across all soils.

Despite the difficulties in interpreting soil tests precisely, as indicated in Section 1.4.3 their results can give us valuable early warning of problems likely to be encountered at a particular site. Also, it is sometimes possible to improve the local precision of soil tests by recalibrating them for local soils.

6.3 Selecting, checking, and recalibrating soil tests

6.3.1 Selecting the test

The first step is to read as much as we can about the various tests that have been used for a particular nutrient element (for some up-to-date accounts, see Peverill et al. 1999). In reading, we should pay particular attention to tests that have worked well either across a wide range of soils, or on soils similar to those with which we will be dealing. It may be that we cannot choose a test on this basis alone, but we can at least narrow down the range of possibilities. At this stage, we need to consider also the laboratory equipment and facilities that will be available to us, to ensure that our choice of test is a practical one.

6.3.2 Checking the correlation and calibration

To see how well a soil test estimates the plant-availability of a nutrient element, we can make use of the results of pot or field experiments across a range of sites differing in the supply of that element. In each case, we will relate the soil test value for a particular nutrient in a sample of soil which has not been fertilised, to the relative yield obtained at the zero level of application of that nutrient on the same soil. We may then fit a mathematical function to the data. The closer the fit, the better the correlation between soil test and plant behaviour. Solving the fitted equation for 90% of maximum yield gives us an estimate of the critical value for deficiency.

Figure 6.2 shows an example in which the results of a series of nutrient omission trials with soils from Tonga (Halavatau 1998) have been used to check the soil test correlation for NH_4OAc-extractable potassium (exchangeable K). Note that the data indicate a critical soil-test value of about 0.5 cmol(+)/kg, which sits just below the start of the 'fertiliser response unlikely' range in Table 6.1. These results served to confirm that the test is suitable for use on Tongan soils.

Table 6.2 Some commonly-used soil tests and their interpretation (Landon 1991).

Test	Extractant	Units	Values	Interpretation
NO_3–N	1:5 soil:water	mg/kg	<5 >20	Very low High
P	H_2SO_4 (acid soils)	"	<20 20–40 >40	Deficient Fertiliser response likely Fertiliser response unlikely
	$NaHCO_3$ (neutral and alkaline soils)	"	<5 5–15 >15	Deficient Fertiliser response likely Fertiliser response unlikely
K	NH_4OAc	cmol(+)/kg	<0.2 >0.6	Fertiliser response likely Fertiliser response unlikely
Mg	"	"	<0.5 >4.0	Low (uptake further depressed by Al^{3+}, Ca^{2+}, and K^+) High
Ca	"	"	<0.2 <4 >10	Probably deficient Low High
Na	"	"	>1	High
S	$Ca(H_2PO_4)_2$	mg/kg	<8	Deficient
B	Hot water	"	<0.1–0.7	Deficient (depends on soil texture)
Fe	DTPA[a] + $CaCl_2$	"	<2.5–4.5	Deficient (but many interacting factors)
Mn	dilute HCl + H_2SO_4	"	<5–9	Deficient (affected by many factors)
Zn	"	"	<0.5–1.0	Deficient (affected by pH and Cu)
Cu	NH_4OAc	"	<0.2	Deficient (affected by N, Zn, and other factors)
Mo	NH_4 oxalate	"	<0.04–0.2	Deficient (affected by pH and other factors)
pH	1:5 soil:water	–	<4.5 4.5–5.0 5.0–5.5 5.5–6.5 6.5–7.0 7.0–8.5 >8.5	Al, Mn, and H toxicities possible Al, Mn toxicity, Mo deficiency possible Mn toxicity, Mo deficiency possible Favourable range for many species Near-neutral Increasing likelihood of deficiencies of P, Fe, Mn, Zn, Cu, or Co Strongly alkaline

[a] DPTA = Diethylenetriaminepentaacetic acid

The results in Figure 6.2 provide some information on the calibration of the potassium soil test for this group of soils. However, if available, data from rate trials in the field would probably allow a more accurate estimation of the critical value. For this purpose, trials would need to be conducted at some sites providing adequate plant-available potassium as well as at deficient sites.

6.4 Conclusions

Soil tests can be valuable predictors of the need to apply additional nutrients at a particular site, and they have the advantage over other methods that they can be conducted before a crop is planted. However, at present we do not have a full battery of soil tests that can be relied upon to work well on all soils. Hence, in any 'new' situation we need to determine by experiment whether or not the test results correlate well with plant behaviour, and whether or not the calibration of the test is correct.

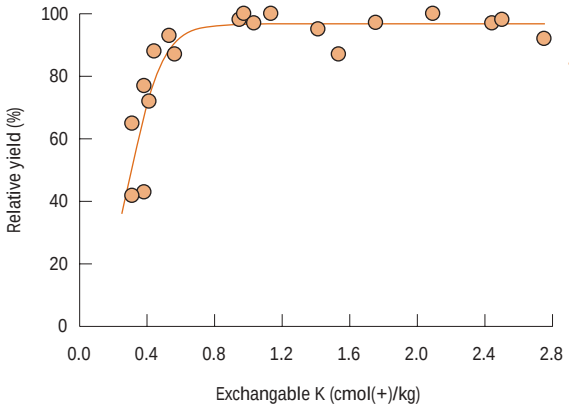

Figure 6.2 Relationship between the amount of potassium extractable with 1M NH_4OAc from 19 unfertilised Tongan soils, and the relative yields of maize plants in the 'all – K' treatments of omission trials conducted with the same soils in the greenhouse.

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APPENDIXES

Appendix 1

Leaf Painting Exercise

Some nutritional disorders produce characteristic and easily recognisable symptoms on some crops, making diagnosis a relatively simple matter. However, in many cases where the symptoms are less clear-cut, or where similar symptoms are produced by more than one nutritional disorder, a tentative diagnosis based on leaf symptoms must be confirmed by other methods. One of these methods is leaf painting.

Principles of leaf painting

Leaf epidermal cells are capable of absorbing mineral nutrients applied to the leaf surface in dilute aqueous solution (see Marschner 1995, pp. 119–128). When mineral-deficient leaves are painted with a solution containing a suitable concentration of the deficient element, they will usually show signs of recovery within a few days (see Figure 1.1, this manual). Hence, by treating portions of affected leaves with solutions containing particular elements and comparing these with untreated 'control' portions, a diagnosis can be made. The method works particularly well with immobile micronutrients such as iron, since recovery is restricted to the treated area.

The concentrations of micronutrient salts needed to give a good recovery of the treated area without burning the leaf will depend on the leaf surface characteristics of the crop. It may depend also on the physiological status of the leaves. For example, there is some evidence that severely iron-deficient maize leaves have thinner-than-normal cuticles, and thus are more easily damaged by

foliar applications of iron salts than are normal leaves. Hence, we need to find by experiment the most suitable concentration to use in a particular situation. However, the concentrations in Table A1.1 provide a useful starting point.

If the salt recommended is not available, you may be able to substitute another salt and adjust the weight of salt so that the concentration of the micronutrient is approximately the same as before. Where leaves are difficult to wet, a wetting agent such as Shirwet® may be added to the leaf painting solution.

Procedure

To test plants that you suspect are iron deficient, follow the steps below.

(a) Carefully apply each ferrous ammonium sulfate test solution to a separate portion of one or more leaves and record the location of the treated areas. Hold the leaf so that the solution does not run onto the 'control' areas.

(b) Label each leaf to show your initials and the solution applied, using tags.

(c) Observe and record the results every 3 or 4 days.

Table A1.1 Suggested composition of solutions for diagnosis of micronutrient deficiencies by leaf painting.

Element	Salt	Molecular weight	Weight of salt (g)/100 mL	Weight of $Ca(OH)_2$ (g)/100 mL
Fe	$(NH_4)_2SO_4.FeSO_4.6H_2O$	392.1	1.0	–
	FeEDTA	367.1	0.1	–
Mn	$MnSO_4.7H_2O$	277.0	1.0	–
B	$Na_2B_4O_7.10H_2O$	381.4	0.5	–
Zn	$ZnSO_4.7H_20$	287.5	0.5	0.25[a]
Cu	$CuSO_4.5H_2O$	249.7	0.5	0.5[a]
Mo	Na_2MoO_4	242.0	0.1	–

[a] Addition of $Ca(OH)_2$ will precipitate some of the metal, reducing the risk of burning the leaf.

Appendix 2

Exercises with Tissue Tests

Tissue tests are rapid, qualitative or semi-quantitative chemical tests that can be performed in the field to determine the nutrient status of plants. Since a deficiency in one essential element often leads to the accumulation of other non-deficient elements in the tissues (by reducing *growth dilution* of these other elements), the precision of testing can often be enhanced by applying several tests to the same plants, e.g. if nitrogen deficiency is suspected, test also for phosphorus deficiency and potassium deficiency.

Tissue tests may be performed using commercially available plant testing kits, using mixtures of reagents that you can prepare yourself, or using test kits intended for testing water samples. We have had only limited experience using commercial plant testing kits, but have had generally good results with the other two methods.[4]

For the present exercise, you will need some healthy plants and some that have had their supply of individual essential elements restricted.

Nitrogen

The tests discussed here are based on the fact that, under field conditions, plants adequately supplied with nitrogen usually have some nitrate-nitrogen in their sap. Note, however, that the tests do not work on legumes that are dependent on symbiotic nitrogen fixation,

[4] We use Merkoquant® water test kits. With other test kits, the directions may be slightly different to those described in this appendix.

because in these plants the nitrogen is transported in the sap in chemical forms other than nitrate.

1. Using nitrate water test strips

Chemical basis of the test

The reaction zone of the test strip contains a reducing agent that reduces nitrate to nitrite. In the presence of an acid buffer the nitrite is then converted to nitrous acid which diazotises an aromatic amine (sulfanilic acid). Coupling with N-[naphthy(1)]-ethylenediamine (NNEDDC) produces a red–violet azo dye.

Procedure

(a) Gently squeeze or press a small amount of plant sap onto the reaction zone of the nitrate test strip. This may be done by laying the piece of plant stem or petiole across the reaction zone and pressing down using a glass rod.

(b) Wait 2 minutes for full colour development then compare the test zone with the colour scale provided with the test strips.

2. Using Bray's nitrate powder

Chemical basis of the test

In this test, the tissue nitrate is reduced to nitrite, using metallic zinc as the reducing agent. At low pH and in the presence of nitrite, the sulfanilic acid undergoes a diazotisation reaction to form a diazo salt. This then couples with 1-napthylamine to form a red–violet dye, as in the case of the test strips described above.

Bray's nitrate powder is a convenient mixture of the reagents needed. Details of its composition and formulation follow.

Caution: 1-naphthylamine is a carcinogen. Use gloves, avoid all skin contact, and avoid inhaling the dust when making or using Bray's nitrate powder.

(i) 4 g sulfanilic acid

(ii) 2 g 1-naphthylamine

(iii) 10 g $MgSO_4.2H_2O$

(iv) 2 g finely powdered zinc

(v) 100 g $BaSO_4$

(vi) 75 g citric acid (for pH control)

All reagents are first ground separately to fine powders, then reagents (i) to (iv) inclusive are mixed separately with small portions of the $BaSO_4$. These portions are then combined and mixed with the citric acid and the remainder of the $BaSO_4$. The mixture is then stored in a black bottle to exclude light.

Procedure

(a) Cut the tissue to be sampled across the vascular bundles to induce bleeding (a diagonal cut will increase the cut area of vascular bundles).

(b) Either sprinkle the powder directly onto the cut surface (if the surface is large, e.g. a maize stem),

or

obtain a sample of sap from the cut surface on a filter paper and sprinkle the nitrate powder onto the moist filter paper. Fold and press the paper until the powder takes up moisture from the sample. Colour should develop in about 1 minute.

(c) If the powder remains white, the tissue is probably nitrogen deficient; if it turns pink the nitrogen status is satisfactory; if it turns dark pink or red excess nitrate is present.

Phosphorus

The test is based on the fact that plants well supplied with phosphorus will usually have detectable amounts of phosphate ions in their sap. It can be applied using a water testing kit, or by using laboratory reagents.

Chemical basis of the test (both tests)

The colour reaction is based on the phosphomolybdenum blue (PMB) reaction. Orthophosphate ions react with isopolymolybdic acid in sulfuric acid solution to give phosphomolybdic acid which is light-yellow in colour. Phosphomolybdic acid is then reduced with ascorbic acid or stannous [tin(II)] chloride to give the intensely blue coloured phospho-molybdate complex.

1. Using a water testing kit

Procedure

(a) Take a piece of plant stem or petiole and, using a glass rod, squeeze 2 or 3 drops of plant sap into the calibrated plastic test vial.

(b) Fill to the 5 mL mark with deionised water.

(c) Add 2 drops of reagent 1 and swirl.

(d) Add 1 level microspoon (provided with the kit) of reagent 2 and dissolve by swirling.

(e) After 2 minutes, place the test vial on the colour card provided and read off the concentration of phosphate in your diluted sap sample.

2. Using laboratory reagents

Procedure

(a) Dissolve 8 g of ammonium molybdate in 200 mL of deionised water and carefully add a mixture of 12 mL concentrated HCl and 74 mL of distilled water. The resulting solution is then diluted 5-fold before use. If necessary, the concentrated stock solution may be stored for extended periods in the refrigerator.

(b) Cut about 0.5 g of fresh tissue into thin slices and place in a clean 15 mL vial.

(c) Add about 10 mL of the *diluted* ammonium molybdate solution, stopper the vial and shake for 1 minute.

(d) Add a *small* amount of stannous chloride or ascorbic acid to the vial and shake again. Adding too much stannous chloride will make the blue colour hard to read.

(e) Observe the colour after about 1 minute. Deficient tissue will usually yield a colourless, yellow, green, or bluish-green solution. Light to medium blue indicates adequate phosphorus.

Potassium

1. Using a water test strip

Chemical basis of the test

Dipicrylamine forms an orange complex with potassium ions in alkaline solution.

Procedure

(a) Place the glass ignition tube (supplied in kit) into the depression in the kit.

(b) Place in it 10 drops of reagent solution.

(c) Take a test strip and gently press a piece of petiole or stem onto the test zone of the strip, taking care not to abrade the reaction zone.

(d) Immerse the reaction zone in the reagent solution in the glass tube for 1 minute and compare the colour of the reaction zone with the colour chart provided.

2. Using laboratory reagents

Chemical basis of the test

Tissue potassium is extracted in Morgan's solution and precipitated as the bright yellow cobaltinitrite salt $(H_nNa_{3-n})\ Co(NO_2)6.xH_2O$. Isopropyl alcohol is used to depress the solubility of this salt.

Procedure

(a) Prepare Morgan's extracting solution by dissolving 100 g of sodium acetate in 500 mL of deionised water, and adding 30 mL of glacial acetic acid. Make up the mixture to 1 L using deionised water.

(b) Prepare the cobaltinitrite reagent as follows:

  (i) Dissolve 5 g of cobalt nitrate in 47.5 mL deionised water plus 2.5 mL of glacial acetic acid.

  (ii) Dissolve 30 g of sodium nitrite in 50 mL deionised water. This solution may be stored in a brown bottle.

  (iii) Mix equal volumes of solutions (i) and (ii) in a bottle and cover with a watch glass, allowing nitrogen dioxide to escape, for about 12 hours. The bottle may then be stoppered and stored in the refrigerator until required.

(c) Prepare an isopropyl alcohol–formaldehyde solution by mixing 90 mL of isopropyl alcohol with 10 mL of neutral formaldehyde.

(d) Cut up about 0.5 g fresh tissue and place in a vial as in the phosphorus test.

(e) Add 5 mL of Morgan's solution and shake for 2 minutes.

(f) Add 0.2 mL of the cobaltinitrite reagent and shake briefly.

(g) Add 2 mL isopropyl alcohol–formaldehyde solution (to depress the solubility of the potassium salt) and shake for 2 minutes.

A clear reddish-brown solution indicates potassium deficiency. A turbid yellowish-brown suspension indicates an adequate potassium status.

Calcium

Chemical basis of the test

The colour reaction is based on the reaction of calcium ions with glyoxalbis-(2-hydroxyanil) to form a red–violet complex.

Procedure

(a) Take a piece of plant stem or petiole and, using a glass rod, squeeze 2 or 3 drops of plant sap into the calibrated plastic test vial provided with the test kit.

(b) Fill to the 5 mL mark with deionised water.

(c) Immerse a test strip briefly in this solution and set aside with the reaction zone facing upwards.

(d) Add 1 level microspoon (provided with the kit) of reagent 1 to the test solution and dissolve.

(e) Add 10 drops of reagent 2 and shake.

(f) Immerse the reaction zone of the test strip in this solution for 45 seconds and then compare with the colour scale.

Magnesium

Chemical basis of the test

Magnesium ions react with 1-azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)-naphthaline-1-(2-hydroxybenzene-5-sodium sulfonate) to form a red dye.

Procedure

(a) Take a piece of plant stem or petiole and, using a glass rod, squeeze 1 large drop of plant sap into the measuring vial.

(b) Add 9 drops of buffer solution and shake.

(c) Use the pipette supplied to transfer 2 drops of this solution to a second measuring vial.

(d) Fill up to the 5 mL mark with the buffer solution.

(e) Add 10 drops of reagent and shake.

(f) After 1 minute, place the measuring vial on the white strip of the colour chart and read off the concentration of magnesium in your diluted sample of sap.

Appendix 3

Iminodiacetic Resin[5] Procedure for Removal of Trace Metals

Chelating resins such as Chelex 100 (BioRad) and Amberlite IRC-748 (Rohm and Haas Co.) have a high affinity for trace metal cations (Cu, Zn, Hg, Pb etc.) relative to the macro-nutrient cations (Ca, K, Mg). Hence, macronutrient stock solutions, including those supplying Ca, K, or Mg, can be freed of micronutrient metal contamination by passing them through a column of chelating resin.

The resin comes as a Na complex. After decanting an appropriate volume of resin (e.g. 20 mL), wash with at least 3 bed volumes of 1 M HCl or HNO_3 to strip off all metals and convert it to the H^+ form. Rinse out the acid with about 5 bed volumes of water. The resin can then be converted to the appropriate cation for the salt you want to purify (Ca, Mg, K etc.) using the chloride salt of that cation.

The main reason for doing this before pouring the resin into the column is that the bed volume changes appreciably depending on the cation bound to the resin. For example, if the resin is converted to the H^+ in the column, it shrinks to almost half the Na volume. When the salt to be purified is added, the resin swells again,

[5]Trade names include Chelex-100® and Amberlite IRC-748®

but tends to become compacted in the column rather than expanding, and this reduces the flow rate.

When the resin is washed, and in the desired cation form, pour into a column. It is best if the inlet end can be sealed onto the tubing so you can apply slight head pressure from gravity or a peristaltic pump. Run the salt to be purified and collect in a clean container (Figure A3.1).

To regenerate the resin, and remove metal salts, run 3 bed volumes of 1 M acid and rinse as before. Convert to cation form again to store it for re-use, as this is more stable than the H^+ form.

Micronutrient binding using iminodiacetic resin

Notes from Riley and Taylor (1968):

Element	pH range for binding	Elution
Cu (and most others)	neutral (5–9)	2 N HNO_3
Mo	5.0 (<6)	4 N NH_4OH
Mn	9.0	2 N HNO_3

Note: Mo contamination — avoid contact with stainless steel.

Figure A3.1 Purification of macronutrient salts for omission trials at the National Institute of Soils and Fertilisers, Hanoi, Vietnam. Note use of blood transfusion drippers to regulate the flow of solution from the reservoirs to the resin columns.

Appendix 4

Soil pH Measurement

As with most soil measurements, the pH value obtained for a soil will vary depending on the procedure used. For example, the measured pH will depend on the soil:solution ratio used (the pH generally increasing as the soil:solution ratio widens) and the composition and concentration of the solution used (salt solutions give lower pH values than water). The pH guide values used in this manual are based on determinations made in a 1:5 soil:water suspension, and we recommend that this soil:water ratio be used.

Measurement of pH is made using glass and reference electrodes (either as separate electrodes or, more commonly, as a 'combination' electrode) and a pH meter. The electrode(s) are calibrated against standard buffer solutions of known pH. Two buffer solutions are required to calibrate the pH electrode and these should ideally span the pH range within which the samples will fall. The pH of solutions will vary slightly with temperature, thus measurements should be made at a constant temperature, e.g. 25°C. Where this is not possible, an automatic temperature correction (ATC) probe should be used, or the solutions allowed to equilibrate to room temperature and this temperature manually measured and set on the pH meter. Extremes of temperature should be avoided.

Reagents

pH buffer solutions

Buffer solutions may be purchased as ready-to-use solutions, prepared from buffer tablets, sachets or by dilution of concentrated solutions, or prepared from analytical reagent (AR) grade chemicals as described below.

Distilled/ deionised water for preparing pH buffer solutions
This water should have a pH of 6.5 to 7.5, which can be obtained by boiling distilled/deionised (DI) water for 15 minutes and cooling under CO_2-free conditions. The electrical conductivity of this water should be less than 1 µS/cm.

pH 4.01 buffer
Dry potassium hydrogen phthalate ($KHC_8H_4O_4$) at 110°C for 2 hours and cool in a desiccator. Dissolve 10.12 g of $KHC_8H_4O_4$ then make up to 1.0 L using the DI water prepared for use in buffer solutions. Protect solution against evaporation and contamination.

pH 6.86 buffer
Dry potassium dihydrogen orthophosphate (KH_2PO_4) and disodium hydrogen orthophosphate (Na_2HPO_4) at 130°C for 2 hours and cool in a desiccator. Dissolve 3.39 g KH_2PO_4 and 3.53 g Na_2HPO_4 then make up to 1.0 L using the DI water prepared for use in buffer solutions. Protect solution against CO_2, evaporation, and contamination.

pH 9.18 buffer
Dry sodium tetraborate ($Na_2B_4O_7.10H_2O$) over a saturated aqueous solution of NaCl and sucrose in a desiccator. Dissolve 3.80 g of $Na_2B_4O_7.10H_2O$ then make up to 1.0 L using the DI water prepared for use in buffer solutions. Protect solution against CO_2, evaporation, and contamination.

Calibration of the pH electrode

Wash the electrode(s) with DI water from a wash bottle, then dry the electrode(s) gently with a soft tissue. Place the electrode(s) in the pH 6.86 buffer solution and stir with a mechanical stirrer or gently swirl the solution by hand. (This will reduce the time required for the reading to stabilise.) Once stable, adjust the meter to read 6.86 using the 'calibrate' (or buffer) control. Remove the electrode(s), rinse well with DI water, dry and place in the pH 4.00 or pH 9.18 buffer. When the reading is stable, adjust to 4.00 or 9.18, as appropriate, using the slope control. Repeat these steps until the meter reads both buffers correctly without adjustment. The pH electrode is now calibrated and can be used to measure the pH of soil suspensions. If a series of measurements is to be made, the calibration should be checked periodically using one of the buffer solutions.

Sample measurement

Prepare a 1:5 soil:water suspension by weighing 20.0 g of air-dry soil into a screw-topped jar and adding

100 mL of DI water. Shake end-over-end for 1 hour, then allow 20–30 minutes for the soil to settle. After calibrating the pH electrode(s) as described above, wash well with DI water, dry, then immerse in the sample suspension. Record the pH value obtained when the meter appears steady while the suspension is being mechanically stirred or gently swirled by hand. Replicate determinations should give results within 0.1 pH unit.

Appendix 5

Adjustment of Soil pH — Constructing a Buffer Curve

If the soil pH is less than 5.2, sufficient lime should be included in the 'all' treatment of an omission trial to bring the soil to about pH 6.0. If the pH lies in the range of 5.2 to 5.5, there is the possibility of yield reductions caused by manganese toxicity, but we do not recommend including lime in the 'all' treatment. Instead, we recommend including an 'all + lime' treatment in the experiment (see Section 3.11.1 of this manual).

To find out the correct amount of liming material, we first need to construct a buffer curve. For this we need to prepare a saturated solution of calcium hydroxide.

Saturated $Ca(OH)_2$ solution

Boil 1 L of deionised/distilled water for 15 minutes to drive off CO_2, then allow to cool slightly. Add 2 g of $Ca(OH)_2$ and mix well. Place the solution in a bottle and cover to prevent CO_2 entry. Allow to settle overnight before use. Note that more $Ca(OH)_2$ is added than will dissolve, so the excess settles to the base of the storage bottle. This sediment should not be disturbed when the saturated $Ca(OH)_2$ solution is decanted for use. Saturated $Ca(OH)_2$ solution has a OH^- concentration of approximately 0.038 M (or 0.019 Ca^{2+}). The accurate OH^- concentration can be

determined by titration with standard acid, but the approximate value of 0.038 M will be sufficiently accurate in most situations.

Next, we proceed as follows :

i. Take 12 clean screw-topped jars.

ii. Weigh 20 g air-dry soil into each jar.

iii. To the first pair of jars, add no $Ca(OH)_2$ solution. To the second pair of jars, accurately add 2.5 mL of saturated $Ca(OH)_2$ solution to the soil in each jar. To the third pair of jars, add 5 mL of $Ca(OH)_2$ solution. To the fourth pair of jars, add 10 mL of $Ca(OH)_2$ solution. To the fifth pair of jars, add 20 mL of $Ca(OH)_2$ solution. To the sixth pair of jars, add 40 mL of $Ca(OH)_2$ solution.

These rates of addition of $Ca(OH)_2$ solution equate to field lime rates of 0.4–6.8 t/ha $CaCO_3$ (assuming a 15 cm depth of incorporation and a bulk density of 1.2 g/cm^3), and should be adequate for most soils. However, the graduated amounts of $Ca(OH)_2$ solution may be adjusted up or down to suit soils with differing pH buffering capacities. For example, lower rates can be used to provide a more accurate lime requirement assessment for poorly buffered sandy soils, and higher rates used for strongly acid clay soils.

iv. Add deionised or distilled water to each jar to bring the total volume of solution to approximately 100 mL, screw on the lids, and shake them to mix the soil, water, and $Ca(OH)_2$.

v. Allow the jars to stand, with occasional shaking, for 24 hours before measuring the pH.

vi. Plot pH against the volume of $Ca(OH)_2$ solution added, and read off the graph the volume of $Ca(OH)_2$ solution needed per 20 g of dry soil to bring the soil to a pH of 6.0. The amount of $Ca(OH)_2$ needed per pot can then be calculated from the following formula:

$$\text{weight of } Ca(OH)_2 \text{ (g/pot)} = V \times C/2 \times 74 \times W/20$$

where V is the volume in mL of $Ca(OH)_2$ solution required to obtain pH 6.0 in 20 g air-dry soil, C is the molar concentration of OH^- in the saturated $Ca(OH)_2$ solution (use 0.038 M unless a more accurate value is determined by titration), 74 is the molecular weight of $Ca(OH)_2$, and W is the weight of air-dry soil in g/pot .

Liming of soils is a practical and effective means of raising the pH of strongly acidic soils. With strongly alkaline soils (pH greater than 8.5) it is possible to lower the soil pH by adding elemental sulfur (which is oxidised to sulfuric acid by soil microorganisms) or by adding aluminium salts such as $AlCl_3$ (used in water treatment in some cities) or $Al_2(SO_4)_3$. Acidification of alkaline soils is only likely to be economic with very high value crops. Hence, we do not usually acidify alkaline soils before conducting pot experiments with them. However, if you wish to acidify a strongly alkaline soil,

perhaps to pH 7.0, you could construct a buffer curve, to determine the amount of acidifying agent needed, by substituting an aluminium salt for $Ca(OH)_2$ in the procedure described earlier.

Note: $Ca(OH)_2$ is a convenient liming material for establishing buffer curves and for adjusting the soil pH in pot experiments. However, chemically equivalent amounts of finely ground $CaCO_3$ many be substituted in pot experiments if desired. The equation above can be altered for $CaCO_3$ calculations, by replacing 74 (the molecular weight of $Ca(OH)_2$) with 100 (the molecular weight of $CaCO_3$).

Appendix 6

Some Calculations Needed before Making Stock Solutions

Start with the rate of application for each element in kg/ha (e.g. Table 3.1 of this manual), and convert this to the rate of application of the chemical compound in kg/ha by using a 'weight conversion factor':

$$\textit{Weight conversion factor} = \frac{\textit{Molecular weight of compound}}{n(\textit{Atomic weight of element in compound})}$$

where n is the number of atoms of the element in the chosen chemical compound.

Use the 'weight conversion factor' to obtain the rate of chemical compound in kg/ha:

$$\textit{Weight of compound (kg/ha)} = (\textit{Weight of element (kg/ha)}) \times (\textit{Weight conversion factor})$$

Next, we convert from 'rate of chemical compound in kg/ha' to 'rate of chemical compound in mg/pot' by either of two methods:

(a)Pot area basis:

Knowing that 1 kg/ha = 1 mg/100 cm^2 of pot area, the rate of application per pot can be calculated:

$$Weight\ of\ compound\ (mg/pot) = \frac{(Weight\ of\ compound\ (kg/ha)) \times (Area\ of\ pot\ (cm^2))}{100}$$

(b)Weight-of-soil basis:

Knowing that 1 ha of soil down to 15 cm has a volume of 1500 m^3 and a mass in kg of ($1.5 \times 10^6 \times$ the bulk density of the soil), the rate of application per pot can be calculated by the following two equations. Firstly, we convert from a weight of chemical compound in kg/ha to a weight of chemical compound per kg of soil:

$$Weight\ of\ compound\ (mg/kg\ soil) = \frac{10^6(Weight\ of\ compound\ (kg/ha))}{1.5 \times 10^6 \times Bulk\ density\ of\ soil}$$

Then we calculate the weight of chemical compound in mg/pot:

$$Weight\ of\ compound\ (mg/pot) = (Weight\ of\ compound\ (mg/kg\ soil)) \times (Weight\ of\ soil\ (kg/pot))$$

Finally, we must calculate the concentration of each stock solution needed to supply the desired rate of application per pot in a volume of solution that is sufficient to obtain a good spread throughout the soil without making the soil too wet. We have found a volume of 5 mL per element to be convenient in most cases.

$$Weight\ of\ compound\ in\ stock\ solution\ (g/L) = \frac{Weight\ of\ compound\ (mg/pot)}{Volume\ of\ stock\ solution\ added\ (mL/pot)}$$

Appendix 7

Methods for Preparing Sulfate-free Chelated Iron

Method 1:

Reference: Steiner and van Winder (1970)

1.1 Dissolve 4.12 g NaOH in approximately 500 mL distilled water

1.2 Warm to 30°C if necessary

1.3 Dissolve 33.3 g Na_2EDTA in this solution

2.1 Add 0.41 mL concentrated HCl to approx. 300 mL distilled water

2.2 Heat to 70°C

2.3 Dissolve 17.8 g $FeCl_2.4H_2O$ in this solution

3.1 Mix solutions 1.3 and 2.3

3.2 Add distilled water to approximately 950 mL

3.3 Aerate vigorously for 12 hours

3.4 Make up to 1000 mL with distilled water

Method 2:

Reference: Adapted from Hewitt (1966)

1.1 Dissolve 37.224 g Na_2EDTA in approximately 350 mL distilled water

1.2 Dissolve 29.036 g $FeCl_3.6H_2O$ in approximately 350 mL distilled water

1.3 Mix solutions 1.1 and 1.2 and adjust to pH 5.0– 5.5

1.4 Bring the volume up to approximately 950 mL with distilled water

1.5 Aerate vigorously for about 12 hours

1.6 Bring the volume up to 1000 mL

1.7 Solution 1.6 contains 6 mg/mL of Fe (107.4 mM)

1.8 For a solution containing 40 mM Fe, dilute to a final volume of 2686 mL

Method 3:

Reference: Clark (1982)

1.1 Dissolve 8.68 g HEDTA [N-2-(hydroxyethyl)-ethylenediaminetriacetic acid] in 200 mL distilled water

1.2 Add 80 mL 1N NaOH to solution 1.1

1.3 Dissolve 13.31 g $Fe(NO_3)_3.9H_2O$ in solution 1.2

1.4 Adjust pH to 4.0 using small additions of 1N NaOH (approx. 50 mL); *add NaOH slowly to prevent precipitation of iron*

1.5 Bring solution 1.4 to final volume of 1000 mL with distilled water

Appendix 8

Tabulating and Analysing the Data from Preliminary Trials

The data can be analysed in more than one way, but the following method, which uses Student's *t* test, allows us to compare the mean yield in each of the highest-yielding treatments with the mean yields in the other treatments. The analysis requires only a scientific calculator. The accompanying worked example comes from an experiment conducted in Vietnam.

With your scientific calculator in the statistical mode, first enter the four values for the no fertiliser ('best guess × 0') treatment, pressing the M+ (or DATA) key after each value. The display should show the number of values entered. Next, read off and record on your data sheet the values for the mean (x → M or $\bar{x}$ key), the standard deviation (MR or S key), and the variance, S^2 (x^2 key). Now repeat these operations in turn for each of the other treatments. Now calculate and record the relative means by multiplying each mean by 100, and dividing by the mean for the treatment with the highest mean yield.

Now calculate and record the difference between the mean of the highest-yielding treatment and each of the other treatments ($\bar{x}_{max.} - \bar{x}_i$).

Next, we need to calculate and record the pooled variances for each combination of the highest-yielding treatment and the other treatments. To do this, we weight the individual variances according to their degrees of freedom. In the case of a trial with four replications, each mean has (4 – 1) = 3 degrees of freedom.

$$\text{Pooled } S^2 = \frac{3\times S^2_{max.} + 3\times S^2_i}{6}$$

Using our values of pooled S^2, we now calculate and record the standard deviation of the difference between the mean yield of the highest-yielding treatment and each of the other treatments, i.e. $S(\bar{x}_{max.} - \bar{x}_i)$.

$$\begin{aligned} S(\bar{x}_{max} - \bar{x}_i) &= \sqrt{Pooled\ S^2}.\sqrt{\frac{4+4}{4\times 4}} \\ &= Pooled\ S.\sqrt{0.5} \\ &= Pooled\ S \times 0.707 \end{aligned}$$

Next, we calculate the *least significant difference* (LSD) for each value of $\bar{x}_{max.} - \bar{x}_i$.

The LSD for the chosen level of probability
$= S(\bar{x}_{max.} - \bar{x}_i) \times t$

In our case, t has 3 + 3 = 6 degrees of freedom. Thus from the table of distribution of t, we have: t = 2.447 at P = 0.05.

First, we calculate the LSD for P = 0.05 for each of the treatments giving less than the highest yield. If the difference between the mean for the highest-yielding treatment and that of another treatment is greater than the LSD, then we conclude that the yield in the latter was significantly less than that in the highest-yielding treatment. The symbol '*' is often used to denote 'significant at P = 0.05'.

Note on missing values

Suppose a pot in the 'i' treatment was destroyed by rats so that for this treatment we had only 3 replications. Then

$$Pooled\ S^2 = \frac{3\times S^2_{max} + 2\times S^2_i}{5}$$

Similarly

$$S(\bar{x}_{max} - \bar{x}_i) = \sqrt{Pooled\ S^2}.\sqrt{\frac{4+3}{4\times 3}}$$

Again the LSD applying to treatment 'i' will have 3 + 2 = 5 degrees of freedom compared with the other undamaged treatments.

Table A8.1 Statistical analysis of a preliminary trial, conducted as part of a training course at the National Institute of Soils and Fertilisers, Hanoi, Vietnam, using Student's *t* Test. The replicate results are dry weights of tops in g/pot.

Soil: Lowland soil from Thanh Xuan Commune, Soc Son Distict, Vietnam
Test plant: Maize
Planted: 7/09/2001 Harvested: 21/09/2001

Treatment	No Fertiliser	Best guess × 0.5	Best guess	Best guess × 2	Best guess × 3	Best guess × 4
Rep A	0.34	0.76	0.86	0.78	0.49	0.38
Rep B	0.39	0.56	0.78	0.82	0.48	0.41
Rep C	0.35	0.67	0.74	0.68	0.71	0.19
Rep D	0.42	0.65	0.51	0.67	0.56	0.28
$\bar{x}$	**0.375**	**0.660**	**0.723**	**0.738**	**0.560**	**0.315**
Relative $\bar{x}$ (%)	50.8	89.4	98.0	100	75.9	42.7
S	0.0370	0.0821	0.1502	0.0741	0.1061	0.1002
S^2	0.00137	0.00673	0.02256	0.00549	0.01127	0.01003
$\bar{x}_{max} - \bar{x}_i$	**0.363**	**0.078**	**0.006**	**0**	**0.178**	**0.423**
$S(\bar{x}_{max} - \bar{x}_i)$	0.04141	0.0553	0.0837	–	0.0648	0.0622
$LSD_{(P=0.05)}$	**0.101**	**0.135**	**0.205**	**–**	**0.159**	**0.152**
Significance	*	ns	ns	–	*	*

Interpretation: Differences were not significant between the best guess × 0.5, best guess, and best guess × 2 treatments, after 2 weeks' growth. However, these differences could be expected to widen with a further week's growth and its corresponding demand on the nutrient supply from each pot. On the other hand, plant dry weights fell off substantially when the nutrient supply was increased beyond best guess × 2. Hence, it is suggested that an 'all' treatment lying between best guess and best guess × 2, would be the most suitable to use in the subsequent omission trial.

Table A8.2 The distribution of *t* (two-tailed tests).

Degrees of freedom	Probability of a larger value (sign ignored)		
	0.05	0.01	0.001
1	12.706	63.657	-
2	4.303	9.925	31.598
3	3.182	5.841	12.941
4	2.776	4.604	8.610
5	2.571	4.032	6.859
6	2.447	3.707	5.959
7	2.365	3.499	5.405
8	2.306	3.355	5.041
9	2.262	3.250	4.781
10	2.228	3.169	4.587
11	2.201	3.106	4.437
12	2.179	3.055	4.318
13	2.160	3.012	4.221
14	2.145	2.977	4.140
15	2.131	2.947	4.073
16	2.120	2.921	4.015
17	2.110	2.898	3.965
18	2.101	2.878	3.922
19	2.093	2.861	3.883
20	2.086	2.845	3.850

Appendix 9

Tabulating and Analysing the Data from Nutrient Omission Trials

The data can be analysed in more than one way, but the following method, which uses Student's *t* test, allows us to compare the mean yield in each of the omission treatments with the mean yield in the 'all' treatment. The analysis requires only a scientific calculator. The accompanying worked example comes from an experiment conducted as part of a training course in Vanuatu.

With your scientific calculator in the statistical mode, first enter the 8 values for the 'all' treatment, pressing the M+ (or DATA) key after each value. The display should show the number of values entered. Next, read off and record on your data sheet the values for the mean (x ➔ M or $\bar{x}$ key), the standard deviation (MR or S key), and the variance, S^2 (x^2 key). Now repeat these operations in turn for each of the omission treatments, starting with the –N treatment. You may now wish to calculate the relative means by multiplying each mean by 100, and dividing by the mean for the 'all' treatment.

Now calculate and record the difference between the mean of the 'all' treatment and the mean of each omission treatment ($\bar{x}_{all} - \bar{x}_i$), starting at the –N treatment. Note that where an omission treatment gave a slightly higher yield than the 'all' treatment

(e.g. the –Mg treatment in the example from Vanuatu), the difference is really a negative value, but for present purposes we shall ignore the sign and record them also as positive values.

Next, we need to calculate and record the pooled variances for each combination of the 'all' and the various omission treatments. To do this, we weight the individual variances according to their degrees of freedom. In a trial with 8 replicates of the 'all' treatment and 4 replicates of each omission treatment we will have:

$$Pooled\ S^2 = \frac{7 \times S_{all}^2 + 3 \times S_i^2}{10}$$

(If there are any missing values, the degrees of freedom must be reduced accordingly (see Appendix 8).)

Using our values of pooled S^2, we now calculate and record the standard deviation of the difference between the mean yield of the 'all' treatment and each omission treatment, i.e. $S(\bar{x}_{all.} - \bar{x}_i)$.

$$S(\bar{x}_{all} - \bar{x}_i) = \sqrt{Pooled\ S^2} . \sqrt{\frac{8+4}{8 \times 4}}$$

$$= Pooled\ S. \sqrt{\frac{12}{32}}$$

$$= Pooled\ S \times 0.612$$

Next, we calculate the *least significant difference* (LSD) for each value of $\bar{x}_{all} - \bar{x}_i$.

The LSD for chosen level of probability
$= S\,(\bar{x}_{all.} - \bar{x}_i) \times t$

In our case, t has 7 + 3 = 10 degrees of freedom. Thus, from the table of distribution of t (Table A8.2) we have:

t = 2.228 at P = 0.05; t = 3.169 at P = 0.01; and t = 4.587 at P = 0.001

In this example, we first calculate the LSD for P = 0.05 for all the omission treatments. If the difference between the mean for the 'all' treatment and that of an omission treatment is greater than the LSD, then we conclude that the yield in the omission treatment was significantly different from that in the 'all' treatment. In the example given, this was true of the –N, –P and –S treatments, but not of any of the other treatments. Hence, we interpret the results as meaning that the soil contained insufficient amounts of plant-available N, P and S for the healthy growth of the test plant. Where a difference is significant at P = 0.05, we may wish to calculate LSD values for higher levels of probability and test them also. Again, in the case of this example from Vanuatu, the very large difference in yield between the 'all' treatment and the –P treatment (4.85 g/pot) was significant at P = 0.001, and the smaller difference with the –N treatment (1.38 g/pot) was significant at P = 0.01. However, the difference in the case of the –S treatment (0.99 g/pot) was significant only at P = 0.05.

Table A9.1 Statistical analysis of nutrient omission trial using Student's *t* test

Soil: Root crops research area, Valeteruru, Espiritu Santo, Vanuatu
Test plant: Hybrid sweet corn cv. 'Punchline'
Planted: 22/06/2000; Harvested: 15/07/2000

Dry weights of tops in g/pot

Treatment	All A	All B	–N	–P	–K	–Ca	–Mg	–S	–Fe	–B	–Mn	–Zn	–Cu	–Mo	–Ni
Replicate 1	6.05	6.22	4.85	1.56	6.16	5.62	7,68	4.80	7.58	6.41	6.43	6.05	7.45	6.55	6.60
Replicate 2	6.29	5.05	4.60	1.53	6.15	3.80	7.83	5.46	5.40	6.31	6.98	6.02	6.00	6.11	5.84
Replicate 3	7.21	6.78	5.17	1.48	7.42	7.50	8.47	6.14	5.60	7.21	7.12	6.60	6.72	7.11	8.59
Replicate 4	6.77	6.58	5.33	1.50	7.80	5.43	5.88	5.13	7.05	5.60	6.20	6.20	7.09	6.70	8.09
$\bar{x}$	**6.37**		**4.99**	**1.52**	**6.88**	**5.59**	**7.47**	**5.38**	**6.41**	**6.38**	**6.68**	**6.22**	**6.82**	**6.62**	**7.28**
Relative $\bar{x}$ (%)	100		78.3	23.9	108	87.8	117	84.4	101	100	105	97.6	107	104	114
S	0.650		0.326	0.035	0.854	1.1514	1.111	0.572	1.073	0.659	0.438	0.2669	0.620	0.409	1.279
S^2	0.422		0.107	0.0012	0.073	0.2293	1.234	0.328	1.152	0.434	0.192	0.071	0.3844	0.1677	1.636
$\bar{x}_{all.} - \bar{x}_i$	–		**1.38**	**4.85**	**0.51**	**0.78**	**1.10**	**0.99**	**0.04**	**0.01**	**0.31**	**0.15**	**0.45**	**0.25**	**0.91**
Sp^2	–		0.328	0.296	0.514	0.964	0.666	0.394	0.641	0.426	0.353	0.317	0.411	0.346	0.786
$S(\bar{x}_{all.} - \bar{x}_i)$	–		0.350	0.333	0.439	0.601	0.507	0.384	0.487	0.399	0.369	0.345	0.392	o.360	0.543
LSD	t = 0.05		0.78	0.74	**0.98**	**1.33**	**1.13**	**0.86**	**1.06**	**0.89**	**0.82**	**0.77**	**0.87**	**0.80**	**1.21**
	t = 0.01		**1.11**	1.05				1.22							
	t =0.001		1.60	**1.53**											
LSD	P = 0.05		*	*	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	P = 0.01		**	**				n.s.							
	P = 0.001		n.s.	***											

n.s. = not significant

Appendix 10

Tabulating and Analysing the Data from Rate Trials in Pots

The data can be analysed in more than one way, but the following method, which uses Student's *t* test, allows us to compare the mean yield in the highest-yielding treatment with the mean yields in the other treatments. The analysis requires only a scientific calculator. The accompanying worked example comes from an experiment conducted in Vanuatu.

With your scientific calculator in the statistical mode, first enter the four values for the no nitrogen ('N_0') treatment, pressing the M+ (or DATA) key after each value. The display should show the number of values entered. Next, read off and record on your data sheet the values for the mean (x → M or $\bar{x}$ key), the standard deviation (MR or S key), and the variance, S^2 (x^2 key). Now repeat these operations in turn for each of the other treatments. Now calculate and record the relative means by multiplying each mean by 100, and dividing by the mean for the treatment with the highest mean yield.

Now calculate and record the difference between the mean of the highest-yielding treatment and each of the other treatments ($\bar{x}_{max.} - \bar{x}_i$).

Next, we need to calculate and record the pooled variances for each combination of the highest-yielding

treatment and the other treatments. Assuming that we have four replications we will have:

$$Pooled\ S^2 = \frac{3 \times S^2_{max} + 3 \times S^2_i}{6}$$

Using our values of pooled S^2, we now calculate and record the standard deviation of the difference between the mean yield of the highest-yielding treatment and each of the other treatments, i.e. $S\,(\bar{x}_{max.} - \bar{x}_i)$.

$$\begin{aligned} S(\bar{x}_{max} - \bar{x}_i) &= \sqrt{Pooled\ S^2} . \sqrt{\frac{4+4}{4 \times 4}} \\ &= Pooled\ S. \sqrt{\frac{8}{16}} \\ &= Pooled\ S \times 0.707 \end{aligned}$$

Next, we calculate the *least significant difference* (LSD) for each value of $\bar{x}_{max.} - \bar{x}_i$

The LSD for chosen level of probability
$= S\,(\bar{x}_{max.} - \bar{x}_i) \times t$

In our case, t has 3 + 3 = 6 degrees of freedom. Thus, from the table of distribution of t, we have: t = 2.447 at P = 0.05 (Table A8.2).

In this example, we first calculate the LSD for P = 0.05 for each of the treatments giving less than the highest yield. If the difference between the mean for the highest-yielding treatment and that of another treatment is greater than the LSD, then we conclude that the yield in the latter was significantly less than that in the highest-yielding treatment.

In this example, the highest-yielding treatment was N_{200}. The yield in this treatment was significantly greater at P = 0.05 than that at N_{100} or any lower N treatment, but not significantly greater than that at $N_{400.}$

Table A10.1 Statistical analysis of rate trial using Student's *t* test

Soil: Mele village area, Efate, Vanuatu
N Source: Urea
Test plant: Hybrid sweet corn cv. Punchline
Planted: 15/06/2000; Harvested: 20/07/2000

Dry weights of tops in g/pot

Treatment	N_0	N_{25}	N_{50}	N_{100}	N_{200}	N_{400}
Replicate 1	4.98	5.70	6.33	9.37	10.18	7.40
Replicate 2	4.67	6.25	6.18	6.53	9.60	7.87
Replicate3	4.48	6.38	6.46	6.65	9.36	8.70
Replicate 4	4.39	5.55	5.44	8.14	9.14	10.25
$\bar{x}$	**4.63**	**5.97**	**6.10**	**7.67**	**9.57**	**8.56**
Relative $\bar{x}$ (%)	48.4	62.4	63.7	80.1	100	89.4
S	0.2609	0.4065	0.4562	1.3479	0.4480	1.2513
S^2	0.0681	0.1653	0.2082	1.8170	0.2007	1.5658
$\bar{x}_{max.} - \bar{x}_i$	**4.94**	**3.60**	**3.47**	**1.90**	**–**	**1.01**
Sp^2	0.1344	0.1830	0.2045	1.0089	–	0.8833
$S(\bar{x}_{max.} - \bar{x}_i)$	0.2591	0.3024	0.3197	0.7101	–	0.6644
$LSD_{(0.05)}$	**0.63**	**0.74**	**0.78**	**1.74**		**1.62**
Significance	*	*	*	*		n.s.

n.s. = not significant