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PROJECT REPORT No. 224

DEVELOPMENT OF ON-FARM PLANT TESTS FOR PHOSPHATE AND POTASSIUM IN WHEAT

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by

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CONTENTS

1 SUMMARY

In an earlier HGCA project (1992-95), critical concentrations of phosphorus (P), inorganic phosphate (Pi) and potassium (K) in soils and plants for maximum grain yield of winter wheat were determined. Plant tests recommended for field use were leaf(1) %P, leaf(1) %K, leaf(1) Kw, and whole shoot %Pi and Piw (Leaf(1) is the newest fully expanded leaf blade, Pi is inorganic orthophosphate, Piw and Kw are Pi and K concentrations in tissue water, respectively). The main aims of the present project (1996-98) were to develop on-farm testing procedures for Piw and Kw in wheat, and to investigate the effects of N and water supply on plant P, Pi and K concentrations.

Field experiments on the responses of winter wheat to soil P and K were conducted at Rothamsted (Hertfordshire), Gleadthorpe (Nottinghamshire), Ropsley (Lincolnshire) and Sedge Fen (Suffolk). At Rothamsted, crops were grown on soils having plant-available P and K levels ranging from very deficient to abundant. At Ropsley, soil P levels ranged from deficient to sufficient. Sedge Fen, ostensibly a K-deficient site, was used to study responses to K fertiliser. The effects of N were studied at Rothamsted, Gleadthorpe and Ropsley. The effects of water supply were studied in irrigation experiments on the light sandy soil at Gleadthorpe. On-farm procedures were developed in the laboratory at Rothamsted and tested under field conditions at Rothamsted and ADAS.

Critical topsoil P and K for 95% maximum grain yield changed little between years, but varied with soil type. Critical soil P averaged 8 mg/kg and 17 mg/l at Rothamsted and Ropsley, respectively. Grain yield responses were 2.1 and 1.6 t/ha relative to Index 0 and 1 soils at Rothamsted and Ropsley, respectively. Critical topsoil K at Rothamsted averaged 88 mg/kg with a yield response of 4.2 t/ha relative to the Index 0 soil. Topsoil K was not a reliable guide to grain yield response at Sedge Fen.

Critical plant concentrations for 95% maximum grain yield generally agreed well with those determined in 1992-95. Over all sites and seasons (1992-98), during stem elongation (GS 31-39), critical leaf(1) %P was in the range 0.23-0.38%.

Similarly, critical whole shoot %Pi was in the range 0.028-0.071% (equivalent Piw 2.7-6.7 mM). Critical leaf(1) %K was in the range 1.61-3.21% (equivalent Kw 126-227 mM).

Applying N to crops already sufficient in N had no effect on plant P, Pi and K concentrations. Differences in N supply in the normal range of fertiliser application rates, 100-250 kg/ha, are unlikely therefore to have any effects on plant P, Pi and K concentrations. In N-deficient crops, both whole shoot and leaf(1) %P and %K were lower, Kw was the same, and Pw, %Pi and Piw were higher than in N-sufficient crops. It is therefore likely that where N is limiting, critical %P and %K for maximum yield will be lower than where N is non-limiting, critical %Pi, Piw, and Kw will be unaffected, and critical Pw will be higher.

Drought reduced dry matter concentrations of %P, %Pi and %K in whole shoots and leaf(1), and critical concentrations of these parameters for maximum yield also appeared to be reduced. In the case of tissue water concentrations (Pw, Piw, Kw), the effects of drought were not consistent, with responses depending on the particular concentration parameter and the plant organ. Critical values in two recommended plant tests, namely leaf(1) Kw and whole shoot Piw, appeared to be largely unaffected by drought.

Critical tissue water concentrations reported in the earlier project were determined by standard laboratory methods not by on-farm methods. The former involved extraction of nutrients from dried, milled plant material and analysis of the extracts by standard laboratory procedures. It was important therefore to establish that any on-farm methods developed in the current project gave the same results as the laboratory methods, if previously determined critical values were to be used as benchmarks for on-farm testing. The on-farm tests involved extraction and dilution of tissue water, addition of reagents and analysis with portable analytical equipment. We assessed two portable analytical instruments, the RQflex meter for Pi and K which uses test strip technology, and the Cardy-K meter which is a miniature ion-specific electrode.

The RQflex meter generally performed well for Pi. The meter gave a linear response with standards up to 60 mg PO4/l (0.63 mM Pi). Tissue water typically needed to be diluted 30 times to bring Pi concentrations into this range. There was good agreement between the RQflex and standard laboratory instruments for analysing Pi in plant extracts.

The Cardy-K meter gave a linear response with standards up to 3,900 mg K/l (100 mM K). Tissue water typically needed to be diluted 3 times to bring K concentrations into this range. The Cardy meter underestimated the standard laboratory instrument (Flame Photometer) on plant extracts, possibly due to interference by other ions.

Extracting a representative sample of tissue water from fresh plant material was not easy, especially from leaves. It was necessary to rupture the plant cells first by freezing and thawing them. Tissue water was then readily extracted by squeezing fully thawed material in a plastic syringe.

For Pi determination, it was essential to analyse the tissue water within 30 minutes of the start of thawing to minimise the conversion of organic to inorganic phosphate. If this was done there was good agreement between the on-farm and laboratory methods for shoot Piw (see Appendix VIII for protocol).

In the case of K, tissue water concentrations obtained by the 'freeze/thaw' method were invariably less than those obtained by extracting oven-dried material with water, irrespective of the pressure used. This may be due to the tenacity with which K is held in plant cell walls. The Cardy meter also underestimated K concentrations compared with the Flame Photometer for all types of extract. More development work is required before an on-farm method can be recommended for K.

2 TERMINOLOGY

P	- Phosphorus
Pi	- P as inorganic orthophosphate ($H_2PO_4^-$)
P_2O_5	- fertiliser "phosphate" (P = P_2O_5 x 0.436)
Olsen-P	- plant-available soil P (mg/kg or mg/l)
%P	- total P content as % of plant dry matter
Pw	- total P concentration in plant tissue water (millimolar, mM)
%Pi	- P content, as Pi, as % of plant dry matter
Piw	- Pi concentration in plant tissue water (mM)
K	- Potassium
K_2O	- fertiliser "potash" (K = K_2O x 0.830)
Kex	- soil exchangeable K (mg/kg or mg/l)
%K	- total K content as % of plant dry matter
Kw	- total K concentration in plant tissue water (mM)
DM	- dry matter
FM	- fresh matter
TW	- tissue water
Sap	- water in vascular tissue (xylem and phloem)
Leaf(1)	- newest fully expanded leaf blade (collar visible)
Leaf(2)	- second newest leaf blade
Shoot	- stem plus leaves
Mainshoot	- first or primary shoot
Tiller	- side shoot
Whole shoots	- mainshoot plus tillers
GS	- growth stage
DFS	- days from sowing
JD	- Julian days (days from 1 Jan)

3 INTRODUCTION

In a previous project funded by HGCA, 'Plant Testing to Determine the P and K status of Wheat' (Barraclough *et al.*, 1997), critical concentrations of P, Pi and K in plants (and soils) for maximum grain yield were determined in a series of field experiments by Rothamsted and ADAS in 1992-95. Twelve plant diagnostic procedures were compared, four each for total-P and -K, and four for inorganic-P (Pi - inorganic orthophosphate), involving comparisons between whole shoots and newest leaves, and between conventional 'dry' tests (based on nutrient content in plant dry matter) and 'wet' tests (based on nutrient concentration in tissue water). The rationale for leaf testing and wet testing is that these approaches should reduce the effects of plant ageing, a common problem of shoot dry matter testing. Wet testing also offers the prospect of *in situ* procedures. The advantage of using Pi as opposed to total-P is that Pi is the major storage pool for P in plants, and its presence should signify that plant P requirements are satisfied irrespective of the growing conditions. The benefits of these approaches to plant testing were demonstrated in the earlier project.

Four plant tests were recommended, two dry tests based on total nutrient content and requiring laboratory facilities - leaf %P and leaf %K, and two wet tests - shoot Pi and leaf K. The wet tests were done in the laboratory but also had *in situ* potential. Critical values for the recommended tests were summarised in HGCA topic sheet No. 19 (1998).

Plant tests that can be carried out *in situ* in the field or at least 'on-farm' are potentially more attractive to farmers than laboratory-based tests as they are cheap and quick to carry out. A major aim of the present project therefore was to develop plant testing procedures suitable for on-farm use. The critical wet concentrations reported in the previous project, which would form the basis of on-farm tests, were not determined directly, i.e. by squeezing water out of fresh plant material, but indirectly via the measurement of tissue water (fresh weight minus dry weight) and extraction of Pi and K from dried material with water. Nutrients in the extracts were analysed by standard laboratory methods. If the

previously determined critical wet concentrations were to form the basis of on-farm testing procedures, it was necessary to show that on-farm methods, which involved the direct extraction and analysis of tissue water, gave the same results as the indirect laboratory methods.

It is important to note the physiological distinction between 'tissue water' and 'sap'. Sap is the solution in vascular tissues (xylem and phloem), although the term is often used generically for any form of plant water. Tissue water, defined as the difference between fresh and dry weights of plant material, includes water in all types of tissue, both intra-cellular (cytosol, vacuole, cell wall) and extra-cellular.

The first problem in developing on-farm tests is how to extract a representative sample of tissue water from a wheat plant, specifically from leaves or whole shoots. Surprisingly little is known about this, although work was done in the 1980s on extracting sap from basal stems of wheat for nitrate testing (Papastylianou *et al.*, 1984; Darby *et al.*, 1986). The work was successful but attracted little commercial interest in the UK. Sap is readily extracted from the basal stems of wheat, but extracting a representative sample of tissue water from leaves or whole shoots is not so simple. Secondly, can tissue water be reliably analysed for Pi and K in the field? Several hand-held analytical instruments have recently appeared on the market which may be suitable. The RQflex meter uses 'test strip' technology for analysing nitrate, phosphate and potassium, whilst the Cardy K-meter utilises specific-ion electrode technology. The suitability of these instruments for analysing plant tissue water, which contains complex mixtures of nutrient ions, was unknown.

In the previous project, there were indications that N and water supply were affecting plant concentration parameters, but there was no direct evidence for this. Short-term changes in water supply, i.e. diurnal fluctuations, are likely to be important in wet testing, as concentrations can increase passively as tissues lose water and turgor on hot days. The major problems confounding the interpretation of plant tests are plant ageing and interactions with growing conditions, including the supply of water and nutrients. Nutrient interaction

effects are well documented for conventional plant tests, i.e. those based on the total nutrient in dry matter (Reuter and Robinson, 1997), but there is little or no information on how interactions affect wet concentrations or storage pools. Leigh and Johnston (1983) claimed that a major benefit of expressing K concentrations on a tissue water basis was the elimination of effects due to N and water supply.

The aims of the present project were (a) to develop methods for measuring Pi and K in wheat plants on-farm, (b) to investigate the effects of N supply and (c) the effects of water supply on nutrient concentrations in wheat plants. The approaches involved field experiments at Rothamsted farm and ADAS sites in 1996-98, complemented by laboratory experiments at Rothamsted. Four field sites were used at Rothamsted - Fosters (N effects), Sawyers I (P site), Exhaustion Land (P site, on-farm methods) and Sawyers III (K site, on-farm methods). Three ADAS sites were used in the study - Ropsley, Lincs. (P site, on-farm methods, N effects), Gleadthorpe, Notts. (N and water effects, on-farm methods) and Sedge Fen, Suffolk (K site). Laboratory work at Rothamsted included development of on-farm methodologies and solution culture studies on the effects of N supply on tissue concentrations. Values of critical P, Pi and K concentrations in plants and soils for maximum grain yield were obtained in all field experiments.

4 METHODS

4.1 Rothamsted laboratory experiments

Laboratory experiments were carried out on the effects of N supply on plant P, Pi and K, and the development of *in situ* testing procedures. The methods used in these experiments are described in the relevant sections.

4.2 Rothamsted field experiments

Rothamsted farm (Hertfordshire, Grid Ref. TL122142) is 128 m above sea level. Mean annual rainfall is 687 mm (see Appendix V for rainfall records). The soil is a free-draining silty clay loam over clay with flints (Batcombe Series). Experiments were carried out on four fields: Fosters (N), Exhaustion Land (P), Sawyers(I) (P) and Sawyers(III) (K).

Rothamsted fields

Fosters (N):	1997 (w. wheat - Hereward)
Sawyers (I) (P):	1996 (w. wheat - Mercia)
	1997 (w. wheat - Mercia)
	1998 (w. wheat - Hereward)
Exhaustion Land (P):	1996 (w. wheat - Hereward)
	1998 (w. wheat - Mercia)
Sawyers (III) (K):	1996 (w. wheat - Mercia)
	1997 (w. wheat - Mercia)
	1998 (w. wheat - Mercia)

N.B. Years correspond to the year of harvest.

Experimental designs and treatments

Fosters (N)

Three blocks of 7 plots (3 m x 20 m). Seven rates of N (0, 50, 100, 150, 200, 250, 300 kg N/ha). Abundant soil P and K.

Exhaustion Land (P)

Twenty plots (6 m x 25.6 m) arranged in five strips of four plots. Plots had a range of unreplicated Olsen-P levels which increased systematically along each strip.

Sawyers(I) (P)

Six blocks of 12 plots (4.3 m x 15 m). Winter wheat was grown on two blocks. The 24 plots had a range of unreplicated Olsen-P levels randomly arranged in the blocks (20 of the plots were sampled).

Sawyers (III) (K)

Two blocks of 20 plots (9 m x 24 m) having a range of unreplicated soil K levels randomly arranged in each block. Winter wheat alternated with winter oats.

Agronomic details

Details of previous crop, variety, sowing date, harvest date, and rate and timing of NPK fertilisers are shown in Appendix I. All crops were precision drilled with 380 seeds/m^2 at 12 cm row spacing, given growth regulator and treated as necessary against weeds, pests and diseases. Straw was chopped and incorporated.

Soil sampling

Soil sampling was undertaken in February. Cores were taken to a depth of 23 cm using a 2 cm diameter auger. Twelve to sixteen cores were taken from each plot depending on the plot size. Cores were taken from two lengthwise transects of each plot and bulked into a composite sample. Field-moist soil was air dried and milled to pass a 2 mm mesh. A subsample of dried soil was analysed for plant-available P and exchangeable-K .

Plant sampling

At Rothamsted, a 3 m wide strip down the centre of each plot was reserved for combine harvesting. Plant samples (whole shoots) were taken at random from either side of this strip leaving a guard of at least 0.5 m between samples and at the edge of each plot. Each sample consisted of 4-8 x 0.5 m length of row (0.24-

0.48m^2). Sampling dates are in Appendix II. The dates do not correspond to the dates that reported growth stages (Zadoks *et al.*, 1974) were attained.

Sampling was carried out between 9 and 11 am to minimise diurnal effects and as quickly as possible to minimize the loss of tissue water. Sampling was not done on rainy days. Shoots were cut at ground level excluding dead leaves. Samples were kept in sealed plastic bags in a cool box. Back in the laboratory, samples were stored in a cold room at 5°C until they could be processed, usually within two hours of cutting. It was shown in a separate study that fresh samples could be stored at 5 °C in sealed plastic bags for up to 4 hours without affecting Piw concentrations.

For each sample, total fresh weight was measured, and subsamples were dried overnight at 80 °C in a conventional oven for total-P and -K analysis, or in a microwave oven for Pi analysis (section 4.2.7). Shoot subsamples were separated into individual leaves and stems as required. Fresh and dry weights of all samples and subsamples were recorded. Dried samples were milled to pass a 1 mm mesh (Glen Creston or 8" Christy Norris mill).

Combine grain yields were taken from the 3 m central strip on each plot. The width of cut from the combine was 2.3 m. Plot length harvested was in the range 12-20 m giving a harvest area of 27-46 m^2. The fresh weight of grain was measured, a subsample was weighed and dried in a conventional oven at 105 °C. Yields were corrected to 85% DM and converted to t/ha.

Extraction and analysis of nutrients

Available soil P

The method used for determining plant-available soil P was essentially that of Olsen *et al.* (1954). 5 g of air-dry soil was shaken with 100 ml of 0.5 M sodium bicarbonate at pH 8.5 for 30 minutes at 20 °C on a reciprocating shaker. The solution was filtered (Whatman No.42) and the first 5 ml discarded. Extracts were analysed immediately for Pi using the molybdate-blue colorimetric method (Murphy and Riley, 1962). Available soil P was expressed as mg P/kg dry soil.

Exchangeable soil K

Exchangeable soil K (Kex) was determined by leaching 5 g of air-dried soil with 100 ml of 1 M ammonium acetate for at least 4 hours. Extracts were analysed for K using an inductively coupled plasma emission spectrometer (ICP) or a flame photometer. Exchangeable K was expressed as mg K/kg dry soil.

Drying of plant material for analysis

Fresh plant material is normally oven-dried at 80 °C prior to extraction and analysis, and this method was used for %P and %K analysis. However, for Pi analysis fresh plant material was dried rapidly in a microwave oven to minimise the transformation of organic-P to Pi. In this method, fresh plant material (30-40 g) was cut into small pieces (5-10 mm) and dried in a domestic microwave oven on full power (750W). The samples were removed from the microwave when they were crisp and brittle to touch. To ensure the material was completely dry, the samples were dried in a conventional oven at 80 °C for a further 4 hours. Drying times in the microwave oven varied with sample size and the plant tissue, but were usually in the range 2-5 minutes. Stems took longer to dry than leaves. To prevent charring of the samples and overheating of the microwave oven, a beaker of water was kept in the oven during drying.

Extraction and analysis of plant Pi

0.25-0.50 g of microwave-dried plant material was shaken with 25 ml of 2% (v/v) acetic acid for 30 minutes (Luckham MultiMix Major, oscillating, rolling bed shaker (setting 5) or other reciprocating shaker). There was no increase in the amount of Pi extracted when the samples were shaken for up to 4 hours. The extract was filtered (Whatman No.6 or No.42) and the first 5 ml discarded. The filtrate could be stored overnight at 5 °C. Pi was determined colorimetrically using the molybdate-blue method (Murphy and Riley, 1962).

Extraction and analysis of plant total-P

0.25-0.50 g of oven-dried, milled plant material was digested in a concentrated nitric/perchloric acid mixture and the residue dissolved in 10% (v/v) hydrochloric acid. Total-P was determined by ICP.

Extraction and analysis of plant K

0.25-0.50 g of oven dried, milled plant material was shaken with 25 ml of distilled water for 30 minutes (Luckham MultiMix Major, oscillating, rolling bed shaker (setting 5) or other reciprocating shaker). The extract was filtered (Whatman No.6 or No.42) discarding the first 5 ml. A 5 ml aliquot was mixed with an equal volume of 10% (v/v) hydrochloric acid and analysed for K by ICP or flame photometer. This water extraction method extracts at least 95% of the total-K extracted by strong acid digestion.

Analysis of plant N

Total N in dried, milled plant material was analysed by the Dumas' combustion method in a Leco N Analyser.

Statistical analysis

Maximum and minimum yields, critical soil values and critical plant concentrations were derived by curve fitting (Genstat 5, Release 3.1 and Fig.P, Version 6.0).

Curve Fitting: The Mitscherlich Function

A successful plant test depends on the determination of critical nutrient concentrations in plant tissue, i.e. concentrations below which growth or yield are limited. Conventionally, critical concentrations are determined for 5-10% reductions in growth or yield (Smith, 1986). In the present study, critical soil and plant values were estimated for 95% of the maximum yield by fitting mathematical functions to the observed relationships. Several functions are available, but a common one used in crop research is the exponential decay function developed by Mitscherlich based on the law of diminishing returns.

The equation is: $y = a*\exp(-bx) + c$ ..... (1)

Where y is yield, x is concentration and a, b and c are parameters. Maximum y is attained (asymptotically) at infinite x and equals c. In biological systems, a more realistic maximum y can be interpolated from the curve at maximum

observed *x*. This equation has been used on all Rothamsted data unless otherwise stated.

Mathematical derivation of maximum yield and critical soil concentration

Maximum grain yield in each experiment was derived from a plot of 'yield' vs. 'soil', where 'soil' represents the concentration of plant-available nutrient in spring or autumn. For example, in the K experiment at Rothamsted in 1997, maximum soil K was 334 mg/kg giving a maximum grain yield of 8.37 t/ha, the same as 'c' in this case as the asymptote was very flat (Fig. 4.1a). 95% maximum yield was therefore 7.95 t/ha, which corresponds to a critical soil K value of 81 mg/kg.

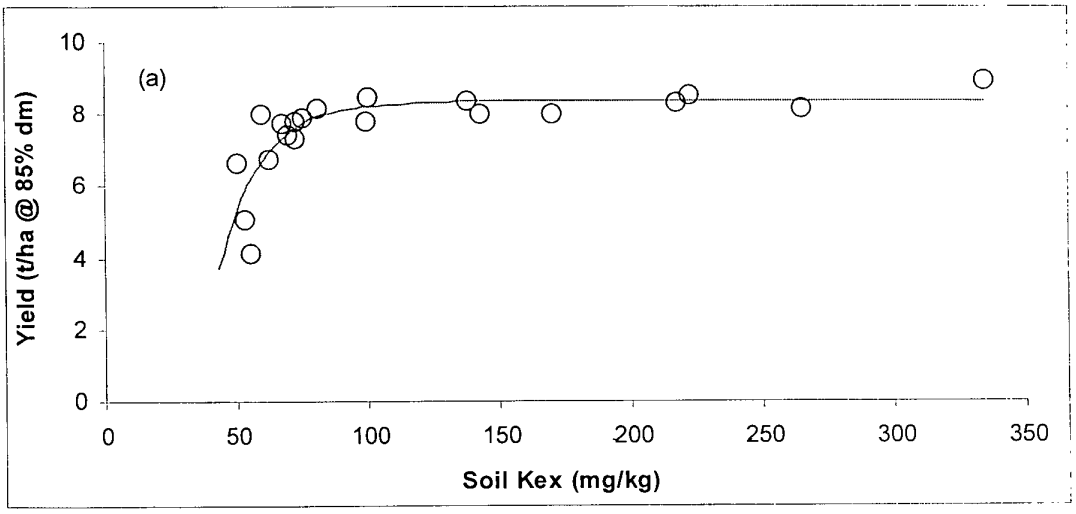
Mathematical derivation of critical plant concentration (yield vs plant route)

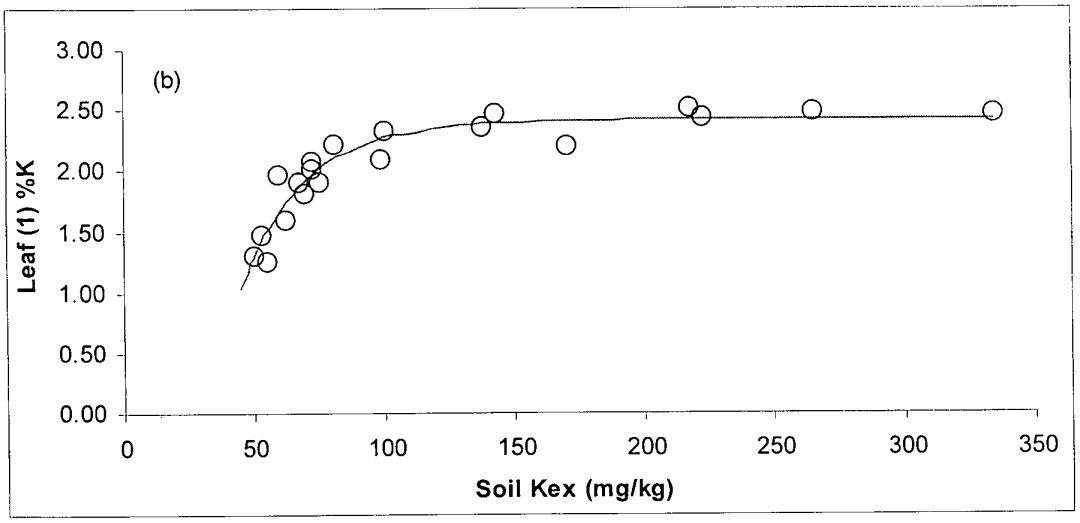
Critical plant concentrations for 95% maximum yield can be derived from plots of 'yield' vs. 'plant' where 'plant' represents the concentration in an appropriate plant part. For example, in the K experiment at Rothamsted in 1997, yield is shown plotted against leaf(1) %K at GS 39 in Fig. 4.1c. There was no yield plateau within the observed range of leaf K implying that yield was limited by K, but examination of Fig. 4.1a,b show this was not the case. Critical leaf K was derived from this relationship as follows: maximum yield (8.44 t/ha) was interpolated from the curve at maximum observed leaf K (2.51%). Critical leaf K for 95% of maximum yield (8.02 t/ha) was then 2.15%.

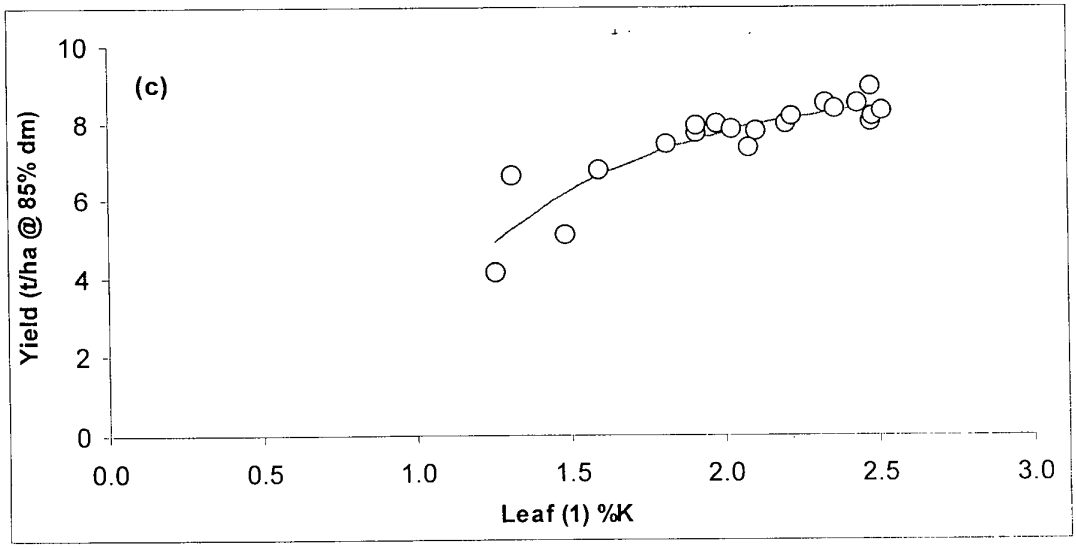
Mathematical derivation of critical plant concentration (plant vs soil route)

Critical plant concentrations can be derived indirectly from 'plant' vs 'soil' relationships. For example, in the K experiment at Rothamsted in 1997, leaf(1) %K is shown plotted against soil K in Fig. 4.1b. Critical leaf K was interpolated from this plot at the critical soil K value of 81 mg/kg derived from the 'yield' vs 'soil' plot described above. The critical value of 2.11% was in good agreement with 2.15% derived via the 'yield' vs 'plant' route.

Figure 4.1 Derivation of critical soil and plant K







4.3 ADAS field experiments

ADAS sites

Ropsley (Lincolnshire. Grid Ref. SK976359): Mean annual rainfall 625 mm (see Appendix VI for details). Sandy clay loam soil (27% clay) over clay (Beccles Association). Used for P, effects of N and *in situ* testing.

Gleadthorpe (Nottinghamshire. Grid Ref. SK589705): Mean annual rainfall 600 mm. Loamy sand soil (7% clay). Used for effects of N, water and *in situ* testing.

Sedge Fen (Suffolk. Grid Ref. TL667847): Silty clay loam soil (19% clay) over silt loam (Willingham Association). Used for K.

Experimental designs and treatments

Ropsley (P in 1996,97,98)

The relationship between plant tissue P concentrations and grain yields was investigated at Ropsley. Here, P fertiliser had been applied at rates of 0, 40, 70 & 100 kg/ha P_2O_5 annually from 1985-1997 to winter wheat grown on soils having different P levels as a result of previous nil, low and high P fertiliser regimes in 1977-1984 (Appendix III). Olsen extractable P concentrations in the topsoil ranged from 11-24 mg/l (ADAS Index 1-2) in autumn 1995. The 11 fertiliser treatments were arranged in a randomised block design, with 3 replicates of each treatment. Winter wheat (cv. Brigadier) was grown for harvests 1996, 97 & 98, with 40 kg/ha N applied in early March and 160 kg/ha N applied in late April. In 1997 and 1998, the early N application (40 kg/ha) was withdrawn from a 1 x 5 m area of treatments 1, 6, 9 & 11, in order to assess the effect of N fertiliser on plant concentrations. This was repeated with the main dressing (160 kg/ha) on a 'new' 1 x 5 m area, with the strip which received no early N discarded.

Sedge Fen (K in 1996)

The relationship between plant tissue K concentrations and grain yields was investigated at Sedge Fen. Six rates of potash fertiliser (0-210 kg/ha) were applied in February 1996 to winter wheat (cv. Soissons). There were 3 replicates of each treatment arranged in a randomised block design.

Gleadthorpe (N and water in 1997,98)

The effect of irrigation and N fertiliser additions on P and K concentrations in winter wheat were investigated at Gleadthorpe. In 1997, the experiments were conducted on winter wheat (cv. Brigadier) grown in Far Kingston field and in 1998 on winter wheat (cv. Riband) grown in Lamb field. Both crops followed sugar beet. Topsoil P status was 44 and 30 mg/l (ADAS Index 3) and K status was 72 and 70 mg/l (ADAS Index 1) in February 1997 and 1998, respectively. Potash was applied at rates of 75 and 95 kg/ha K_2O in March 1997 and February 1998, respectively.

The treatments were with or without irrigation, with irrigation applied to maintain a maximum soil moisture deficit of 50 mm. There were four replicates of each treatment arranged in a randomised block design. An early dressing of 40 kg/ha N was applied to the whole experimental area each year, except for a 1 x 6 m strip within each plot, which was used to assess the effect of N fertiliser on plant P and K concentrations. Similarly, the main N fertiliser dressing (140 kg/ha in 1997 & 160 kg/ha in 1998) was withheld from a 'new' 1 x 6 m strip within each plot, with the strip that received no early N discarded.

Soil sampling

Soil nutrient status was determined at each site every year by taking topsoil samples (0-15 cm) in September at Ropsley (1996-98) and February at Sedge Fen (1996) and Gleadthorpe (1997 & 98). At Ropsley, approximately 25 bulked cores were taken from each plot following ploughing, but before drilling, except in 1998, when they were sampled prior to ploughing. At Sedge Fen and Gleadthorpe, samples were taken from each plot, prior to the application of treatments, and bulked to give a single sample per block. Additional topsoil (0-30 cm) samples were taken at each plant sampling for the determination of gravimetric moisture content (3 cores per plot).

Plant sampling

At Ropsley, whole shoots were sampled 6 times between tillering and anthesis in 1996, and 3 times in 1997 and 1998, with leaf(1) and leaf(2) samples taken on some occasions (Appendix IV). In 1998, samples were taken from treatments

1, 3, 6, 7, 9 and 11 only. At GS 32 & 39, samples of whole shoots and leaf(1) were taken in the morning (9 am) and again in late afternoon (4 pm) from treatments 1, 6, 8 & 11 in 1996 and 1, 5 & 11 in 1997, to determine the effect of sample timing on plant nutrient concentrations. In 1997 and 1998, additional samples of whole shoots and leaf(1) were also taken at GS 23 and 39 from the areas which had not received N fertiliser, for comparison with those taken from the conventionally fertilised areas. Samples were taken approximately 2 weeks after N fertiliser had been applied.

At Sedge Fen, whole shoots were sampled 6 times between tillering and anthesis in 1996, with leaf(1) and leaf(2) samples taken 4 and 2 times respectively, during this period (Appendix IV). At GS 32 and 65, samples of whole shoots, leaf(1) and leaf(2) (GS 65 only) were taken in the morning and again in late afternoon, from plots which had received 0, 90 or 210 kg/ha potash.

Similarly at Gleadthorpe, whole shoots were sampled 6 times in 1997 between tillering and anthesis and 3 times in 1998, with leaf(1) samples taken on some occasions (Appendix IV). At GS 32 and 47 in 1997, samples of whole shoots and leaf(1) were taken in the morning (9 am) and again in afternoon (2 pm) to determine the effect of sample timing on plant nutrient concentrations. Whole shoot samples were also taken from the areas which had not received N fertiliser at GS 24 and 32 in 1997, and GS 30 and 39 in 1998 (approximately 2-3 weeks following fertiliser application).

At each site, whole plants were cut at ground level from a 0.50-0.75 m^2 sample area, with leaf(1) and (2) samples selected from 50 tillers and analysed separately. Fresh weights of all samples were recorded and the samples dried. As samples for inorganic P analysis (Pi) required rapid drying, all samples from Ropsley and Gleadthorpe were rapidly dried in a microwave oven for approximately 2 minutes (until brittle), followed by at least 4 hours drying in a conventional oven at 80 or 100 °C. All other samples were dried in a conventional oven (100 °C) overnight.

At each site, whole crop 'grab' samples were taken a few days prior to harvest and divided into ears and straw for determination of P and K, and crop P and K offtakes. Plots were then harvested by combine for determination of grain yields.

Nutrient analysis

Soils

Topsoil samples were analysed for extractable-P_{2}, -K and -Mg, pH, total-N and organic matter according to standard analytical techniques (MAFF, 1986).

Plants

Total plant phosphorus concentrations were determined by strong acid digestion on whole shoot and leaf samples from Ropsley and Gleadthorpe only. Phosphorus concentrations were determined by the molybdate-blue method and results expressed as mg P/kg dry matter.

Plant potassium concentrations were also determined by strong acid digestion, except at Sedge Fen, where they were determined by shaking 0.5 g of oven-dried plant material with 25 ml of deionised water for 30 minutes. This technique extracts at least 95% of the total K extracted by acid digestion. Potassium concentrations in the extracts were determined by ICP and expressed as %K in dm or K in the tissue water (Kw, millimolar).

Inorganic plant phosphorus concentrations (Pi) were determined by shaking 0.5 g of dried and milled plant material with 25 ml of 2% acetic acid for 30 minutes on a reciprocating shaker, followed by filtration through a Whatman No 40 filter paper. Pi concentrations in the filtered extract were then determined by the molybdate-blue method and expressed as %P, as Pi, in DM or on a tissue water basis (Piw, millimolar).

Evaluation of in situ plant testing methodologies

In 1998, subsamples of the whole shoot, leaf(1) and leaf(2) from each sampling occasion at Ropsley and Gleadthorpe were frozen. Initially they were chopped into 2 cm pieces and placed in a syringe prior to freezing, but after the first sampling, it proved easier to freeze whole plant samples and chop them into 1

cm pieces on defrosting and place them in syringes. The syringes were filled to 30 ml and the tissue water extracted by depressing the plunger. The tissue water was then diluted 50 fold for analysis of Pi and K by the RQflex and Cardy meters respectively, and by 5-10 fold for analysis of K by RQflex. Samples were analysed within 30-50 minutes of defrosting. A sample of the diluted tissue water was also sent for laboratory analysis of total-P and -K.

5 CRITICAL SOIL P AND K

5.1 Introduction

Critical values of plant-available soil P for near-maximum grain yield were determined on Sawyers(I) field at Rothamsted and at ADAS-Ropsley. At Rothamsted, the variety Mercia was grown in 1996,97 and Hereward in 1998. The variety Brigadier was grown at Ropsley. Critical soil K was determined on Sawyers(III) field at Rothamsted with variety Mercia.

5.2 Yields, responses and critical soil P and K

Yield ranges for the main P and K experiments at all sites and seasons are shown in Table 5.1. Values were interpolated from fitted curves at the maximum and minimum plot values of soil P and K in each experiment. Soil P at Rothamsted, measured in February, ranged from 4-34, 4-29 and 3-26 mg/kg in 1996,97,98, respectively. Equivalent ranges at Ropsley, measured after harvest, were 10-30, 7-25 and 10-29 mg/l, respectively. Soil K at Rothamsted, measured in February, ranged from 51-281, 50-334 and 50-371 mg/kg in 1996,97,98, respectively.

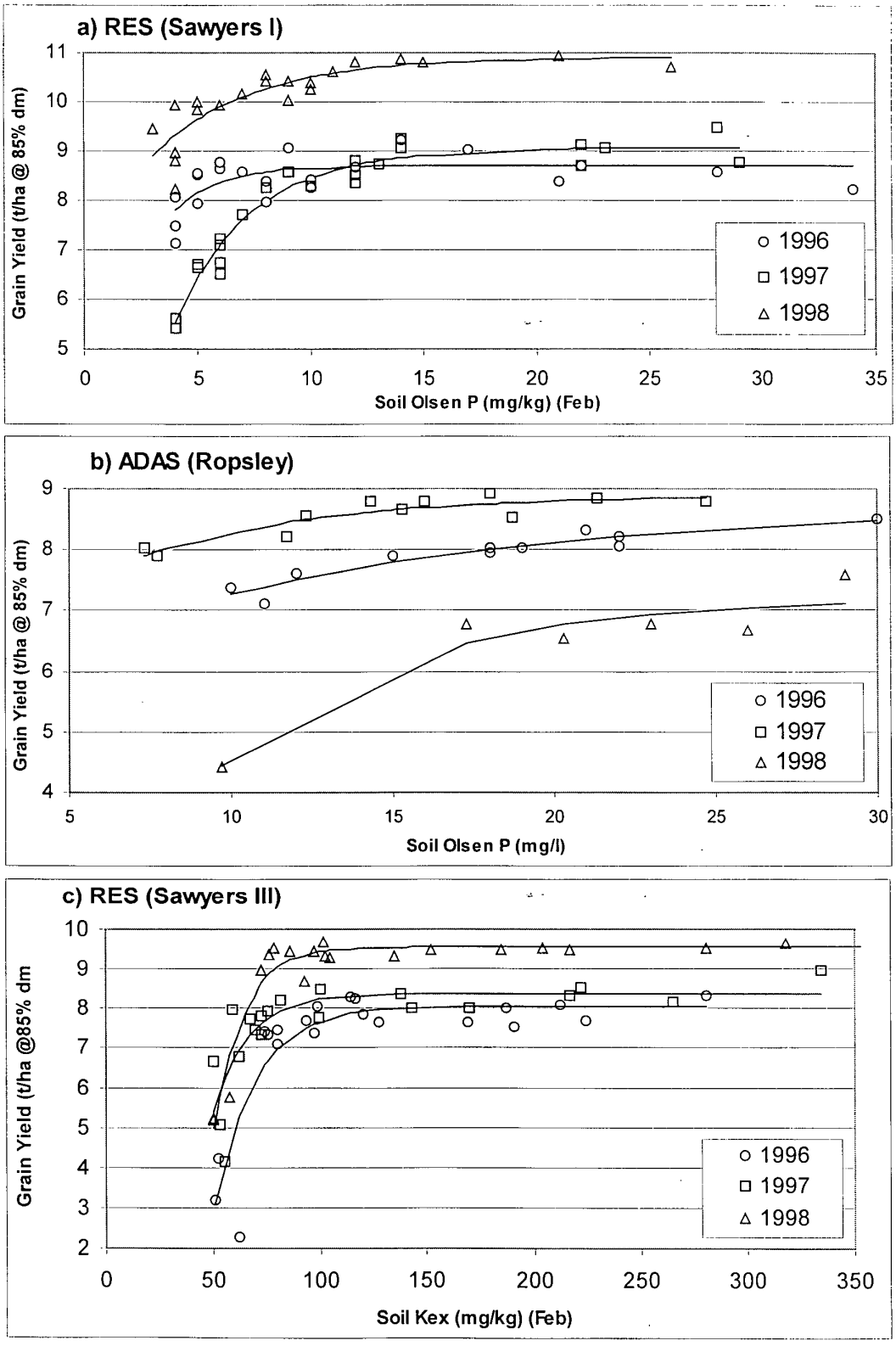
Table 5.1 Grain yield ranges (t/ha @ 85%dm)

SITE	1996	1997	1998
ROTHAMSTED - P	7.80-8.71	5.56-9.07	8.91-10.90
ROPSLEY - P	7.26-8.47	7.90-8.84	4.43-7.11
ROTHAMSTED - K	3.05-8.06	5.36-8.37	4.99-9.55

Maximum yields in the Rothamsted P experiments were 8.71, 9.07 and 10.90 t/ha (mean 9.56 t/ha) in 1996,97,98, respectively. Yield responses to soil P were 0.91, 3.51 and 1.99 t/ha (mean 2.14 t/ha), respectively. Critical soil P for 95% maximum yield was 5.5, 11.0 and 8.6 mg/kg (mean 8.4 mg/kg), respectively

(Fig. 5.1a). This compares with a mean critical soil P at Rothamsted of 10 mg/kg for the period 1992-95.

Figure 5.1 Grain yield and available soil P and K



Maximum yields in the P experiments at Ropsley were 8.47, 8.84 and 7.11 t/ha (mean 8.14 t/ha) in 1996,97,98, respectively. Yield responses to fresh P fertiliser and residual soil P were 1.21, 0.94 and 2.68 t/ha (mean 1.61 t/ha), respectively. Critical soil P for 95% maximum yield was 18.8, 11.0 and 20.1 mg/l (mean 16.6 mg/l), respectively (Fig. 5.1b). For the period 1992-95, mean critical soil P at Ropsley was 15.5 mg/l (equivalent to 13.6 mg/kg for a laboratory bulk density of 1.14 kg/l).

Maximum yields in the Rothamsted K experiments were 8.06, 8.37 and 9.55 t/ha (mean 8.66 t/ha) in 1996,97,98, respectively. Yield responses to soil K were 5.01, 3.01 and 4.56 t/ha (mean 4.19 t/ha), respectively. Critical soil K for 95% maximum yield was 100, 81 and 81 mg/kg (mean 88 mg/kg), respectively (Fig. 5.1c). For the period 1993-95, mean critical soil K at Rothamsted was 83 mg/kg.

The winter wheat crop at Sedge Fen (ADAS) in 1996 did not show a grain yield response to K fertiliser ($P>0.05$). Soil K status prior to sowing was 38 mg/l (ADAS Index 0), suggesting that a yield response to K fertiliser could be expected. However, K concentrations in shoots and leaves were consistently greater than the critical concentrations derived in the previous HGCA project (Barraclough *et al.*, 1997).

5.3 Conclusions

On the silty clay loam at Rothamsted, mean critical Olsen-P for 95% maximum grain yield was 9 mg/kg (range 6-11 mg/kg) in the period 1992-98, with a mean yield response, relative to the Index 0 soil, of 2.67 t/ha. On the sandy clay loam at Ropsley for the same period, mean critical Olsen-P was 16 mg/l (range 11-20 mg/l) (equivalent to 14 mg/kg), with a mean yield response, relative to the Index 1 soil, of 1.25 t/ha. There were no differences in critical soil P values between Brigadier (1996-98) and Mercia (1992-95). Mean critical exchangeable-K at Rothamsted in the same period was 85 mg/kg (range 78-100 mg/kg), with a mean yield response relative to the Index 0 soil of 3.29 t/ha. The Sedge Fen experiment demonstrated that yield responses to K fertiliser are not guaranteed even on Index 0 soils.

6 CRITICAL PLANT P, Pi AND K

6.1 Introduction

Critical plant P and Pi concentrations for near-maximum grain yield were determined on Exhaustion Land field at Rothamsted in 1996 (cv Hereward) and 1998 (cv Mercia), and at ADAS-Ropsley in 1996-98 (cv Brigadier). At Rothamsted, Exhaustion Land field was used in preference to Sawyers(1), which had been used for the critical soil P work (Section 5), because there were indications that soil P had moved across plots during cultivations on Sawyers(1), although not as far as the central combine area. Critical plant K was determined on Sawyers(III) field at Rothamsted (cv Mercia), and at ADAS Sedge Fen (cv Soissons) in 1996.

6.2 Rothamsted P experiments

Critical plant P and Pi concentrations at Rothamsted (Exhaustion Land) are shown in Table 6.1. Critical values were derived in two ways as described in Section 4. Those derived from yield/plant relationships are shown under the heading 'plant' in the Table. Those derived indirectly from plant/soil relationships are shown under the heading 'soil' in the Table. This was the method used in the previous HGCA project. There was generally good agreement between the two methods. Critical values at Rothamsted and ADAS-Ropsley in the period 1992-95 are also shown in Table 6.1 for comparison.

Critical shoot %Pi, determined by the 'soil' method, was in the range 0.028-0.069% during stem elongation, compared with 0.047-0.071% in 1992-95. Critical shoot Piw was 2.7-6.2 mM compared with 3.6-5.8 mM in 1992-95. Critical leaf(1) %P was 0.238-0.329%, lower than that found in 1992-95 (0.28-0.38%). It is noteworthy that the critical values in 1996 when Hereward was grown were lower than in all other years when Mercia was grown. However, 1996 was also a particularly dry season.

Table 6.1 Critical plant P and Pi concentrations at Rothamsted (Exhaustion Land)

Year	GS	Shoot %Pi		Shoot Piw (mM)		Leaf(1) %P	
		'Soil'	'Plant'	'Soil'	'Plant'	'Soil'	'Plant'
1996	31	0.047	0.049	3.9	4.0	0.245	0.265
	32	0.036	0.030	4.0	3.5	0.238	0.262
	39	0.028	0.029	2.7	2.6	0.270	0.264
	61	0.039	0.044	5.2	5.7	0.236	0.242
1998	32	0.065	0.066	4.5	4.4	0.290	0.327
	41	0.069	0.073	6.2	6.1	0.329	0.358
1992-1995	31-39	0.047-0.071		3.6-5.8		0.28-0.38	

Table 6.2 Critical Leaf(1) K concentrations at Rothamsted (Sawyers(III))

Year	GS	Leaf(1) %K		Leaf(1) Kw (mM)	
		'Soil'	'Plant'	'Soil'	'Plant'
1996	31	3.13	2.96	189	185
	32	2.37	2.43	188	184
	37	2.15	2.44	181	201
	39	2.02	2.13	157	161
1997	39	2.11	2.15	199	196
1998	32	1.81	1.93	133	147
	41	1.97	2.09	151	159
1992-95	31-41	1.60-3.21		130-227	

6.3 Rothamsted K experiments

Critical leaf(1) K values are shown in Table 6.2. Critical %K, determined by the 'soil' method, was in the range 1.81-3.13% during stem elongation (1.60-3.21% in 1992-95). Critical Kw range was 133-199 mM (130-227 mM in 1992-95).

In the previous HGCA project, limited measurements were made on leaf(2) K, and critical values were found to be more stable than those in leaf(1). A comprehensive programme of measurements was made on leaf(2) in this study and critical values for the period 1992-98 are shown in Table 6.3. Critical leaf(2) %K, determined by the 'soil' method, was in the range 1.72-2.98% and Kw in the range 131-204 mM, slightly narrower ranges than for leaf(1).

Table 6.3 Critical Leaf(2) K concentrations at Rothamsted (Sawyers(III))

Year	GS	Leaf(2) %K		Leaf(2) Kw (mM)	
		'Soil'	'Plant'	'Soil'	'Plant'
1993	41	1.72		150	
1995	31	1.81	1.81	163	
	32	1.79	1.82	151	
	37	1.81	1.66	170	
	39	1.72	1.70	185	
1996	31	2.98	2.62	184	173
	32	2.49	2.28	165	159
	37	2.09	2.13	152	156
	39	2.27	2.10	177	158
1997	39	1.96	1.92	204	192
1998	32	2.24	2.15	148	150
	41	1.75	1.78	131	136

6.4 ADAS P experiments

Critical shoot %P at Ropsley declined during the growing season in all years due mainly to dilution of P with structural dry matter (not shown). In 1998, critical shoot %P was much lower (0.20% & 0.15% at GS 32 and 39, respectively) than any previously observed values at Ropsley (typically 0.20-0.35% at GS 31-39).

Critical leaf(1) %P contents were more stable than those in whole shoots with a range of 0.23-0.37% at GS 32-39, but declined by anthesis. The critical value at GS 32 in 1998, 0.23%, was lower than any observed previously (Table 6.4).

Critical shoot Piw concentrations were variable early in the 1996 growing season (GS 22-24), but much more stable at GS 30-39 in the 1997 and 1998 growing seasons (Table 6.4). Critical leaf(1) Piw concentrations (not shown) increased during the growing season from 5.5-11.4 mM in 1996 (GS 30-59) and 5.0-10.1 mM in 1998 (GS 32-39). Critical leaf(1) Piw concentration was 10.9 mM at GS 39 in 1997.

These results generally support the critical values found in the previous HGCA project, although critical plant %P concentrations (shoot & leaf) were very low in 1998. The 1998 growing season was much wetter than previous years, particularly during April when critical concentrations were assessed, consequently, damp, overcast conditions may have been the factor which limited crop growth rather than P supply.

Table 6.4 Critical leaf(1) %P and shoot Piw at Ropsley 1996-1998

GS	Leaf(1) %P			Shoot Piw (mM)		
	1996	1997	1998	1996	1997	1998
30	0.39	Nd	Nd	nd	nd	4.5
32	0.35	0.30	0.23	nd	5.1	3.7
39	0.37	0.33	0.28	nd	6.7	5.9
59	0.31	Nd	Nd	nd	nd	nd

6.5 ADAS K experiments

The winter wheat crop at Sedge Fen in 1996 did not show any grain yield response to K fertiliser. The soil K status was 38 mg/l (Index 0), suggesting that a yield response was highly probable. However, K concentrations in the newest leaf (Table 6.5) were greater than the critical concentrations reported previously.

Table 6.5 Plant K on the control plots at Sedge Fen 1996

GS	Part	%K	Kw (mM)
32	leaf(1)	2.59	215
39	leaf(1)	2.44	198
65	leaf(1)	2.10	209

6.6 Conclusions

Critical plant concentrations during stem elongation in the period 1996-98 agreed well with those for 1992-95, although values were very low for P at Rothamsted in 1996 and at Ropsley in 1998. Critical concentration ranges for leaf(2) K were slightly narrower than for leaf(1). Critical concentration ranges for all sites and seasons in 1992-98 (GS 31-41) are summarised in Table 6.6.

Table 6.6 Critical plant concentration ranges for 95% maximum grain yield

Plant part	Test	Critical range
Leaf(1)	%P	0.23-0.38%
Leaf(1)	%K	1.61-3.21%
Leaf(1)	Kw	126-227 mM
Leaf(2)	%K	1.72-2.98%
Leaf(2)	Kw	131-204 mM
Shoot	%Pi	0.028-0.071%
Shoot	Piw	2.7-6.7 mM

7 EFFECT OF N ON PLANT P, Pi AND K CONCENTRATIONS

7.1 Introduction

In the previous HGCA project, we observed considerable site/season variation in plant P, Pi and K concentrations in early spring, probably caused by differences in growing conditions such as soil N supply. We also observed sharp increases in P, Pi and K concentrations following the application of N fertiliser in some years, although it was not clear whether the N had caused the increases. We need to know how concentration parameters are affected by N supply, what happens to the concentrations when N is applied to crops and whether the effects of N are ameliorated by expressing plant concentrations on a tissue water basis. This was done with a combination of field experiments, at Rothamsted in 1997 and ADAS sites in 1997 and 1998, and a controlled environment experiment at Rothamsted.

7.2 Rothamsted growth room experiment

Materials and methods

Winter wheat, cv. Mercia, was germinated in moist sand. Seedlings were transplanted into solution culture pots 8 days after sowing (9 plants per 1.2 l pot). Seedlings were about 10 cm tall with the second leaf just emerging. There were 4 N treatments x 4 harvests x 3 replicates (48 pots) randomly arranged in a growth room. Growing conditions were 20/16 °C (day/night), 16 h daylength, and light levels of 325-500 μmol/m^2/s. Nutrient solutions were changed every other day. N was supplied as calcium nitrate and the nutrient solutions contained basal levels of P (0.1 mM), K (3 mM) and all other essential elements. Earlier work had shown that P and K supply were adequate under this regime.

N treatments

- 1mM (deficient)
- 1mM increased to 4 mM 17 days after transplanting
- 5mM (abundant)
- 5mM increased to 8 mM 17 days after transplanting

Harvests and plant analysis

Plants were harvested 16, 19, 21 and 23 days after transplanting. Shoot fresh and dry weights (microwave) were measured. Dried shoots were milled and analysed for total N, P, Pi and K.

Results

The 1 mM N treatment was deficient for growth (Fig. 7.1b) and shoot %N declined in this treatment during the experiment. In the other N treatments, shoot %N was maintained close to 5% (Fig. 7.1a).

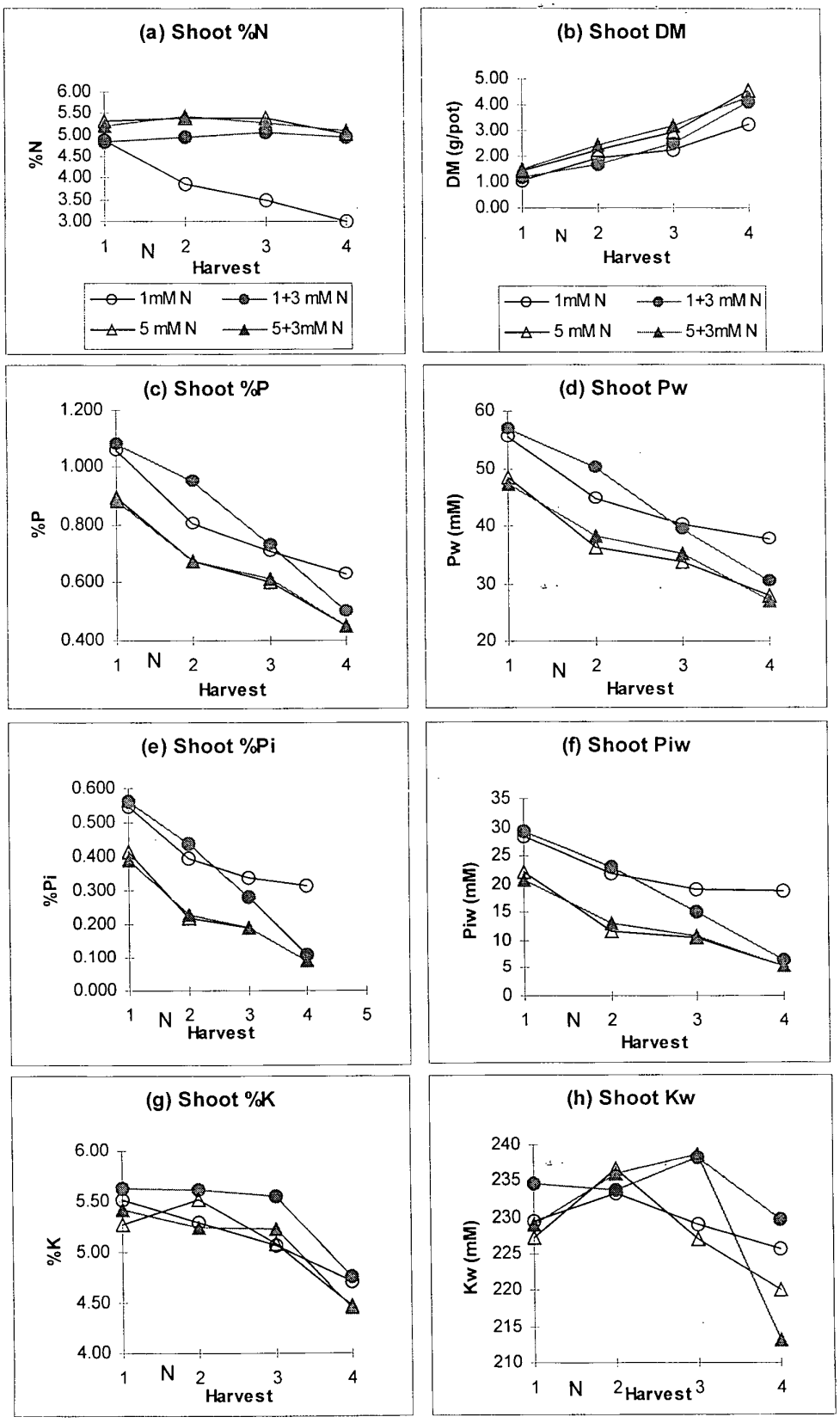
Shoot P and Pi (wet and dry) declined during the experiment and were consistently greater at low N than high N (Fig. 7.1c-f). Increasing N from 1 to 4 mM initially increased shoot P and Pi, although the increase in Pi was not significant, before both P and Pi declined to values similar to the high N treatments at final harvest. Increasing N from 5 to 8 mM had no effect on P and Pi.

Shoot %K and Kw changed little during the experiment and there were no significant treatment effects. All %K values were in the range 4.5-5.5% and all Kw values were in the range 220-240 mM. Increasing N from 1 to 4 mM increased shoot %K and Kw initially, but the effects were small and not significant (Fig. 7.1g-h).

Conclusions

Shoot P and Pi concentrations, wet or dry, were all higher when growth was limited by N. When N was supplied to N-deficient plants, shoot %P and Pw increased then declined to the same level as in N-sufficient plants. Shoot %Pi and Piw showed no initial increase, but declined to the same level as in N-sufficient plants. Shoot %K and Kw in N-deficient plants increased when N was added, but the effects were small and not significant. Overall, there were no significant effects on K concentrations in this experiment Adding N to N-sufficient plants had no effect on any of the nutrient concentration parameters.

Figure 7.1 Effect of N on shoot nutrient concentrations: hydroponics



7.3 Rothamsted field experiment

Materials and methods

The effect of different rates of N fertiliser on plant P, Pi and K concentrations was studied in a N response experiment on Fosters field at Rothamsted in 1997 (see Appendix I for agronomic details). There were seven N rates (0-300 kg/ha) applied as a split dressing of ammonium nitrate, 50 kg/ha on 10 March (pre-GS 24) and the remainder on 11 April (GS 31). Treatments were randomly arranged in three blocks. Two N rates (0 and 200 kg/ha) were sampled five times between late-tillering and anthesis (GS 24, 30, 32, 39 and 68). Mainshoot leaf(1) was sampled for all seven N rates at GS 37. Soil available P and K were not limiting, with Olsen-P in the range 35-51 mg/kg and Kex in the range 145-229 mg/kg.

At each harvest, three adjacent 0.5 m rows were taken at random from each plot. On the first three occasions, plants were dug up and the roots cut off. Thereafter, shoots were cut at ground level. Subsamples were taken of whole shoots and mainshoot leaf(1). Fresh and dry weights (microwave) were measured. Dried material was milled and analysed for total P, Pi and K by the standard methods (see Section 4).

Results

Increasing the N rate increased shoot %N, shoot water (Fig. 7.2 a,b) and grain yield. Yield was 4.7 and 9.4 t/ha at N0 and N200, respectively. N rate had no significant effect on shoot %P at any stage of the experiment. All other shoot P parameters - Pw, %Pi and Piw - were reduced following application of the second dose of N (150 kgN/ha) in the N200 treatment (Fig. 7.2 c-f). Shoot %K increased following the N application, but there was no effect on shoot Kw except at anthesis (Fig. 7.2 g,h). Leaf(1) concentrations showed similar responses to N as whole shoots (Fig. 7.3).

Figure 7.2 Effect of N on shoot nutrient concentrations at Rothamsted in 1997

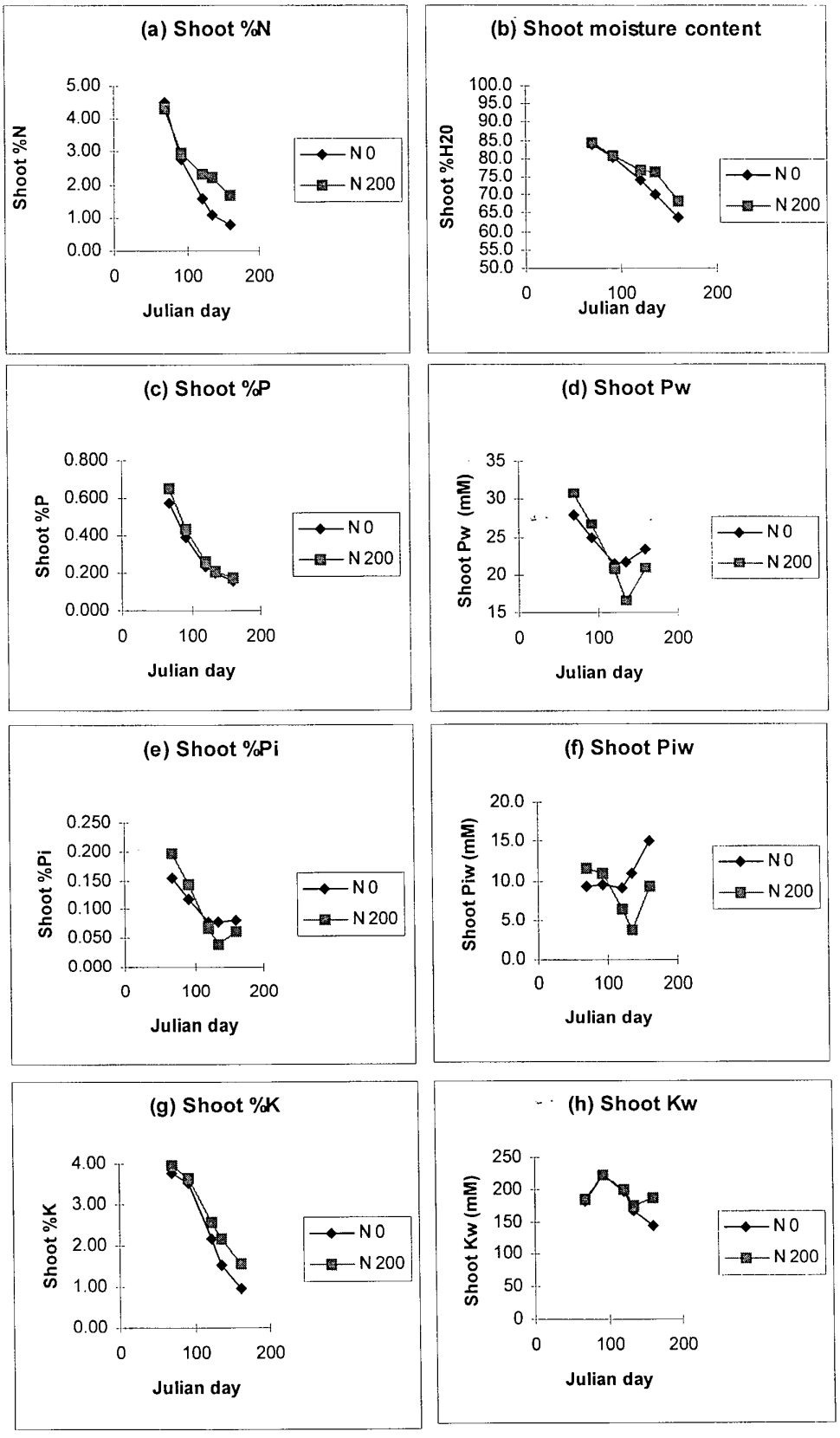
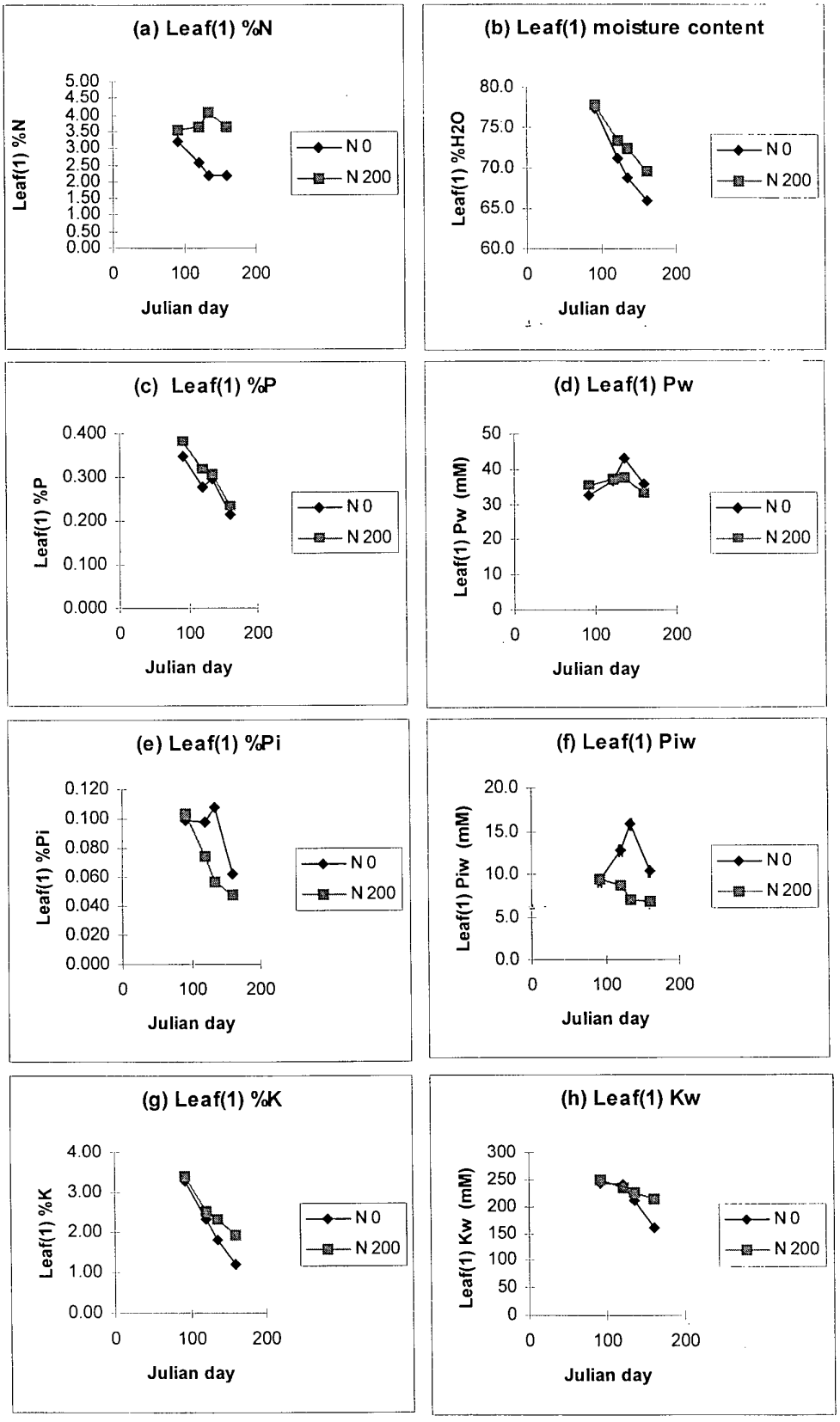


Figure 7.3 Effect of N on leaf(1) nutrient concentrations at Rothamsted in 1997

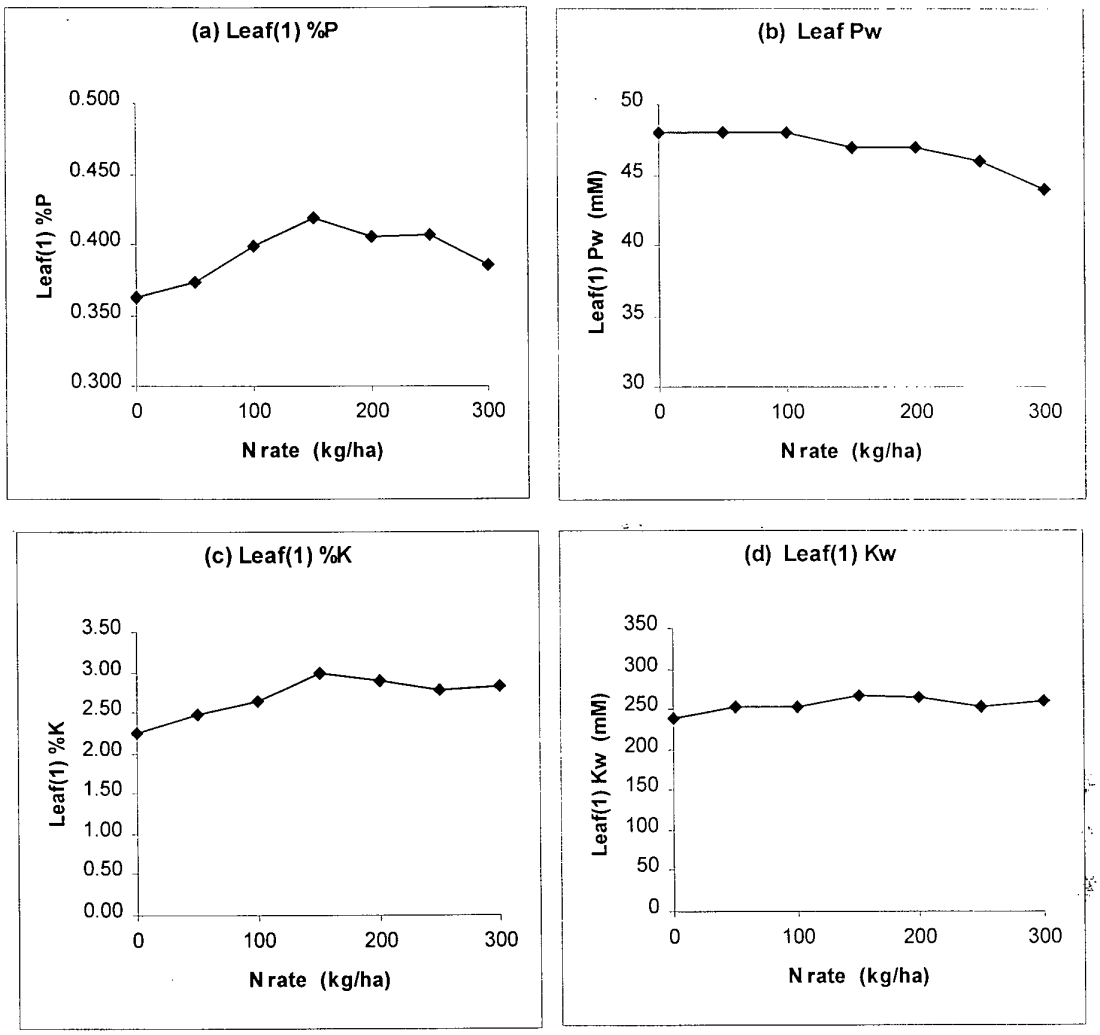


Other studies (Gregory *et al.*, 1979; Barraclough, 1986; Leigh and Johnston, 1983, 1986) have shown increases in shoot %P and %K following the application of N fertiliser (probably to crops that were N-deficient), and that N-deficient crops had smaller %P and %K values than N-sufficient crops. This appears to be at odds with the growth room results for %P where N-deficient plants had *higher* concentrations. Leigh and Johnston working with spring barley concluded that N supply had no effect on shoot Kw (1983) and reduced Pw (1986) as was found in the present study. However, close inspection of their results suggests that initial increases in both parameters following N addition may have occurred.

At GS 37, leaf(1) was sampled for all 7 N rates. The relationships between leaf(1) P and leaf(1) K and N rate were complicated (Fig. 7.4). Leaf(1) %P and %K increased with increasing N rate up to 150 kg/ha then declined at higher N rates. The pattern was similar for Kw, but the effect of N was greatly attenuated. In the case of leaf(1) Pw, this was constant at 48 mM up to 100 kgN/ha then declined gradually to 44 mM at 300 kgN/ha. The effect of N on leaf %K was studied in France (Loue, 1987) with similarly complex responses reported. In the French study, leaf(1) %K increased with N at all K rates, whilst leaf(2) %K decreased with N at low K, was constant at intermediate K, and increased at high K.

The above leaf results help to explain the discrepancy between growth room and field data for the effect of N on %P. In the field, the low (N0) and high (N200) N treatments represented very deficient and adequate N, respectively, with %P being lower at N0. In the growth room, the low (1 mM) and high (5 mM) N treatments represented just-deficient and abundant N, respectively, leading to %P being higher at 'deficient' N.

Figure 7.4 Effect of N on leaf(1) concentrations at Rothamsted in 1997 (GS 37)



Conclusions

No sudden increases or 'spikes' occurred in any concentration parameter in any organ when the main N dose was applied to a crop already sufficient in N. Shoot storage pool concentrations, %Pi and Piw, as well as Pw, were higher in the N-deficient crop (N0) compared with the N-sufficient crop (N200), whilst %P was the same. Shoot %K was lower, whilst Kw was the same. Expressing K concentrations on a tissue water basis eliminated the effects of N. Concentrations in leaf(1) were affected in a similar way to whole shoots. Differences in N rates in the field, at least in the normal application range of 100-250 kg/ha, are unlikely to have any major effects on plant P, Pi and K concentrations during stem elongation.

7.4 ADAS field experiments

Ropsley P experiment

An early application of 40 kg/ha N did not increase plant dry matter production at Ropsley in either 1997 (GS 23) or 1998 (GS 30), probably due to the presence of adequate soil N reserves to sustain early growth. Consequently, whole shoot P and K contents remained fairly constant with or without N fertiliser. However, shoot %Pi and Piw contents decreased following N fertiliser application ($P<0.05$), typically by 0.01% and 1-2 mM, respectively. This was associated with a slight (0.5-1.0%) increase in tissue water content with N fertiliser ($P<0.05$) in 1997.

Following the main N application (160 kg/ha), shoot dry matter and tissue water contents increased in both seasons at GS 39 ($P<0.06$). This was associated with a mean increase in shoot %P of 0.02% ($P<0.01$ in 1997), and 0.25-0.27% ($P<0.05$) in shoot %K. There was no increase in shoot Kw. Shoot %Pi and Piw decreased ($P<0.01$) following N application by up to 0.008% or 2 mM, respectively, in 1997. The application of 160 kg/ha N fertiliser also increased leaf(1) %P by 0.02% and %K by 0.2% in 1997 ($P<0.01$), but had no effect on leaf(1) concentrations in 1998. Leaf(1) Pi concentrations were not affected.

Gleadthorpe

In 1997, the crop was drilled late (27/11/96) and was slow to establish on a poor seedbed. The early application of 40 kg/ha N did not affect whole shoot dry matter production or P, Pi and K concentrations at GS 24 in 1997 (Table 7.1). By contrast, the 1998 crop was drilled a month earlier and established well, with rapid early growth due to the warm conditions in February which depleted soil N reserves early in the season. In 1998, dry matter production at GS 30 was significantly increased by the early N fertiliser application ($P<0.01$). This was associated with increased shoot %P, %Pi and %K contents ($P<0.01$; Table 7.1). Expressing Pi and K concentrations on a tissue water basis eliminated the effects of N.

Increased dry matter production and tissue water contents were observed following the main (140/160 kg/ha) N application in both 1997 and 1998

(P<0.05; Table 7.2). This was associated with increased shoot %P and %K (P<0.05; Table 7.2), but %Pi, Piw and Kw were not affected. Leaf(1) was not sampled at Gleadthorpe.

Table 7.1 Effect of an early application of 40 kgN/ha on whole shoot P and K concentrations at ADAS-Gleadthorpe.

Parameter	1997 (GS 24)		1998 (GS 30)	
	+ N	- N	+ N	- N
P (%)	0.57	0.56	0.57	0.46
K (%)	3.31	3.26	3.73	3.47
Pi (%)	0.27	0.27	0.29	0.26
Piw (mM)	18.5	20.2	20.6	20.7
Kw (mM)	230	278	206	218
DM (t/ha)	0.81	0.83	0.96	0.77
TW (%)	82.7	81.4	82.2	80.3

Table 7.2 Effect of the main application of 160 kgN/ha on whole shoot P and K concentrations at ADAS-Gleadthorpe.

Parameter	1997 (GS 32)		1998 (GS 39)*	
	+ N	- N	+ N	- N
P (%)	0.49	0.44	0.28	0.24
K (%)	3.56	3.23	1.91	1.69
Pi (%)	0.24	0.23	0.13	0.12
Piw (mM)	16.0	17.6	10.6	11.9
Kw (mM)	249	250	127	133
DM (t/ha)	3.3	3.0	9.4	7.9
TW (%)	82.6	81.1	79.0	76.4

* average of irrigation treatments

Conclusions

When the application of N fertiliser resulted in increased tissue water content and dry matter production, shoot %P and %K were increased. Early in the season, this was probably dependent on the level of soil N reserves, with no differences observed where N reserves were adequate (although this was not measured). Shoot Kw was not affected. Shoot %Pi and Piw were unaffected by N application at Gleadthorpe, but decreased at Ropsley.

7.5 Conclusions

- The interaction of plant nutrient concentration parameters with N supply was complex, although shoot and leaf(1) concentrations responded to N in a similar way.

- Applying N to an N-sufficient crop had no effect on the concentration parameters.

- In N-deficient crops, shoot and leaf %P and %K were lower, Kw was the same, and Pw, %Pi and Piw were higher than in N-sufficient crops. Accordingly, applying N to an N-deficient crop increased %P and %K, had no affect on Kw, and decreased %Pi and Piw. Testing for %P and %K should not be carried out if N deficiency is suspected.

- Expressing K concentrations on a tissue water basis eliminated N effects.

- In the normal application range of field N rates (100-250 kgN/ha), differences in N supply are unlikely to have significant effects on plant PK concentration parameters.

8 EFFECT OF WATER SUPPLY ON PLANT P, Pi and K CONCENTRATIONS

8.1 Introduction

Plant concentrations can be affected in both the short and long-term by changes in soil water supply. Firstly, there may be short-term passive increases in wet concentrations arising from diurnal fluctuations in transpiration and water supply. Secondly, concentrations could be affected by long-term differences in water supply as occurs in wet and dry years for example. In the previous HGCA project, we observed fluctuations in plant P, Pi and K concentrations during the season at a given site, and variations between sites and seasons. Some of the variation was probably due to differences in soil water content affecting P and K availability and plant growth. We need to know how the various concentration parameters are affected by water supply, and whether the effects are ameliorated by expressing plant concentrations on a tissue water basis. This was done in a series of irrigation experiments on the light sandy soil at Gleadthorpe in 1997 and 1998, and by considering previous results obtained in contrasting years at Rothamsted (1994 and 1995).

8.2 Diurnal variation

Phosphorus (Ropsley)

Sampling was carried out at GS 30 in 1996 and GS 32 in 1997 on dry, sunny days with maximum temperatures up to 12 °C. There were no significant changes in shoot %P and leaf(1) %P between morning and afternoon samplings in both years. Whole shoot Pi concentrations were also unaffected in 1996, but increased by up to 0.03% or 2.5 mM in the afternoon in 1997 ($P<0.05$). Leaf(1) Pi concentrations increased in the afternoon in both years by up to 0.06% or 4 mM in 1996, and 0.04% or 4.8 mM in 1997, depending on the treatment ($P<0.05$). Changes in leaf(1) Pi were associated with a decrease in leaf tissue water content in 1997 ($P<0.05$), but not in 1996.

In 1996, sampling at GS 39 was carried out on a hot sunny day (26 °C), with the crop visibly wilting by the afternoon. Despite this, shoot and leaf(1) %P and Pi

concentrations did not change during the day ($P>0.05$). By contrast, sampling at GS 39 in 1997 was carried out on an overcast day with a maximum temperature of just 15 °C. There was no change in shoot and leaf tissue moisture contents or leaf(1) concentrations during the day ($P>0.05$), but whole shoot %P, %Pi and Piw all increased in the afternoon by up to 0.03%, 0.02% and 1.5 mM, respectively.

Phosphorus (Gleadthorpe)

Sampling was carried out in 1997 at GS 32 and 47 on cloudy days. Whole shoot and leaf(1) tissue water contents decreased during the day, by up to 1% on both occasions ($P<0.05$). However, in contrast to Ropsley, these decreases were associated with a decrease in plant P concentrations: whole shoot Pi decreased by up to 0.03% or 1.6 mM at GS 32 ($P<0.05$ dry basis only), and 0.05% or 5 mM at GS 47 ($P<0.01$); shoot %P decreased by 0.02% at GS 47 only ($P<0.05$). Leaf(1) %P also decreased by 0.08 and 0.02% at GS 32 and 47, respectively. There was no significant change in leaf(1) Pi concentrations at GS 32, but at GS 47 decreases of up to 0.08% or 8 mM were observed.

Potassium (Sedge Fen and Gleadthorpe)

There was no significant change in shoot, leaf(1) or leaf(2) moisture contents or K concentrations between morning and afternoon samplings at GS 32 and 65 at Sedge Fen in 1996. Similarly at Gleadthorpe in 1997, K concentrations did not change during the day at either GS 32 or 47, except for leaf(1) %K at GS 32, which decreased by up to 0.4% in the afternoon ($P<0.05$).

Potassium (Rothamsted)

Diurnal measurements were made at GS 61 at Rothamsted in 1994 (K site - Sawyers III). Flag leaves were sampled from three contrasting K plots, Kex 133, 83 and 49 mg/kg. Sampling was done at 9 am and 3 pm on a cool, humid overcast day (maximum temperature 19 °C). Reductions in tissue water and %K occurred on all three plots (Table 8.1), whilst Kw increased by an average of 4.3%. The exercise was repeated a few days later on the 83 mg/kg K plot on a hotter day (maximum 22 °C). Kw increased by just 1.5%.

Table 8.1 Diurnal changes in flag leaf water content, %K and Kw at Rothamsted in 1994

Time	Kex 133 ppm			Kex 83 ppm			Kex 49 ppm		
	$\%H_2O$ in FW	%K in DM	Kw (mM)	$\%H_2O$ in FW	%K in DM	Kw (mM)	$\%H_2O$ in FW	%K in DM	Kw (mM)
am	76.2	2.49	200	75.9	2.32	189	74.8	1.18	101
pm	74.8	2.41	209	73.8	2.14	194	73.3	1.14	107

Conclusions

The effect of sample timing on plant P and K concentrations was inconsistent between sites and nutrients. Generally there were no changes or increases in P and Pi at Ropsley, decreases in P and Pi at Gleadthorpe, no changes in K at Gleadthorpe and Sedge Fen, and decreases in %K and increases in Kw at Rothamsted. In the light of these results, it is recommended that sampling be confined to the morning as was the case in the previous HGCA project.

8.3 Irrigation experiments at ADAS Gleadthorpe

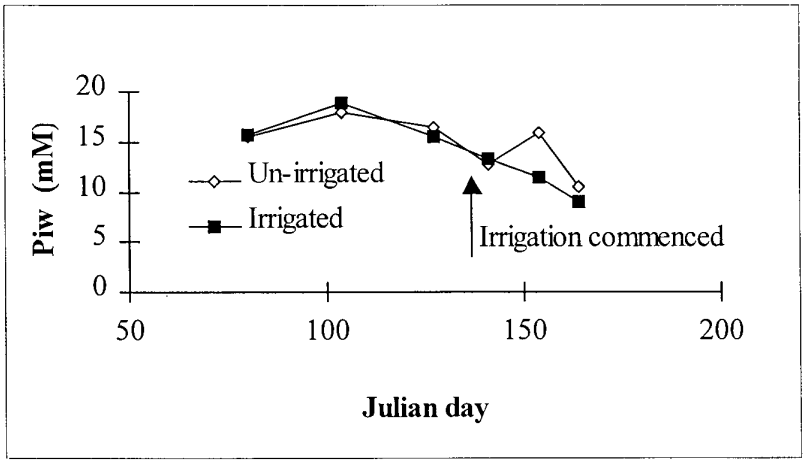
The effects of irrigation were studied on the light sandy soil at Gleadthorpe in 1997 and 1998. In both years, soil moisture was not limiting early in the season so irrigation was not required until May. A total of 98 and 69 mm of irrigation was applied up to late May/early June in 1997 and 1998, respectively, which significantly increased soil moisture content in both seasons by 2-3% ($P<0.05$). Rainfall in both seasons exceeded the long-term average by over 200%. As a result, irrigation treatments were only imposed 6-12 days prior to sampling at GS 39 in both years. Thus soil water supply was only likely to have had an effect at GS 39, 47 and 65 in 1997, and at GS 39 in 1998.

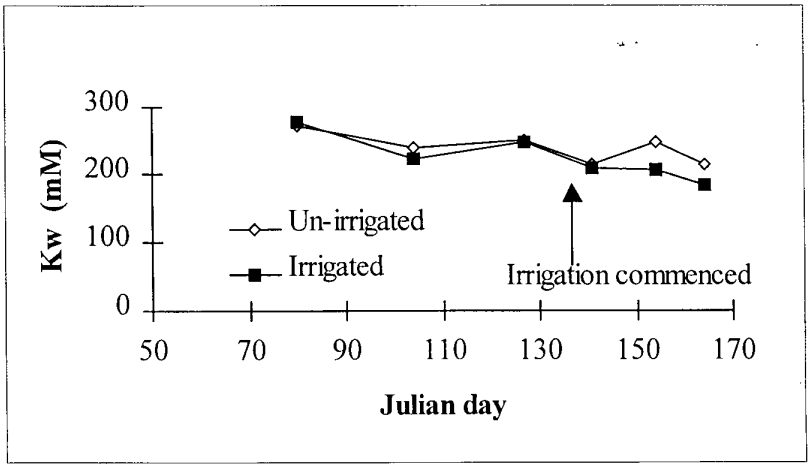
Total dry matter production was not affected by irrigation in either season. Irrigation increased grain and straw yields by 0.4 t/ha and 1.6 t/ha, respectively in 1997, but in 1998 the combination of high rainfall and supplemental irrigation actually decreased yield (Table 8.2). Irrigation had no effect on grain and straw %P or grain %K. Uptakes of P and K at harvest were unaffected by irrigation in 1997, but reduced in 1998 (Table 8.2).

Table 8.2 Effect of irrigation at final harvest at ADAS-Gleadthorpe (1997 & 1998)

Parameter	1997		1998	
	Irrigated	U-irrigated	Irrigated	U-irrigated
Yield (t/ha @85 % dm)	6.79	6.37	7.91	9.54
Grain % P	0.31	0.34	0.35	0.34
Straw % P	0.10	0.10	0.12	0.11
Grain % K	0.42	0.46	0.46	0.45
Straw % K	0.36	0.45	1.49	1.29
Total P uptake (kg/ha)	24.0	23.2	33.1	37.1
Total K uptake (kg/ha)	46.4	45.7	136.8	147.0

Figure 8.1 Effect of irrigation on shoot Piw and Kw at Gleadthorpe, 1997





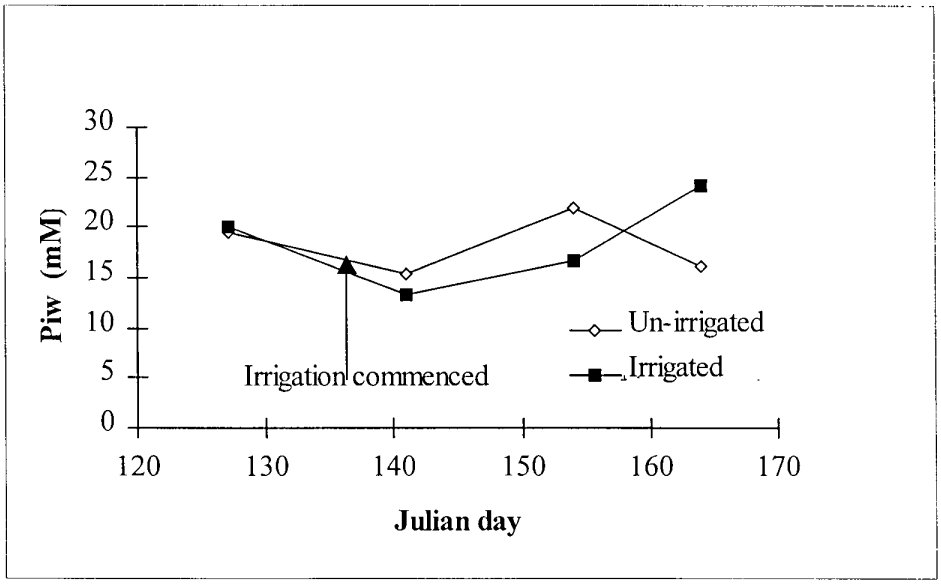
Shoot %P, %Pi and %K were not affected by the dry conditions. However, shoot Piw and Kw were greater in the unirrigated treatment at GS 47 in 1997 ($P<0.05$) (Fig. 8.1) and at GS 39 in 1998 (Table 8.3). Most studies have reported that drought reduces shoot %N, %P, %K, %Ca and %Mg (spring barley - Day (1981), Leigh & Johnston (1983 &1986); winter wheat - Barraclough (unpublished)). Leigh & Johnston reported that Pw in spring barley shoots was slightly reduced by drought (1986) whilst Kw was slightly increased (1983). It is doubtful if the crops in the present were truly 'droughted'.

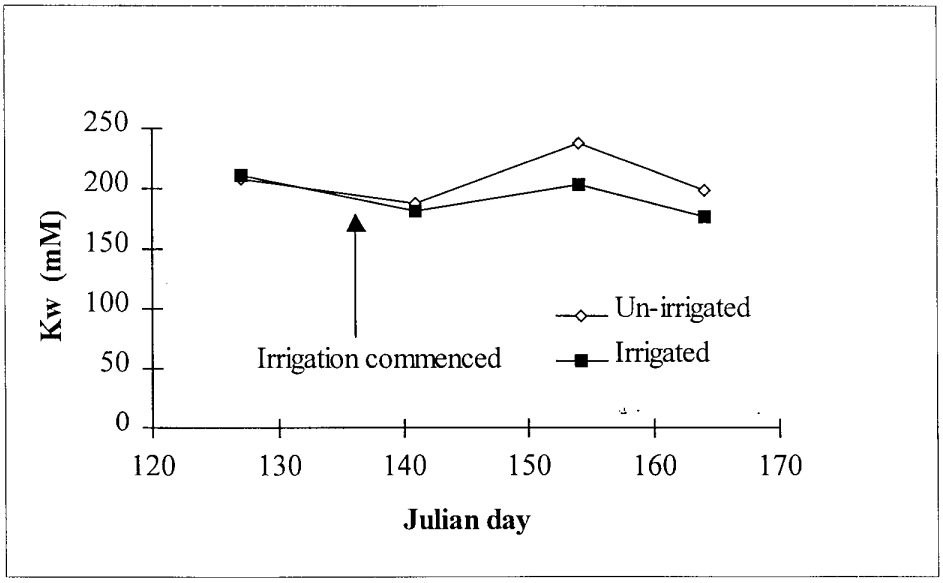
Table 8.3 Effect of irrigation on plant P and K concentrations at GS 39 (ADAS-Gleadthorpe, 1998)

Nutrient	Whole shoots		Leaf(1)	
	Irrigated	Un-irrigated	Irrigated	Un-irrigated
K (%)	1.89	1.93	2.09	2.45
P (%)	0.28	0.29	0.36	0.39
Pi (%)	0.12	0.14	0.10	0.15
Piw (mM)	9.46	11.8	10.4	15.5
Kw (mM)	121	133	169	167

Leaves are more likely to be affected by water stress than whole shoots. At Gleadthorpe, leaf(1) tissue water contents were higher following irrigation at GS 47 & 65 in 1997 ($P<0.05$), but were unaffected at GS 39 in both seasons. Irrigation had no effect on leaf(1) %P and %K in 1997, but significantly reduced them, and %Pi, in 1998 ($P<0.05$; Table 8.3). Leaf(1) Piw and Kw concentrations were also lower with irrigation at GS 47 in 1997 as was Piw, but not Kw, at GS 39 in 1998 ($P<0.05$; Fig. 8.2; Table 8.3).

Figure 8.2 Effect of irrigation on leaf(1) Piw and Kw at Gleadthorpe, 1997





8.4 Effects of drought in the previous HGCA project (1992-95)

In the previous HGCA project, no formal experiments were set up to study the effects of drought, but contrasting conditions did occur at Rothamsted in 1994 and 1995. Long-term rainfall at Rothamsted between April and July is 210 mm; 1994 was near normal (185 mm) whilst 1995 was dry (86 mm). There were marked differences in plant water contents in the two years. The effect of the dry

conditions in 1995 on mean shoot and leaf concentrations (GS 24-61) compared with the much wetter conditions in 1994 are shown in Table 8.4. 'Dry' plant concentrations (%) were lower under drought, in line with literature reports, whilst 'wet' concentrations (w) tended to be unaffected. Shoots and leaf(1) were affected in the same way by drought. In the current Gleadthorpe experiments, nutrient concentrations in DM were unaffected or higher where irrigation was withheld, whilst wet concentrations were higher (Table 8.4). The contrast between these results illustrates the complexity of drought effects.

Table 8.4 Effect of drought on PK concentration parameters at Rothamsted (1994 vs 1995) and Gleadthorpe (irrigated vs non-irrigated)

Parameter	Shoots		Leaf(1)	
	Rothamsted	Gleadthorpe	Rothamsted	Gleadthorpe
%P	lower	Same	Lower	Same/higher
Pw	same	n/a	Same	n/a
%Pi	lower	Same	Lower	Higher
Piw	same	Higher	Same	Higher
%K	lower	Same	Lower	Same/higher
Kw	higher	Higher	Same	Higher/same

In the previous project, critical 'dry' concentrations at Rothamsted were lower in both shoots and leaf(1) (Table 8.5). The situation with wet concentrations was less predictable. Critical values showed a variable response to drought depending on the concentration parameter and plant organ (Table 8.5).

Table 8.5 Effect of drought on critical PK concentration parameters at GS 39 at Rothamsted (1994 vs 1995)

Parameter	Shoots	Leaf(1)
%P	Lower	lower
Pw	Higher	same
%Pi	Lower	lower
Piw	Same	higher
%K	Lower	lower
Kw	Lower	same

8.5 Conclusions

Drought affects plant concentration parameters in complex ways. Critical dry matter contents (%P, %Pi, %K) in both shoots and leaf(1), are likely to be reduced by drought. In contrast, the effect on critical wet concentrations (Pw, Piw, Kw) is not so predictable, with responses depending on the particular concentration parameter and the plant organ. For two of the plant tests recommended in the previous HGCA project, namely leaf(1) Kw and shoot Piw, concentrations appeared to be largely unaffected by drought. In the case of short-term diurnal effects, inconsistent results were obtained between sites and nutrients. As a general rule, plant testing is best carried out in the mornings.

9 LEAF POSITION

9.1 Introduction

Mainshoots cannot be unequivocally identified at all growth stages in the field, consequently samples of leaf (1) invariably consist of a mixture of mainshoot and tiller leaves. It is important to establish if there are differences in nutrient concentrations between leaves from different stems. Measurements made at Rothamsted in 1994 showed that leaf(1) Kw at GS 41 was marginally less in tiller(1) than in the mainshoot (73 and 78 mM, respectively) on K-deficient soil, and marginally greater (173 vs 161 mM, respectively) on K-sufficient soil.

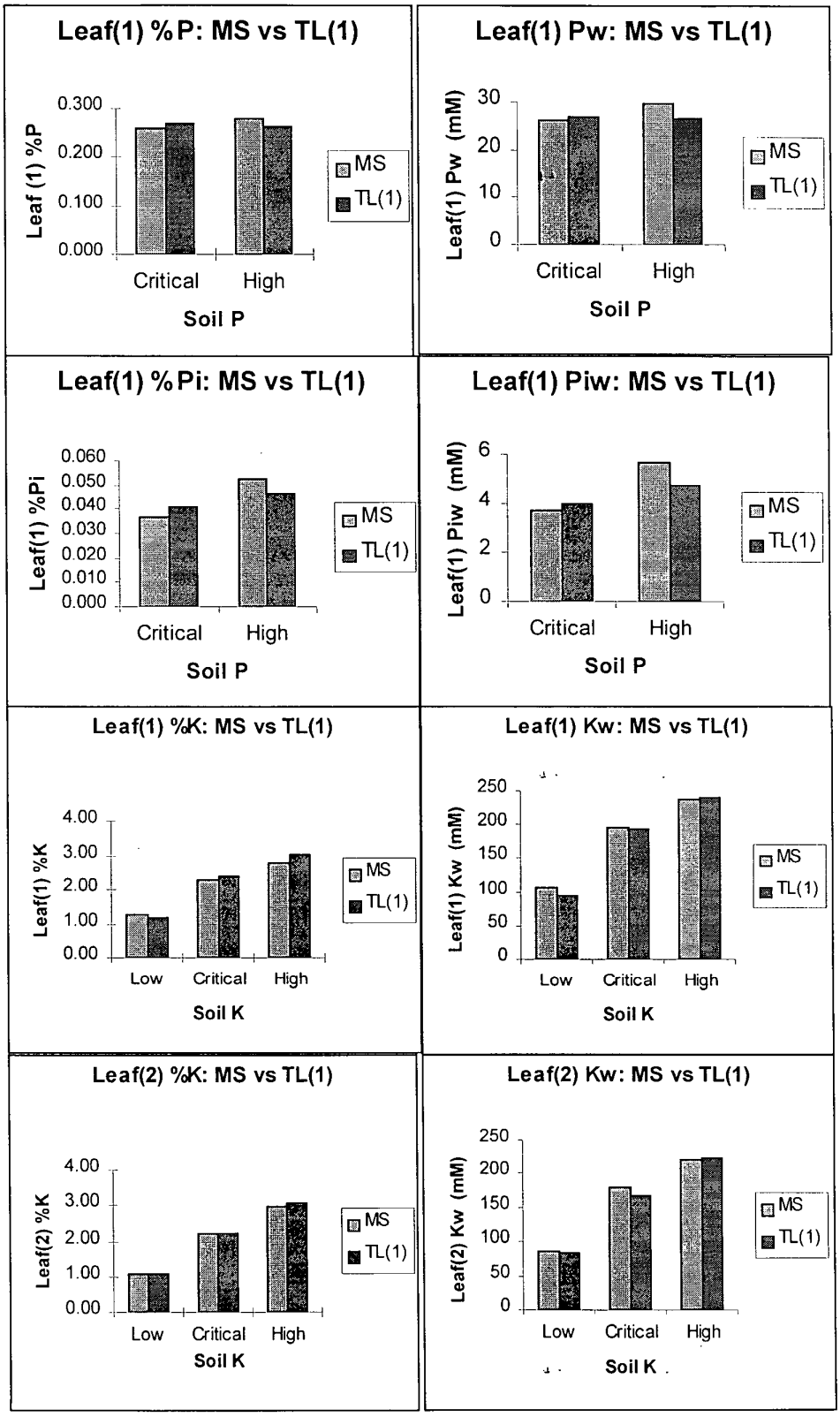
9.2 Materials and Methods

Whole plants were dug up (4 x 0.5 cm rows) at GS 30 from the P (Sawyers I) and K (Sawyers III) experiments at Rothamsted in 1997. Six plots were sampled in the P experiment, three at critical soil P (mean 9 mg/kg) and three at high soil P (mean 23 mg/kg). Nine plots were sampled in the K experiment, three at low soil K (mean 47 mg/kg), three at critical soil K (mean 72 mg/kg), and three at high soil K (mean 140 mg/kg). Soil was washed from the roots and leaf(1) samples taken from the mainshoot and first tiller (tiller(1)). Leaf(2) samples were also taken in the K experiment. Soil and plant nutrients were analysed by standard laboratory methods (Section 4).

9.3 Results

Soil P had little effect on leaf(1) P and Pi concentrations, and there were no significant differences between the mainshoot and tiller(1) (Fig. 9.1). Leaf(1) and (2) K increased with increasing soil K, but again there were no significant differences between the mainshoot and tiller(1) K concentrations (Fig. 9.1). It appears that samples of leaf(1) for testing purposes can consist of mixtures of mainshoot and tiller(1) leaves.

Figure 9.1 Effect of leaf(1) position and soil PK level on nutrient concentrations



10 ON-FARM PLANT TESTS

10.1 Introduction

Plant tests that can be carried out *in situ* in the field or at least 'on-farm' can be more attractive to farmers than laboratory-based tests as they cost less and results are available immediately. On-farm tests involve extraction of sap or tissue water, dilution of tissue water to bring concentrations into the working range of analytical instruments, addition of reagents where necessary and analysis for orthophosphate and potassium using portable analytical equipment.

In the previous HGCA project, critical 'wet' concentrations were based on tissue water not sap. These were determined indirectly from measured tissue water (fresh weight minus dry weight) followed by extraction of nutrients from dried plant material with water and analysis by standard laboratory procedures. Consequently, it is important that on-farm methods give the same results as the laboratory methods if the critical 'wet' values determined in the previous project are to be valid. In this section we consider two portable analytical instruments suitable for on-farm use, the RQflex and Cardy-K meters, practical aspects of extracting tissue water on-farm, and present comparisons of on-farm and standard laboratory methods conducted by Rothamsted and ADAS.

10.2 The Merck Reflectoquant System

Introduction

'Test strip' technology has been available for many years for the semi-quantitative analysis of simple ionic species such as NO_3^-, $H_2PO_4^-$, K^+, Mg^{2+}, Ca^{2+} etc. The recently launched Reflectoquant system (manufactured by Merck, Darmstadt, Germany and supplied by BDH, UK) takes test strip technology a stage further by allowing the quantitative determination of these ions. In the present project, the system was assessed for inorganic orthophosphate (Pi). The system comprises an RQflex© reflectometer and Reflectoquant® plastic strips specific for each analyte. The strips are impregnated with reagents which react with the analyte to produce specific colours at an intensity which is proportional to the concentration of the analyte. The RQflex is a small portable

instrument which measures the difference in intensity between incident and reflected light from the coloured strips thereby allowing the analyte to be determined quantitatively. The RQflex is programmed for each ion using a barcode system.

Standard laboratory analysis of Pi

The concentration of Pi is usually determined in the laboratory using the phosphomolybdate blue method (Murphy and Riley, 1962). The solution to be analysed is first acidified with sulphuric acid, orthophosphate ions (PO_4^{3-}) and molybdate ions then form molybdophosphoric acid. This is reduced to phosphomolybdenum blue. The intensity of the blue coloration is proportional to the phosphate concentration and is determined colorimetrically. The analytical section at Rothamsted uses a Technicon Autoanalyser system coupled to a Cecil Instruments spectrophotometer.

Analysis of Pi by Reflectoquant

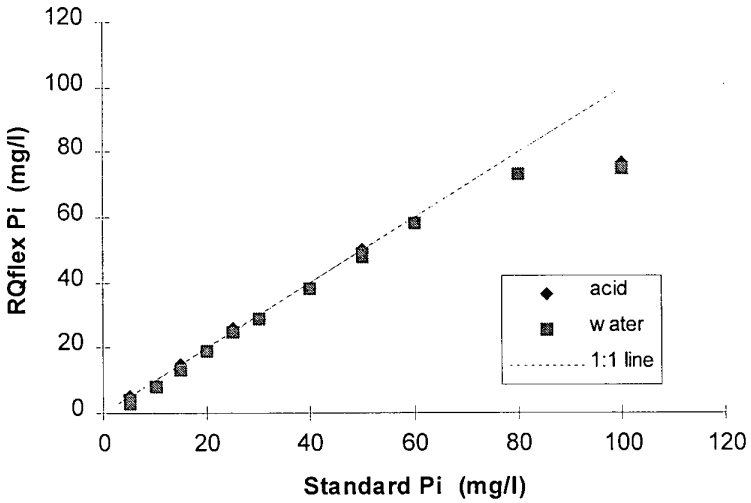
The Reflectoquant system also uses the phosphomolybdate blue method. Samples to be analysed should have a pH between 4-11. Ten drops of a liquid reagent are added to 5 ml of diluted sap. A phosphate test strip is dipped in the sample and the start button on the RQflex simultaneously pressed. After 2 seconds the strip is removed and the excess solution shaken off. The instrument counts down for 90 seconds. During the last 5 seconds, the strip is inserted into the instrument and the concentration of phosphate in mg/l is displayed on the screen. Merck claim an overall measuring range of 5-120 mg/l PO_4^{3-}, with a resolution of 1 mg/l.

Calibration of RQflex

Standard Pi solutions in the range 5-100 mg/l (0.05-1.05 mM) were prepared using oven-dried potassium orthophosphate (KH_2PO_4, Analar) and distilled water or 2% acetic acid (v/v). The standards were measured using the Reflectoquant system following the manufacturer's instructions. The equipment performed reasonably well with a linear response up to about 60 mg/l (Fig. 10.1). At 100 mg/l, values were considerably lower (75 mg/l) than the standard. The pH of the acetic acid standards was about 3, but this had no effect on the

results. We conclude that the working range of the system is 5-60 mg/l phosphate. Occasionally, meter readings were up to 2 mg/l below standards. As Pi concentrations in the tissue water of field-grown plants are likely to range up to 1900 mg/l (20 mM), samples may need to be diluted 30 times to bring them into range. Major interfering ions likely to be encountered in plant sap are nitrate, chloride, potassium, magnesium and calcium. According to the manufacturer the critical interference level for these ions is 1000 mg/l, equivalent to 16-42 mM.

Figure 10.1 RQflex standard curve for Pi



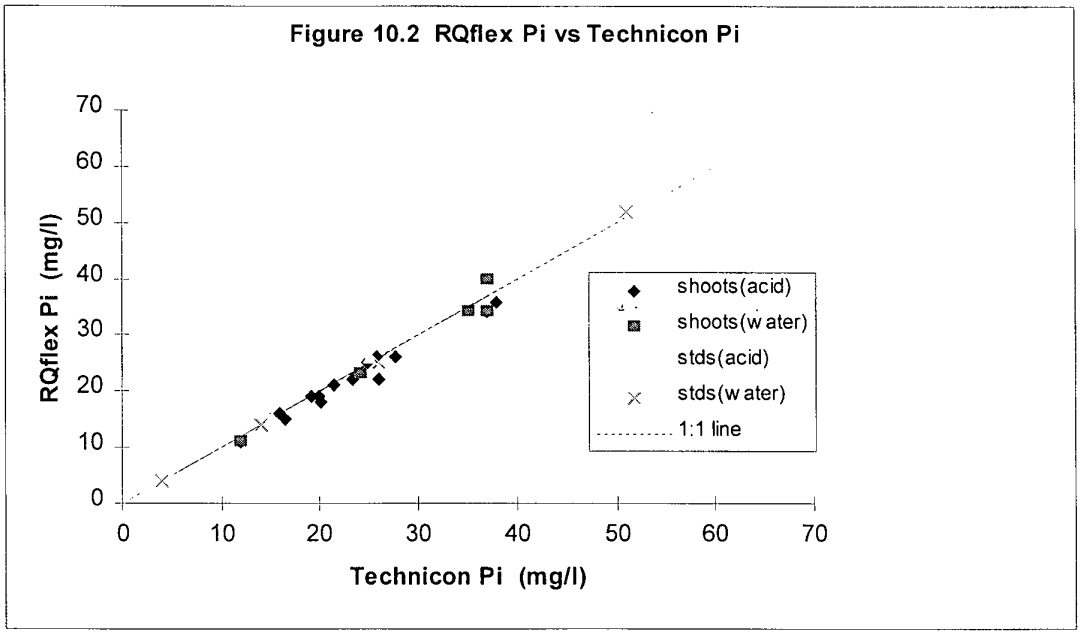
Variability of phosphate test strips

A standard solution of 20 mg/l phosphate in distilled water was measured ten times by RQflex using ten strips from the same batch. The mean concentration (±sd) was 19±1 mg/l phosphate. Tissue water pressed from a frozen/thawed sample of wheat leaves was diluted and also measured ten times, the mean concentration (±sd) was 31±1 mg/l phosphate. For comparison, a sample of tissue water pressed from frozen/thawed wheat shoots was measured ten times by the standard laboratory procedure. The mean phosphate concentration (±sd) was 44 ± 0.2 mg/l.

RQflex vs. Technicon

Comparisons were made between the Technicon system and the Reflectoquant system in three runs. In the first run, microwave-dried shoots were extracted with water and acetic acid, in the second run microwave-dried shoots were extracted with acetic acid, and in the third run water and acid standards were compared. RQflex results were not statistically different to those of the Technicon, nor were there any differences between the acid and water extracts (Fig. 10.2).

Figure 10.2 RQflex Pi vs Technicon Pi



Conclusions

The Reflectoquant system provides a convenient portable method for measuring phosphate in plants. There was a direct linear response up to 60 mg/l Pi (0.66 mM Pi) which typically would require plant tissue water to be diluted 30 times. The RQflex compared favourably with the laboratory-based Technicon system.

10.3 Cardy K meter

Introduction

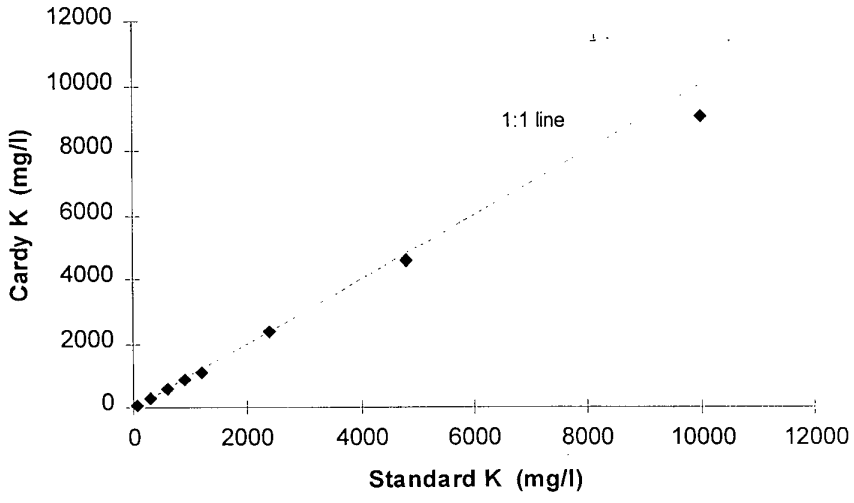
The Cardy K meter (manufactured by Horiba, Japan and supplied by Spectrum Technologies Inc. of the USA) is a compact credit-card sized meter for

measuring K^+ ions in solution. The meter consists of a miniaturised ion-specific electrode sensitive to K^+ ions and a voltmeter with LCD digital display. The meter has three measuring ranges (resolution in brackets): 0-100 (1), 100-1,000 (10) and 1,000-10,000 (100) mg/l K, equivalent to 0-2.6 (0.026), 2.6-25.6 (0.26) and 25.6-256 (2.56) mM K. The manufacturers claim a linear working range of 39-3,900 mg/l (1-100 mM). The range of K in the tissue water of field-grown wheat is likely to be 50-300 mM, consequently samples would need to be diluted three times to bring concentrations into the working range of the instrument. No information was available on the potential effects of interfering ions.

Calibration

Standard K solutions ranging from 0 to 10,000 mg/l K (0 to 256 mM) were prepared from oven-dried potassium chloride (KCl, Analar). The calibration curve was linear in the guaranteed range (Fig. 10.3). The Cardy readings could be volatile especially at high K concentrations and stabilisation could take up to two minutes.

Figure 10.3 Cardy standard curve for K



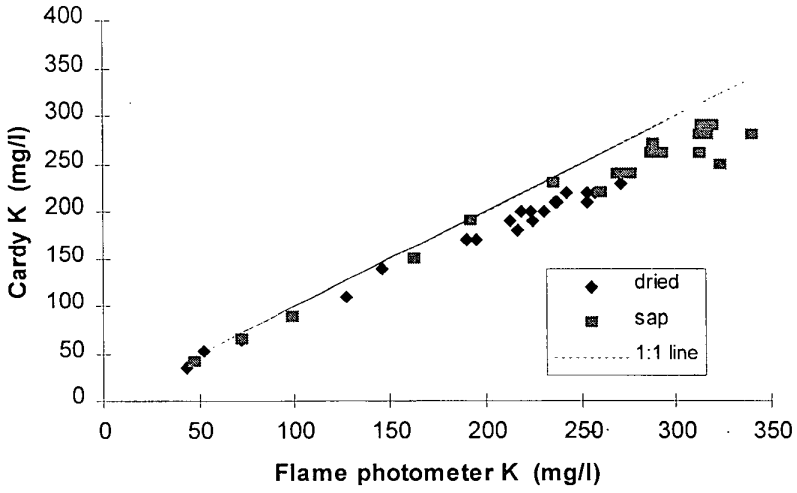
Reproducibility

Potassium standards of 300 and 400 mg/l and a sample of diluted plant tissue water were each measured ten times using the Cardy meter. The 300 mg/l standard measured 290 ± 6 mg/l (mean ± sd), the 400 mg/l standard measured 404 ± 8 mg/l, and the tissue water sample 185 ± 5 mg/l.

Cardy vs. Flame photometer

Several comparisons between the Cardy meter and a flame photometer (Jenway, PFP7) were made using water extracts of dried plant material and pressed tissue water from frozen/thawed material. The flame photometer is linear in the range 0-5 mg/l (0-0.13 mM), consequently solutions measured on the flame photometer had to be diluted 1,000 times more than for the Cardy meter. Readings from the two instruments were highly correlated (Fig. 10.4), but Cardy readings were less than the flame readings by 10% above 200 mg/l.

Figure 10.4 Cardy K vs Flame photometer K



Conclusions

The Cardy meter provided a quick and simple method for the determination of K concentration. The meter gave a linear response over a wide range of standard K solutions (1-100 mM), but underestimated K concentrations in tissue water by

up to 10% compared with the flame photometer, although the results were highly correlated.

10.4 Factors affecting on-farm determination of Pi and K

Introduction

The first problem in carrying out an *in situ* test is to obtain a representative sample of tissue water. Unlike conventional sap testing which involves petioles and stems which are naturally 'sappy', the 'wet' tests in the current project involved leaf blades and whole shoots, and less was known about the extractability of water from these plant parts. A series of experiments was carried out to identify the best way of extracting a representative sample of tissue water either from leaves (for the Kw test) or shoots (for the Piw test).

1. NUTRIENT EXTRACTION METHOD

Five methods of extracting Pi and K from plants were assessed:

A Microwave dry, mill, extract with 2% acetic acid (standard lab method for Pi)

B Oven dry (80°C), mill, extract with 2% acetic acid (standard lab method for K)

C Freeze (-18°C for 1 h), thaw at room temperature, extract tissue water by screw press

D Freeze (-18°C for 48 h), thaw at room temperature, extract tissue water by screw press

E Extract tissue water from fresh material by screw press

Methods

Shoots were cut from a plot (Kex 99 mg/kg) of Sawyers(III) at GS 30 in 1997. The sample was well mixed (but not chopped) then divided into five subsamples. Ten replicates (10 g) were assessed by each method. In methods A and B, tissue water content was determined from fresh and dry weights. In methods C, D and E, tissue water was extracted using a bench-mounted screw press constructed in the Rothamsted workshops. The press had a 10 cm handle

and was fully tightened by hand. Acetic acid extracts and pressed tissue water were analysed for Pi (RQflex) and K (Cardy) after appropriate dilution.

Results

The highest mean Pi concentration, 16.5 mM, was obtained by conventional oven drying (Table 10.1). This was more than twice the mean 7.5 mM obtained by microwave drying. Oven drying enhances the enzyme-mediated transformation of phosphate esters to Pi in the early stages of drying (Bollons & Barraclough, 1997), whereas rapid drying in a microwave oven minimises the transformations.

Table 10 .1 Shoot Piw extracted by different methods

Shoot Piw (mM)					
Rep	A Microwave	B Oven	C Freeze(1)	D Freeze(48)	E Fresh
1	5.5	15.1	9.5	8.2	4.1
2	6.9	17.3	9.7	7.8	4.2
3	7.4	15.6	10.7	10.1	5.2
4	7.2	14.1	10.7	8.4	4.9
5	5.7	15.6	9.3	9.5	3.4
6	8.7	18.8	8.2	10.3	4.7
7	9.5	16.7	10.7	10.1	3.7
8	9.3	15.1	11.4	11.2	4.0
9	7.9	19.0	11.2	11.8	3.6
10	6.6	17.1	11.2	14.1	5.1
Mean	**7.5**	**16.5**	**10.3**	**10.1**	**4.3**
P<0.001, df = 45, sed = 0.62					

Freeze/thaw resulted in significantly higher Pi values (c.10 mM) than microwave drying, probably because transformations occurred during thawing. All samples were taken out of the freezer at the same time and thawed at room temperature, and it was at least two hours before the last sample was analysed. A trend of increasing Pi is obvious between replicates 1 to 10 (Table 10.1). There was no effect of prolonging the freezing period to 48 h. In a further experiment, no changes in Pi and K in shoots were found after three months storage at -18°C.

It was very difficult to extract tissue water from fresh plant material. Only about 10% of the total water could be extracted compared with about 50% from frozen/thawed material. Pi in tissue water pressed from fresh material (4.3 mM) was considerably lower than that obtained by any other method. It is probable that tissue water was preferentially extracted from the vascular tissues of fresh material, i.e. true sap was preferentially extracted.

The greatest mean K concentrations (163 mM) were obtained by drying, but the method of drying was unimportant (Table 10.2). Potassium concentrations from frozen/thawed samples (138-147 mM) were about 13% less than from dried material. Potassium in tissue water from fresh material (84 mM) was considerably lower than by all other methods.

Conclusions

Concentrations of Pi and K from fresh material were about half those by other methods. It was difficult to press tissue water from fresh material and the fraction obtained was probably vascular water (i.e. true sap) and not representative of the tissue water as a whole. To get a representative sample of tissue water, the cells had to be ruptured by freezing and thawing. The duration of freezing was not important, but the freeze/thaw method resulted in higher Pi concentrations than microwave drying and lower K concentrations than oven drying. In the case of Pi, this was probably because enzymic transformations increased Pi during the prolonged thawing period. Microwave drying resulted in much lower Pi concentrations than oven drying but had no effect on K concentrations.

Table 10.2 Shoot Kw extracted by different methods

Shoot Kw (mM)					
Rep	A Microwave	B Oven	C Freeze(1)	D Freeze (48)	E Fresh
1	168	178	141	133	99
2	160	169	156	141	79
3	171	161	149	149	87
4	156	157	156	133	87
5	169	164	149	133	72
6	170	164	133	141	87
7	159	159	138	149	79
8	167	162	138	146	82
9	157	161	151	138	81
10	151	156	156	121	88
Mean	**163**	**163**	**147**	**138**	**84**
P<0.001, df =45, sed = 3.4					

2. PLANT PART

Methods

A further comparison of extraction methods was made on different plant parts. Potassium was used in preference to Pi to eliminate complications due to transformations between organic and inorganic forms. Glasshouse-grown spring wheat (cv. Avans) harvested at GS 39 was used for this experiment. Subsamples (2-4 g) of whole shoots, leaves and stems were taken. There were three treatments and five replicates. Samples were either dried at 80°C and extracted with water, frozen/thawed/pressed, or pressed fresh. The screw press was fully tightened by hand. Potassium was analysed by flame photometer.

Results

Table 10.3 Effect of extraction method on Kw in different plant parts

Kw (mM)									
Rep	Shoots			Stems			Leaves		
	Dried	Frozen	Fresh	Dried	Frozen	Fresh	Dried	Frozen	Fresh
1	257	235	119	232	248	173	248	278	113
2	244	231	154	240	311	124	219	255	88
3	239	252	152	238	243	166	266	243	117
4	244	247	125	242	260	160	250	252	121
5	240	255	149	255	252	145	259	267	157
mean	**245**	**244**	**140**	**241**	**263**	**154**	**249**	**259**	**119**
	P<0.001, df = 12 sed = 7.6			P<0.001, df = 12 sed = 12.7			P<0.001, df = 12 sed = 12.2		

Potassium concentrations in shoots, stems and leaves were not significantly different in frozen/thawed and dried materials (in fact concentrations in frozen/thawed stems and leaves were higher, the opposite of what was found in the previous experiment). Concentrations were considerably less in tissue water pressed from fresh material of all plant parts (Table 10.3).

Conclusions

This experiment confirmed the difficulty of extracting a representative sample of tissue water from all plant parts by pressing fresh plant material. The frozen/thawed samples had similar K concentrations to dried samples.

3. PROPORTION OF TISSUE WATER EXTRACTED

Methods

Two experiments were carried out using field or glasshouse plants. In the field, winter wheat shoots were cut at GS 41. In the glasshouse, spring wheat (cv. Avans) shoots were cut at GS 39. Subsamples of whole shoots, stems and leaves were taken. There were 3 replicates in the field and 5 in the glasshouse studies. Samples were either frozen/thawed/pressed or pressed fresh. The screw press was fully tightened by hand. Samples were weighed before and after pressing, and the pressed material dried at 80°C so that the percentage of total tissue water extracted could be calculated.

Results

For the field plants, the percentage of total tissue water pressed from fresh shoots, stems and leaves was in the range 4-8% compared with 50-57% from frozen/thawed material (Table 10.4). Equivalent values for glasshouse plants were higher at 11-18% and 55-68%, respectively. Differences between the field and glasshouse were probably due to differences in shoot tissue water contents (81% for the field compared with 85% for the glasshouse), and possibly to different amounts of material being pressed (10 g for the field compared with 2-4 g for the glasshouse).

Table 10.4 Percent of total tissue water pressed from plant material

Percent of tissue water extracted						
Expt.	Shoots		Stems		Leaves	
	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh
Field	50	8	50	7	57	4
Glass	68	12	55	18	61	11

4. AMOUNT OF PLANT MATERIAL

Methods & Results

Spring wheat shoots (GS 39). Screw press fully hand tightened.

For fresh material, 22% of the water was extracted from 2 g and 9% from 10 g.

For frozen/thawed material, 52% was extracted from 2 g and 64% from 10 g (Table 10.5).

Table 10.5 Effect of sample weight on percent tissue water extracted

Weight of fresh plant material (g)	Total water (g)	Tissue water extracted	
		Fresh	Frozen
2	1.8	22%	52%
5	4.4	17%	58%
10	8.6	9%	64%

5. DURATION OF THAWING

Methods

Two experiments were conducted on shoots and leaves. Shoots were collected from a high P plot (Olsen 35 mg/kg) at GS 39. The sample was chopped into 2 cm pieces, mixed, sealed in a plastic bag and frozen at -18°C. Leaves were collected, mixed and frozen. Three replicate subsamples (5 g) were taken from the freezer and pressed 0, 0.25, 0.5, 1, 2 and 4 hours after removal from the freezer. Samples were fully thawed after about 30 minutes at room temperature, though still cold to touch. Tissue water was pressed from the shoots using hand pressure and from leaves at a torque of 30 Nm. Tissue water was analysed for Pi by RQflex and K by Cardy after suitable dilution.

Results

Shoot Pi was reasonably constant at about 4 mM for up to 30 minutes of thawing time, but increased thereafter to 9 mM after 4 hours (Fig. 10.5a). There was a gradual increase in Pi in leaves, from 20 to 28 mM, with thawing time (Fig. 10.5b).

Figure 10.5(a) Changes in shoot Pi with thawing time

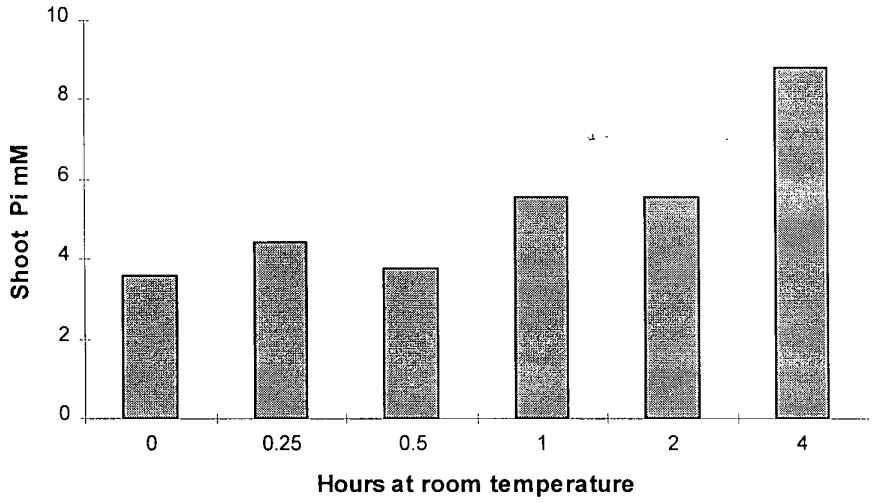
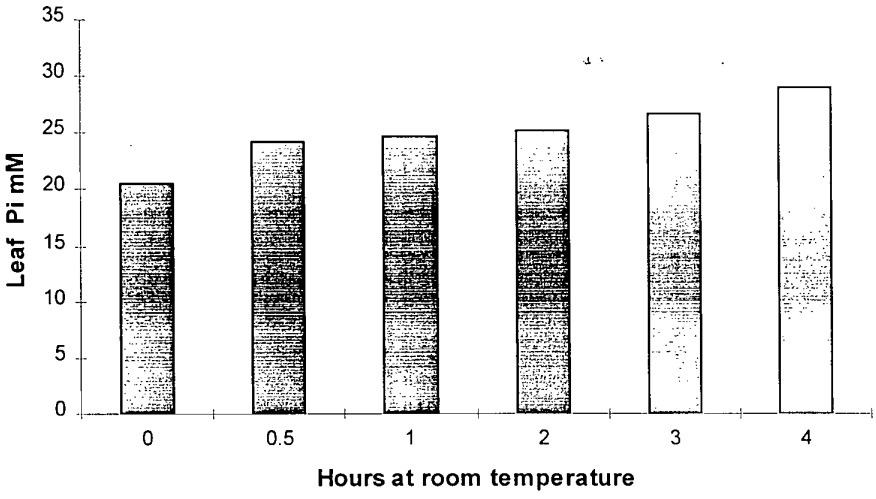


Figure 10.5(b) Changes in leaf Pi with thawing time



There were no significant differences in K concentrations in shoots or leaves with thawing time (Fig. 10.6a,b). Samples which were pressed whilst still frozen had lower Pi and K concentrations than those pressed after 30 minutes of thawing (though differences were not statistically significant), which suggested

that a representative sample of tissue water cannot be pressed from material that is still frozen.

Figure 10.6(a) Changes in shoot K with thawing time

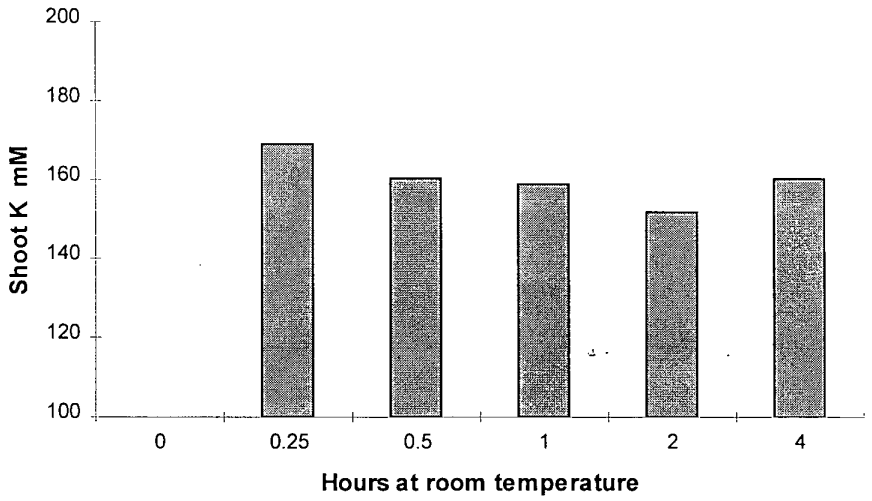
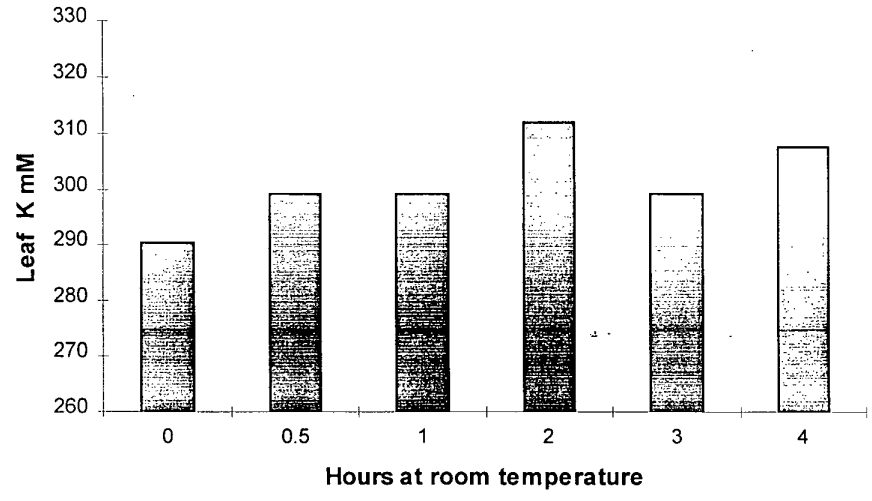


Figure 10.6(b) Changes in leaf K with thawing time



Conclusions

Frozen samples must be thawed completely before pressing and tissue water should be analysed for Pi within 30 minutes of the sample being removed from the freezer.

6. EFFECT OF PRESSURE

Introduction

Increasing the pressure on a sample increased the amount of tissue water extracted. A series of experiments was conducted to see if the fractions of tissue water pressed from frozen/thawed samples at different pressures were representative of the tissue water as a whole with respect to Pi and K concentrations.

Expt. 1 Effect of high pressures (torque) on K in leaves

Methods

Spring wheat leaves from the glasshouse at GS 39. Frozen/thawed samples, 1-2 cm pieces, 4 x 5 g replicates per treatment. Tissue water was extracted using the screw press tightened with the standard 10 cm lever (manual treatment) or with a torque wrench (10 to 50 Nm). Fresh samples were also oven-dried and extracted with water. Potassium was determined by flame photometer.

Results

The percentage of tissue water extracted increased from 55 to 70% with increasing torque (Table 10.6). Manual tightening released 59% of the water, equivalent to a torque of about 15 Nm. There was no effect of torque on K concentration with a mean of 182 mM, some 15% less than the value for oven-dried material (215 mM).

Torque and pressure are not related in a simple way because of frictional forces in the screw press. In the present system, 10 Nm of torque was calculated to be equivalent to about 15 MPa (about 150 atmospheres) of pressure assuming no friction (i.e. 100% mechanical efficiency). Assuming a more realistic efficiency of 33% leads to a calculated pressure of 5 MPa which is still very large. What happens if much smaller pressures are applied?

Table 10.6 Effect of torque on % water extracted and K in leaves

Torque (Nm)	K (mM)	% water extracted
10	180	55
20	185	64
30	184	63
40	179	66
50	182	70
Manual	186	59
Oven-dried	215	100
	P<0.001, df = 22 sed = 4.2	P<0.001, df = 18 sed = 2.4

Expt. 2 Effect of low pressures on K in leaves

Methods

Winter wheat leaves. Frozen/thawed samples, 1-2 cm pieces, 3 x 5 g replicates per treatment. Low pressures were obtained by applying weights (4-80 kg) to the 22 cm^2 cell (equivalent to pressures of 20-370 kPa) until tissue water ceased to flow (after about 5 minutes). Potassium analysed by Cardy.

Results

At least 20 kPa (0.2 atmospheres) was needed to extract tissue water (20%). At maximum pressure (370 kPa), about 50% of tissue water was extracted which is approaching that obtained at 10 Nm torque. Potassium concentrations were not affected by the pressure applied (Fig 10.7).

Expt.3 Comparison of low and high pressure for K in leaves

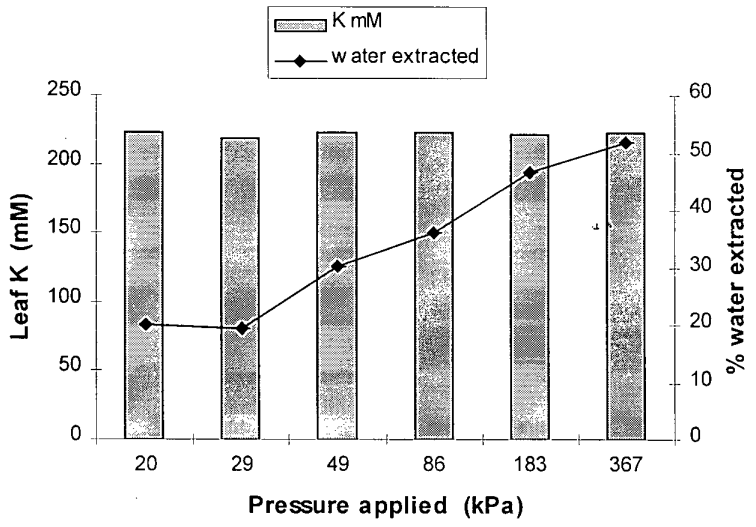
Methods

Winter wheat leaves from Sawyers(III) in May 1998. Frozen/thawed samples, 1-2 cm pieces, 5 x 5 g replicates per treatment. Low pressure was 50 kPa, high pressure was a torque of 40 Nm (c. 20 MPa). Potassium analysed by Cardy.

Results

On average, 61% of the total tissue water was extracted at high pressure compared with 17% at low pressure. The difference in mean K concentrations, 202 mM at high pressure and 196 mM at low pressure, was not significant. This was an important experiment as it showed that pressure and the percentage of tissue water extracted, at least in the range 20-70%, had no affect on K concentrations in frozen/thawed plant material.

Figure 10.7 Leaf K and water extracted under different pressures



Expt. 4 Extraction of tissue water with a syringe and K in leaves

Methods

Spring wheat leaves from the glasshouse at GS 39. Three treatments: (1) frozen/thawed hand-pressed in a 50 ml syringe, (2) frozen/thawed pressed in the screw press (manual), and (3) microwave-dried and extracted with water. Samples were 1-2 cm pieces, 10 x 5 g replicates per treatment. Potassium was determined by Cardy.

Results

Some 43% of the tissue water was extracted by the syringe compared with 68% by the screw press. Potassium concentrations were 208, 229 and 213 mM (LSD (5%): 10mM) for syringe, press and dry methods, respectively.

Expt. 5 Effect of low pressures on Pi in leaves

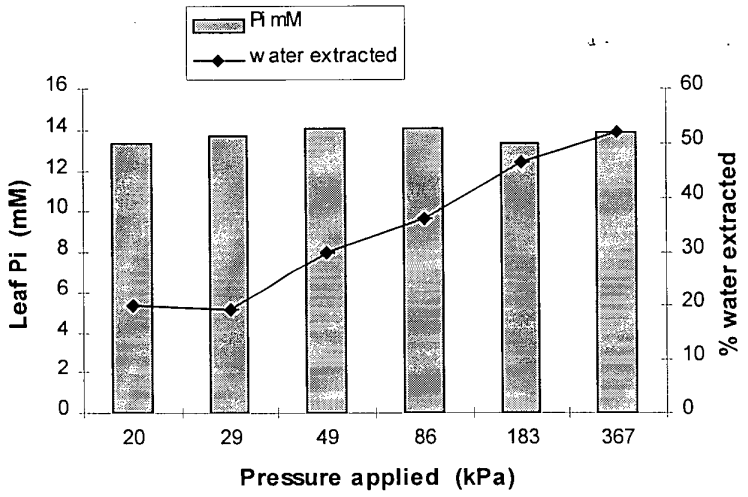
Methods

Winter wheat leaves. Frozen/thawed samples, 1-2 cm pieces, 3 x 5 g replicates per treatment. Low pressures were obtained by applying weights (4-80 kg) to the 22 cm^2 cell (equivalent to pressures of 20-370 kPa) until tissue water ceased to flow (after about 5 minutes). Phosphate analysed by RQflex.

Results

At least 20 kPa (0.2 atmospheres) was needed to extract tissue water (20%). At maximum pressure (370 kPa) about 50% of water was extracted which is approaching that obtained at 10 Nm torque. Phosphate concentrations were not affected by the pressure applied (Fig 10.8).

Figure 10.8 Leaf Pi and water extracted under different pressures



Expt. 6 Comparison of low and high pressure for Pi in shoots (1)

Methods

Winter wheat shoots from Exhaustion Land at GS 32 in 1998. Frozen/thawed shoots cut into 2 cm pieces before subsampling. 5 x 5 g replicates per treatment. Low pressure was 25 kPa, high pressure was torque of 30 Nm (c. 15 MPa). Phosphate analysed by RQflex.

Results

The percentage of tissue water extracted was 65% and 28%, and Pi concentrations were 7.0 and 5.9 mM at high and low pressures, respectively. Both differences were significant ($P < 0.05$). However, there was a wide variation in Pi in individual samples and on closer inspection it was clear that the low pressure samples contained a preponderance of stems whilst high pressure samples contained a preponderance of leaves. This was despite efforts to ensure good mixing of the subsamples. As leaves and stems generally differ in Pi concentration, incorrect mixing could have led to biased results.

Expt. 7 Comparison of low and high pressure for Pi in shoots (2)

Methods

Experiment 6 was repeated, but the shoots were chopped up after subsampling. Winter wheat shoots were from a plot (Kex 135 mg/kg) on Sawyers(III) at GS 41 in 1998. The sample had been frozen in a sealed polythene bag for 5 months. Whole shoots were subsampled (2 whole stems) to preserve the correct ratio of stem to leaf material. 8 x 10 g replicates per treatment. Low pressure was 50 kPa, high pressure was a torque of 40 Nm (c. 20 MPa). Phosphate analysed by RQflex.

Results

Again, significantly different amounts of tissue water were extracted. On average, 21% was extracted under low pressure compared with 65% at high pressure. The Pi concentrations in the tissue water from the two pressure treatments were significantly different ($P < 0.001$), but the difference was small. Low pressure extracts had a mean Pi concentration of 8.7 mM compared with 8.2 mM under high pressure (Table 10.7).

Table 10.7 Effect of pressure on % water extracted and Pi in shoots

Rep	High pressure		Low pressure	
	Pi (mM)	% water	Pi (mM)	% water
1	10.1	70	8.8	24
2	4.6	65	7.6	25
3	8.4	66	8.4	18
4	6.7	68	8.8	23
5	10.1	66	8.8	22
6	5.9	60	7.6	21
7	9.7	64	9.3	19
8	9.7	64	10.1	18
Mean	**8.2**	**65**	**8.7**	**21**
			Pi: P<0.001, df =14 sed = 1.4	

Expt. 8 Extraction of tissue water with a syringe and Pi in shoots

Methods

Spring wheat shoots and leaves from the glasshouse at GS 39. Three treatments: (1) frozen/thawed hand-pressed in a 50 ml syringe, (2) frozen/thawed pressed in the screw press (30 Nm), and (3) microwave-dried and extracted with water. Samples were 1-2 cm pieces, 10 x 5 g replicates per treatment. Phosphate was determined by RQflex.

Results

In leaves, 43% of the tissue water was extracted by the syringe compared with 68% by the screw press. Equivalent values for shoots were 46% and 63%, respectively. For a given organ, there were no significant differences in Pi concentrations (Table 10.8).

Table 10.8 Effect of extraction method on Pi (mM) in shoots and leaves

Part	Syringe	Press	Dried
Leaves	17.6	18.4	18.2
Shoots	16.4	16.4	16.8

10.5 On-farm vs. laboratory at Rothamsted

1. SHOOT Pi

Methods

Whole shoots, excluding dead leaves, were cut from random positions in plots of Exhaustion Land at GS 32 in 1998. For the laboratory procedure, a 30 g subsample from each plot was dried in a microwave-oven followed by a further 4 h at 80 °C. Dried material was milled (<1 mm) and Pi extracted with distilled water. A second 30 g subsample, for the on-farm procedure, was sealed in a plastic bag and frozen in a domestic deepfreezer. The samples remained in the freezer for five months. Five shoots were selected, thawed, chopped into 1-2 cm pieces and pressed by hand in a 50 ml plastic syringe. The pressed tissue water (TW(fz)) and water extracts (TW(dr)) were diluted as required and analysed for Pi by the RQflex and Skalar laboratory systems.

Results

There was good agreement between on-farm and laboratory procedures with no significant deviation from the 1:1 line (Fig. 10.9). However, the close agreement was fortuitous as there were differences between tissue water and dried extracts for a given instrument (Fig. 10.10), and between RQflex and Skalar for a given extract (Fig. 10.11). The occasional underperformance of the RQflex, with readings up to 2 mg/l below standards, had already been noted in Section 10.2. The reasons for this are unknown, but strip age may be responsible. It appears strip performance can decline during storage even when the strips are within the manufacturer's recommended date. A 2 mg/l difference is equivalent to 0.7 mM in tissue water diluted 30 times, similar to the difference in Fig. 10.11. The slightly higher readings in tissue water compared with dried extracts for a given

instrument (Fig. 10.10) were most likely due to increases in Pi by enzymic action during thawing. Samples were processed as rapidly as possible, but on average the whole procedure took 45 mins per sample, rather longer than the 30 min guideline previously established as desirable. Samples had also been stored frozen for 5 months which might have contributed to the differences.

Figure 10.9 Comparison of *in situ* (TW(fz)/RQflex) and laboratory (TW(d)/Skalar) methods for shoot Pi

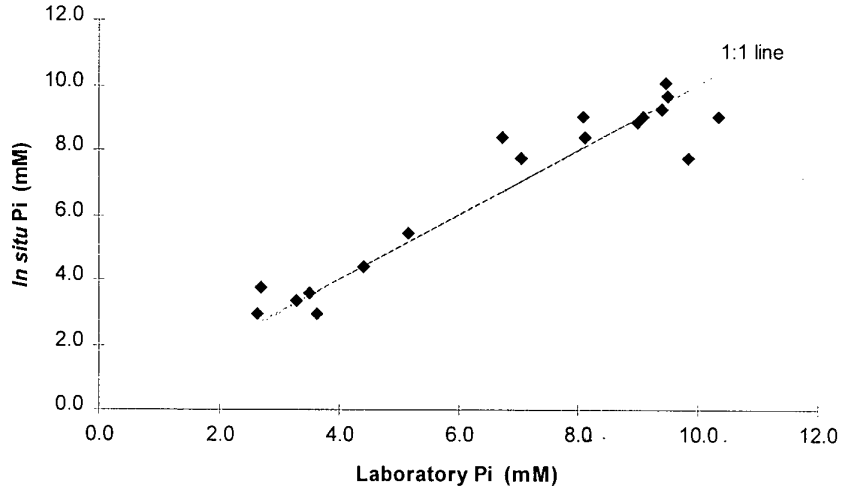


Figure 10.10 Comparison of Pi in tissue water extracts measured by RQflex

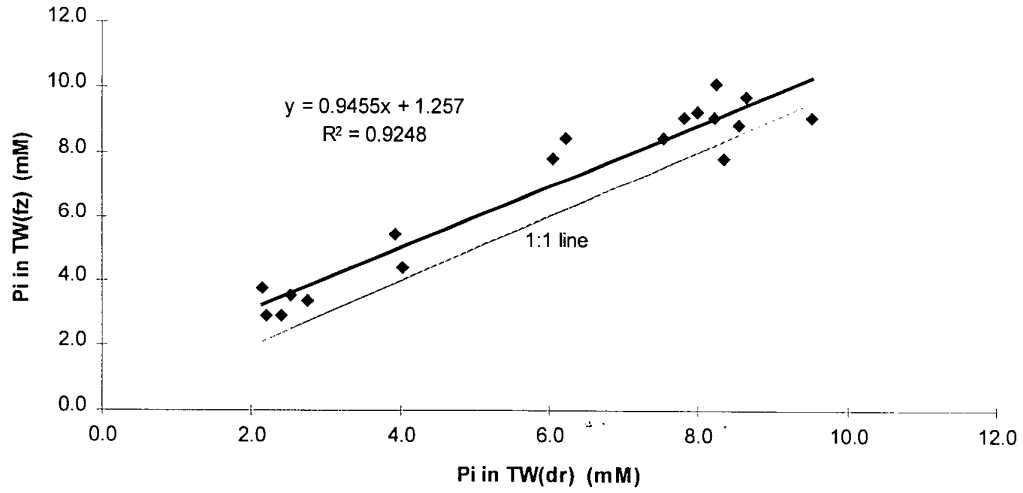
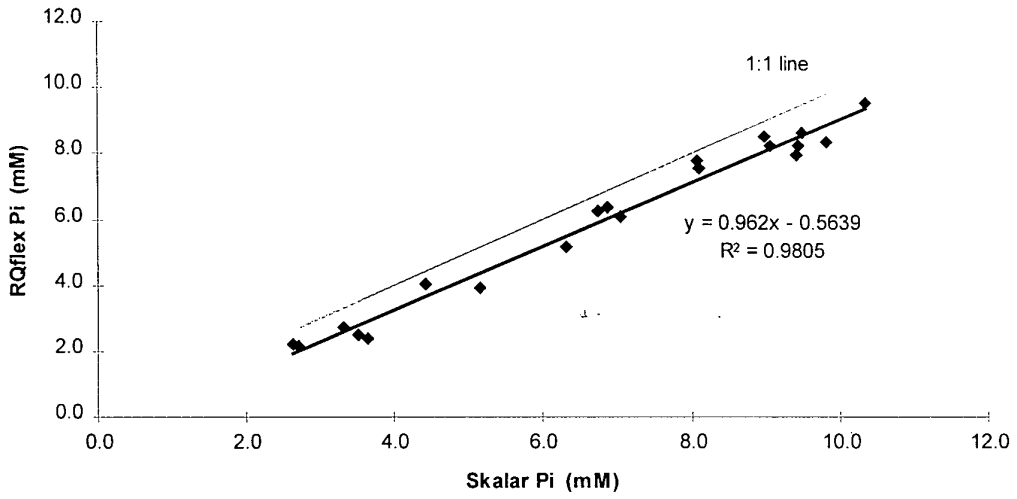


Figure 10.11 Comparison of RQflex and Skalar for measuring Pi in dried TW extracts



Conclusions

For Pi concentrations determined on-farm to be comparable with those obtained by the standard microwave drying method, it is crucial that tissue water is pressed from fully thawed material and analysed within 30 minutes of removing a sample from the freezer. Ideally, new RQ strips should be used for the analysis. Strips should always be checked against standard solutions before use even if they are within the manufacturer's use-by date.

2. LEAF K

Methods

Whole shoots, excluding dead leaves, were cut from random positions in plots of Sawyers(III) at GS 32 in 1998. For the laboratory procedure, mainshoots were selected at random and leaf(1) removed. Leaves were dried at 80°C overnight, milled (<1 mm) and K extracted with distilled water. A second sample of leaves, for the on-farm procedure, was sealed in a plastic bag and frozen in a domestic deepfreezer for five months. Frozen samples were thawed, chopped into 1-2 cm pieces and tissue water pressed using a 50 ml plastic syringe. The pressed

tissue water and water extracts were diluted and analysed for K by flame photometer and Cardy meter.

Results

The on-farm method (Frozen/Cardy) underestimated K concentrations compared with the laboratory method (Dried/Flame) (Fig. 10.12). For a laboratory value of 200 mM, on-farm K averaged 156 mM (78%). Differences between extraction methods and instruments no doubt contributed to this overall difference. When analysed by Flame photometer for example, K in frozen extracts averaged 176 mM (88%) compared with a dried extract value of 200 mM (Fig. 10.13). Similarly, when dried extracts were analysed, Cardy K averaged 174 mM (87%) compared with a Flame K value of 200 mM (Fig. 10.14) (see also Section 10.3).

Figure 10.12 Comparison of *in situ* (TW(fz)/Cardy) and laboratory (TW(dr)/Flame) methods for K

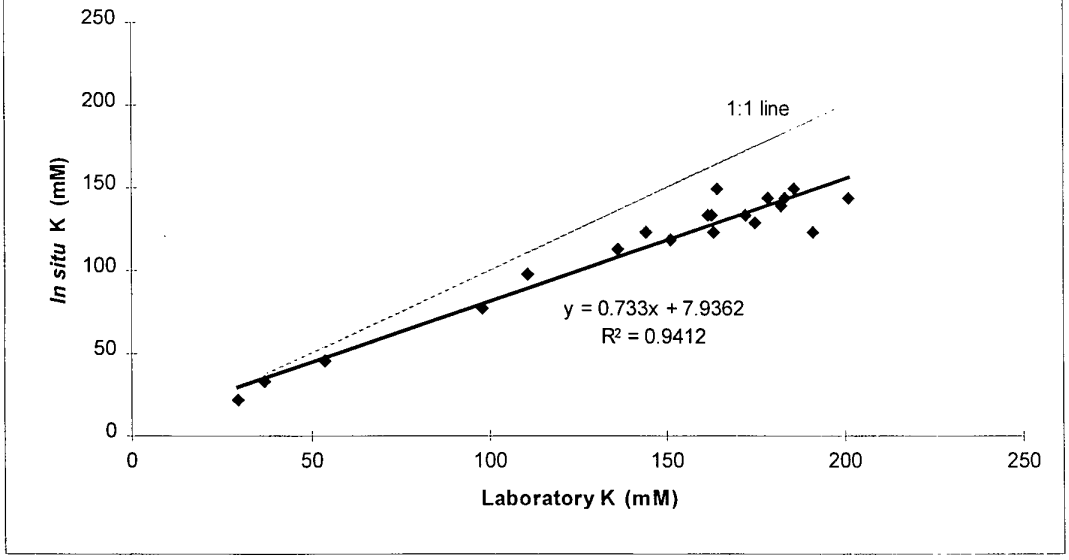


Figure 10.13 Comparison of TW extracts of K measured by Flame photometer

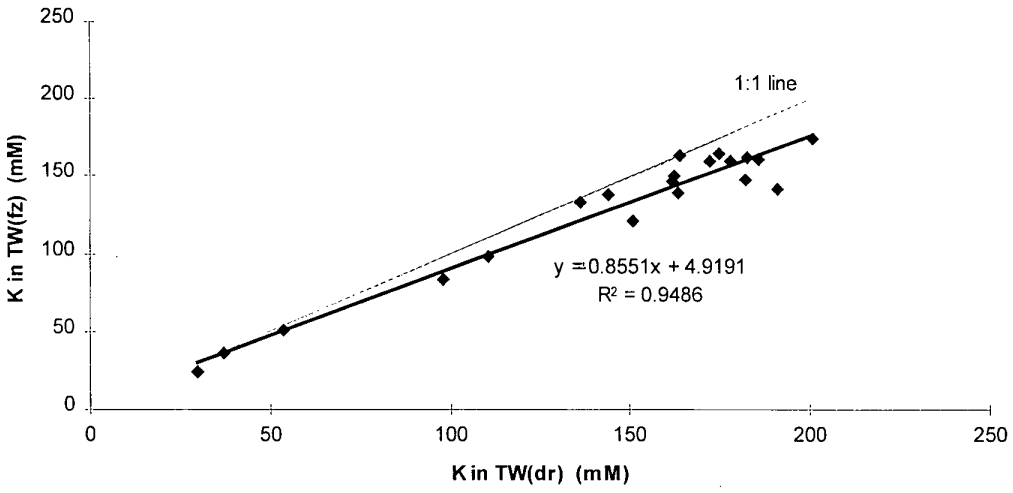
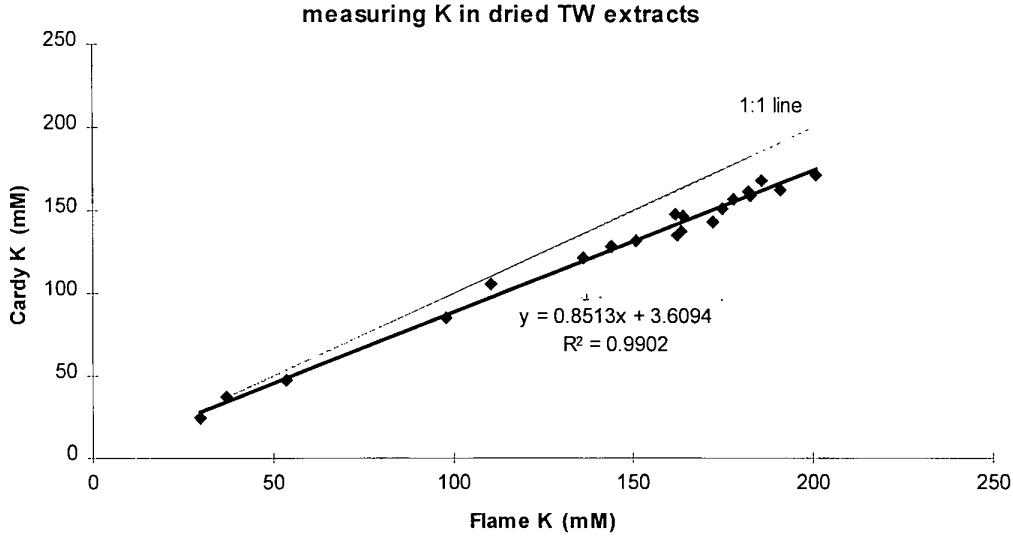


Figure 10.14 Comparison of Cardy meter and Flame photometer for measuring K in dried TW extracts



Conclusions

There were unresolved discrepancies between the Cardy meter and Flame photometer for the analysis of K in tissue water, with the Cardy meter giving lower readings than the Flame photometer. The reasons for this are unknown, but could be due to interfering ions present in plant tissue water. As well as differences between analytical instruments, there appear to be fundamental

differences in the amount of K that can be extracted by different methods. Tissue water from frozen samples nearly always had lower K concentrations than water extracts from dried material. This may be due to K in cell walls not being released in pressed tissue water.

10.6 On-farm vs. laboratory by ADAS

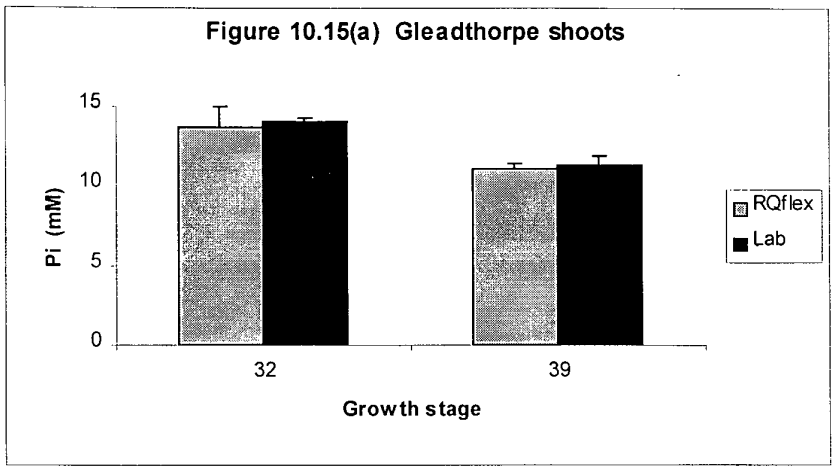
Methods

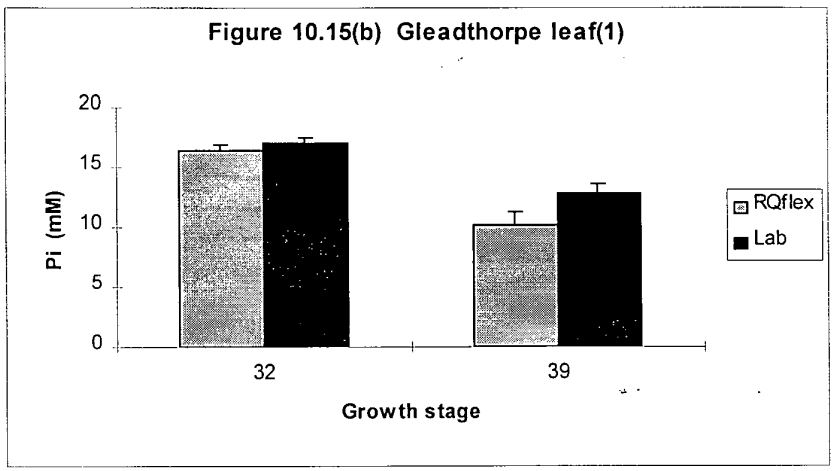
On-farm methods of measuring Pi and K in plants developed by Rothamsted were tested at Gleadthorpe and Ropsley. The techniques required extraction and dilution of tissue water, followed by analysis for Pi by RQflex and K by Cardy meter. The RQflex was also used to measure K. Extraction of tissue water was achieved by freezing a sample of shoots or leaves, chopping into 1 cm pieces (larger pieces proved difficult to extract), thawing and squeezing out the tissue water in a 50 ml syringe. The first set of samples from Gleadthorpe (GS 30) were frozen in the syringes. However, this resulted in uneven defrosting of the sample, which was unsatisfactory as tissue water was extracted preferentially from those plant parts which defrosted the quickest. Subsequently, samples were frozen in plastic bags then chopped and transferred to syringes on defrosting.

The performance of the on-farm methods was assessed by comparison of the full data set at each growth stage with results from the standard laboratory analysis of plant material using t tests (ie. ignoring any treatment effects). The pressed tissue water was also analysed by standard laboratory methods.

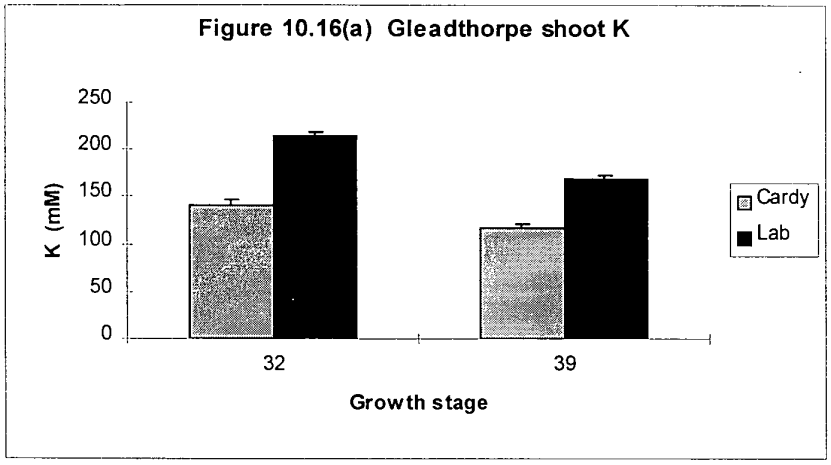
1. GLEADTHORPE

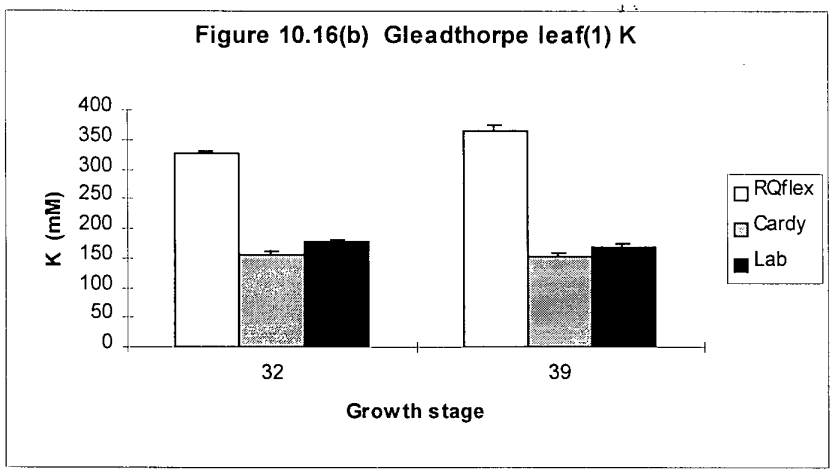
At GS 32 and 39, whole shoot Pi concentrations measured by the RQflex were similar to those determined by the laboratory method (Lab) ($P>0.05$; Fig. 10.15a). For the newest leaf samples, the RQflex measured similar Pi concentrations to the Lab at GS 32 ($P>0.05$), but at GS 39 RQflex concentrations were 2 mM lower on average ($P<0.05$; Fig. 10.15b).





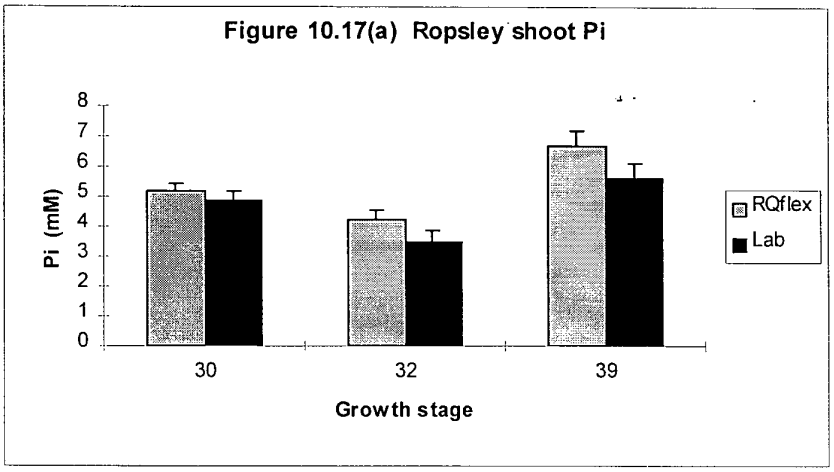
Whole shoot K concentrations measured by the Cardy meter were significantly lower than by the Lab method at both growth stages by about 50 mM ($P<0.001$; Fig. 10.16a). Better agreement was obtained with leaves although Cardy values were still about 20 mM lower ($P>0.05$; Fig. 10.16b). The RQflex measured much higher K concentrations than either the Cardy or Lab at both growth stages ($P<0.001$; Fig. 10.16b).

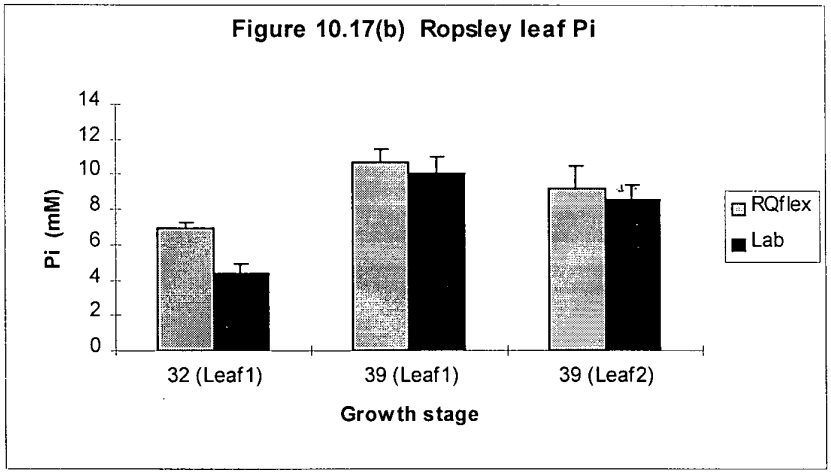




2. ROPSLEY

The Pi results from Ropsley were more variable than those from Gleadthorpe, with the RQflex consistently measuring higher Pi concentrations than the lab method in all plant parts and on virtually all occasions, except for those measured in whole shoots at GS 30 and leaf(1) at GS 39 ($P<0.05$; Figs. 10.17a,b). In shoots, average differences were small at 1.1 mM, although differences in individual samples of up to 2 mM at GS 30 and 32, and up to 4 mM at GS 39 were observed. Differences were greater in the newest leaf (Fig. 10.17b), with the RQflex measuring up to 6 mM more Pi than the lab in individual cases. The results generally support those obtained from Gleadthorpe, which suggests the RQflex could be used as an alternative to lab techniques for the measurement of Pi concentrations in whole shoots.





The Cardy meter measured consistently lower Kw concentrations than the Lab in all plant parts and on all occasions ($P<0.05$; Figs. 10.18a,b). Differences in individual samples were up to 65 mM in whole shoots, 100 mM in leaf(1) and 140 mM in leaf(2). The RQflex was used to determine K concentrations in leaf(1) and leaf(2) at GS 39, where it was in reasonable agreement with the Cardy meter. The Cardy meter (and RQflex) consistently underestimated plant K concentrations, by amounts that were considerably larger than those observed at Gleadthorpe, which would significantly affect the assessment of K concentrations. Strong acid digestion was used as the standard laboratory extractant for determination of K in plant material, with the results expressed on a tissue water basis. This may therefore have included extraction of K not

contained in the tissue water (although this would also have been the case at Gleadthorpe).

Figure 10.18(a) Ropsley shoot K

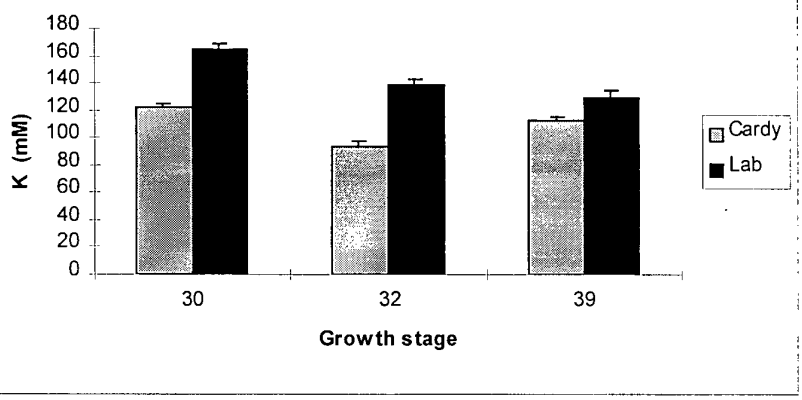
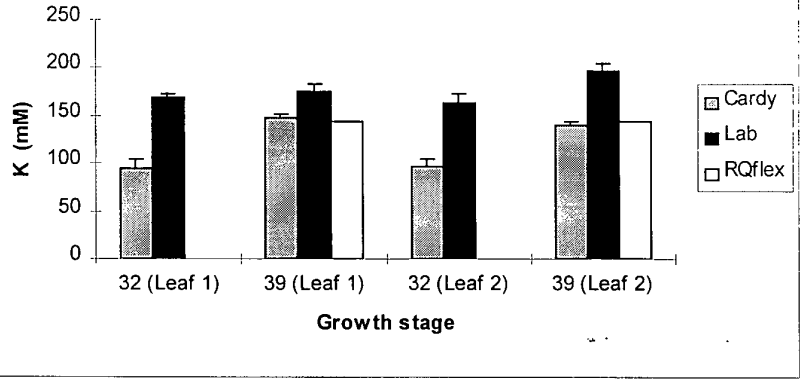


Figure 10.18(b) Ropsley leaf K



Conclusions

On-farm analysis of Pi in tissue water by the RQflex was generally comparable to the standard laboratory method, although differences were evident in some situations. The RQflex was not suitable for the measurement of K in tissue water, although performance with standard K solutions suggested it was measuring accurately. It was disappointing that the two nutrients could not be accurately analysed by the same instrument, as the purchase of two pieces of equipment is less attractive. The RQflex procedures for both Pi and K were quite laborious, involving different dilutions of the tissue water samples for each nutrient. The Cardy ion meter, by comparison, was much easier to use, with a

much wider measurement range and therefore more attractive as an *in situ* tool, although dilution of the tissue water was still necessary. However, K concentrations were underestimated by the Cardy compared with the standard laboratory method.

10.7 Conclusions

The RQflex generally performed well against KH_2PO_4 standards with a linear response to Pi up to 60 mg/l. Plant tissue water would typically need to be diluted 30 times to bring Pi into this range. There was good agreement between the RQflex and the standard laboratory instruments (Technicon) for analysing Pi in a given type of extract.

The Cardy performed well against KCl standards with a linear response up to 4,000 mg/l K. Tissue water would typically need to be diluted 3 times to bring K into this range. The Cardy underestimated the standard laboratory instrument (Flame photometer) for all types of extract, possibly due to interference by other ions.

Pressing tissue water from fresh plant material was not easy. Yields were poor and Pi and K concentrations were only half those obtained with microwave-dried or frozen extracts irrespective of the organ or pressure applied. It is likely that water was only being expressed from vascular tissue. To obtain a sufficient and representative tissue water sample by pressing, it was necessary to rupture plant cells by freezing and thawing them. The duration of freezing was not important and, once frozen, samples could be stored for 3 months and maybe longer. Samples must be fully thawed (no ice) before pressing. With frozen/thawed samples, any low pressure above about 0.2 atmospheres was adequate and hand-pressed plastic syringes were successfully used in the present work.

Representative sampling of plant material at field level is obviously important, but correct subsampling especially of shoots was equally important as Pi and K concentrations differ between organs. Shoots should be subsampled whole to maintain the correct proportion of stems to leaves.

In the case of Pi, it was essential to analyse frozen/thawed extracts within 30 minutes of the start of thawing to minimise the effects of conversions from organic to inorganic phosphate. Providing this was done, there was acceptable agreement between Pi in frozen extracts and Pi in extracts from microwave-dried material.

Agreement between on-farm and laboratory methods for shoot Pi was generally good and a recommended protocol for this test is given in Appendix VIII.

In the case of K, concentrations in frozen/thawed extracts were invariably less than those in extracts from dried material irrespective of the pressure used. This may be due to the difficulty of extracting cell wall K from frozen extracts. The Cardy meter also underestimated K compared with the Flame photometer for all types of extract. Consequently, more development work on the on-farm K method is required before it can be recommended.

11 CONCLUSIONS

Critical soil P for maximum yield

On the silty clay loam soil at Rothamsted, critical soil P (Olsen-P) in the period 1992-98 for 95% maximum grain yield averaged 9 mg/kg (range 6-11 mg/kg). The mean yield response, relative to soil P Index 0, was 2.67 t/ha. On the sandy clay loam soil at Ropsley in the same period, critical Olsen-P averaged 16 mg/l (range 11-20 mg/l) (equivalent to 14 mg/kg), with a mean yield response, relative to soil P Index 1, of 1.25 t/ha.

Critical soil K for maximum yield

Critical soil exchangeable-K at Rothamsted in the period 1992-98 for 95% maximum grain yield averaged 85 mg/kg (range 78-100 mg/kg). The mean yield response, relative to soil K Index 0, was 3.29 t/ha. There was no yield response to K fertiliser on the Index 0 soil at Sedge Fen, which demonstrated the unreliability of soil analysis for predicting yield responses to K fertiliser.

Critical plant concentrations for maximum yield

Critical plant concentrations, for 95% maximum grain yield, generally agreed well with those determined in 1992-95. Over all sites and seasons (1992-98) during stem elongation (GS 31-39), critical leaf(1) %P was in the range 0.23-0.38%. Similarly, critical shoot %Pi was in the range 0.028-0.071% (equivalent Piw range 2.7-6.7 mM). Critical leaf(1) %K was in the range 1.61-3.21% (equivalent Kw range 126-227 mM).

Effect of N supply on plant concentrations

Total-P, Pi and K concentrations in whole shoots and leaf(1) responded similarly to N. Applying N to N-sufficient crops had no effect on plant P, Pi and K concentrations. Hence, differences in N supply in the normal application range (100-250 kg/ha) are unlikely to have any effects on plant P, Pi and K concentrations. In N-deficient crops, whole shoot and leaf(1) %P and %K were lower, Kw was the same, and Pw, %Pi and Piw were higher than in N-sufficient

crops. It is likely that critical %P and %K, for 95% maximum grain yield, will be lower where N is limiting, but that critical %Pi, Piw, and Kw will be unaffected.

Effect of water supply on plant concentrations

Concentrations of %P, %Pi, and %K in shoot and leaf(1) dry matter were reduced by drought, and critical dry matter concentrations for 95% maximum grain yield also appeared to be reduced. In the case of tissue water concentrations (Pw, Piw, Kw), the effects of drought were not consistent, with responses depending on the particular concentration parameter and the plant organ. However, critical values in two recommended plant tests, namely leaf(1) Kw and shoot Piw, appeared to be largely unaffected by drought.

Plant sampling for tissue testing

Plant sampling should ideally be carried out in the morning to minimise the effects of diurnal variations in tissue concentrations. Leaf(1) samples should come from mainshoots where possible, but the inclusion of some tiller(1) leaves should not affect nutrient concentrations unduly.

On-farm testing

The RQflex meter generally performed well for Pi but was not thoroughly assessed for K. The meter gave a linear response to Pi standards (KH_2PO_4) up to 60 mg PO4/l (0.63 mM Pi). Plant tissue water would typically need to be diluted 30 times to bring Pi concentrations into this range. There was good agreement between the RQflex and standard laboratory instruments for analysing Pi in plant extracts.

The Cardy-K meter performed well against potassium chloride (KCl) standards with a linear response up to 3,900 mg/l K (100 mM K). Tissue water would typically need to be diluted 3 times to bring K concentrations into this range. The Cardy meter underestimated the standard laboratory instrument (flame photometer) for all types of plant extract, possibly due to interference by other ions.

Extracting tissue water from fresh wheat leaves was not easy. Quantities of tissue water were generally low and Pi and K concentrations were only half those obtained with microwave-dried or frozen/thawed material irrespective of the organ or pressure applied. In the case of fresh plants, it is likely that water was being expressed primarily from vascular tissue. To obtain sufficient quantities of representative tissue water by pressing, it was necessary to rupture plant cells by freezing and thawing them. The duration of freezing was not important and, once frozen, samples could be stored safely for 3 months. Samples had to be fully thawed (no ice) before they were pressed. Low pressures above c. 0.2 atmospheres were adequate for water extraction, and hand-pressed plastic syringes can be used for this.

Representative sampling of plants in the field was important, but correct subsampling was equally important, especially of whole shoots, as Pi and K concentrations differed between organs. Whole shoots should be subsampled intact to maintain the correct proportion of stem to leaf material.

In the case of Pi, it was essential to analyse frozen/thawed extracts within 30 minutes of the start of thawing to minimise the conversion of organic to inorganic phosphate. When this was done, there was good agreement between Pi from frozen extracts and microwave-dried extracts. Agreement between on-farm and laboratory methods for shoot Pi was generally good, and a recommended protocol for a shoot Piw test is given in Appendix VIII.

In the case of K, concentrations in tissue water obtained by the 'freeze/thaw' method were invariably less than those in water extracts from oven-dried material, irrespective of the pressure used. This may be due to the difficulty of extracting K from cell walls in frozen/thawed material. The Cardy meter also underestimated K compared with a flame photometer for all types of extract. Consequently, more development work on the on-farm K method is required before it can be recommended.

12 FURTHER WORK

In the present project, we obtained information on how P, Pi and K concentration parameters in different plant parts were affected by differences in N and water supply, but we still do not know for certain how critical P, Pi and K concentrations change with N and water supply. There is a need for multifactorial field experiments involving N, P, K and water. The difficulties and costs of setting up such experiments, especially those involving full control of water supply (shelters/irrigation), are considerable.

The *in situ* analytical instruments, RQflex and Cardy-K, performed very well with standard solutions, as did the RQflex for Pi in plant extracts. However, more work is needed on comparisons between RQflex/Cardy/Flame/ICPS for analysing plant K extracts. As well as inconsistencies between instruments in analysing for K, there were inconsistencies between laboratory and *in situ* extraction procedures for K which need to be resolved. It seems that only 80-90% of the K extracted by standard laboratory procedures (shaking dried milled plant material with water) can be extracted by freezing/thawing/pressing plant tissue.

There are many interesting physiological questions remaining concerning the nature of phosphate storage pools in plants. Current studies have focussed on the inorganic pool mainly because this is easily measured *in situ*. However, there is also the organic phosphate pool to consider (mainly sugar phosphates) which is in dynamic equilibrium with the inorganic pool via enzymic activity. A case could be made for classifying all of this phosphate, organic as well as inorganic, as the true storage pool.

All of our work on plant testing to date has been conducted by scientists using small scale field plots. There is a need to validate plant tests, ideally alongside soil tests, under farm conditions. Tests need to be carried out by farmers and consultants rather than by scientists, so that any practical problems can be identified and rectified.

13 ACKNOWLEDGEMENTS

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Appendix I Rothamsted agronomy

Rothamsted (Fosters - N)

Harvest Year	Previous crop	Sowing date/ Harvest date	Variety	N Fertiliser	Soil P/ P fertiliser	Soil K/ K fertiliser
1997	W. rape	26 Sept 96 12 Aug 97	Hereward	Range 0-300 as split dressing: 50kg 10 Mar Rem 11 Apr	Olsen 42 ppm	Kex 191 ppm

Rothamsted (Sawyers(I) – P)

Harvest Year	Previous crop	Sowing date/ Harvest date	Variety	N Fertiliser	Soil P/ P fertiliser	Soil K/ K fertiliser
1996	Lupin/bean	28 Sept 95 9 Aug 96	Mercia	40 7 Mar 160 2 Apr	Range None	Sept 96 152-258 100 kg K/ha to stubble
1997	Lupin/bean	3 Oct 96 12 Aug 97	Mercia	40 11 Mar 160 11 Apr	Range None	Sept 96 152-258 100 kg K/ha to stubble
1998	W. rape	9 Oct 97 18 Aug 98	Hereward	40 13 Mar 160 28 Apr	Range √ (Aut 97)	Sept 96 152-258 100 kg K/ha to stubble

Rothamsted (Exhaustion Land – P)

Harvest Year	Previous crop	Sowing date/ Harvest date	Variety	N Fertiliser	Soil P/ P fertiliser	Soil K/ K fertiliser
1996	W. wheat	29 Sept 95 15 Aug 96	Hereward	192 Kg 15 Apr	Range None	83 kg K/ha in Autumn
1997	W. wheat	8 Oct 96 13 Aug 97	Hereward	192 kg 11 Apr	Range None	83 kg K/ha in Autumn
1998	W. wheat	24 Oct 97 18 Aug 98	Mercia	192 kg 24 Mar	Range None	83 kg K/ha in Autumn

Rothamsted (Sawyers(III) – K)

Harvest Year	Previous crop	Sowing date/ Harvest date	Variety	N Fertiliser	Soil P/ P fertiliser	Soil K/ K fertiliser
1996	W. oats	12 Oct 95 4 Aug 96	Mercia	40 8 Mar 160 2 Apr	None 200 kg/ha TSP	Range Some plots
1997	W. oats	7 Oct 96 22 Aug 97	Mercia	40 10 Mar 160 11 Apr	None 200 kg/ha TSP	Range Some plots
1998	W. oats	24 Oct 97 7 Aug 98	Mercia	40 13 Mar 160 29 Apr	None 200 kg/ha TSP	Range Some plots

Appendix II Rothamsted sampling dates

Rothamsted (Fosters – N)

GS	Cut	Date	JD	DFS	Shoots	Leaf (1)
24	1	10 Mar	69	161	√	
30	2	2 Apr	92	184	√	√
32	3	1 May	121	213	√	√
37	4	8 May	128	220		√
39	5	15 May	135	227	√	√
68	6	9 Jun	160	252	√	√

Rothamsted (Exhaustion Land – P)

Harvest Year	GS	Cut	Date	JD	DFS	Shoots	Leaf (1)
1996	31	1	30 Apr	121	214	√	√
	32	2	16 May	137	230	√	√
	39	3	31 May	152	245	√	√
	61	4	14 Jun	166	259	√	√
1998	32	1	7 May	127	195	√	√
	41	2	20 May	140	208	√	√

Rothamsted (Sawyers(III) – K)

Harvest Year	GS	Cut	Date	JD	DFS	Shoots	Leaf (1)	Leaf (2)
1996	24	1	7 Mar	67	147	√		
	31	2	24 Apr	115	195	√	√	√
	32	3	15 May	136	216	√	√	√
	37	4	20 May	141	221	√	√	√
	39	5	29 May	150	230	√	√	√
	61	6	12 Jun	164	244	√	√	√
1997	39	1	14 May	134	220		√	√
1998	32	1	8 May	128	197		√	√
	41	2	19 May	139	208		√	√

Appendix III ADAS (Ropsley) P treatments

Treatment No	Phosphate fertiliser policy 1977 – 84	Annual phosphate 1985 - 98 (kg/ha)	Code	Extractable P autumn 1995 (mg/l)
1	NIL	0	N-0	11
2	NIL	70	N-70	16
3	NIL	100	N-100	19
4	LOW	0	L-0	11
5	LOW	40	L-40	16
6	LOW	70	L-70	18
7	LOW	100	L-100	24
8	HIGH	0	L-0	13
9	HIGH	40	L-40	17
10	HIGH	70	L-70	21
11	HIGH	100	L-100	23

Nil = No fertiliser P; Low = Annual P to balance offtake; High = Annual low P plus 50%

Appendix IV ADAS sampling dates

ADAS (Ropsley)

Date	Growth stage	Whole plant	Leaf 1	Leaf 2
25/3/96	22	–		
3/4/96	24	–		
5/5/96*	30	–	–	
5/6/98*	32	–	–	–
21/5/96	39	–	–	–
18/6/96	59	–	–	–
20/8/96	harvest	–		
2/4/97+	23	–		
29/4/97*	32	–	–	
28/5/97*+	39	–	–	
20/8/97	harvest	–		
7/4/98+	30	–		
28/4/98	32	–	–	–
30/5/98+	39	–	–	–
18/8/98	harvest	–		

* Samples taken in the morning and afternoon
+ Samples taken from areas with and without N fertiliser

ADAS (Sedge Fen)

Date	Growth stage	Whole plants	Leaf 1	Leaf 2
17/4/96	22	–		
3/5/96	30	–		
20/5/96*	32	–	–	
2/6/96	39	–		
17/6/96*	65	–	–	–
1/7/96	71	–		
4/9/96	Harvest	–		

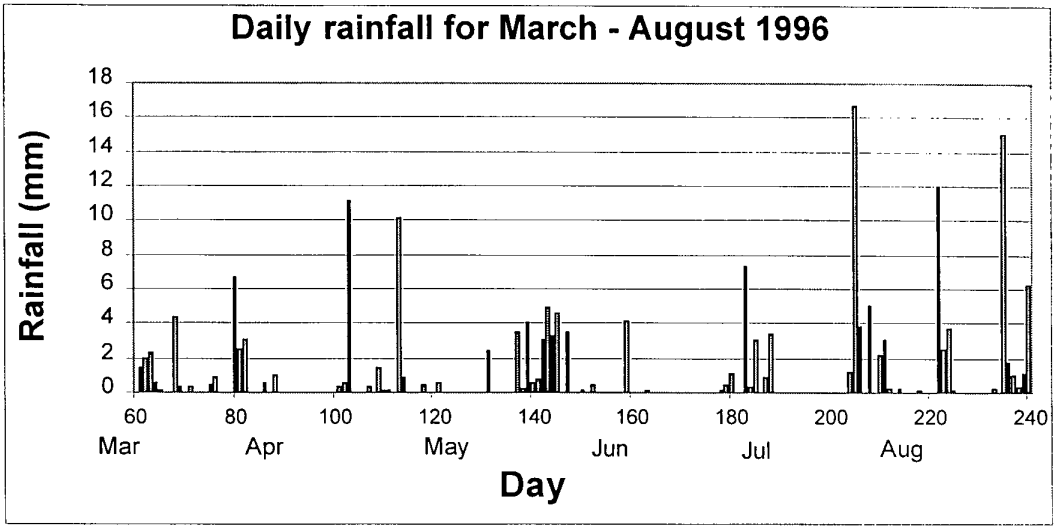
* Samples taken in the morning and afternoon

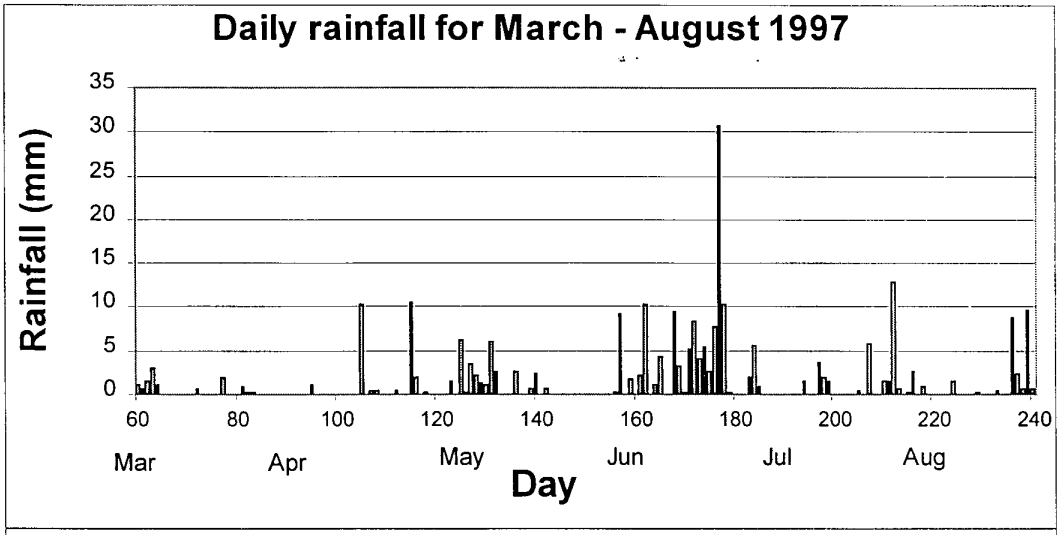
ADAS (Gleadthorpe)

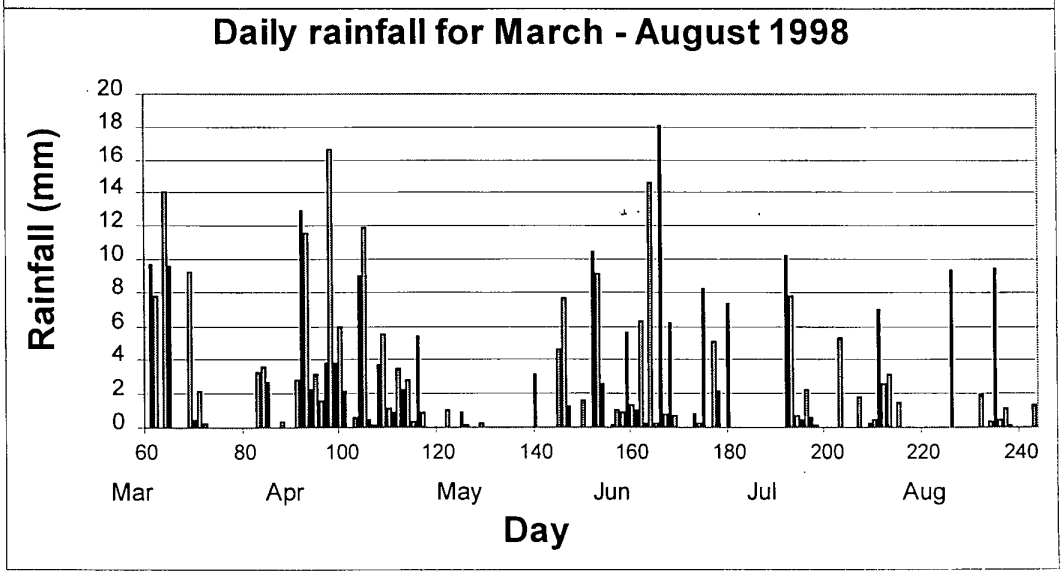
Date	Growth stage	Whole plant	Leaf 1
21/3/97	14/23	–	
14/4/97+	24/5	–	
7/5/97*+	32	–	–
21/5/97	37-39	–	–
3/6/97*	47	–	–
13/6/97	65	–	–
2/9/97	harvest	–	
1/4/98+	30	–	
24/4/98	30/31	–	–
27/5/98+	39	–	–
11/8/98	harvest	–	

* Samples taken in the morning and afternoon
+ Samples taken from areas with and without N fertiliser

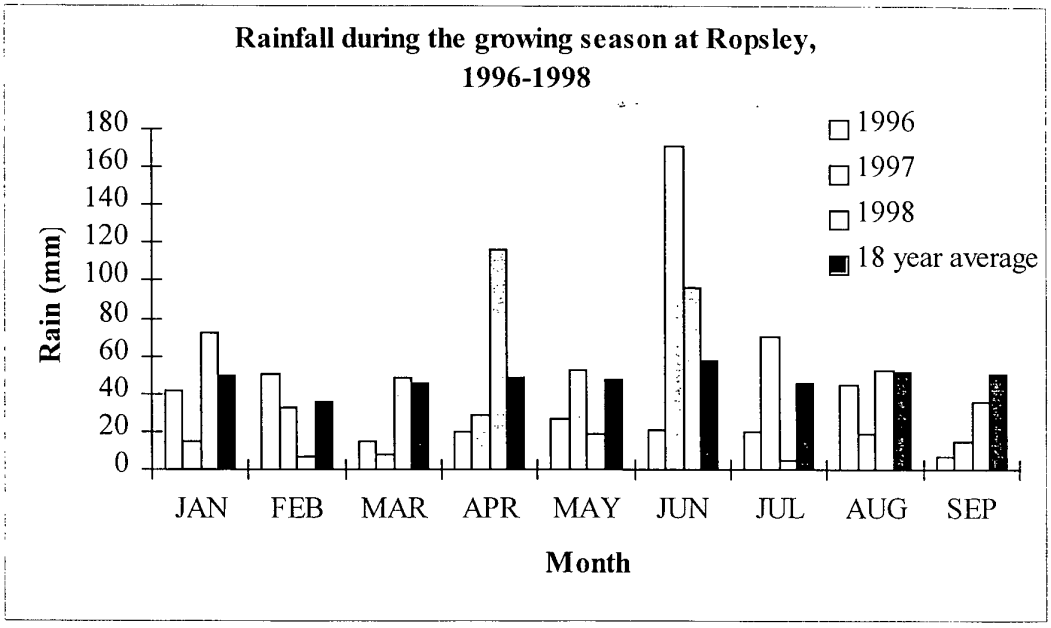
Appendix V Rothamsted rainfall







Appendix VI ADAS (Ropsley) Rainfall



Appendix VII Equipment for on-farm plant testing

MERCK RQflex system for Pi (K & NO3)

RQflex-2 Reflectometer (cat. no. 31902 3U)	£502 (incl. VAT)
Phosphate test strips (cat. no. 31923 3F) 50 strips	£49 (incl. VAT)
Potassium test strips (cat. no. 31929 3R) 50 strips	£49 (incl. VAT)

Supplier: Merck/BDH (Tel. 0800 22 33 44)
Also supplies syringes/measuring cylinders etc. for extracting/diluting sap

CARDY ion meter for K

Cardy compact ion meter for K (C-131)	£220 (estimate)

Supplier: Spectrum Technologies Inc., 23839 W. Andrew Rd. Plainfield, IL 60544 USA Tel. 800-248-8873/815-436-4440 Fax 815-436-4460

Appendix VIII On-farm protocol for whole shoot phosphate (Pi)

01 Collect a representative sample of whole shoots
Preferably before 10 am. Do not allow shoots to dry out

02 Select two subsamples (5 whole shoots - intact stems and green leaves)

03 Seal in polythene bag and place in deepfreezer (-18 °C)

04 Samples must be frozen, but can then be stored for several months

05 Thaw completely (no ice) at room temperature
Pi analysis must be completed within 30 min of removing sample from freezer

06 Cut shoots into 2 cm pieces and pack into a plastic syringe
Keep correct proportion of stems to leaves as these organs have different phosphate concentrations

07 Squeeze out tissue water into a clean vessel

08 Dilute aliquot of tissue water 30 times with distilled/deionised water
Water for car batteries/steam irons can be used - check phosphate level

09 30 times dilution may not be appropriate for all samples
Experiment to bring sample into the linear range of the RQflex

10 Analyse for phosphate by RQflex following manufacturer's instructions
Use fresh test strips. Multiply result by dilution factor (e.g. x 30)

N.B. 1 mg/l PO_4 is equal to 0.011 mM Pi