PROJECT REPORT No. OS58

PLANT AND SOIL TESTING TO ASSESS THE ADEQUACY OF PHOSPHORUS SUPPLY TO WINTER OILSEED RAPE

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PLANT AND SOIL TESTING TO ASSESS THE ADEQUACY OF PHOSPHORUS SUPPLY TO WINTER OILSEED RAPE

by

P B BARRACLOUGH and B J MAJOR

Agriculture and Environment Division, IACR-Rothamsted
Harpenden, Hertfordshire, AL5 2JQ

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Abstract

Winter oilseed rape is a common cereal break crop in farm rotations, yet relatively little is known about the phosphorus (P) requirements of this crop under UK conditions. With farm profit margins being squeezed and continuing concern for the environment, P fertilisers need to be used more precisely than in the past to ensure that soil supply and crop demand are closely matched at all times.

Soil testing is the usual way of assessing the adequacy of P supply to arable crops and for making fertiliser recommendations, but there is no published information on the response of rape yield to soil P or on critical soil P for maximum seed yield in the UK. Plant testing could also be used to help with fertiliser planning, but again information is lacking on critical plant concentrations for maximum yield under UK conditions.

Maximum rape seed yields (4-5 t ha^{-1}) were attained with a soil Olsen-P of 9 ppm (top of MAFF Index 0). Yield reductions of 50% were observed where soil P was below 5 ppm. Crops are likely to respond to P fertiliser on Index 0 soils. A much higher soil P level of 27 ppm (bottom of Index 3) was needed for maximum canopy growth. Large autumn canopies may be beneficial where pigeon damage is a recurring problem, but lush canopies were not essential for maximum seed yield. If soils have luxury P levels (Index 3 and above), growers can save money by withholding P in the short-term. But if pigeon damage is a recurring problem, growers may wish to maintain soil P at high Index 2 to encourage autumn canopy growth.

Tissue testing can be used to confirm the adequacy of soil P supply to rape crops. Traditionally, %P in leaf dry matter is used for assessing plant P status, a service offered by commercial testing laboratories. At the rosette stage in the autumn, leaf-blade samples taken from the middle of the plant canopy need a P content of 0.45% for maximum seed yield. Alternatively, growers can do their own tissue tests by measuring how much P has been stored as phosphate in leaf tissue water (the Pi test). Kits are available for carrying out this test. Between the rosette stage in autumn and the yellow bud stage in spring, leaf-blade samples taken from the middle of the plant canopy need Pi values greater than 4 mM for maximum seed yield.

If a plant test indicates P deficiency, even if it is carried out in the autumn, a grower may still not be able to take remedial action for that crop. Crops are unlikely to respond to top-dressings of P in the way that they do to N and S because of the poor mobility of P in soil. Equally, it is uncertain if foliar applications of P can be beneficial on deficient rape. At least the test will alert the grower to the need for action in subsequent crops.

Critical soil P values for high yielding crops of winter wheat (10 t ha^{-1}) and oilseed rape (5 t ha^{-1}) are very similar at 9 ppm (Olsen method), and the crops remove similar amounts of P in grain and seed (26 kg ha^{-1} P). Therefore, wheat and rape can be considered as identical crops in the rotation when it comes to planning a P fertiliser strategy. If soils are adequately fertilised for wheat, they should also be adequate for rape.

1. Summary Report

1.1 Aims

Winter oilseed rape is a common cereal break crop in farm rotations, yet relatively little is known about the P requirements of this crop under UK conditions. There is a feeling that rape is particularly sensitive to soil P, but there is little hard evidence for this. The P requirements of any crop can easily be met by prophylactic fertilising. This practice has probably contributed to the general P enrichment of soils in England and Wales, with more than half of all arable soils now being at Index 3 or above (>25 ppm). The national cost of P fertiliser for rape is considerable. An average application of 30 kg P/ha with P at 60p /kg will cost £18/ha, giving a national fertiliser bill of £9M pa for 0.5M ha of rape. In the current climate of tight farm profit margins and concern for the environment, fertilisers need to be used much more precisely than in the past to ensure that crop demand and soil supply are closely matched. What is lacking is reliable information on the response of oilseed rape crops to soil P and methods for assessing whether rape crops are adequately supplied with P.

Soil testing is the usual method for assessing the adequacy of P supply to crops and for making fertiliser recommendations. In the MAFF Soil Index System, a soil P Index of 2 (16-25 ppm Olsen-P) is the recommended target for all combinable crops including rape. However, there is no published information on critical soil-P for maximum rape seed yield in the UK. One of the objectives of this project was to establish a critical benchmark soil-P value for the growth and yield of oilseed rape. A weakness of soil testing is that critical soil values depend on soil type, and a comprehensive testing programme involving representative soils is very difficult to set up and run. Soil testing indicates the potential availability of a nutrient, it does not tell you if the nutrient is getting into the plant to satisfy crop requirements; that requires a plant test.

As with soil testing, there is no published information on critical plant P concentrations for maximum rape seed yield in the UK. A second aim of this project was to develop a reliable plant test for confirming the adequacy of soil P supply to rape crops independent of soil type. Traditional plant tests are based on total-P in dry matter (%P), but this is difficult to interpret because of complications from plant growth stage and interactions with other nutrients. The 'storage pool' offers an alternative approach to plant testing. Generally, plants over-supplied with P store the excess as simple inorganic orthophosphate ions (Pi) in cell vacuoles, and this has proved to be a far more useful diagnostic indicator of the P status of wheat than total-P (HGCA Topic Sheet No. 19). In this project, we wanted to see if Pi could also be used to indicate the P status of rape and to develop a Pi plant test suitable for on-farm use.

1.2 Methods

A combination of pot and field experiments was used in this project. In the laboratory, different methods of extracting and measuring Pi in rape were investigated including novel on-farm testing technology. Pot experiments utilising hydroponic sand culture in a growth room were used to investigate the dynamics and distribution of Pi in rape plants to enable the best organ to be chosen for diagnostic purposes. In the field, rape was grown in plots having a wide range of established soil P levels to identify critical soil P and critical plant P and Pi concentrations for maximum growth and seed yield.

1.2.1 Field experiments

Field experiments were undertaken in two seasons, 1999/00 and 2000/01, and the results are given in this report. Rape is being grown for a third season (2001/02) and the results will be available at a later date. For simplicity, growing seasons will hereafter be designated by the harvest year. The experiment was sited on the long term P plots on Sawyers I field at Rothamsted Experimental Station in Hertfordshire (51^o 49' N, 0^o 22' W, 128 m above sea level). The soil is a free-draining flinty silty clay loam over clay. The experiment consisted of 2 blocks of 12 plots, each plot measuring 9 x 15 m and each having a different plant-available soil P. The plots were not replicated, but the wide range of soil P values allowed a curve fitting approach to be applied to the data. Winter oilseed rape (*Brassica napus* cv. Apex) was grown in alternating rotation with winter wheat (*Triticum aestivum* cv. Hereward) on the two blocks. Rape was sown at 90-120 seeds m^{-2} in late August and netted until spring. Crop protection was used as required. No P fertiliser was applied to the site after the start of the experiment.

Soils were sampled annually in August before sowing the crop. The soil was air-dried, milled and available-P was extracted by the Olsen method (0.5 M $NaHCO_3$ solution). Crops were sampled at four growth stages, rosette in November (2,0), green-bud in April (3,0), yellow-bud in May (3,3) and at maturity in August (6,9). Measurements included plant counts, shoot fresh and dry weights, canopy LAI, and yield components at maturity. The %P in dry matter and Pi in tissue water in leaves, petioles, stems and flower buds were measured. A central strip down each plot (36 m^2) was left for combine-harvesting. Plots were combined using a Sampo plot combine-harvester directly after spraying with a desiccant. Harvested seed yields (t ha^{-1}) were expressed on a 91% dry matter basis.

1.2.2 Hydroponic experiments

The accumulation and distribution of P and Pi in oilseed rape plants and the identification of a suitable indicator organ for diagnosing plant P status were determined in sand-culture hydroponics in a growth room. The plant material was also used for examining different extraction and analytical methods for measuring Pi.

Winter oilseed rape (*Brassica napus* cv. Apex) was grown in 5 litre, 20 cm diameter pots (four plants per pot) filled with 5 kg of washed sand. The growth room was maintained at 20 ^{o}C in a 16 hour light period and at 16 ^{o}C in the dark period. Light intensity was 440-550 *u*E m^{-2} s^{-1}. Relative humidity was maintained at 75 %. In each experiment, a randomised block design was used with four replicates per treatment. Treatments varied from very deficient (0.1 mM external P), to luxurious (10 mM external P). Other essential nutrients were non-limiting.

Plants were harvested at the rosette stage before stem extension. Shoots were broken down into three categories - top, middle and bottom canopy - and then split into leaf-blades, petioles and stems. Fresh and dry weights, total %P in dry matter and Pi in tissue water were determined in all components.

1.2.3 Analytical methods

Three principal methods were used to analyse plant material for total-P and Pi in this project:

Total-P in plant dry matter (%P) was measured by a standard laboratory method using inductively-coupled plasma (ICP) atomic emission spectrometry after the dried, milled samples had been digested with concentrated perchloric and nitric acids.

In the laboratory, inorganic orthophosphate (Pi) in tissue water or aqueous extracts was determined colorimetrically using the molybdate-blue method. The reagent is formed by sequentially adding solutions of concentrated sulphuric acid (H_2SO_4), antimony (III) potassium oxide tartrate hemihydrate ($K(SbO)C_4H_40_6.½H_20$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_20$) and ascorbic acid ($C_6H_8O_6$). For analysis, 5 ml of sample was added to 20 ml of the reagent. The absorbance of the blue solution was measured after 20 minutes at 880 nm using a Jenway 6400 spectrophotometer. A calibration curve using KH_2PO_4 standards was linear in the range 0-3.1 mg PO_4 l^{-1} (0-0.03 mM Pi).

The potential for on-farm Pi analysis using the Reflectoquant system (Merck UK Ltd.) was investigated. This consists of a hand-held reflectometer (RQflex2®) and ion-specific test-strips. The reflectometer is programmed using a barcode strip supplied with each batch of test-strips. The phosphate sensitive test-strips use molybdate-blue chemistry (as above) with all reagents (except sulphuric acid) bound to a plastic strip. Ten drops of acid reagent were shaken with 5 ml of sample solution and a test-strip dipped in the solution. After 2 seconds the test-strip was removed and excess solution shaken off. After 85 seconds the test-strip was inserted into the reflectometer and the concentration in mg PO_4 l^{-1} was displayed 5 seconds later. The measuring range of the system was quoted as 5-120 mg PO_4 l^{-1} (0.05-1.3 mM Pi). The accuracy, precision and specificity of this assay were investigated in this project.

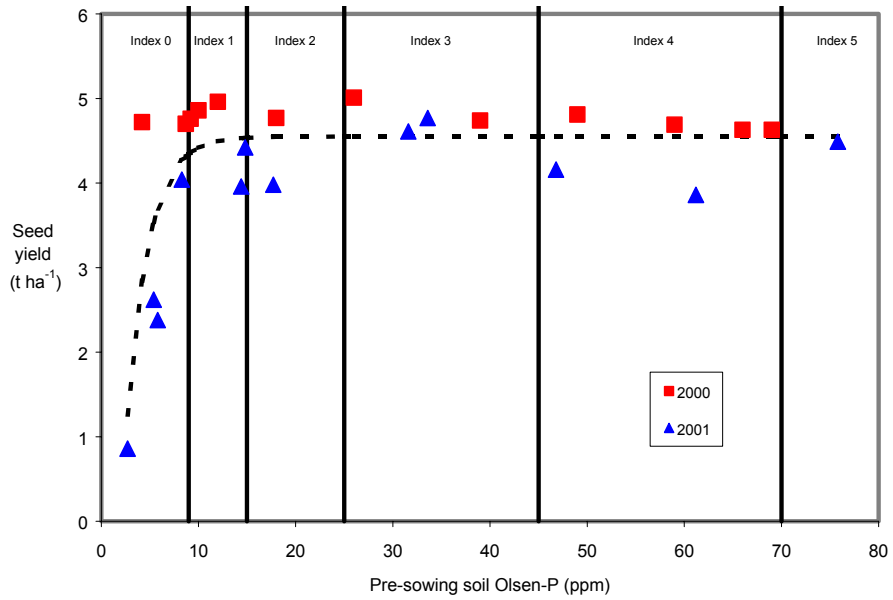
1.3 Key results

Key results from the project including crop P uptake, effects of P deficiency on growth and yield components, critical soil P for maximum yield, critical plant P and Pi for maximum yield, and on-farm testing for plant Pi are summarised below.

1.3.1 Critical soil Olsen-P for maximum seed yield

Seed yield was unaffected by soil P supply in 2000. All plot yields were in a narrow range of 4.6-5.0 t ha $^{-1}$ (91% DM) across a soil Olsen-P range of 4-69 ppm. In 2001, seed yields were affected by P supply, varying from 0.9-4.5 t ha $^{-1}$ across a soil Olsen-P range of 3-76 ppm (Index 0-5). Yield was reduced by 50% at Olsen-P less than 5 ppm. Critical soil Olsen-P for 95% maximum seed yield was 9 ppm (top of Index 0) averaged over the two years (Figure 1.1).

Figure 1.1 Effect of pre-sowing soil Olsen-P on final oilseed rape yield (t ha^{-1})



1.3.2 Canopy growth

There were no marked effects of soil Olsen-P on the number of plants established even on single figure Olsen plots. Maximum yield was obtained from plant populations of 65 m^{-2}. Rape can be difficult to establish, but the present results rule out low soil P as a factor. Seedbed preparation is usually the key factor affecting establishment, but pigeons and other pests also contribute to poor plant establishment, so the current experiment was netted throughout the autumn and winter to prevent this.

Plant growth was significantly affected by soil Olsen-P, but visible symptoms of P deficiency only occurred at very low soil P levels (<5 ppm). The difference in growth caused by soil P (ground cover and dry matter production) in the autumn was dramatic (Figure 1.2). The critical Olsen-P for growth at the rosette stage was 27 ppm (Index 3), three times the 9 ppm (Index 0) needed for maximum seed yield. Maximum yield was obtained with LAIs above 2 at the yellow bud stage. Phosphorus uptake by these 4-5 t ha^{-1} crops was 43 kg P ha^{-1} with 26 kg of that being in the seed at final harvest.

Figure 1.2 Effect of soil Olsen-P on rape growth at the rosette stage in autumn 1999
(left - 9 ppm, critical for yield; right - 69 ppm, luxury supply)

1.3.3 Yield components

Yield loss induced by P deficiency was principally due to fewer pods per plant. The number of seed bearing branches and seed bearing pods per plant decreased with decreasing soil Olsen-P. A maximum pod density of 12,500 pods m^{-2} was attained at an Olsen-P of 24 ppm, but the number of seeds per pod decreased with increasing pod number. Maximum seed yield was thus obtained with 8,500 pods m^{-2}, and a canopy LAI of 2. This accounts for why no increase in seed yield was seen above a soil Olsen-P of 9 ppm, despite the large differences in biomass production. Thousand seed weight (100% DM) varied little with soil P, being 4.65-4.95g in 2000, and 4.09-4.38g in 2001, although it was much lower (3.43g) in the very low Olsen-P plot of 2.7 ppm in 2001.

Percentage oil content in the seed varied from 42.1 to 44.2% in 2000 and from 39.8 to 44.3% in 2001. There was no clearly identifiable trend due to soil Olsen-P, therefore the oil yield data reflected the seed yield data with 1.95-2.15 t ha^{-1} being obtained in 2000 and 0.34-2.04 t ha^{-1} in 2001.

1.3.4 Plant testing

Traditional plant tests based on %P in dry matter cannot be done on-farm and there are also problems with the interpretation of results from such tests. Measuring the 'storage pool' (Pi in tissue water) in plants has advantages over conventional dry matter testing including the ability to test on-farm. The sand culture experiments showed that rape excessively supplied with P accumulated Pi to concentrations of 20 mM or more without suffering toxic growth reductions.

A suitable indicator organ must accurately represent the P status of the plant, must be easy to identify and sample, have easy extraction of nutrients, must be sensitive to P supply, and exhibit stable concentrations over an extended period of time. Traditionally, the youngest fully-expanded leaf is favoured in plant tests, but it proved very difficult to pinpoint exactly when a leaf was fully expanded in rape. Analysis of individual leaves and petioles in rape plants showed that P and Pi concentrations were generally most responsive yet stable in mature fully-unfurled leaf-blades from the central third of the plant canopy.

1.3.5 Critical plant P and Pi concentrations for maximum seed yield

Critical %P values in mature indicator leaves for maximum seed yield in the field varied with crop growth stage (Table 1.1). Concentrations in the indicator leaf-blade gave the best relationships to seed yield, but critical values for the whole leaf (blade plus petiole) are also included as this organ is commonly tested by commercial laboratories. Relationships between Pi concentrations in the tissue water of mature indicator leaves and seed yield were better than those obtained with %P, but critical Pi values also declined with growth stage (Table 1.1).

Table 1.1 Critical leaf P and Pi concentrations for maximum seed yield

Leaf from middle of plant canopy (fully unfurled, no senescence). Standard laboratory analysis

Growth Stage	Sampling Date	Critical leaf P and Pi for seed yield		
		Leaf-blade %P	Leaf-blade Pi (mM)	Leaf -blade plus petiole %P
Rosette (2,0)	Nov-Dec	0.45	3.5	0.38
Green bud (3,3)	Mar-Apr	0.29	2.8	0.28
Yellow bud (3,7)	Apr-May	0.22	1.9	0.22

1.3.6 On-farm plant testing

On-farm diagnostics invariably involve some form of 'sap' extraction and a simple means of analysing the sap. The physiological basis of our novel approach to P testing required a measurement of 'stored-phosphate' in leaf vacuoles. In practice, this is Pi in leaf tissue water rather than sap *per se,* as vacuolar water cannot be isolated. Physiologically speaking, 'sap' is that fraction of the tissue water in the vascular tissues (petioles and stems). Storage testing is also quite distinct from conventional petiole testing. The problem with using petioles is that sap concentrations are constantly changing as nutrients are always in transit to other organs. So the first problem was how to extract a representative sample of tissue water from rape leaves whilst preserving Pi concentrations at the time of sampling.

Five different methods of extracting Pi from leaf-blades were investigated - pressing fresh material, pressing frozen/thawed material, and aqueous extracts of freeze-dried, microwave-dried and oven-dried material. Extraction method had a significant effect on Pi concentrations which were measured by a standard laboratory method (Figure 1.3). Obtaining a good yield of tissue water from fresh leaves was difficult and this method gave the lowest Pi value of 6 mM, probably because the 'tissue water' was largely 'sap' from the leaf mid-ribs. Using a freeze-thaw technique (deep freezer) produced a good yield of tissue water with a Pi concentration of 15 mM. The quick freeze-dry technique (liquid nitrogen) probably best represented the true Pi concentration at the time of sampling. This, and the microwave-dry method, gave similar Pi concentrations to the (slower) freeze/thaw method. Conventional oven-drying gave Pi values above 20 mM, probably because organic phosphates were enzymatically converted to inorganic forms during the early stages of drying. The freeze-thaw method is practical to do on-farm and produced true leaf Pi concentrations (not changed by the extraction method).

On-farm analysis has always been a problem for DIY testing, but Merck has recently introduced the Reflectoquant system. This consists of a hand-held reflectometer (RQflex2) and test strips for different nutrients (strips are available for nitrate, phosphate and potassium). The chemistry behind the phosphate test strips is based on the molybdate-blue reaction. RQflex gave a linear response in the range 0-1.3 mM Pi, meaning that plant tissue water will typically need to be diluted some 40 times to bring it into the measuring range of the instrument. Dilution was advantageous as it minimised the effects of potential interfering agents (chloride, nitrate, chlorophyll). Test strips were quite variable in performance and at least two strips per sample are recommended. Overall, this system was comparable with the standard laboratory method (Figure 1.3). An on-farm Pi test is now possible using a Reflectoquant kit (Figure 1.4).

Figure 1.3 Effect of extraction method on Pi concentrations in oilseed rape leaf blades

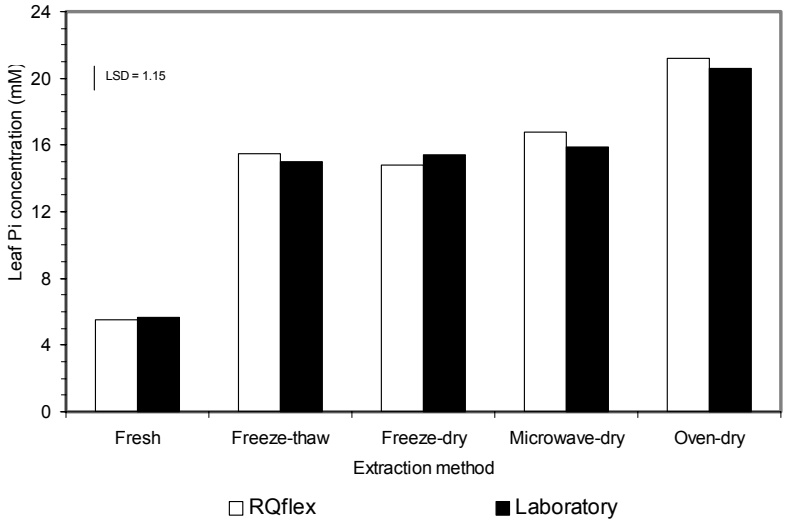
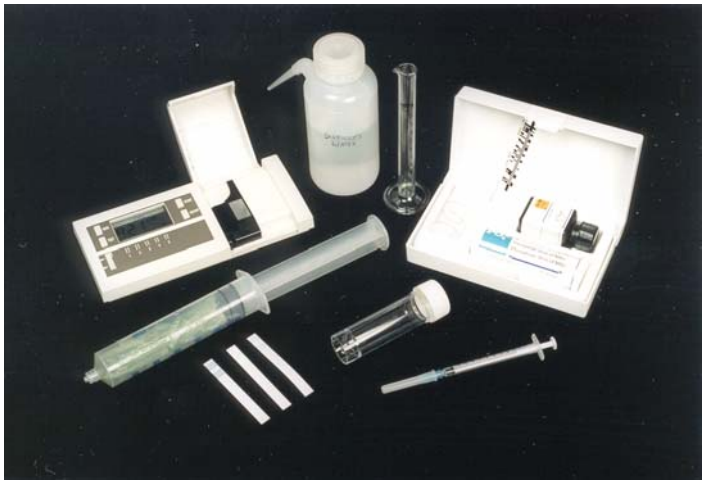


Figure 1.4 On-farm test kit for Pi analysis: RQflex2 reflectometer, Pi test strips, syringe



To perform an on-farm Pi test, a representative sample of twenty indicator leaves (mature green leaf-blades, no petioles, from the mid-canopy) is recommended. The sample should be cut up and mixed, frozen in a domestic freezer (duration not important), thawed quickly and tissue water pressed out in a syringe barrel. The tissue water is then diluted typically 40 times for testing. Results should be compared with the critical Pi values in Table 1.1.

1.4 Implications for levy payers

Marked symptoms of P deficiency in rape only occurred at very low soil P levels (<5 ppm). If growers are concerned about the adequacy of P supply to oilseed rape crops, the first thing to check is the soil. In the present study, maximum seed yields were attained with a soil Olsen-P of 9 ppm (top of Index 0), so the target Soil Index of 2 recommended by MAFF (16-25 ppm) has a good measure of safety built-in for yield. Maximum seed yields were obtained with 65 plants m^{-2}, canopy LAI of 2, and 8,500 pods m^{-2}. A much higher level of soil P of 27 ppm (bottom of Index 3) was needed for maximum canopy growth. Large autumn canopies may be beneficial where pigeon damage is a recurring problem, but lush canopies are not essential for maximum seed yield. The critical soil Olsen-P values at Rothamsted may not be applicable to all soil types, and plant testing which is independent of soil type can have a useful complementary role to play.

Standard laboratory testing for %P in leaf dry matter can be used to diagnose the P status of rape. This service is provided by commercial testing laboratories. At the rosette stage in the autumn, leaf-blade samples taken from the middle of the plant canopy having a P content greater than 0.45% indicated that P supply was sufficient for maximum seed yield. On-farm plant testing for Pi (stored-phosphate) in leaf tissue water is also an option using the Merck Reflectoquant system which consists of a hand-held reflectometer (RQflex2) and phosphate test-strips. The reflectometer is not cheap, so on-farm testing may be more attractive to farmer groups and consultants than to individual farmers. Between the rosette stage in autumn and yellow bud stage in spring, leaf-blade samples taken from the middle of the plant canopy having Pi values greater than 4 mM indicated that P supply was sufficient for maximum seed yield.

If a soil is P deficient (Index 0), then a crop will probably respond to P fertiliser applied before sowing. If a plant test indicates P deficiency, even if it is carried out in the autumn, a grower may still not be able to remedy the situation for that crop. Crops are unlikely to respond to top-dressings of P in the way that they do to N and S because of the poor mobility of P in soil, although evidence on this is scant. Equally, there is no information on the efficacy of foliar P for deficient rape. At least the test will alert the grower to the need for action in subsequent crops. If soils have luxury P levels (Index 3 and above), growers can save money by withholding P in the short-term. But if pigeon damage is a recurring problem, growers may wish to maintain soils at high Index 2 to encourage autumn growth.

Critical soil P values for high yielding winter wheat (10 t ha^{-1}) and oilseed rape crops (5 t ha^{-1}) are very similar at 9 ppm (Olsen method), and the crops remove similar amounts of P in grain and seed (26 kg ha^{-1} P). Therefore, wheat and rape can be considered as identical crops in the rotation when it comes to planning a P fertiliser strategy. If soils are adequately fertilised for wheat, they should also be adequate for rape.

2. Main Report

2.1 Development of an on-farm plant testing method

2.1.1 Introduction

The limited plant tests on oilseed rape that have been undertaken have focused on measuring total P as %P in dry weight in plant organs (Pinkerton *et al.*, 1989; Reuter and Robinson, 1997). An alternative approach is to measure P on a wet basis by measuring inorganic phosphate ($H_2PO_4^-$ or Pi) within the plant tissue. Pi is required in a metabolic role to maintain the equilibrium between numerous key biochemical pathways in the cell, affecting the P balance of the whole plant (Mimura, 1999). If P supply and uptake by the plant is high (luxury consumption), simple Pi ions are segregated from the cell cytoplasm into the vacuole where they form a storage pool (Bieleski, 1973). Maintaining a concentration of nutrients in the storage pool, such that the plant can balance its supply and demand for nutrients with optimum growth should, theoretically, correspond to optimal plant nutrition. Measuring the storage pool concentration of Pi thus has high potential as a diagnostic indicator of the P nutritional status of the plant (Barraclough, 1993). Measurement of Pi concentrations in plant cell vacuoles under laboratory conditions is possible using single-cell sampling (Takeshige *et al.*, 1992) but is generally not applicable for use in measuring Pi concentrations in field crop samples. A more practical alternative is to measure Pi concentration in tissue water extracts from whole organs. Although this approach does not account for the sub-cellular distribution and localisation of Pi this method has been successfully used in winter wheat (*Triticum aestivum*) to determine critical Pi values for yield (Bollons and Barraclough, 1999).

On-farm analysis has been given a boost with the introduction of the Reflectoquant analysis system (Merck UK Ltd.) which allows nutrient ion concentrations to be determined quantitatively using test-strips and a small portable reflectometer, the RQflex. Phosphate sensitive test-strips are based on conventional molybdate-blue chemistry (Murphy and Riley, 1962). The system has the potential to measure Pi in aqueous plant extracts *in situ* given a suitable plant extraction method. The first objective of our work was to compare the Reflectoquant system with a conventional laboratory assay for measuring Pi. The second objective was to compare different methods of extracting Pi from rape to identify the most practical and accurate method as a basis for *in situ* testing. To achieve these objectives three experiments were undertaken. Firstly, the accuracy and precision of the Reflectoquant system was investigated using standard phosphate solutions. Secondly, the effects of common solutes and metabolites in plant tissue water such as nitrate, organic acids and P-esters with the potential to interfere with the Pi assay were investigated. Thirdly, the effect of different preservation and extraction methods on Pi concentrations in oilseed rape leaves was compared.

2.1.2 Materials and methods

2.1.2.1 Conventional Laboratory Analysis of Pi

Phosphate was determined colorimetrically using the molybdate-blue method (Murphy and Riley, 1962) and a procedure adopted by Greenberg *et al.*, (1993). The reagent is formed by adding sequentially - 100 ml of 0.14% (v/v) concentrated sulphuric acid (H_2SO_4, 5N), 10 ml of 0.008 M antimony (III) potassium oxide tartrate hemihydrate solution ($K(SbO)C_4H_40_6.½H_20$), 30 ml of 0.032 M ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_20$) and 60 ml of 0.1 M ascorbic acid ($C_6H_8O_6$). For analysis, 5 ml of sample was added to 20 ml of the reagent. After 20 minutes the absorbance was measured at 880 nm using a Jenway 6400 spectrophotometer (Jenway Ltd., Essex, UK.) using a reagent blank as the reference solution. Absorbance readings were in the range 0-0.65. A calibration curve was prepared using KH_2PO_4 standards. This was linear ($r^2 \geq 0.99$) in the range 0-3.1 mg $PO_4\ l^{-1}$ (0-0.03 mM Pi).

2.1.2.2 Analysis of Pi by Reflectoquant

The Reflectoquant system (Merck UK Ltd.) consists of a hand-held reflectometer (RQflex2®) and ion-specific test-strips. The reflectometer is programmed using a barcode strip supplied with each batch of test-strips. The phosphate sensitive test-strips use molybdate-blue chemistry (as above) with all reagents (except sulphuric acid) bound to a plastic strip. Ten drops of acid reagent were shaken with 5 ml of sample solution and a test-strip dipped in the solution. After 2 seconds the test-strip was removed and excess solution shaken off. After 85 seconds the test-strip was inserted into the reflectometer and the concentration in mg $PO_4\ l^{-1}$ was displayed 5 seconds later. The measuring range of the system was quoted as 5-120 mg $PO_4\ l^{-1}$ (0.05-1.3 mM Pi)

2.1.2.3 Experiment 1: Accuracy, Precision and Calibration

Standard solutions of KH_2PO_4 spanning the range 0-120 mg $PO_4\ l^{-1}$ were analysed with five test-strips per solution. The solutions were then diluted 50 times to bring them into the measuring range of the Jenway spectrometer and each measured five times using the laboratory method. Linear regression lines were fitted to the data using Genstat 5 (Payne *et al*., 1993). The variation in test-strip performance was examined by using an entire tube of new test-strips to measure Pi in a standard solution (50 mg $PO_4\ l^{-1}$). Similarly, a standard solution (1.75 mg $PO_4\ l^{-1}$) was analysed 50 times by the laboratory method.

2.1.2.4 Experiment 2: Specificity of Pi Assay

A large number of P-esters are present in plant tissue, which could amount to a concentration of up to 7 mM Pi if all the P-esters were to undergo hydrolysis (Bieleski, 1973). To see if P-esters were detected by the analysis methods, adenosine triphosphate (ATP) ($C_{10}H_{14}N_5O_{13}P_3Na_2$) (200 uM), fructose-6-phosphate (F6P) ($C_6H_{11}O_9PK_2.2H_20$) (120 uM) and nicotinamide adenine diphosphate (NADP) ($C_{21}H_{27}N_7O_{17}P_3Na$) (30 uM) were made up in water at the typical plant concentrations shown in brackets (Bieleski, 1973), and analysed

by the standard laboratory method. Much higher concentrations of these solutions (1.6 mM) were used for assessing the effects on the Reflectoquant system due to its higher operating range.

Anions such Cl^- and NO_3^- can be present in relatively high concentrations in plant tissue (Marschner, 1995), and may interfere with the measurement of Pi. Similarly, concentrations of organic acids such as citric acid may also influence the reaction (Hoffland *et al*., 1989). The effects of concentrations of 150 mM Cl^- (KCl), 100 mM NO_3^- (KNO_3), and 0.1 mM citric acid on the measurement of a 2.45 and 50 mg PO_4 l^{-1} (0.53 mM Pi) standard solution was assessed using both methods respectively.

2.1.2.5 Experiment 3: Extraction Methods

Oilseed rape (*Brassica napus* cv. Apex) plants were grown to the rosette stage (Sylvester-Bradley, 1985) with a luxury supply of P (5 mM) in sand culture. The upper leaves, which were still unfurling and had crinkled edges, and the lower leaves showing signs of senescence were discarded. The remaining 3 or 4 large leaves per plant were used for analysis. The leaf-blades (including mid-ribs) were separated from the petioles. The leaf-blades were then cut into pieces, thoroughly mixed and divided into four replicate samples.

Leaf samples were then divided into 5 sub-samples (each approximately 60 g fresh weight) with each undergoing a different extraction method: i) fresh, ii) freeze-thaw, iii) freeze-dry, iv) microwave-dry and v) oven-dry. Fresh leaves were extracted and analysed within 1 hour of cutting. Leaves to be frozen were immediately put in a freezer at -20 ^oC for 24 hours. On removal from the freezer, the leaves were broken up and thawed for approximately 12 minutes before extraction of tissue water was possible. Leaves to be freeze-dried were immediately immersed in liquid nitrogen and then freeze-dried (-60^oC) for 5 days. Leaves to be microwaved were heated on full power (750 W) for five minutes in a domestic microwave-oven. This removed approximately 50% of the tissue water. The leaves were then oven-dried at 80 ^oC for 24 hours to remove the remaining moisture. Leaves for oven-drying were dried at 80 ^oC for 24 hours in a conventional oven. Dried leaves were milled using a Glen Creston mill fitted with a 1 mm sieve.

Tissue water was extracted from fresh leaves using a hand-tightened screw press, and from freeze-thaw samples in a 60 ml plastic syringe. The tissue water was analysed directly following suitable dilution by factors of 500 and 1000 respectively for laboratory analysis, and by factors of 20 and 40 respectively for analysis by Reflectoquant. For microwave-, oven- and freeze-dried leaves, 0.25 g of dried milled material was shaken with 25 ml of 2% (v/v) acetic acid for 30 minutes. The extract was filtered through Whatman No.42 filter paper, discarding the first 5 ml. The filtrate was diluted by a factor of 100 for analysis by the standard laboratory method and by a factor of 6 for analysis by Reflectoquant. In the case of dried samples, Pi concentrations in tissue water were calculated from the extract concentrations and tissue water content determined from the fresh weight minus the dry weight of the sample. All results were analysed by ANOVA using Genstat 5 (Payne *et al.*, 1993).

2.1.3 Results and discussion

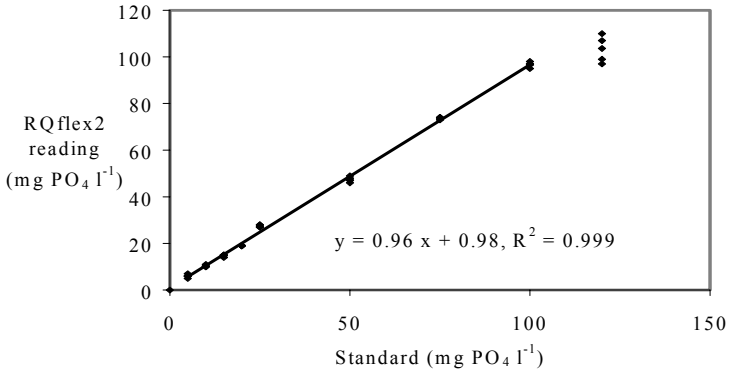
2.1.3.1 Experiment 1: Accuracy, Precision and Calibration

A standard potassium phosphate solution, 1.75 mg PO_4 l^{-1}, was analysed 50 times by the standard laboratory method. The mean value was 1.75 mg PO_4 l^{-1} in a range of 1.67-1.81 mg PO_4 l^{-1} (SD = 0.010, CV=1.7%). In practice, any single analysis of a tissue-water sample having a Pi concentration of 10 mM might range from 9.6-10.3 mM. Any single analysis would thus be within 95% (± 0.5 mM) of the true concentration.

A new tube of strips (Batch No. 92166066, Expiry date 01/04/2001, Analysis date 26/09/2000) contained 52 test-strips. Colour failed to develop properly on one test-strip due to uneven colouration of the reaction pads and this was discarded from the analysis. A standard solution, 50 mg PO_4 l^{-1}, was analysed with the remaining 51 test-strips. The mean value was 48.6 mg PO_4 l^{-1} in a range of 44-54 mg PO_4 l^{-1} (SD = 2.02, CV = 4.2%). The phosphate test-strips therefore under-estimated the true value by 2.8 %. In practice, any single analysis of a tissue-water sample having a Pi concentration of 10 mM might range from 8.8 -10.8 mM. To obtain a value within ± 1 mM of the mean with 95% confidence would require the use of 2 test-strips per sample.

Both analysis methods showed good linearity. The laboratory method gave a linear response in the range 0-3 mg PO_4 l^{-1} (Absorbance = 0.180x, R^2=0.997) although results were more variable at the higher end of the range. The phosphate test-strips slightly under-estimated Pi, but had a larger linear range of 5-100 mg PO_4 l^{-1} (Absorbance = 0.96x + 0.98 , R^2=0.999) (Figure 2.1).

Figure 2.1 : Accuracy of the Reflectoquant system with Pi standards



Tissue-water samples and extracts will need to be diluted by at least a factor of forty to bring them into the linear measurement range of the RQflex2 reflectometer and avoid any interference effects (Section 2.1.3.3). A slight decline in the accuracy of the test-strips occurred once the expiry date of the tube had passed at 96 weeks. It is recommended that a new tube of test-strips should always be used were possible, and that test-strips past their expiry date should be discarded.

2.1.3.2 Experiment 2: Specificity of Pi Assay

Table 2.1 illustrates the effects of P-ester solutions and anions on the measurement of a standard Pi solution. Both $H_2PO_4^-$ and HPO_4^{2-} ions were detected by the assay methods, which is important as both these forms of the phosphate anion are present in plant cells (Bieleski, 1973). Using the laboratory method, approximately 5.3% of the total P in F6P was detected. In ATP and NADP, approximately 2.5% and 0.3% of the respective total P was detected. These compounds therefore had very little effect on the measurement of a Pi standard, causing only minor increases in measured values (Table 2.1).

Table 2.1 : Effect of sap constituents on Pi analysis

	Standard laboratory analysis			Reflectoquant analysis		
Compound	Concentration examined	Possible full hydrolysis concentration (mg PO_4 l^{-1})	% Effect on Pi standard[d]	Concentration examined	Possible full hydrolysis concentration (mg PO_4 l^{-1})	% Effect on Pi standard[e]
$H_2PO_4^-$	26 uM	2.47	+100	550 uM	50	+100
ATP	200 uM[a]	19.0	+0.45	1.6 mM	153	+2.4
F-6-P	120 uM[a]	11.4	+1.8	1.6 mM	153	+2.6
NADP	30 uM[a]	2.85	+0.1	1.6 mM	153	-0.8
Cl^-	150 mM[b]		+0.8	150 mM		-1.4
NO_3^-	100 mM[b]		+2.8	100 mM		-1.4
Citric acid	100 uM[c]		+0.1	0.1 mM		0

Concentrations commonly found in plant tissue, [a] Bieleski, 1973, [b] Marschner, 1995, [c] Hoffland *et al.*, 1989.
Pi standard [d]2.45 mg PO_4 l^{-1}, [e]50 mg PO_4 l^{-1}

The partial hydrolysis of the P-esters may explain the low levels of Pi detected. It appears that both the laboratory method and the Reflectoquant phosphate test-strips only detect Pi. Typical plant tissue concentrations of chloride, nitrate and citric acid had no significant effect on the laboratory method or Reflectoquant phosphate test-strips. In practical plant analysis, dilution of sap is also likely to nullify any interference effects. For example in wheat extracts, a dilution factor of thirty is used to bring tissue water samples within measurement range (Barraclough *et al*., 2000). Thus, P-esters, anions and organic acids at normal plant concentrations are very unlikely to have any adverse effects on Pi measurement using phosphate test-strips.

2.1.3.3 Experiment 3: Extraction Methods

There was no significant difference ($P=0.324$) between using the laboratory method or reflectoquant technology for determining Pi in the samples (Table 2.2). Extraction method, however, had a significant effect ($P<0.001$) on Pi measurement. Fresh samples gave the lowest Pi value of 5.6 mM, with freeze dried and frozen samples giving values approximately three times higher at 15.1 and 15.3 mM Pi respectively. Microwave and oven dried samples gave significantly ($P<0.05$) higher values at 16.3 mM, with the highest values obtained from oven dried samples at 20.9 mM.

Table 2.2 : Effect of extraction methods on Pi concentrations measured in oilseed rape leaves using Reflectoquant technology and a standard laboratory method

Extraction method	Analysis method		Mean (Pi mM)
	Laboratory (Pi mM)	Reflectoquant (Pi mM)	
Fresh	5.7	5.5	5.6
Freeze-thaw	15.0	15.5	15.3
Freeze-dry	15.4	14.8	15.1
Microwave-dry	15.9	16.8	16.3
Oven-dry	20.6	21.2	20.9
Mean	14.5	14.7	14.6

LSD - 0.81 (extraction), 0.52 (analysis), 1.15 (extraction x analysis), df = 27, CV = 5.4%

This follows the trend seen in other studies (Saarela, 1990; Bollons and Barraclough, 1997). The higher values recorded in frozen as compared to fresh material are not unexpected. Freezing causes expansion of tissue water which ruptures cell walls and releases Pi stored in vacuoles, therefore increasing the availability of extractable inorganic P forms upon thawing (Raun *et al.*, 1989). Saarela (1990), however, found only a one and half times difference between the Pi in fresh and frozen oilseed rape leaves. This small difference can be attributed to the inclusion of leaf-petioles in his study, which would have had a diluting effect on the amount of Pi extracted, as it is the vacuoles in cells of the leaf-laminae which store most Pi (Mimura, 1999). Some problems were encountered when measuring Pi in freeze-thaw samples using the phosphate test-strips. Differing dilution levels and filtration of the samples lead to significantly different results (Table 2.3). As no significant chemical effects of the sap constituents had been seen in Experiment 2 (Table 2.1), it is suggested that unknown constituents in the tissue-water samples were having an inhibitory effect on the performance of the phosphate test-strips. After diluting samples forty fold, filtration was shown to have no significant effect on Pi measurement, suggesting the inhibitory effects can be overcome by dilution alone. However, by employing a dilution factor of forty, a reduction in the lower measurement range of the phosphate test-strips occurs down to 2.2 mM Pi. This may not be a problem in practice as critical values for yield are likely to be higher than 2.2 mM, for example the critical shoot Pi for wheat yield is in the range 4-6 mM (Bollons and Barraclough, 1999). Pi values below 2.2 mM would thus simply be recorded as 'Lo' by the RQflex indicating P deficiency.

The variation in results obtained using different extraction methods highlights the importance of the choice of method used for determining Pi. Extraction method must be taken into account when comparing critical Pi values from other studies. This probably accounts for much of the disparity between reported critical values in the literature for different crops (Reuter and Robinson, 1997).

Table 2.3 : Effect of diluting and filtering freeze-thaw tissue samples on Pi measurement using Reflectoquant phosphate test-strips.

Dilution of sample	Reflectoquant Pi measurement (mM) Filtration of sample		Mean (mM)
	Yes	No	
x 20	13.0	10.3	11.7
x 40	17.2	16.9	17.0
Mean (mM)	15.1	13.6	

LSD (dilution) = 1.05, (filtration) = 1.05, (dilution x filtration) = 1.49, df =12 , C.V. = 7.5%

2.1.4 Conclusions

The freeze-dried sample perhaps best represents the true Pi status of the tissue at the time of sampling because of the speed with which ester hydrolysis would be halted by this method (Raun *et al.*, 1989), but this is not a practical technique for on-farm use. The fact that the freeze-thaw samples gave similar Pi values to the freeze-dried samples is encouraging for the development of a practical yet physiologically sound on-farm test. Freeze-thaw appears to be the most practical technique to use in measuring Pi content in oilseed rape leaves in on-farm tests using the Reflectoquant phosphate test-strips. Direct comparisons with an established laboratory method are also possible as a reference. Dilution of tissue water samples is however critical, with samples needing to be diluted by a factor of 40 at least to ensure interfering effects on the phosphate test-strips are minimised. The use of two test strips per sample should ensure sufficient accuracy within ±10%. Full details of the method recommended for on-farm testing are provided in Appendix 2.

2.2 Hydroponics: distribution of phosphate in plants and critical concentrations for maximum growth

2.2.1 Introduction

Phosphorus is a key element for plant growth, being a component of many compounds within plant cells. It plays an important structural role, being present in the sugar-phosphate backbone of DNA and RNA, and in the phospholipids which form the basic building blocks of cell membranes. Additionally, it plays an important metabolic and energetic role, with phosphorylation and dephosphorylation acting as major regulatory mechanisms (Bieleski, 1973; Mimura, 1999). Oilseed rape (*Brassica napus*) has a high demand for phosphorus (Barraclough, 1989). In young seedlings the oldest leaves die prematurely under severe deficiency, and yellowing and anthocyanin formation is evident in all but the youngest leaves giving the leaves a distinctive purple colouration around the leaf edge, which is most visible on the underside of leaf (Paul and Rawlinson, 1992). Under less severe P deficiency, plants simply tend to be smaller than those supplied with sufficient P (Pinkerton *et al*., 1989). As development progresses, P deficiency can delay flowering and the ripening of seed causing delays in harvesting the crop (Holmes, 1980). Traditionally, soil testing has provided the basis for assessing the adequacy of P supply to oilseed rape (Anon, 2000). Plant

testing is little used for making fertiliser recommendations, even though it is the only way of knowing if plant needs are actually being satisfied.

Measurement of stored Pi has been successfully used to diagnose the P status of winter wheat (*Triticum aestivum*) (Bollons and Barraclough, 1999). Attention now turns to oilseed rape, which is an important break crop in UK cereal rotations. To evaluate the potential of plant testing methods for assessing the adequacy of P supply to oilseed rape seedlings three controlled environment experiments (hereafter referred to as experiments 4, 5 and 6) were undertaken focusing on two principal objectives. Firstly, to establish the distribution of P and Pi within the different organs of oilseed rape seedlings (experiment 4) to allow the identification of a suitable target organ for plant testing, and secondly to determine critical P and Pi values in the target organ for maximum growth (experiments 5 and 6).

2.2.2 Materials and methods

2.2.2.1 Growing conditions

Six seeds of winter oilseed rape (*Brassica napus*) cv. Apex , (thousand grain weight 5.17g and total P content 0.58%), were sown into 5 litre, 20 cm diameter pots filled with 5 kg of 2EW washed sand. These pots contained mesh bottoms, to allow retention of the sand and easy draining of excess nutrient solution. Black plastic beads (diameter 4 mm) were placed on the sand surface to a depth of 15 mm to reduce evaporation. Seedlings were grown in a plant growth room, with a temperature of 20^{o}C in a 16 hour light period and 16^{o}C in an 8 hour dark period (±3^{o}C), at light intensities of 438, 425 and 550 *u* E m^{-2} s^{-1} , in Experiments 4, 5 and 6, respectively. Relative humidity was maintained at 75 % (± 10%). Seedlings were thinned to 4 per pot before nutrient application occurred. In Experiment 4, four replicates were used, with four pots for each of the two P treatments used in a randomised block design to generate sufficient material for analysis. In Experiments 5 and 6, a randomised block design was again employed with four replicates of eight P treatments.

2.2.2.2 Nutrient treatments

In Experiment 4, two nutrient treatments were used, a deficient P supply (0.1 mM) and a luxuriant P supply (5 mM). In Experiments 5 and 6, eight nutrient treatments were used varying from 0.1-10 mM P. The concentrations of other essential nutrients used in the solution (Table 1) were modified from Pinkerton (1991). Preliminary experiments (1-3) had shown that these concentrations were non-limiting to seedling growth (unpublished data). The pH of the nutrient solutions was adjusted to 5.7 using NaOH. Nutrient solution was applied to the surface of the pots, avoiding contact with the foliage. In Experiment 4, the seedlings received de-mineralised water until 9 days after emergence (9 DAE) (growth stage 1,01 (Sylvester-Bradley, 1985)) and were then flooded with excess nutrient solution every other day until 43 DAE (1,08-1,11). Thereafter, they received daily nutrient changes until harvest at 63 DAE (1,12-1,14). In Experiments 5 and 6, the seedlings received de-mineralised water until 11 DAE (1,01), and were then flooded with nutrient solution every other day until 38 DAE (1,05-1,08). Thereafter, they received daily nutrient changes until

harvest at 52 DAE (1,09-1,13). In all experiments pots were flushed through every 7 days with de-mineralised water to prevent build-up of unused nutrients.

2.2.2.3 Plant sampling

2.2.2.3.1 Experiment 4 - Distribution of %P and Pi

Whole shoots were cut at bead level. The fresh weight of the shoots per pot was recorded and the number of leaves on each of the plants counted. A leaf was counted as soon as it separated from the central leaf cluster (generally greater than 10 mm in length), with leaf 1 the first to emerge and the oldest leaf, and leaf 2 the next leaf up the leaf cluster and so on). Small subsidiary leaves subtending from branch points, and not from the main spiral development of leaves were not counted as main leaves. The stump remaining after leaves had been removed was retained. The main leaves were then split into (a) laminar and midrib and (b) petioles, with the fresh weight of each recorded. The samples were then microwave oven dried (750 KW, high power) for 4-5 minutes, turning once after 2 minutes, and then oven dried at 80 ^{o}C overnight. The dry weight was then measured and the samples milled to < 2mm using a Glen Creston Mill.

2.2.2.3.2 Experiments 5 and 6 - Critical %P and Pi

The number of main leaves on the shoot rosette was recorded and the leaves of the plants divided up into upper, middle and lower canopy categories. The leaf-laminar (including mid-rib), petioles and stalk (remaining tissue) were then split up and their fresh weight measured. The samples were then dried as in Experiment 4 and the dry weights recorded.

2.2.2.4 Plant analysis

Pi was determined colorimetrically using the molybdate-blue method (Murphy and Riley, 1962) and a procedure adopted by Greenberg *et al.*, 1993. The reagent is formed by adding sequentially, 100 ml of 0.14% (v/v) concentrated sulphuric acid (H_2SO_4, 5N), 10 ml of 0.008 M antimony (III) potassium oxide tartrate hemihydrate solution ($K(SbO)C_4H_40_6.½H_20$), 30 ml of 0.032 M ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_20$) and 60 ml of 0.1 M ascorbic acid ($C_6H_8O_6$). A standard calibration curve was prepared using KH_2PO_4, giving a linear relationship ($r^2 \geq 0.99$) between 0-1 mg P l^{-1} and absorbance readings in the range 0-0.65.

Pi concentration was determined on 0.25g of dried material using 25 ml of 2%acetic acid as an extractant. Analysis involved a 5 ml sample being added to 20 ml of the reagent. After 20 minutes the absorbance was measured at 880 nm using a Jenway 6400 spectrophotometer (Jenway Ltd., Essex, UK.) using a reagent blank as the reference solution. Pi concentrations (mM) were calculated using the previously measured % moisture content of the samples.

Total-P (%P in dry matter) was measured by inductively-coupled plasma (ICP) atomic emission spectrometry after the samples had been digested with concentrated perchloric and nitric acids (Zarcinas *et al*., 1987).

2.2.2.5 Statistical analysis

Analysis of variance was undertaken using Genstat 5 (Payne *et al.*, 1993). The dry weight (g m^{-2}) of the shoots (*y*) was related to tissue Pi and %P concentrations (*x*) by fitting Mitscherlich curves to the data (Ware *et al.*, 1982). Critical values were calculated on a 95% threshold basis.

2.2.3 Results and discussion

2.2.3.1 Experiment 4: Distribution of %P and Pi in seedlings

At harvest, the P-deficient plants (supplied with 0.1 mM P) had a significantly ($P<0.004$) lower number of leaves (10.3 *cf.* 13.4) compared to the P-sufficient plants (supplied with 5 mM P), and also a significantly ($P<0.002$) lower overall fresh weight (56.1 *cf.* 131.2g). However, the only visible symptoms in the P-deficient plants were some classical purple tipping on leaves 3 and 4 (Paul and Rawlinson, 1992).

Total %P and Pi values were significantly ($P<0.001$) higher in the leaf-lamina compared to the petioles. In the P-deficient plants, total %P and Pi measurements followed a similar trend, with significantly ($P<0.001$) higher values in newly emerged leaf laminae and petioles, compared to the older maturing and fully mature organs. In the P-sufficient plants, there were smaller differences in %P and Pi measurements between newly emerged and maturing organs (Table 2.4). In selecting leaves for testing purposes, careful choice of leaf position must be made to minimise variability in the results. Selecting the youngest mature leaf as in some previous studies (Pinkerton *et al*., 1989), was not really practical as the majority of leaves were still expanding when the plants were harvested. Selecting the youngest mature leaf in oilseed rape seedlings has also been problematic in other studies (Hocking, 2001). The distribution of %P and Pi in leaves measured here however, showed that this selection problem can be largely overcome, without losing accuracy in the results, by dividing the canopy up into top, middle and bottom categories, enabling a relatively accurate distinction of P and Pi contents to be obtained.

A comparison between P contents of a fully expanded leaf-lamina (leaf 4) receiving a deficient or sufficient P supply revealed a difference in total %P content by a factor of 8, and a difference in Pi concentration by a factor of 21. Similarly, in comparing %P contents in petioles in sufficient and deficient plants, %P values differed by a factor of 6 and Pi values by a factor of 23. Measuring Pi concentrations (mM) rather than total P (% dry matter) content in organs thus appears to offer a better method of discriminating between different P levels in plants.

Additionally, greater variability was seen when measuring %P and Pi in petioles compared to leaf-laminae (Table2.4). This is not surprising, as the petiole largely consists of vascular tissues transporting solutes to and from the leaf, so concentrations in the tissue water are naturally likely to fluctuate more widely in this tissue than in the leaf lamina, were excess Pi is stored in leaf vacuoles (Bieleski, 1973).

Measurement of Pi is thus likely to be more reliable and repeatable in leaf laminar tissue as opposed to whole leaves or petioles. By separating the leaf-lamina from the petiole we are also overcoming the problem of ensuring that a representative amount of petiole and leaf-laminar tissue is included in the sample when whole leaves are analysed.

Table 2.4 : Total %P and Pi concentrations (mM) in oilseed rape tissue receiving either deficient or sufficient P supply.

Leaf	Leaf- laminar				Petiole			
	P deficient[1]		P sufficient[2]		P deficient		P sufficient	
	Total %P	Pi mM	Total %P	Pi mM	Total %P	Pi MM	Total %P	Pi MM
1(old)	*	*	*	*	*	*	*	*
2	0.07	0.3	0.85	8.8	0.09	0.5	0.80	12.1
3	0.10	0.5	0.88	13.6	0.08	0.2	0.66	8.6
4	0.12	0.7	0.93	14.9	0.09	0.3	0.56	7.0
5	0.16	0.8	0.94	16.2	0.07	0.2	0.59	8.2
6	0.23	1.8	0.96	18.3	0.12	0.3	0.53	7.4
7	0.28	2.6	0.93	18.8	0.16	0.5	0.50	7.4
8	0.37	4.0	0.92	20.9	0.22	0.7	0.52	7.6
9	0.54	6.3	0.89	20.7	0.37	2.8	0.51	7.5
10	0.43	4.3	0.89	22.0	0.42	1.5	0.55	8.1
11	0.62	7.6	0.90	20.5	0.30	1.4	0.57	8.4
12	*	1.1	0.93	20.7	*	*	0.63	8.8
13	*	*	0.99	20.6	*	*	0.69	9.0
14	*	*	1.09	19.2	*	*	0.78	10.0
15	*	*	1.10	18.9	*	*	0.86	10.1
16 (new)	*	*	1.06	*	*	*	0.90	12.7
Stump	*	*	*	*	0.34	1.5	0.69	14.6
Shoot	0.29	2.2	0.93	18.8	0.22	1.0	0.61	8.2
Range[3]	Leaf 3-10		Leaf 3-14		Leaf 3-10		Leaf 4-14	
FProb	<0.001	<0.001	<0.001	<0.001	<0.001	0.012	<0.001	0.012
LSD	0.145	2.62	0.064	1.49	0.148	0.64	0.067	1.48
% CV	34.3	63.9	4.8	5.5	52.9	81.9	8.0	12.6

P-supply 0.1 mM[1], P-supply 5 mM[2], Statistical comparisons only in this range[3].

2.2.3.2 Experiments 5 and 6 - Critical %P and Pi

Using a wide range of external P solution concentrations produced a range of shoot dry weights. Across both Experiments 5 and 6 the shoot dry weights at harvest varied from 2.0-10.0 g plant^{-1}. In this growing system, a critical external P supply of 1.0 mM generated 95% of the maximum shoot dry weight (Figure 2.2). Despite the large differences in shoot dry weight, the only visual symptoms of P deficiency were slight purple tipping of the older mature leaves in the low 0.1 mM P treatment as also seen in Experiment 4. There was no obvious evidence of phyto-toxic growth reductions in the plants at the highest P supply of 10 mM.

Total shoot P content varied from 0.14-0.88%P and total shoot Pi content varied from 1.6-13.9 mM across the experiments (Figure 2.3). The %P and Pi content in the leaf-blades and petioles varied according to leaf age, with older more mature leaves at the bottom of the canopy having lower values (Table 2.5). The %P figures fall within the typical nutrient concentration range of oilseed rape as quoted in several studies (Reuter

Figure 2.2 : Relationship between P supply in sand culture and relative shoot dry weight of oilseed rape plants.

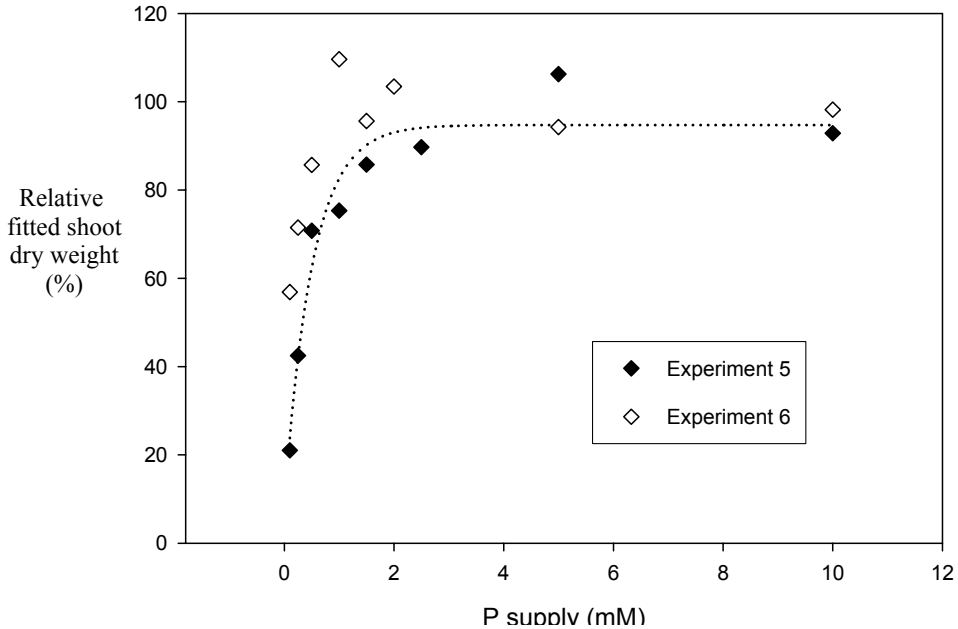
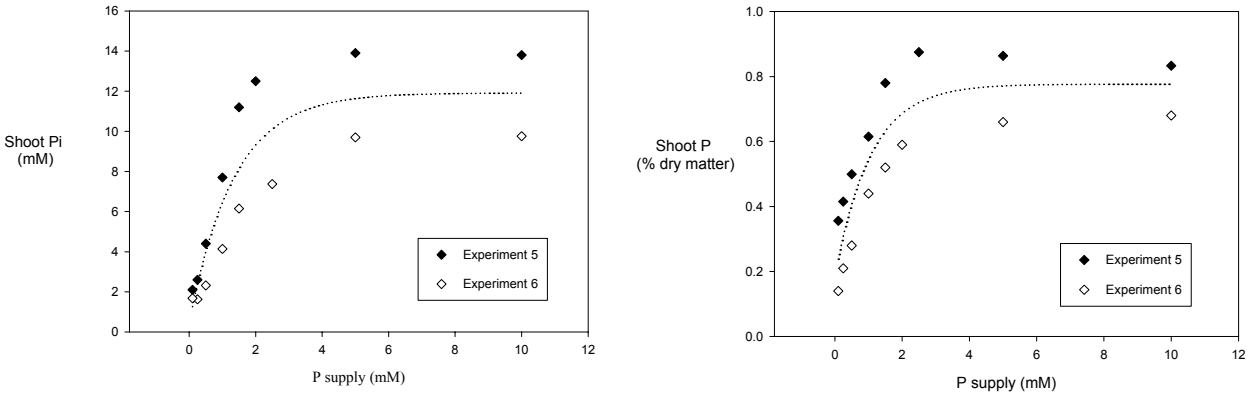


Figure 2.3 : Relationship between P supply and shoot Pi and %P concentrations in sand culture.



and Robinson, 1997). Few studies have measured Pi concentrations in leaves of oilseed rape, but the values reported here are in the same range as those reported by Saarela (1990).

The differences in P concentrations in the plants in the two experiments, which is particularly noticeable at the higher levels of P supply, is surprising given that they were grown under similar conditions (Figure 2.3). The highest shoot Pi and %P contents were however, obtained at a similar external P supply in the two experiments. The only major difference between conditions in the experiments was the higher light intensity in Experiment 6, which lead to faster growth of the plants. Differences in Pi concentrations could be due to

different water contents in the plant tissues, although there was no evidence for any differences in the moisture contents.

Table 2.5: Ranges of %P and Pi concentrations in oilseed rape plants grown in sand culture with external P supplies ranging from 0.1-10 mM.

Tissue analysed	Position in canopy	%P		Pi (mM)	
		Minimum	Maximum	Minimum	Maximum
Leaf-blade	Top	0.47	1.23	4.3	23.4
	Middle	0.15	1.07	1.3	20.6
	Bottom	0.08	1.00	0.5	15.6
Petiole	Top	0.19	0.74	3.6	10.0
	Middle	0.10	0.64	1.2	9.2
	Bottom	0.06	0.66	0.7	9.1
Stalk		0.20	0.81	3.2	14.9
Whole shoot		0.14	0.83	1.6	13.9

By relating tissue Pi and %P concentrations to shoot dry weights, critical values for maximum shoot growth can be determined (Figure 2.4). The critical Pi and %P values for maximum shoot growth in different organs are shown in Table 2.6. Using Pi gave curve fits a good deal better than using %P values, with a critical Pi mM in the middle leaf-blade calculated as 10.9 mM from the fitted curve. The critical %P for the middle leaf-blade was calculated as 0.85%P, but the poor R^2 value of 0.45, means this should be treated with some caution.

Figure 2.4 : Relationships between Pi and %P concentration in middle leaf-blades and shoot dry weight at the rosette stage.

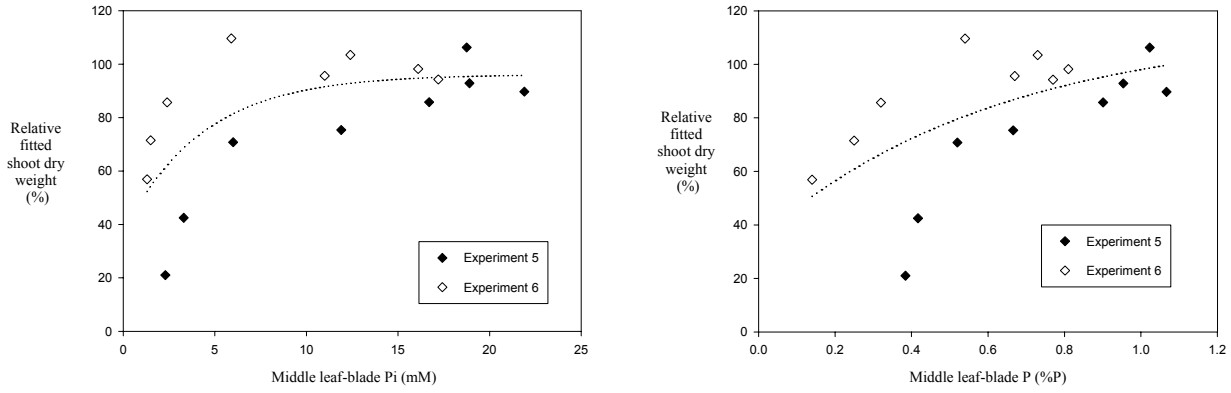


Table 2.6 : Mitscherlich growth curve parameters and critical %P and Pi in different tissues for 95% maximum shoot growth at the rosette stage.

Measure	Plant part from middle canopy	Miterschlich curve ($y = a*\exp(-bx)+c$			F Prob	R^2	Critical value
		a	*B*	*c*			
Pi	Leaf-blade	-59.2	0.223	96.1	0.0238	0.92	10.9
	Petiole	-78.7	0.624	96.3	0.0093	0.54	4.4
	Whole leaf	-66.9	0.511	99.9	0.0001	0.74	5.0
%P	Leaf-blade	-79.2	1.61	113.9	0.0052	0.45	0.85
	Petiole	-66.7	4.33	100.2	0.0403	0.41	0.49
	Whole leaf	-142.0	0.756	180.2	0.0207	0.45	0.72

2.2.4 Conclusions

Measuring the Pi concentration in the leaf-laminae of maturing leaves from the middle of the plant canopy is recommended for establishing the P status of oilseed rape. Care must be taken when selecting tissue for analysis as %P and Pi values vary within the canopy and between different plant tissues. A critical leaf Pi value of 11 mM was calculated as optimal for plant growth at the leaf production stage. Values below this indicated the crop was P-deficient for growth, whereas values above this indicated the crop was P-sufficient for growth. Sampling oilseed rape plants in the autumn could thus be used to determine whether P supply has affected the growth and establishment of oilseed rape plants. If P supply is identified as deficient, action could be taken to remedy the situation for the following crop. Remedial action in the form of soil applied P to the current crop may not be beneficial. If P supply is identified as sufficient, then attention can focus onto managing the crop canopy, as optimal autumn growth does not always translate to maximum seed yields (see below).

2.3 Field experiments: critical soil and plant concentrations for maximum seed yield

2.3.1 Introduction

There are increasing economic and environmental pressures to use phosphorus (P) fertilisers more sparingly in UK agriculture, with the inefficient use of P fertilisers being blamed for pollution and eutrophication in some water courses (Haygarth and Jarvis, 1999). Oilseed rape (*Brassica napus*) has a high P requirement (Barraclough, 1989), but it also seems to be fairly efficient at taking up P compared to some other crop species (Bolland, 1997). Slightly lower P fertiliser application rates are recommended for oilseed rape compared to wheat in the UK (Appendix D), with P fertilisers applied to 61% of the UK oilseed rape crop at an average rate of 19 kg ha^{-1} P (Anon., 2000). A reported 0.6-0.9 kg P ha^{-1} yr^{-1} is lost to water courses (Johnes *et al.*, 1996). On the basis of soil testing, 14% of UK fields probably require P fertiliser to avoid yield limitations in combinable crops, with 31% of fields having sufficient P, and 55% probably having excessive P (Skinner and Todd, 1998). It should be noted however, that there is scant evidence on critical

soil P levels for rape yield under UK conditions. Earlier observations at Rothamsted suggested that rape was particularly sensitive to soil P level, with low or even no yields on some low P plots (unpublished data). A possible explanation for this could be poor pigeon control, as the trials were sited largely in isolation from other oilseed rape crops (Inglis *et al.*, 1989). Pigeons prefer landing on bare ground or in areas of poor crop growth, as caused by low soil P for example, thereby exacerbating the differences caused by low soil P. In this project, the crops were netted to eliminate the effects of pigeon damage. Traditionally, growers' long-term P fertiliser strategy is based on soil tests every 3-4 years. This is not always the case however, with intuition and habit often playing as big a role as rational quantitative soil testing.

Simple plant tissue tests were also investigated as an alternative to soil testing to give growers confirmation of the efficacy of their P fertiliser strategy on different soils. Traditionally, tissue testing has focused on measuring total element concentration in plant dry matter (e.g. %P). This requires expensive and time consuming laboratory analysis, but more worryingly such tests are difficult to interpret. Measuring physiologically important nutrient pools in plants such as the storage pool (Pi or $H_2PO_4^-$ in the case of P) may give a better indication of plant nutrient status than total-P (Barraclough, 1993). Such simple ions also have the benefit of being relatively easy to measure, offering the prospect of simple rapid on-the-spot tests.

The fieldwork aimed to determine critical soil and plant P for maximum rapeseed yield. The effects of soil P supply (not P fertiliser additions) on the concentration of %P and Pi within rape plants and how these related to canopy growth and seed yield were studied.

2.3.2 Materials and methods

2.3.2.1 Field site and experimental design

Field experiments were undertaken in 1999/0 and 2000/1 (hereafter 2000 and 2001) using the long term P plots in Sawyers I field at Rothamsted Experimental Station in Hertfordshire (51^o 49' N, 0^o 22' W, 128 m above sea level). The soil is a free-draining silty clay loam over clay. The field consisted of 2 blocks of 12 plots, each plot measuring 9 x 15 m and each plot having a different plant available soil-P. Oilseed rape (*Brassica napus* cv. Apex) was grown in rotation with winter wheat (*Triticum aestivum* cv. Hereward), with 1 block of 12 plots being sown to each crop each year. The plots were not replicated, but the wide range of soil-P values allowed a curve fitting approach to be applied to the data (Ware *et al.*, 1982).

2.3.2.2 Weather

The 2000 growing season was warmer than the thirty year average (Appendix A). Above average rainfall was experienced in the spring, but below average rainfall was seen in the autumn and summer. There was above average sunshine in the autumn and winter, but below average summer sunshine. Temperatures in the 2001 growing season were very similar to those in 2000, although slightly cooler. The autumn began very dry, and irrigation was needed to aid crop establishment, but it then became very wet with above average rainfall in all four seasons. There was above average winter and summer sunshine.

2.3.2.3. Soil sampling and analysis

Soil samples were taken before sowing on the 17th August 1999 and 16th August 2000. Each plot was notionally split into four strips, and in 1999 five 23cm deep cores (2.5 cm diameter) were taken and bulked together for each of the central two strips, giving 2 samples per plot. In 2000, five cores were taken from each of the four strips and bulked together to give four samples per plot. The soils were air dried and then milled to pass a 2 mm sieve. Available-P was extracted by the Olsen method (5g dry soil shaken for 30 minutes with 100 ml 0.5 M $NaHCO_3$ (pH 8.5 at 20 ^oC)(Olsen *et al.*, 1954). Phosphate in the extract was measured using an adaptation of the molybdate-blue colorimetric method (Greenberg *et al.*, 1993).

2.3.2.4 Crop husbandry

Winter oilseed rape (*Brassica napus* cv. Apex) was drilled on the 31st August 1999 and the 22nd August 2000 using an Accord drill at a seed rate of 120 and 90 seeds m^{-2}, respectively. Establishment was good in both years, with 72 plants m^{-2} established in 1999 and 69 plants m^{-2} in 2000. Fertiliser in the form of 210 kg N ha^{-1} and 45 kg S ha^{-1} was applied in 2000, and 200 kg N ha^{-1} and 50 kg S ha^{-1} in 2001, with herbicides and pesticides applied as needed (Appendix A). To counter possible pigeon damage, the plots were netted over-winter with a 7.5 cm nylon mesh up until the yellow-bud stage (3,3). Crops were harvested on the 22nd July 2000 and 27th July 2001, respectively, using a Sampo plot combine harvester, with 1 kg of seed retained for the determination of dry weight, thousand seed weight and percentage oil content.

2.3.2.5 Plant sampling and analysis

Whole shoots were sampled at the rosette growth stage (2,0), green-bud stage (3,0) and yellow-bud stage (3,3) and at harvest ripeness (6,9) (Sylvester-Bradley, 1985). In 2000, three randomly assigned 0.25 m^2 quadrats were taken from each plot, and four 0.25 m^2 quadrats in 2001. A central 2.3 m strip of the plot was reserved for combine-harvesting. Plant numbers, and shoot fresh and dry weights were determined. The %P and Pi concentrations in the leaf-laminae, petioles, stems and flower buds were measured and combined to determine whole shoot %P and Pi concentrations. The largest-leaf which was generally equivalent to the youngest fully mature leaf or leaves from the middle of the plant canopy, which have been shown to have a stable Pi status (Section 2.2.3.1) were tested as potential indicator organs of plant P status.

Samples were either rapidly dried in a microwave-oven to prevent conversion of organic-P compounds to Pi (Bollons and Barraclough, 1997) or frozen at -20 ^oC in a domestic freezer. A modified molybdate-blue method (Greenberg *et al.*, 1993) was used to measure extracted Pi from dried milled plant samples using 2% acetic acid as extractant, with Pi concentration being calculated using the sample moisture content. Pi was also measured directly in tissue-water, after thawing frozen tissue and squeezing it in a syringe, to extract the tissue water. The tissue-water was analysed immediately, after appropriate dilution, using a modified molybdate-blue method (Greenberg *et al.*, 1993). Other samples were oven dried at 80 ^oC for 48 hours, milled, and the total %P in dry matter measured by inductively-coupled plasma (ICP) atomic emission spectrometry after digesting the samples in concentrated perchloric and nitric acids (Zarcinas *et al*., 1987).

At harvest ripeness, 25 plants were assessed for the number of pods per plant and seeds per pod. Thousand seed weight was determined and the oil content of the seeds measured using NMR. Seed yield was determined from the combine harvesting of a central 36 m^2 strip from the plot.

2.3.2.6 Statistics

Statistical analysis of data was performed using Genstat 5 (Payne *et al.*, 1993). The final seed yield and dry weight (g m^{-2}) of the shoot (*y*) were related to soil Olsen-P, Pi mM and %P concentration (*x*) by fitting Mitscherlich growth curves to the data (Ware *et al.*, 1982). Critical values for growth were calculated on a 95% threshold basis.

2.3.3 Results and discussion

2.3.3.1 Critical Soil Olsen-P

In 2000, total seed yield was unaffected by soil P, varying from 4.6-5.0 t ha^{-1} (at 9% moisture content) across a soil Olsen-P range of 4-69 ppm. In 2001, overall seed yields were lower but responsive to soil P varying from 0.86-4.5 t ha^{-1} across a soil Olsen-P range of 3-76 ppm. A critical Olsen-P for seed yield averaged over both years was calculated as 9 ppm (top of ADAS Index 0) (Figure 2.5). Critical soil values are however dependent on soil type and will usually vary between soil types. Theoretically, heavy clay soils with high buffer capacities are expected to have higher critical Olsen-P levels than light sandy soils (Holford and Mattingly, 1976). The low critical Olsen-P for seed yield at Rothamsted is supported by evidence from P-fertiliser studies on Chernozem soils in Canada, were no seed yield response was seen on soils with an Olsen-P above 10 ppm (Soper, 1971). Studies on red earths in Australia however, found that soils with an Olsen-P between 10-15 ppm responded well to P-fertiliser application (Osborne and Batten, 1978). A critical soil Olsen-P for autumn canopy growth at Rothamsted was calculated as 27 ppm (Figure 2.6), bottom of ADAS Index 3. Previous studies based on diffusion theory have shown that an Olsen-P of between 21-23 ppm should be sufficient to ensure adequate P uptake by high-yielding crops (Barraclough, 1989). This is much higher than the critical Olsen-P for seed yield and highlights the importance of canopy management in oilseed rape (Lunn *et al.*, 2001). Although higher soil Olsen-P leads to larger lusher canopies, it did not improve seed yield. Sparser canopies on plots with Olsen-P values between 10-26 ppm were just as effective for seed yields (Figure 2.5).

Percentage oil content varied from 42.1 to 44.2% in 2000 and from 39.8 to 44.3% in 2001. There was no clear trend due to soil Olsen-P, therefore the oil yield data reflected the seed yield data with 1.95-2.15 t ha^{-1} being obtained in 2000 and 0.34-2.04 t ha^{-1} in 2001. Other field studies (Holmes and Ainsley, 1978;

Figure 2.5 : Effect of soil Olsen-P on rape seed yield

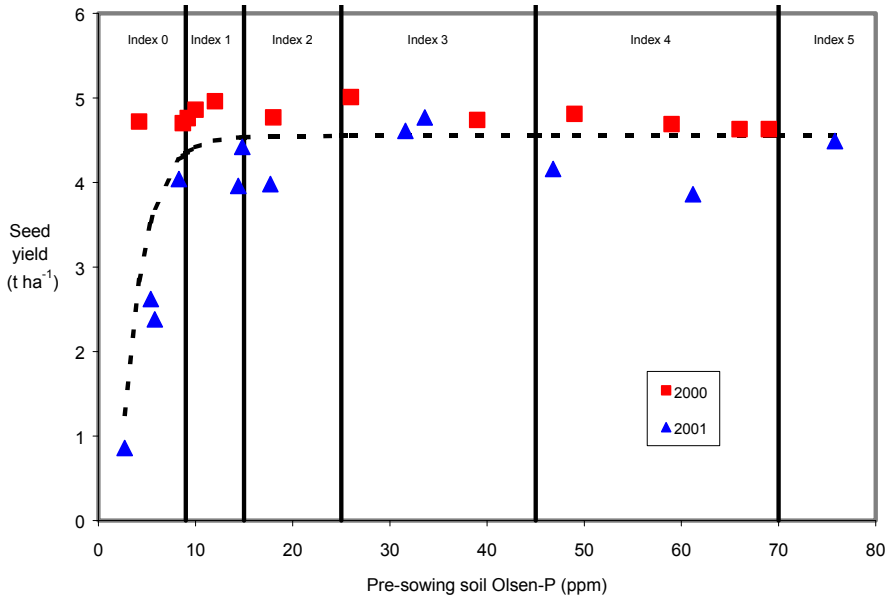
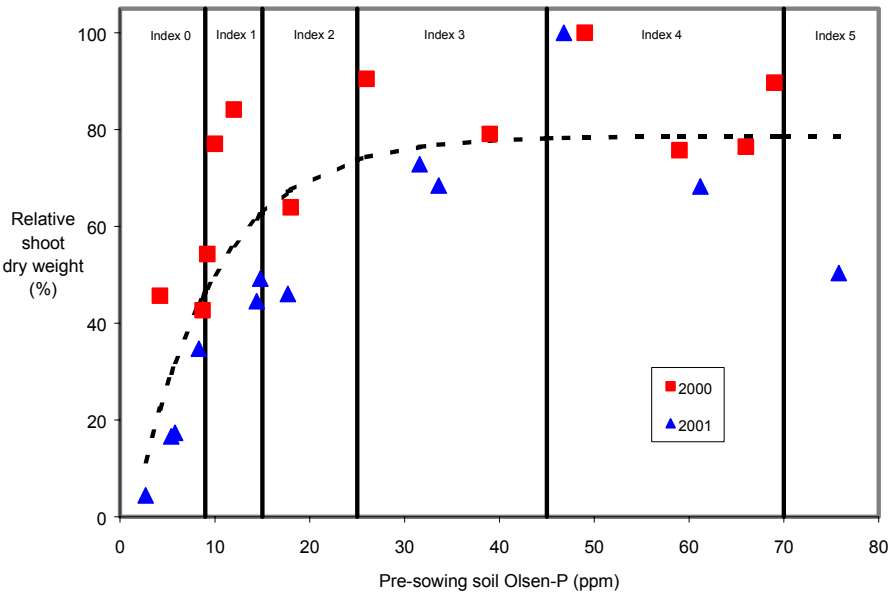


Figure 2.6 : Effect of soil Olsen-P on oilseed rape shoot growth at the rosette stage



Lewis *et al.*, 1987; Cheema *et al.*, 2001) and glasshouse studies (Lickfett *et al.*, 1999) have also shown no clear trend between phosphorus supply and seed oil concentration. Oil yield will be largely unaffected by any effects of P on seed oil content.

Thousand seed weight (TSW)(at 100% DM) varied from 4.65-4.95g and 4.09-4.38g in 2000 and 2001, respectively. However, a very low Olsen-P of 2.7 ppm produced a distinctly lower thousand seed weight of 3.43 g in 2001. Studies on Indian soils with an Olsen-P of 6.4 ppm, showed that increasing P fertiliser application rate increased TSW (Cheema *et al.*, 2001), although at higher soil Olsen-Ps no effect of P-fertiliser on TGW was seen (Majumdar and Sandhu, 1964). Seed TSW thus only appears to be affected on soils with a very low Olsen-P, with seed yields being predominantly lower due to the reduction in the number of pods produced on low Olsen-P soils.

The number of seeds per pod declined and the pod density per plot increased as soil Olsen-P increased. This is a commonly reported phenomenon in oilseed rape and accounts for why canopies bearing many pods often show no benefit over sparser pod bearing canopies (Lunn *et al.*, 2001).

2.3.3.2 Critical indicator leaf %P and Pi for seed yield

The effects of indicator leaf Pi and leaf %P on seed yield are illustrated in Figures 2.7 and 2.8 respectively, and parameters for the fitted curves are shown in Table 2.7. Critical leaf %P values declined with crop growth, which is a commonly observed trait due to the accumulation of starch and cellulose in the plant which has a diluting effect when measuring the concentrations of nutrients in dry matter (Reuter and Robinson, 1997). Critical Pi values also declined with growth stage, although not to such a large extent as critical %P values. Indicator leaf Pi had a better correlation to final seed yield than leaf %P at all the growth stages examined. There was little difference in the strength of the correlation at the rosette or green bud stages, but the strongest correlation occurred at the yellow bud stage (Table 2.7).

Table 2.7 : Equations showing relationship between indicator leaf blade Pi (mM) and %P and final seed yield at different growth stages.

Growth stage	Indicator Leaf	Critical value	Mitscherlich curve (y =*a**exp(-*b*x)+*c*			F Prob	R^2
			A	*b*	*C*		
Rosette	Pi	3.5 mM	-2070	1.72	97.4	<0.0001	0.75
	P	0.45%	-54099	20.7	92.5	<0.0001	0.65
Greenbud	Pi	2.8 mM	-5992	2.52	98.7	<0.0001	0.71
	P	0.29%	-3855	22.8	93.2	<0.0001	0.65
Yellowbud	Pi	1.9 mM	-3741	3.48	98.6	<0.0001	0.94
	P	0.22%	-4957	32.2	93.0	<0.0001	0.80

There are no studies which allow direct comparison with the critical plant values quoted here. Other studies have generally used whole leaves i.e. leaf-blade and petiole when calculating critical values (Pinkerton,

1991;. Rashid and Bughio,1993). This will have a diluting effect on the critical values as petioles contain less Pi than leaf blades (Section 2.2.3.1).

Figure 2.7 : Seed yield of oilseed rape in relation to indicator leaf Pi status at different growth stages

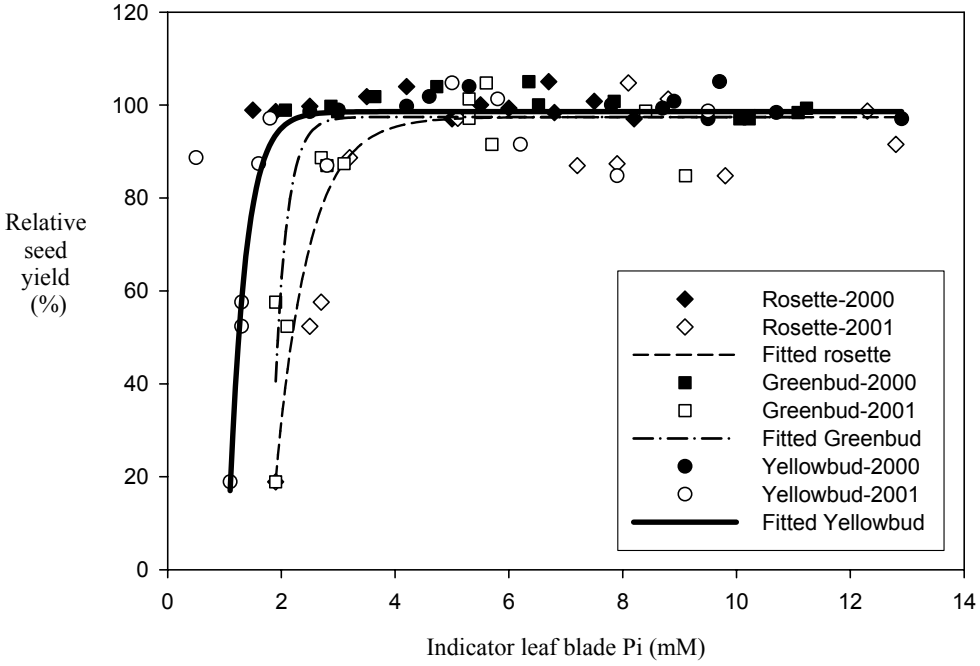
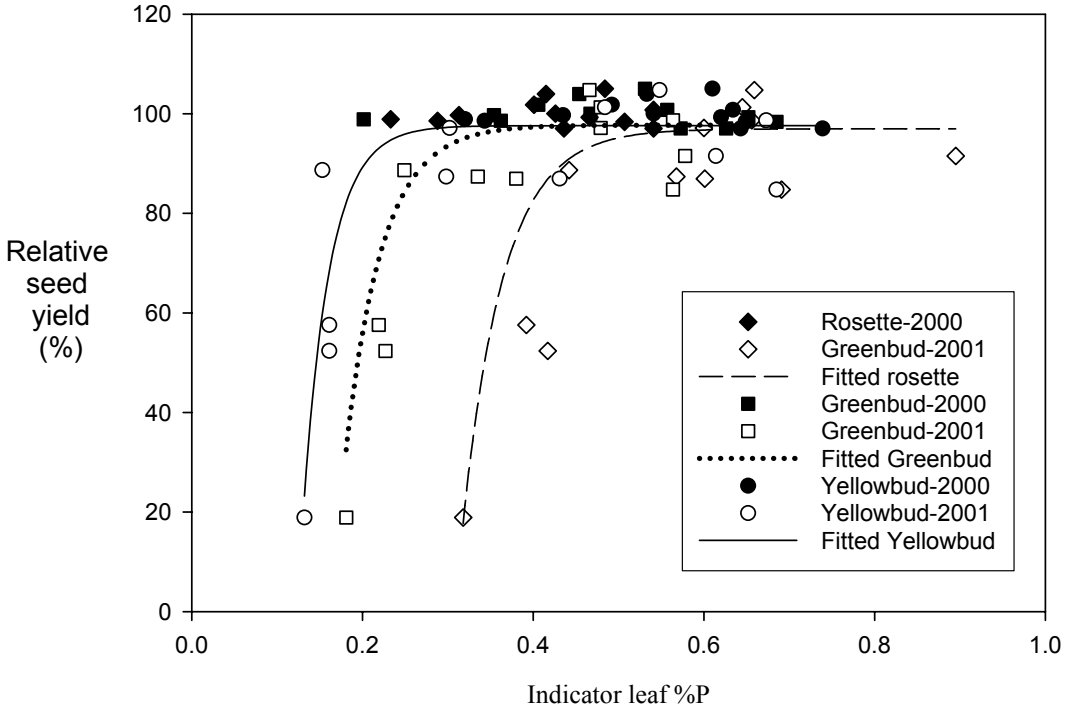


Figure 2.8 : Seed yield of oilseed rape in relation to indicator leaf %P status at different growth stages



Additionally, many workers calculate critical plant values on a 90% maximum yield basis, rather than a 95% basis (Pinkerton, 1991). Although this aids the accuracy of the calculation of critical values due to the inherent properties of the Mitscherlich curve, its agronomic usefulness is questionable, i.e. if a target crop yield is 4 t ha^{-1}, a 90% critical value equates to 3.6 t ha^{-1}, whereas a 95% critical value equates to 3.8 t ha^{-1}, which is a far more acceptable target for growers.

In Australian experiments, Pinkerton (1991) quotes a critical P in the youngest fully emerged leaf (YFEL - blade + petiole) as 0.33%P at the rosette stage for 90% yield. This compares to our critical values of 0.45%P for 95% yield and 0.42% for 90% yield, much higher than the 0.33%P in Australia, even allowing for the diluting effect of including petioles in the YFEL sample. The big differences in critical values may simply be due to the inability of the crop to reach its full potential under Australian conditions due to water limitations, and to higher temperatures experienced during pod fill (Pinkerton, 1991). Rashid and Bughio (1993) quoted a 95% critical value in the YFEL of 0.26%P at flower head initiation, presumably equivalent to the green bud stage. This compares reasonably well with our figure of 0.29%P at the green bud stage, if you take into account the diluting effect the inclusion of petioles in their leaf samples is likely to have had. At the yellow bud stage, Pinkerton (1991) quoted a critical P (90%) of 0.17% in the youngest fully emerged leaf compared to our critical values of 0.22%P (95%) and 0.20%P (90%). By taking account of the diluting effect of petioles in Pinkerton's samples, the critical values are reasonably close. Whilst critical %P in plants has been determined in relatively few studies world-wide, even fewer workers have measured critical Pi. A critical concentration of 128 mg Pi/ kg fresh weight for fresh leaves taken from the middle of *Brassica campestris* plants prior to flowering was reported by Saarela (1990). If we assume the dry weight of the leaves accounted for approximately 10% of the total fresh weight, then this equates to a concentration of 142 mg Pi per litre of tissue water or 1.5 mM. As Figure 2.1 shows, extraction method is important when measuring Pi. Saarela measured Pi on fresh acid digested leaves, but found frozen leaves gave values 30-35% higher. If we correct Saarela's results on this basis to compare with our own, then Saarela's critical value becomes 2.0 mM, very close to our critical Pi of 1.9 mM for the yellow bud stage.

The results reported here are the first critical %P and Pi values determined solely in leaf-blade tissue for oilseed rape seed yield prognosis. Using this tissue gave better relationships to final seed yield compared to using whole leaf samples consisting of leaf-blades and petioles.

2.3.3.3 Critical shoot %P and Pi for seed yield

Critical values of %P and Pi in whole shoots can also be used diagnostically, but care is needed to ensure the correct proportions of the various shoot organs are included in any sub-samples for analysis. The critical values in Table 2.8 were determined by analysing the separate organs and then combining the data to give a figure for whole shoots. The critical values declined as the plants aged following a similar pattern to that in leaf-blades. Critical shoot %P values were in good agreement with other studies taking into account differences in growth stages quoted. Critical shoot P of 0.27% (95%) was quoted for plants less than or equal

to 30 cm high, which probably equates to the green bud stage (Rashid and Bughio, 1993). Similarly Brennan and Bolland (2001), gave a critical shoot P of 0.30%, for shoots sampled 3 months before harvest in Australia, which also probably equates to the green bud stage. In general, at flowering, shoot %P levels below 0.15% have been considered deficient and levels above 0.8%P excessive for seed yield, with a target shoot concentration of 0.25-0.50%P (Grant and Bailey, 1993), which compares reasonably well with our results. No references could be found critical shoot Pi in oilseed rape in the world literature.

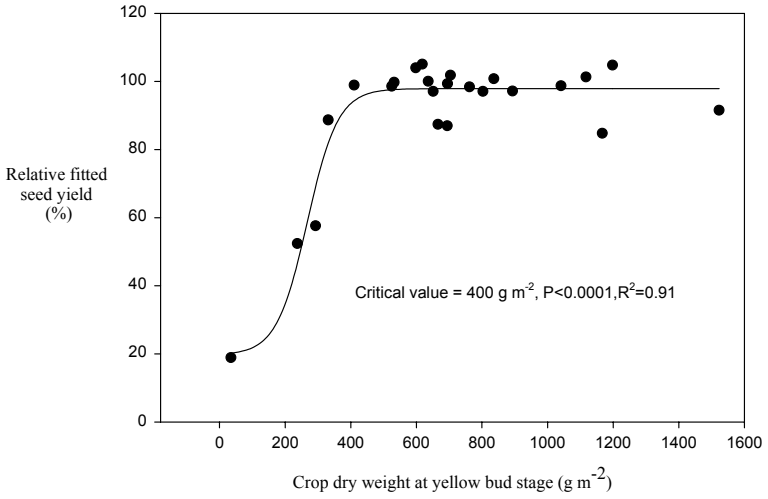
Table 2.8 : Critical shoot %P and Pi for 95% maximum seed yield

Growth stage	Sampling date	Critical Shoot %P	Critical Shoot Pi (mM)
Rosette stage (2,0)	Nov-Dec	0.66	4.3
Green bud (3,3)	Mar-Apr	0.28	2.8
Yellow bud (3,7)	Apr-May	0.21	2.4

2.3.3.4 Effects of soil Olsen-P on canopy management

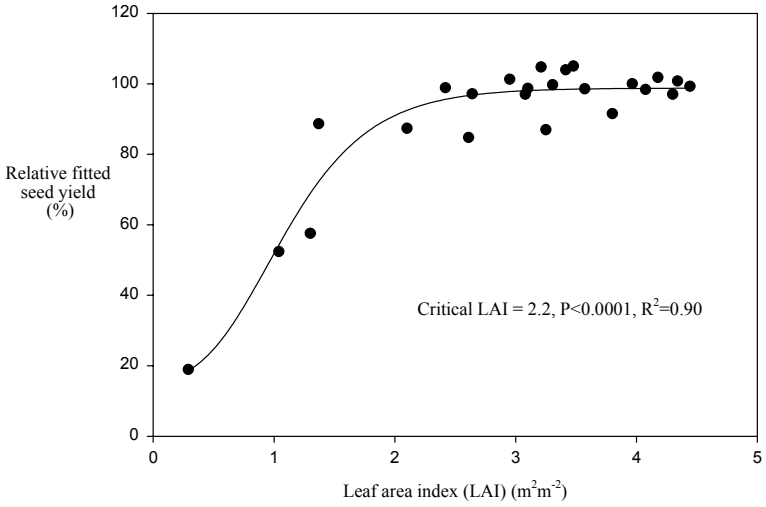
Soil Olsen-P had no discernible effects on the number of plants established m^{-2}. The number of seed bearing branches per plant increased with increasing soil Olsen-P, with more seed bearing branches in 2000 than in 2001. The number of seed bearing pods per plant also increased with increasing soil Olsen-P. However, this was counteracted by the number of seeds per pod decreasing with increasing soil Olsen-P. Thousand seed weight (TSW) remained relatively stable above an Olsen-P of 5 ppm. This explains why there was no increase in seed yield above 9 ppm Olsen despite large increases in vegetative biomass up to 27 ppm. Similar effects were noted on experiments in India on brown sarson (*Brassica campestris*), with plant height, number of pod bearing branches and number of pods per plant increased by P fertiliser, but with no effects on seed number or TSW (Majumdar and Sandhu, 1964). Crop dry weights greater than 400 g m^{-2} (4 t ha^{-1}) at the yellow bud stage were critical for seed yield however (Figure 2.9), in agreement with Mendham *et al.* (1981) who showed that about 500 g m^{-2} (5t ha^{-1}) was required at flowering to ensure optimal seed yield.

Figure 2.9 : Relationship between crop dry weight at the yellow bud stage and harvest seed yield.



A leaf area index (LAI) greater than 2.2 at the yellow bud stage was required to ensure yield was not compromised, with LAIs greater than this not showing any improvements in yield (Figure 2.10). This data supports that of Habekotte (1997) and Lunn *et al.* (2001) who showed that no yield benefit occurred in crops with pre-flowering LAI greater than 1.75. The effects of pre-flowering canopy size on eventual yield could largely be explained by the number of pod bearing branches that developed, these in turn restricted the number of pods that developed on a plant.

Figure 2.10 : Relationship between leaf area index at the yellow bud stage and harvest seed yield



Pod numbers above about 10,000 pods m^{-2} seemed to give no additional yield. The potential increase in yield by producing an increased number of pods was cancelled out by the pods containing fewer seeds, with seed weight being largely unaffected. A critical pod density of 8,500 pods m^{-2} was calculated, although the limited number of data points in this study means this figure should be treated with caution (Figure 2.11). This value is however close to the optimum pod canopy of 7,500 pods m^{-2} quoted by Lunn *et al.* (2001). In our study, soils with an Olsen-P greater than 9 ppm were able to generate a non-yield-limiting pod density of 8,500 pods m^{-2} from an average plant population of 65 plants m^{-2}, equating to 130 pods per plant.

Figure 2.11 : Relationship between pod density in June and harvest seed yield

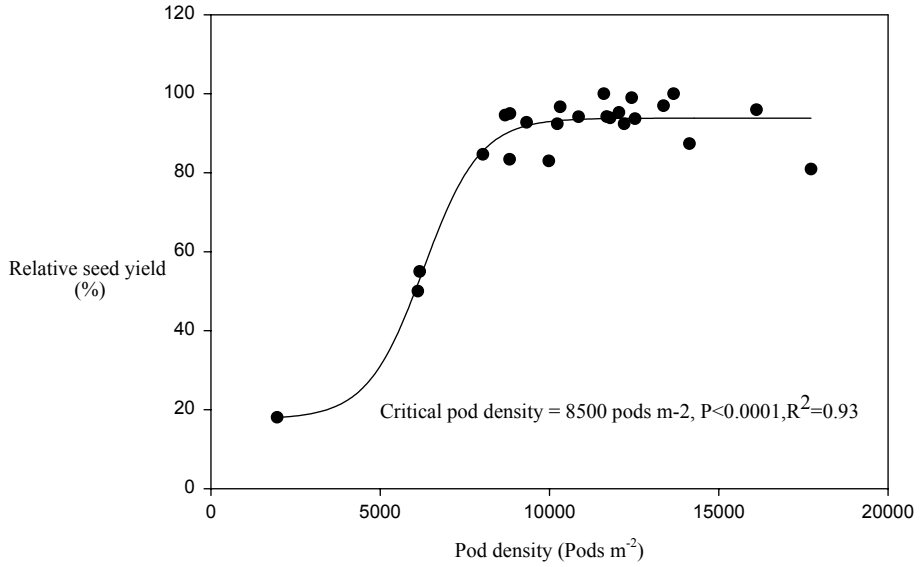
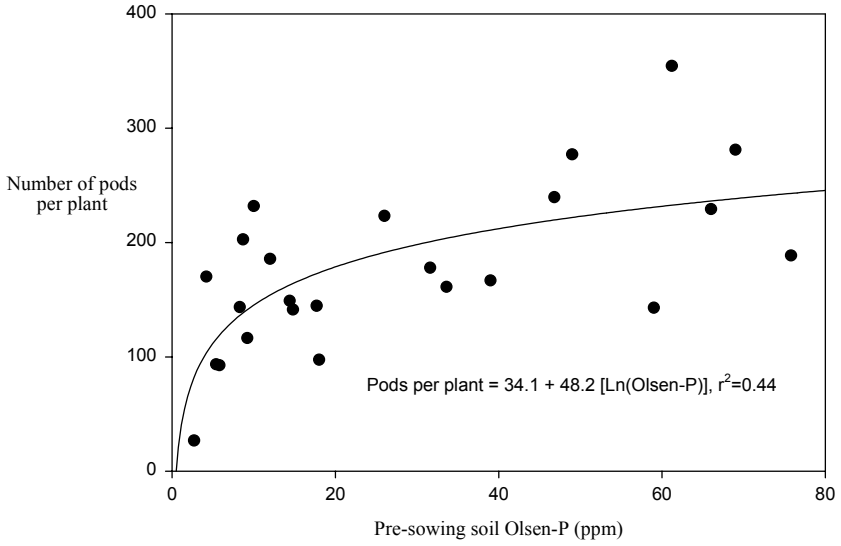


Figure 2.12 : The relationship between soil Olsen-P and the number of pods per plant at harvest



In plots with higher Olsen-Ps, the number of pods per plant increased (Figure 2.12), therefore lower plant populations could have been grown on these plots with no adverse effects on yield. In theory, seed rates could be adjusted in line with soil Olsen-P status with lower rates being possible on higher Olsen-P soils.

For example, a crop growing on a soil with Olsen-P of 12 ppm (ADAS Index 1), would require 55 plants m^{-2} to generate a pod canopy of 8500 pods m^{-2}, whereas a crop on a soil with an Olsen-P of 24 ppm (ADAS Index 1) would require only 45 plants m^{-2} to generate a similar pod canopy, an 18% difference. This

indicates potential savings that could be made by tailoring seed rates to soil Olsen-P status, although other factors such as seedbed conditions will have a much larger influence on determining seed rates (McWilliam *et al*., 1998).

2.3.3.5 Comparison between P requirements of winter oilseed rape and winter wheat

The critical soil Olsen-P for oilseed rape yield of 9 ppm is the same as that previously identified for wheat on the same site (Bollons and Barraclough, 1999). Although critical soil Olsen-P is likely to vary between soil types, it is unlikely that the critical values will differ markedly between wheat and oilseed rape on each soil type. At Rothamsted, an optimum 4.5 t ha^{-1} oilseed rape crop took up 43 kg P ha^{-1}, of which 26 kg was in the seed, equivalent to an offtake of 60 kg ha^{-1} of P_20_5. This is similar to 8 t ha^{-1} wheat crops on the same site, which contained 24 kg P ha^{-1} in the grain, equivalent to an offtake of 55 kg ha^{-1} of P_20_5 (Bollons, 1995).

The P offtake in rapeseed equates to a removal of 13.2 kg P_2O_5 per tonne of harvested seed. This figure is in close agreement with the Potash Development Association guidelines for phosphate removal by oilseed rape of 14 kg P_2O_5 per tonne of harvested seed (PDA, 1997). When adopting a maintenance approach to P fertiliser application, this figure provides a good guide as to the amount required and can be used with some confidence.

It is difficult to compare critical plant concentrations between wheat and oilseed rape as different tissues have been used in the studies and the growth stages are not directly comparable. For wheat, the current recommendations are to sample plants at stem extension (GS 31-39), whereas for oilseed rape the recommendation is to sample at the rosette stage (Table 2.9).

Table 2.9 : Critical %P and Pi concentrations in winter wheat and winter oilseed rape for 95% maximum seed yield.

Crop	Growth stage	Tissue analysed	Measure	Critical concentrations		
				Deficient	Borderline	Adequate
Winter wheat	Stem extension (GS 31-39)	Leaf 1 Whole shoot	%P	< 0.28	0.28-0.38	> 0.38
			Pi (mM)	< 4	4-6	> 6
Winter oilseed rape	Rosette Stage (1,04 -2,0)	Indicator leaf-blade	%P	< 0.40	0.40-0.50	> 0.50
			Pi (mM)	< 3	3-4	> 4

2.3.4 Conclusions

If available soil P concentrations (Olsen P) are maintained at levels sufficient for wheat production, then oilseed rape is unlikely to suffer from lack of P, as its critical Olsen-P was found to be very similar to that of wheat on Rothamsted soils (9 ppm). If you are applying enough P in the rotation to cover the needs of winter wheat, then you are unlikely to be limiting oilseed rape yield even if rape growth appears poor. If you need confirmation that soil P supplies are adequate for oilseed rape yield on different soils, then leaf tests for %P or Pi can be used.

If establishment exacerbated by pigeon damage is a problem, higher Olsen-P levels up to 27 ppm (bottom of ADAS Index 3) will encourage larger bushier plants more able to recover from pigeon damage. Olsen-P values above this showed no additional growth or yield benefits. The large canopies generated on high Olsen-P plots did not necessarily give the highest seed yields. This highlights the importance of canopy management in oilseed rape. Small canopies did not necessarily yield less.

If %P or Pi levels in rape leaves are identified as being sufficient, then the P management strategy being followed is sound. Additionally, there may be scope to omit or reduce P maintenance applications in the short term by utilising accumulated soil P reserves, thus saving on input costs with no adverse effects on yield. In such situations, growers can focus their attention on other nutrients which are more likely to be limiting such as sulphur.

Conversely, if plant %P or Pi levels are identified as being deficient in the crop, there is little growers can do to change the P status of the soil, or the plant for the current crop as deficient crops are unlikely to respond fully to a top dressing of P as they do for N and S. The recommended course of action would, therefore, be to perform a soil test to establish the actual soil Olsen-P to allow the calculation of the amount of P fertiliser to be added to the soil for subsequent crops. It would probably be best to leave the current crop to over-winter and reassess the situation in the spring. Oilseed rape has a remarkable compensatory growth habit, so depending on the plant population all may not be lost. If things haven't improved in the spring, the crop can be ploughed in, P fertiliser applied to the seedbed, and spring oilseed rape drilled.

2.4 Future research

Critical soil Olsen-P values for maximum seed yield are expected to be specific to soil-type. Ideally, soil P response trials need to be repeated on a range of soil types, but suitable sites having a range of plant-available soil P are hard to find.

Another question often asked by farmers is how long fertiliser applications can be withheld on 'luxury' soils of Index 3 or more? This again is soil type dependent as it is determined by soil buffer power. The half-life of Olsen P at Rothamsted for example is nine years. 'Run-down' is expected to be slower on 'heavier' soils than on 'lighter' soils. Again, this would require long term cropping sequences to be followed over many years on a range of soil types each having luxury P levels.

For plant testing, we do not know to what extent critical plant P and Pi concentrations in rape are affected by variations in N supply under field conditions.

There is much interest, but little or no published information, on the efficacy of P top-dressings or foliar-applied P to rectify P deficiencies diagnosed during the growing season.

2.5 Acknowledgements

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Appendix A

Weather data in 1999/0 and 2000/1 compared to long term mean

Season	Months	Mean temperature (°C)			Total rainfall (mm)			Radiation (Total sun hours)		
		1999/0	2000/1	[a]1961-90	1999/0	2000/1	1961-90	1999/0	2000/1	1961-90
Autumn	Sept-Nov	11.3	10.6	10.0	152.6	[b]395.0	184.8	391.0	277.4	308.7
Winter	Dec-Feb	4.9	4.4	3.4	195.9	294.2	182.4	238.5	223.1	163.7
Spring	Mar-May	9.0	8.5	7.9	236.0	225.2	163.1	426.0	424.8	432.3
Summer	Jun-Aug	15.9	16.3	15.2	133.8	230.1	157.7	536.2	629.4	557.8
Year		10.3	10.0	9.1	718.3	1117.5	688.0	1591.7	1554.7	1462.5

[a] Long term thirty year mean 1961-1990, [b] Includes 42 mm applied by irrigation.

Dates of agronomic activities and sampling in 1999/0 and 2000/1

Activity (Growth stage)	Year	
	1999/0	2000/1
Pre-sowing soil Olsen-P test	17/08/99	16/08/00
Sown	31/08/99	22/08/00
Autumn N	19/10/99 - 30 kg N ha^{-1}	14/09/00 - 30 kg N ha^{-1} - 7.6 kg S ha^{-1}
Rosette stage sample (1,12 -1,14)	22/12/99	06/12/01
Spring N (1st dose)	23/02/00 - 50 kg N ha^{-1} - 12.5 kg S ha^{-1}	15/02/01 - 50 kg N ha^{-1} - 12.5 kg S ha^{-1}
Green-bud stage sample (3.3-3.5)	16/03/00	04/04/01
Spring N (2nd dose)	17/03/00 - 130 kg N ha^{-1} - 33 kg S ha^{-1}	04/04/01 - 120 kg N ha^{-1} - 30 kg S ha^{-1}
Yellow bud stage sample (3.6-4.0)	06/04/00	24/04/01
Pod stage sample (6.2-6.3)	08/06/00	20/06/01
Desiccant applied (6.5-6.8)	12/07/00	14/07/01
Hand-harvest sample (6.5-6.8)	17/07/00	19/07/01
Combine harvested (6.9)	23/07/00	24/07/01

Appendix B

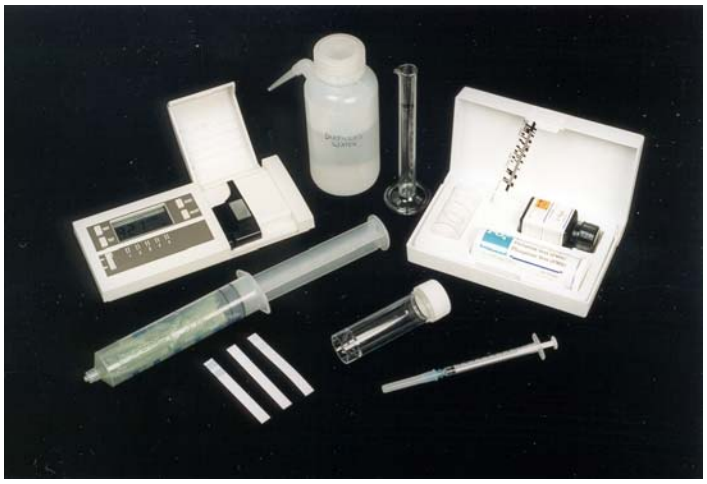
Equipment and protocol for on-farm plant testing of Pi

Reflectoquant system (Merck Ltd)
RQflex-2 Reflectometer (Cat No. 31902 3U) £502 (inc. VAT)
Phosphate test strips (Cat No 31923 3F0 50 strips = £ 54 (inc. VAT)
(test-strips are also available for other elements e.g. NO_3^- and K^+)

Supplier : Merck Ltd, Merck House, Poole, Dorset, BH15 1TD.

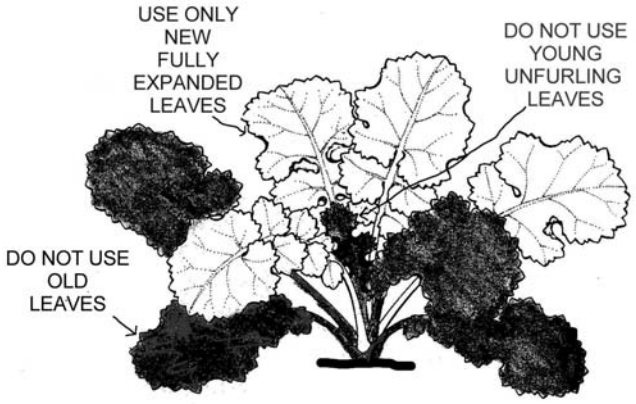
Tel : 01202 669 700, Fax : 01202 665 599 web : http://www.merckeurolab.ltd.uk

Measuring cylinder, syringes for extracting and diluting sap are also available from this supplier.

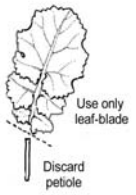


On-farm protocol for leaf-blade Pi at rosette growth stage

1) Select leaves from the middle third of the canopy, i.e. leaves that have fully unfurled and show no signs of crinkling around the leaf edge. Ensure a representative sample of leaves is selected (one leaf from twenty plants will provide plenty of material for testing).



2) Remove petiole (stalk) from the leaf, leaving leaf-blade and mid-rib.



3) Seal leaf-blade in a polythene bag and place in a freezer (-18 ^{0}C).

4) Samples must be frozen before analysis to aid sap extraction. (45 minutes in a domestic freezer is normally suitable).

5) Thaw samples at room temperature, so no ice is remaining on samples (generally 10-15 minutes is sufficient). **Do not leave for longer than this before analysing**.

6) Cut leaf-blades into 2 cm square pieces and pack into a 60 ml plastic syringe.

7) Using hand-pressure only, squeeze the sample to remove the tissue-water into a clean vessel.

8) Dilute the sap 40 times with distilled/de-ionised water and mix well (water for car batteries and steam irons can be used) i.e. 1 ml of sap + 39 ml of water.

9) Analyse for phosphate by using RQflex meter and Reflectoquant ® test strips following the manufacturers' instructions supplied with the test-strips (see below).

Phosphate test using RQflex meter and Reflectoquant ® test strips

Measurement

Calibration

1. Press the “ON/OFF” button, so that the screen display lights up.
   (Note : The machine switches itself off after 2 minutes if no button is pushed)
2. Press the “TEST” button until the arrow points to where the new method will be stored (you can also choose to overwrite an old method).
3. From a new pack of Reflectoquant ® test strips remove the specific barcode.
4. Insert the barcode into the machine from left to right until it disappears, and then carefully pull it out. Follow the arrows on the machine.
5. The coding process is complete when 3 digits are displayed and the beeper sounds. The digits should match the first 3 digits of the tube Batch number.
6. Place the barcode back into the pack, (**do not put the barcode in the strip tube)**.

Phosphate test

7. Rinse the reaction vessel with the diluted test sample.
8. Add 5ml of the diluted test sample to the reaction vessel using a syringe.
9. Add 10 drops of the PO_4 reagent from the dropper bottle to the reaction vessel, and swirl the contents.
10. Press “ON/OFF” to turn machine on and Press “START”. The display should read “90 sec”.
11. Remove one test strip from the tube and replace the cap immediately.
12. Press “START” and at the same time immerse the test strip in the reaction vessel, ensuring both reaction pads on the test strips are fully immersed, for approximately 2 seconds. Remove the strip from the reaction vessel when the display reads “87” as it counts down from “90 sec”.

13. Shake off excess liquid from the test strip. **Do not touch the reaction pads.**
14. As the counter reaches "10 sec", align the strip with the strip adapter ready for insertion into the meter. The reaction pads face towards the digital display on the left hand side.
15. When the beeper sounds (5 seconds from the end of the reaction time), insert the strip all the way into the strip adapter by sliding it open and then closing it (can be a bit fiddly).
16. At "0 sec" the beeper sounds and the result is displayed as mg l^{-1} PO_4^{3-}.

If you fail to insert the strip properly, repeat the test, as timing is crucial.

17. To measure another sample, press "TEST" and "START" and "90 sec" will again be displayed.
18. At the end of the analysis press "MEM" sequentially to display all of the measurements made.
19. At the end of the analysis, dismantle the strip adapter carefully into 3 bits, and clean the reflectant piece with water and a non-abrasive cloth. Dry all the parts and reassemble into the adapter, and carefully slide back into the RQflex machine.

For an explanation of the error messages and further details refer to the Merck RQflex manual

10) Use at least two strips for each test sample, and take the average value.

11) Multiply result by x 40, and then multiply by 0.011 to get results in mM

Volume of sap used	Volume of distilled water needed	Dilution factor	RQflex2 reading (mg l^{-1} PO_4^{3-})		
			Pi Deficient	Pi Borderline	Pi Adequate
1 ml	39 ml	x 40	Lo - 6	7 - 9	10 - Hi
			Equivalent mM concentrations		
			< 2.6 mM	3.1-3.5 mM	> 3.9 mM

Critical Pi mM values for winter oilseed rape during rosette growth stage
(more than 6 leaves emerged)

Pi Status	Pi mM (middle canopy leaf- blade)	Recommend
Deficient	< 3 mM	P fertiliser maintenance dressings require increasing (Soil test before subsequent crops essential).
Borderline	3-4 mM	P fertiliser maintenance dressings require review, possibly increasing rates (Soil test before subsequent crops advisable).
Adequate	> 4 mM	P fertiliser maintenance dressings are okay. There is scope to reduce/withhold P inputs in the short term.

Appendix C

Commercial laboratory analysis of plant P

Plant samples can be sent to a commercial laboratory for total %P analysis. The cost of these services varies depending on the suite of elements analysed and whether the results are linked to specific fertiliser recommendations relating to commercial products. Costs generally rise from approximately £16 a sample.

Below are the critical values established in winter oilseed rape *at the rosette growth stage* for maximum seed yield. Critical values will be different at other growth stages.

Sample	Total %P in dry matter		
	Deficient	Borderline	Adequate
Leaf-blade only	< 0.40	0.40-0.50	> 0.50
Whole leaf for comparison (commonly used by commercial laboratories)	< 0.33	0.33-0.43	> 0.43

Some contact numbers of commercial laboratories offering plant analysis services :-

ADAS Laboratories,
Werg Road,
Wolverhampton,
West Midlands,
WV6 8TQ.

Tel : 01902 693 290
http://www.adas.co.uk/labs

Phosyn Laboratories
Manor Place,
The Airfield,
Pocklington,
York,
YO4 2NR.

Tel : 01759 302 545
Fax : 01759 303 650
http://www.phosyn.com

Natural Resource Management Ltd.,
Coopers Bridge,
Braziers Lane,
Bracknell,
Berkshire,
RG42 6NS.

Tel: 01344 886 338
Fax : 01344 890 972
http://www.nrm.co.uk

Omex Agriculture
Bardney Airfield,
Bardney,
Lincoln,
LN3 5TP.

Tel : 01526 396 000
Fax : 01526 396 001
http://www.omex.co.uk

Appendix D

Fertiliser recommendations for winter oilseed rape (MAFF RB 209)

Soil Index	Soil phosphorus (mg l^{-1} or ppm)	Recommended P_20_5 application for rape (kg ha^{-1})	Equivalent amount of P (kg ha^{-1})
0	0-9	100	44
1	10-15	75	33
2	16-25	50 (Maintenance)	22
3	26-45	50 (Maintenance)	22
4	46-70	Nil	Nil
5 and above	>70	Nil	Nil

Appendix E

Publications from this project

Major, B.J. and Barraclough, P.B. (2000) Preliminary studies on inorganic orthophosphate in winter oilseed rape, In *Resource Allocation in Crop Plants*, A conference organised by the Association of Applied Biologists, Institute of Grassland and Environmental Research, Aberystwyth, 11-13 Sept, 2000 (Abstract).

Barraclough, P.B. and Major, B.J. (2001) Phosphorus requirements of winter wheat and winter oilseed rape. *ARIA Newsletter*, Issue 4, Oct 2001.

Major, B.J. and Barraclough, P.B. (2001) Critical phosphorus (P) and inorganic phosphate (P_i) concentrations for the growth of winter oilseed rape (*Brassica napus*). In *Plant nutrition - Food security and sustainability of agro-ecosystems*. Eds. Horst *et al.*, Published by Kluwer Academic Publishers, Netherlands, pp 714-715.

Major, B.J. and Barraclough, P.B. (submitted) Measuring inorganic orthophosphate in oilseed rape using Reflectoquant technology. *Communications in Soil Science and Plant Analysis*

Other publications from HGCA on P and K management:

'Phosphorus and potassium requirements of cereals'
HGCA Research Review No. 16 (1990)

'Plant testing to determine the P and K status of wheat'
HGCA Project Report No. 137 (1997)

'Optimising P and K use on winter wheat: plant and soil testing'
HGCA Agronomy Roadshows (1998)

'Diagnosing P and K requirements of winter wheat'
HGCA Topic Sheet No. 19 (1999)

'Phosphate and potash fertiliser recommendations for cereals: current issues and future needs'
HGCA Research Review No. 40 (1999)

'P & K fertiliser planning'
HGCA booklet (2000)

'Development of on-farm plant tests for phosphate and potassium in wheat'
HGCA Project Report No. 224 (2000)

'Wheat Management for the Autumn'
HGCA interactive CD (2001)