NITROGEN, PHOSPHORUS AND POTASSIUM REDISTRIBUTION IN HIGH-YIELDING COTTON

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Declaration of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma at this or any other University and to the best of my knowledge, contains no copy, paraphrase of material previously published or written by any other person except where due reference is made in the text of this thesis.

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Abstract

The redistribution of nutrients from one tissue to another is an important process in cotton plants, supplying bolls with the resources required for growth and development. Cotton growth models generally describe redistribution as a supplementary physiological mechanism to supply developing bolls with nutrients when root uptake is limited or inadequate. Despite its acknowledged importance, redistribution is a poorly-described process. The potential remobilisation and subsequent redistribution of vegetative nutrients has been estimated at between 40 and 70% for Nitrogen (N), and has not been quantified for Phosphorus (P) or Potassium (K). The variability of this process in different parts of the cotton plant and the effect of agronomic and environmental factors on this process has not been quantified. Increasing the understanding of how cotton plants use N, P and K, describing the accumulation of these nutrients in different tissues and assessing how the management of the plants affects their distribution and redistribution will help develop systems to maximise nutrient use efficiency, and to link nutrient inputs with the physiological processes of the plants to which they are applied.

From 2007 – 2011, eight field experiments were carried out at three sites in northern NSW, Australia, with three main aims. Firstly, to quantify N, P and K redistribution in high-yielding cotton plants; secondly, to examine the effect of plant phenotype on nutrient redistribution; and thirdly, identify the crop management practices that limit nutrient redistribution. Redistribution was calculated at a single leaf and boll scale, in five node segments up the mainstem of the cotton plant, and at the whole plant scale under various treatments.

To quantify the redistribution of N, P and K in whole plants, and to compare it between different crops, a novel method for the calculation of redistribution at a whole plant scale was developed, comparing the daily demand for N, P and K from bolls with the daily uptake of nutrients in the whole plant. This was then used to evaluate six high-yielding crops grown in Narrabri and Moree in north-west NSW, Australia in the 2007 – 08, 2008-09 and 2009-10 cotton seasons. A great deal of variability in the redistribution of N, P and K from vegetative to reproductive plants organs was measured, even between crops of a similar size, yield, and nutrient content. Within whole plants, between 6 and 52% of total plant N was redistributed from vegetative to reproductive tissue, 0 and 18% of plant P and 0 and 39% of plant K,

highlighting the wide variety in whole plant measurements. Redistribution was not linked to yield or to the ratio of reproductive to vegetative tissue (R:V) for P or K, but for N there was a positive correlation between the R:V ratio and redistribution. Root uptake, redistribution of nutrients from vegetative to vegetative organs and management or environmental stresses were proposed as factors which may have resulted in the variability of N, P and K redistribution in these crops.

To define the maximum potential redistribution, and quantify the contribution of redistribution to a single boll, ^{15}N and Rb were applied directly to mainstem and 1st position leaves, and used as tracers to measure the redistribution of N and K individual leaves. The accumulation of N, P and K in each tissue along a single sympodial branch was also described. The relative contribution of the subtending leaves to the 1st position boll on the corresponding node was calculated, and the transport of N and K from the single leaves to leaves and fruit in removed sites throughout the plant defined. A potential redistribution of 75% of leaf N and 85% of leaf K was calculated. The distribution patterns of remobilised N and K from the mainstem and 1st position leaves were different, and both N and K were transported throughout the whole plant. The relative contribution of the mainstem leaf to the 1st position seed was around 5% of the total N and none of the K, while the 1st position sympodial leaf supplied almost 7% of the seed N and 2% of the K. Contributions to the boll wall, second position leaf and leaves and fruit throughout the canopy were calculated. It was hypothesised that the remainder of the seed N and K was supplied from remote sites or root uptake.

Comparisons of the redistribution of N, P and K in different parts of the canopy were made by measuring the ^{15}N, Rb (applied in solution to the soil) and P uptake and distribution from flowering to maturity. Plants were divided into 5 node segments and the N, P and K distribution and redistribution within and between sections described. Significant variation between the plant parts for K and P were measured, with the middle portion of the canopy exporting much more P and K than the top and bottom portions. N redistribution reached the predicted potential in the bottom sections of the canopy, but not the top, indicating that there was more redistribution of vegetative nutrients from lower in the canopy. Root uptake accounted for more of the nutrients in mature bolls at the bottom of the plant than the top.

The relationship between nutrient and water supply or shortage and N, P and K redistribution was assessed in experiments providing different rates of N, P and K fertiliser and watering the plants at different soil water deficits. High (200 kg N ha^{-1}) and low (50 kg N ha^{-1}) N rates, and high (60 kg P ha^{-1} and 160 kg K ha^{-1}) and low (no fertiliser) P and K rates were applied pre-planting, with a side-dressing application of N in the high N treatment. Irrigation treatments were applied by watering the plants at two soil water deficits, "wet" at a 40 mm deficit, and "dry" at a 120 mm deficit. Nutrient stress increased the redistribution of N, P and K from vegetative to reproductive plant parts, and water stress decreased it. Unfortunately the inevitable challenges of field experiments meant that some of the treatments were confounded by rainfall or variability in soil nutrient supply.

In all experiments the redistribution of N, P or K from leaves and stems was not correlated with yield or with the ratio of reproductive to vegetative tissue. These data contradict the widely held hypothesis that a high boll load or a large ratio of reproductive to vegetative structures places excessive demands on leaf nutrient resources. The lack of relationship between harvest index, or R:V and N, P or K redistribution to bolls indicates that management stresses, nutrient supply to the roots or excess water supply may have more impact on the redistribution process than the boll load of the crop.

The thesis describes the nutrient allocation patterns and demands of high-yielding cotton, and helps to explain the physiological basis for variations in nutrient use efficiency between different crops. This data contributes to the understanding of how high-yielding cotton crops use N, P and K and how this understanding can be used to predict and explain the nutrient requirements of cotton plants.

TABLE OF CONTENTS

Declaration of Originality ii
Abstract iii
List of Tables ix
List of Figures xii
List of Equations xviii
Abbreviations xix
Acknowledgements xx
1 General Introduction 1
1.1 Nutrition in Australian cotton farming systems 2
1.2 Nutrient distribution and redistribution in cotton plants 3
1.3 Objectives 5
2 Literature Review 7
2.1 Introduction 7
2.2 The role of N, P and K 8
2.2.1 Nitrogen 8
2.2.2 Phosphorus 10
2.2.3 Potassium 11
2.3 N, P and K remobilisation and redistribution 12
2.3.1 Quantification of redistribution 13
2.3.2 Remobilisation efficiencies of leaf nutrients 14
2.4 Mineral nutrition and cotton growth and development 15
2.4.1 Cotton growth and development 15
2.4.2 Nutrient uptake and distribution 18
2.4.3 Nutrient distribution and cotton plant development 27
2.4.4 Nutrient redistribution in cotton plants 31
2.4.5 Factors affecting cotton nutrient uptake, distribution and redistribution 33
2.5 Summary 37
3 General Materials and Methods 39
3.1 Site descriptions 39
3.1.1 Australian Cotton Research Institute (ACRI), Narrabri 40
3.1.2 ‘Cardale’, Narrabri 40
3.1.3 ‘Keytah’, Moree 40
3.2 Cultivar 41
3.3 Standard procedures 41
3.3.1 Biomass sampling 41
3.3.2 Nutrient analysis 41
3.3.3 Lint yield 42
3.4 Experiment descriptions 43
3.4.1 Field experiment 1 – 2007-08, ACRI, ‘Keytah’ and ‘Cardale’. 43

3.4.2 Experiment 2 – N fertiliser rates ... 44
3.4.3 Experiment 3 – P and K fertiliser rates ... 46
3.4.4 Experiment 4 – Deficit irrigation rates ... 48
3.4.5 Experiment 5 – Nitrogen fertiliser rates ... 51
3.4.6 Experiment 6 – Application of ^{15}N isotope and RbCl to soil ... 52
3.4.7 Experiment 7 - Nutrient partitioning along a sympodial branch ... 56
3.4.8 Experiment 8 – ^{15}N and RbCl application and distribution along a sympodial branch 58
4 Nutrient uptake and distribution in non-stressed high-yielding cotton ... 62
4.1 Introduction ... 62
4.2 Materials and methods ... 63
4.2.1 Plant Sampling and analysis ... 64
4.2.2 Data Analysis ... 64
4.3 Results ... 65
4.3.1 Crop growth and development ... 65
4.3.2 Yield, boll size and boll number ... 65
4.3.3 Total biomass and nutrient uptake ... 66
4.3.4 Biomass and nutrient partitioning ... 69
4.3.5 Nutrient redistribution ... 77
4.4 Discussion ... 85
4.4.1 Total biomass and nutrient uptake ... 85
4.4.2 Biomass and Nutrient Partitioning ... 87
4.4.3 Quantifying redistribution ... 88
4.4.4 N distribution and redistribution ... 89
4.4.5 P distribution and redistribution ... 90
4.4.6 K distribution and redistribution ... 91
4.4.7 Conclusions ... 92
5 Redistribution of N, P and K along a single sympodial branch ... 94
5.1 Introduction ... 94
5.2 Materials and methods ... 97
5.2.1 Experiment 8 – N, P and K partitioning along a sympodial branch ... 97
5.2.2 Experiment 9 - ^{15}N and RbCl application and distribution along a sympodial branch 98
5.3 Results ... 99
5.3.1 Dry weight ... 99
5.3.2 Nitrogen ... 103
5.3.3 Phosphorus ... 122
5.3.4 Potassium ... 128
5.4 Discussion ... 150
5.4.1 Nutrient distribution and growth patterns along the sympodial branch ... 151
5.4.2 Nutrient redistribution from individual leaves ... 155
5.4.3 Conclusions ... 163
6 N, P and K accumulation and redistribution in relation to position in the canopy of high-yielding cotton plants ... 165
6.1 Introduction ... 165

6.2 Materials and methods ... 167
6.2.1 Treatments and sampling method ... 167
6.2.2 Data analysis ... 169
6.3 Results ... 169
6.3.1 Plant growth and development ... 169
6.3.2 Biomass accumulation ... 170
6.3.3 Uptake of N, P, K from soil ... 176
6.3.4 Distribution of nutrients ... 178
6.3.5 Redistribution of N, P and K ... 184
6.4 Discussion ... 192
6.4.1 Root uptake through the fruiting period and its contribution to boll development 192
6.4.2 Variability in redistribution in different parts of the canopy ... 194
6.4.3 Conclusions ... 199
7 Nutrient redistribution in high-yielding cotton grown with varying levels of N, P and K supply ... 201
7.1 Introduction ... 201
7.2 Materials and Methods ... 203
7.2.1 N rates ... 204
7.2.2 P and K rates ... 204
7.2.3 Plant sampling and analysis ... 204
7.2.4 Data analysis ... 205
7.3 Results ... 205
7.3.1 Crop growth and development ... 205
7.3.2 N rates experiments (2 and 5) ... 206
7.3.3 P and K fertiliser ... 213
7.4 Discussion ... 223
7.4.1 N, P and K supply and the accumulation and partitioning of N, P, K and biomass 223
7.4.2 N, P and K supply and redistribution ... 226
7.4.3 Conclusions ... 231
8 Nutrient redistribution in high-yielding cotton grown under varying soil water deficit irrigation ... 232
8.1 Introduction ... 232
8.2 Materials and Methods ... 235
8.2.1 Irrigation treatments ... 235
8.2.2 Plant sampling and analysis ... 236
8.2.3 Data Analysis ... 236
8.3 Results ... 236
8.3.1 Irrigation treatments ... 236
8.3.2 Yield, boll number and size ... 237
8.3.3 Total biomass and nutrient uptake ... 237
8.3.4 Nutrient and biomass partitioning ... 240
8.3.5 N, P and K redistribution ... 245
8.4 Discussion ... 250

8.4.1 Biomass and nutrient uptake and distribution ... 250
8.4.2 Nutrient redistribution ... 254
8.4.3 Conclusions ... 257
9 General Discussion ... 258
9.1 Quantifying redistribution ... 259
9.1.1 Nitrogen redistribution ... 260
9.1.2 Phosphorus redistribution ... 262
9.1.3 Potassium redistribution ... 263
9.2 The accumulation and source of N, P and K in bolls ... 264
9.3 How does agronomic management effect N, P and K redistribution? ... 266
9.4 The source-sink ratio in cotton plants and its effect on N, P and K redistribution. 267
9.5 Suggested future work ... 273
9.6 Conclusions ... 274
10 References ... 277

List of Tables

Table 1.1 Nutrient uptake in cotton crops yielding between 6 and 16 bales (227 kg) ha^{-1}, and the proportion of Australian growers applying different nutrients as fertilisers each year (adapted from data presented in Australian cotton cooperative research centre (2001). ... 2
Table 2.1 Reported proportional remobilisation of N, P and K from leaves in a variety of crop species ... 15
Table 2.2 Published nutrient uptake, nutrient efficiencies (kg uptake / 100kg lint ha^{-1}), nutrient removal in seed cotton, fertiliser and irrigation treatments and uptake rates and proportions at different growth stages (Planting, Flowering, End of Effective Flowering (that is, the last flower produced that will reach maturity, EEF) and Maturity). A = Total uptake (kg ha^{-1}), B = Uptake rate (kg ha^{-1} day^{-1}) and C = Proportion of total taken up in this period. ... 20
Table 2.3 Calculated amounts of N redistributed from leaves (k ha^{-1}) and as a proportion of total leaf nutrients at the peak content (%) from published nutrient uptake studies ... 32
Table 3.1 Location and year of each field experiment ... 39
Table 3.2 The dates, DAS and day degrees after sowing of each whole plant sample taken from ACRI, Cardale and Keytah in 2007-08. Day Degrees calculated using 12°C as base temperature. ... 44
Table 3.3 Sampling dates, DAS and day degrees after sowing for experiment 2 ... 46
Table 3.4 Sampling dates, DAS and day degrees after sowing for experiment 3 ... 47
Table 3.5 Dates, DAS and day degrees after sowing of irrigation treatments applied in experiment 4 ... 50
Table 3.6 Sampling dates, DAS and day degrees after sowing for experiment 4 ... 50
Table 3.7 Sampling dates, DAS and day degrees after sowing for experiment 5 ... 51
Table 3.8 Sampling dates experiment 6 ... 54
Table 3.9 Sampling dates, and days from sowing, day degrees from sowing, and days from tagging and day degrees from tagging for experiment 7 in the 2008-09 cotton season. ... 57
Table 3.10 Treatments applied to leaves on the tagged branches in experiment 9 ... 59

Table 3.11 Sampling dates, days from sowing, day degrees from sowing and days and day degrees from the application of the treatment solutions in experiment 8 60
Table 4.1 Details of the location and names of each site in chapter 4, and the references to the detailed materials and methods references from chapter 3 64
Table 4.2 Development and timing of key growth stages at the six sites in experiments 1, 2, 4 and 5. 65
Table 4.3 Yield, boll number and average boll weight. Means followed by the same letter are not significantly different at $P = 0.05$ 66
Table 4.4 Dry weight, N, P and K content m^{-2} at the six sites at flowering, 4 NAWF and maturity. * = $P < 0.05$, ** = $P < 0.001$. Means followed by the same letter are not significantly different at $P = 0.05$. 68
Table 4.5 The proportional accumulation (%) of biomass, N, P and K at flowering, 4 NAWF, and maturity for the six sites, and the mean of the pooled data from all sites. 70
Table 4.6 Estimation of redistributed N, P and K by three methods, leaf nutrient decline, vegetative nutrient decline and the comparison of total nutrient accumulation and fruit nutrient accumulation.. 81
Table 4.7 Mean amount of redistributed N (mg N m^{-2}) and the proportion of fruit N supplied by redistribution at the six different sites. Means followed by the same letter are not significantly different at $P < 0.05$. 82
Table 4.8 Correlation P values between redistributed N (mg m^{-2}), other growth parameters and N uptake data. A significant correlation (P < 0.05) is highlighted in bold text. 82
Table 4.9 Mean amount of redistributed P (mg P m^{-2}) and the proportion of fruit P supplied by redistribution at the six different sites. Means followed by the same letter are not significantly different at $P < 0.05$ 83
Table 4.10 Correlation P values between redistributed P (mg m^{-2}), other growth parameters and P uptake data. A significant correlation (P < 0.05) is highlighted in bold text. 83
Table 4.11 Mean amount of redistributed K (mg m^{-2}) and the proportion of fruit K supplied by redistribution at the six different sites. Means followed by the same letter are not significantly different at $P < 0.05$. 84
Table 4.12 Correlation P values between redistributed K (mg m^{-2}), other growth parameters and K uptake data. A significant correlation (P < 0.05) is highlighted in bold text. 84
Table 4.13 Correlation P values between redistributed K (mg m^{-2}), other growth parameters and P uptake data 85
Table 5.1 Treatments applied to leaves on the tagged branches in experiment 8 98
Table 5.2 Tissues from the main stem, position 1 (1) and position 2 (2) classified as a source or a sink, and the total amount of N imported or exported from each group from flowering at position 1 to maturity at position 2 109
Table 5.3 The mean ^{15}N excess content (mg) in each tissue of the mainstem leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the total ^{15}N excess in each. 116
Table 5.4 The equivalent supply and relative contribution of redistribution from the mainstem leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 26.05 mg 117
Table 5.5 The mean ^{15}N excess content (mg) in each tissue of the 1st position leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the total ^{15}N excess in each. 121
Table 5.6 The equivalent supply and relative contribution of redistribution from the 1st position leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 17.25 mg.. 122

Table 5.7 Tissues classified as a source or a sink, and the total amount of P imported or exported from each group.. 127
Table 5.8 Tissues classified as a source or a sink, and the total amount of K imported or exported from each group.. 135
Table 5.9 The mean adjusted Rb content (mg) in each tissue which had a higher Rb content than the control (at a significance of $P < 0.05$) of the mainstem leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the Rb in each. 142
Table 5.10 The equivalent supply and relative contribution of K redistribution from the mainstem leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 7.89 mg 143
Table 5.11 The mean adjusted Rb content (mg) in each tissue which had a higher Rb content than the control (at $P < 0.05$) of the 1st position leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the Rb in each. 149
Table 5.12 The equivalent supply and relative contribution of K redistribution from the 1st position leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 7.89 mg.. 150
Table 6.1 Mean N (mg) redistributed from each tissue in each section, and the combined tissues in each section, extrapolated from the remobilisation of ^{15}N from each tissue from the peak ^{15}N excess content until maturity... 187
Table 6.2 Mean mg P redistributed from each tissue in each section, and the combined tissues in each section from the peak P content until maturity.. 188
Table 6.3 Mean mg K redistributed from each tissue in each section, and the combined tissues in each section, extrapolated from the remobilisation of Rb from each tissue from the peak content until maturity. 191
Table 7.1 Dates of key developmental stages reached in the three experiments................. 205
Table 7.2 Mean yield (kg lint ha^{-1}), number of bolls m^{-2}, boll weight (g) and gin turnout from each N rate. Significance calculated at a 0.05 level.. 206
Table 7.3 The proportional uptake (%) of the total N and total biomass at flowering, 4 NAWF and maturity. 209
Table 7.4 The proportional distribution (%) between the leaves, stems (including petioles) and fruit (including boll walls, bracts, seeds and lint) of N and biomass at flowering, 4 NAWF and maturity.. 210
Table 7.5 The total amount of N redistributed from vegetative to fruit tissue (mg m^{-2}) and the proportion (%) of the fruit N at maturity supplied by redistribution 213
Table 7.6 Mean lint yield (kg lint ha^{-1}) and % lint of seed cotton from plants given no P and K fertiliser or 60 kg P and 160 kg K ha^{-1} fertiliser. Significance measured at $P = 0.05$....... 213
Table 7.7 The proportional uptake (%) of the total P, total K and total biomass at flowering, 4 NAWF and maturity.. 216
Table 7.8 The proportional uptake (%) of the total P, total K and total biomass distributed between the leaf, stem and fruit at (A) flowering, (B) 4 NAWF and (C) maturity. 221
Table 7.9 The total amount of P and K redistributed from vegetative to fruit tissue (mg m^{-2}) and the proportion (%) of the fruit P and K at maturity supplied by redistribution 223
Table 8.1 Measured soil water deficits to which the cotton was irrigated in the 2008-09 cotton season 237
Table 8.2 Mean lint yield (kg lint ha^{-1}), number of bolls m^{-2}, boll weight (g) and lint % from each irrigation treatment. Significance calculated at $P < 0.05$. 237
Table 8.3 The proportional uptake (%) of the total N, P and K and total biomass at flowering, 4 NAWF and maturity.. 240

Table 8.4 The mean proportional distribution (%) between the leaves, stems (including petioles) and fruit (including boll walls, bracts, seeds and lint) of N and biomass at flowering, 4 NAWF and maturity............ 241
Table 8.5 The ratio of reproductive to vegetative (R:V) biomass, N, P and K at flowering, 4 NAWF and maturity............ 242
Table 8.6 Total N redistribution and the proportion of fruit N supplied through redistribution 247
Table 8.7 Total P redistribution and the proportion of fruit P supplied through redistribution 249
Table 8.8 Total K redistribution and the proportion of fruit K supplied through redistribution 250

List of Figures

Figure 1.1 Map of cotton production areas in Australia (Australian Natural Resources Atlas (ANRA) 2002)............ 1
Figure 2.1 Schematic diagram of a cotton plant with 23 main stem nodes, showing the main stem, monopodial and sympodial branches............ 16
Figure 2.2 Cotton plant diagram showing the positioning of bolls along a fruiting branch, and up the plant, with open bolls, green bolls, flowers and squares at different stages of development............ 17
Figure 2.3 Example of dry matter and N accumulation and partitioning over time in a cotton plant, fertilised with 0 – 240 kg N ha^{-1} from Halevy *et al.* (1976). 28
Figure 3.1 Location of the three experimental sites in north-west NSW, at ACRI, Narrabri, 'Cardale', Narrabri and 'Keytah', Moree. The sites are marked in red on the map. 39
Figure 3.2 Experimental design, showing all 9 levels of N-application in the larger experiment, and highlighted are the 0 (0 kg N ha^{-1}), 5 (125 kg N ha^{-1}) and 8 (200 kg N ha^{-1}) plots sampled in experiment 2............ 45
Figure 3.3 Experimental design experiment 3 showing plots with P and K fertiliser or no P and K added............ 47
Figure 3.4 Experimental design of experiment 4, showing the six applied irrigation treatments, of which treatments 1 (frequent irrigation at a 40 mm deficit) and 3 (extended irrigation at a 120 mm deficit) were investigated for nutrient uptake, distribution and redistribution (highlighted in grey)............ 49
Figure 3.5 Schematic diagram of ^{15}N and Rb solution placement............ 53
Figure 3.6 (a) pipette used to apply an equal volume of ^{15}N urea and Rb solution to the soil, (b) the 30cm deep trench dug 15cm from the base of the cotton plants and (c) top view of the application trench showing the marker tape used to mark 10cm intervals for fertiliser solution application 53
Figure 3.7 Schematic diagram of plant sub-sampling sections for experiment 8............ 55
Figure 3.8 Tagging method of branched with white flowers in first position............ 56
Figure 3.9 Diagram of a sympodial branch of a cotton plant, showing the fruiting positions and various tissues sampled in experiment 7. Diagram from (Zhu and Oosterhuis 1992)..... 57
Figure 3.10 Injection of ^{15}N and RbCl solution into the Main stem and 1st position leaves... 59
Figure 4.1 (a) Total biomass accumulation (g m^{-2}), (b) N accumulation (mg m^{-2}), (c) P accumulation (mg m^{-2}) and (d) K accumulation (mg m^{-2}) and fitted sigmoidal curves at ACRI (—●—), Cardale (····○·····), Keytah (– –▼– –), F6 (···–△·–··), B3 (– ■ –) and A3 (··–□–·–). Vertical bar represents the least significant difference $P < 0.05$. 67

Figure 4.2 Leaf (a), stem (b) and fruit (c) dry matter at ACRI ( ), Cardale ( ), Keytah ( ), F6 ( ), B3 ( ) and A3 ( ) at flowering, 4 NAWF and maturity. 71
Figure 4.3 Leaf (a and b), stem (c and d) and fruit (e and f) N content (mg m^{-2}) (a, c and e) and concentration (mg g^{-1} m^{-2}) (b, d and f) at flowering, 4 NAWF and maturity at ACRI ( ), Cardale ( ), Keytah ( ), F6 ( ), B3 ( ) and A3 ( ). Error bars represent the LSD at $P = 0.05$. 73
Figure 4.4 Leaf (a and b), stem (c and d) and fruit (e and f) P content (mg m^{-2}) and concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity at ACRI ( ), Cardale ( ), Keytah ( ), F6 ( ), B3 ( ) and A3 ( ).Error bars represent the LSD at $P = 0.05$......... 74
Figure 4.5 Leaf (a and b), stem (c and d) and fruit (e and f) K content (mg m^{-2}) and concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity at ACRI ( ), Cardale ( ), Keytah ( ), F6 ( ), B3 ( ) and A3 ( ).Error bars represent the LSD at $P = 0.05$......... 76
Figure 4.6 Derived N accumulation curves for total N accumulation (mg m^{-2}) and fruit N accumulation (mg m^{-2}) for control plots at (a) ACRI 07-08, (b) Cardale 07-08, (c) Keytah 07-08, (d) F6 08-09, (e) B3 08-09 and (f) A3 08-09......... 78
Figure 4.7 Derived P accumulation curves for total P accumulation (mg m^{-2}) and fruit P accumulation (mg m^{-2}) for control plots at (a) ACRI 07-08, (b) Cardale 07-08, (c) Keytah 07-08, (d) F6 08-09, (e) B3 08-09 and (f) A3 08-09......... 79
Figure 4.8 Derived K accumulation curves for total K accumulation (mg m^{-2}) and fruit K accumulation (mg m^{-2}) for control plots at (a) ACRI 07-08, (b) Cardale 07-08, (c) Keytah 07-08, (d) F6 08-09, (e) B3 08-09 and (f) A3 08-09......... 80
Figure 5.1 The accumulation of dry weight in the mainstem node and leaf ( ), combined position 1 tissues ( ) and combined position 2 tissues ( ) from flowering at position 1 (day 0) to maturity at position 2 (day 70). Error bars represent +/- one standard error of the mean. 100
Figure 5.2 The (a) leaf, (b) stem, (c) petiole and (d) fruit dry weight in the mainstem section ( ), at position 1 ( ) and position 2( ) from flowering at position 1 to maturity at position 2. Error bars represent +/- one standard error of the mean. 101
Figure 5.3 (a) Position 1 and (b) position 2 boll component dry weights; boll wall ( ), seed ( ), lint ( ), petiolule ( ) and bract ( ). Error bars represent +/- one standard error of the mean. 102
Figure 5.4 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total dry weight, (b) seed and lint to total dry weight, (c) reproductive tissue to vegetative tissue (leaf, petiole and stem), and (d) seed and lint to vegetative dry weight (including the bract and boll wall) at position 1 ( ) and position 2 ( ). The red line in (c) and (d) indicates the 1:1 ratio. Error bars represent the L.S.D at $P = 0.05$. 103
Figure 5.5 The (a) N content (mg) and (b) concentration (mg g^{-1}) in the mainstem node and leaf ( ), position 1 tissue ( ) and position 2 tissue ( ) from flowering at position 1 to maturity at position 2. Error bars represent +/- one standard error of the mean. 104
Figure 5.6 The N content (mg) (a), (c), (e) and (g) and concentration (mg g^{-1}) (b), (d), (f) and (h) of the leaves (a and b), stems (c and d), petiole (e and f) and fruit (g and h) in the mainstem ( ), position 1 tissue ( ) and position 2 tissue ( ). Error bars represent +/- one standard error of the mean. 106

Figure 5.7 The N content (mg) (a) and (c) and concentration (mg g^{-1}) (b) and (d) in the wall ( ), seed ( ), lint ( ), bract ( ) at position 1 (a and b) and position 2 (c and d). Error bars represent +/- one standard error of the mean. 107
Figure 5.8 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total N content, (b) seed and lint to total N content, (c) reproductive tissue to vegetative tissue (leaf, petiole and stem), and (d) seed and lint to vegetative N content (including the bract and boll wall) at position 1 ( ) and position 2 ( ). The red line in (c) and (d) indicates the 1:1 ratio. Error bars represent the L.S.D. at *P* = 0.05. 108
Figure 5.9 The mean N (a) and ^{15}N (b) content (mg) of the mainstem leaf in the branches to which ^{15}N was applied to the mainstem leaf, and the control branches at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean. Error bars represent the L.S.D at *P* = 0.05. 110
Figure 5.10 The N content (mg) (a, c, e and g) and ^{15}N excess content (mg) (b, d, f and h) of the position 1 leaf (a and b), stem (c and d), petiole (e and f) and position 2 leaf (g and h) in the branch to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, letters above the bars represent a significant difference at *P* < 0.05. 111
Figure 5.11 The N content (mg) (a and c) and ^{15}N excess content (mg) (b and d) of the position 1 seed (a and b) and boll walls (including the petiole and bracts) (c and d) in the branch to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments. 112
Figure 5.12 The N content (mg) (a, c, e and g) and ^{15}N excess content (mg) (b, d, f and h) in the leaves at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments. 113
Figure 5.13 The N content (mg) (a, c, e and g) and ^{15}N excess content (mg) (b, d, f and h) in the fruit at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments. 114
Figure 5.14 The mean N (a) and ^{15}N excess (b) content (mg) of the 1st position leaf in the branches to which ^{15}N was applied to the 1st position leaf, and the control branches at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments. 118
Figure 5.15 The N content (mg) (a, c, e and g) and ^{15}N content (mg) (b, d, f and h) of the mainstem leaf (a and b), mainstem node segment (c and d), position 2 leaf (e and f) and position 1 stem (g and h) in the branch to which ^{15}N was applied to the 1st position leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments. 119
Figure 5.16 The N content (mg) (a and c) and ^{15}N content (mg) (b and d) of the position 1 seed (a and b) and boll walls (including the petiolule and bracts) (c and d) in the branch to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments. 120
Figure 5.17 The (a) P content (mg) and (b) concentration (mg g^{-1}) in the mainstem node and leaf ( ), combined position 1 tissue ( ) and combined position 2 tissue ( )

from flowering at position 1 to maturity at position 2. The error bar represents +/- one standard error of the mean. 123
Figure 5.18 The P content (mg) (a), (c), (e) and (g) and concentration (mg g^{-1}) (b), (d), (f) and (h) of the leaves (a and b), stems (c and d), petiole (e and f) and fruit (g and h) in the mainstem (–▪–), position 1 tissue (—●—) and position 2 tissue (- –▽ —). The error bar represents +/- one standard error of the mean. 124
Figure 5.19 The P content (mg) (a) and (c) and concentration (mg g^{-1}) (b) and (d) in the wall (—●—), seed (- –▽ –), lint (- —▪— -), bract (- –◇– – -) at position 1 (a and b) and position 2 (c and d). The error bar represents +/- one standard error of the mean. 125
Figure 5.20 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total P content, (b) seed and lint to total P content, (c) reproductive tissue P to vegetative tissue (leaf, petiole and stem) P, and (d) seed and lint to vegetative P content (including the bract and boll wall) at position 1 (▬) and position 2 (▭). The red line in (c) and (d) indicates the 1:1 ratio. The error bar represents the standard error of the mean. 126
Figure 5.21 The (a) K content (mg) and (b) concentration (mg g^{-1}) in the mainstem node and leaf (–▪–), position 1 tissue (—●—) and position 2 tissue (- –▽ —) from flowering at position 1 to maturity at position 2. The error bars represent +/- one standard error of the mean. 129
Figure 5.22 The K content (mg) (a), (c), (e) and (g) and concentration (mg g^{-1}) (b), (d), (f) and (h) of the leaves (a and b), stems (c and d), petiole (e and f) and fruit (g and h) in the mainstem (–▪–), position 1 tissue (—●—) and position 2 tissue (- –▽ —). The error bar represents +/- one standard error of the mean. 131
Figure 5.23 The K content (mg) (a) and (c) and concentration (mg g^{-1}) (b) and (d) in the wall (—●—), seed (- –▽ –), lint (- —▪— -), bract (- –◇– – -) at position 1 (a and b) and position 2 (c and d). The error bar represents +/- one standard error of the mean. 132
Figure 5.24 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total K content, (b) seed and lint to total K content, (c) reproductive tissue K to vegetative tissue (leaf, petiole and stem) P, and (d) seed and lint to vegetative K content (including the bract and boll wall) at position 1 (▬) and position 2 (▭). The red line in (c) and (d) indicates the 1:1 ratio. The error bar represents one standard error of the mean. 133
Figure 5.25 The mean K (a) and Rb (b) content (mg) of the mainstem leaf in the branches to which RbCl was applied to the mainstem leaf, and the control branches at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments. 136
Figure 5.26 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) of the position 1 leaf (a and b), position 1 stem (c and d), position 1 petiole (e and f) and position 2 leaf (g and h) in the branch to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments. 138
Figure 5.27 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the fruit at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments. 140
Figure 5.28 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the leaves at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments. 141

Figure 5.29 The mean K (a) and Rb (b) content (mg) of the 1st position leaf in the branches to which RbCl was applied to the 1st position leaf, and the control branches at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments. 144
Figure 5.30 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) of the mainstem leaf (a and b), mainstem node segment (c and d), position 2 leaf (e and f) and position 1 stem (g and h) in the branch to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments. 145
Figure 5.31 The K content (mg) (a and c) and Rb content (mg) (b and d) of the position 1 seed (a and b) and position 1 boll walls and bracts (c and d) in the branch to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments. 146
Figure 5.32 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the leaves at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments.................................... 147
Figure 5.33 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the fruit at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments.................................... 148
Figure 6.1 Schematic diagram of plant sub-sampling sections for experiment 6................. 168
Figure 6.2 The mean number of nodes of the plants in the labelled plots from flowering to maturity. The error bar represents +/- one standard error of the mean. 170
Figure 6.3 Biomass accumulation of treated plants from application of labelled fertiliser at flowering to maturity. Error bars represent +/- one standard error of the mean.................. 170
Figure 6.4 Percentage (%) of the total biomass in sections 1 ( ), 2( ), 3 ( ), 4 ( ) and 5 ( ). 171
Figure 6.5 The mean leaf ( ), stem ( ) and fruit ( ) dry weight in sections 1-4 of the labelled plants. The error bar represents +/- one standard error of the mean. 173
Figure 6.6 The mean number of flowers ( ), squares ( ), green bolls ( ), and open bolls ( ) in sections 1 – 5. The error bar is +/- one standard error of the mean. 174
Figure 6.7 The ratio of reproductive to vegetative tissue in sections 1-4. The error bar represents +/- one standard error of the mean and the dotted line represents a 1:1 ratio...... 175
Figure 6.8 The mean (a) N content and (b) ^{15}N content of the labelled plants. The error bar represents +/- one standard error of the mean. 176
Figure 6.9 The mean (a) K content and (b) Rb content of the labelled plants. The error bar represents +/- one standard error of the mean. 177
Figure 6.10 The mean P content of the labelled plants. The error bar represents +/- one standard error of the mean. 178
Figure 6.11 The proportion of total (a) N, (b) P and (c) K in section 1 ( ), 2( ), 3( ), 4 ( ) and 5 ( ). 179

Figure 6.12 The mean N content (mg) (a) and N concentration (mg g^{-1}) (b) of the leaf (—●—), stem (—◇—) and fruit (–▼–) in sections 1, 2, 3 and 4. The error bar represents +/- one standard error of the mean. ... 180
Figure 6.13 The mean K content mg) (a) and K concentration (m g^{-1}) (b) of the leaf (—●—), stem (—◇—) and fruit (–▼–) in sections 1, 2, 3 and 4. The error bar represents +/- one standard error of the mean. ... 182
Figure 6.14 The mean P content (mg) (a) and P concentration (mg g^{-1}) (b) of the leaf (—●—), stem (—◇—) and fruit (–▼–) in section 1 (g and h), 2 (e and f), 3 (c and d) and 4 (a and b). The error bar represents +/- one standard error of the mean. ... 183
Figure 6.15 The mean ^{15}N excess content (mg) (a) and ^{15}N concentration (mg g^{-1}) (b) of the leaf (—●—), stem (—◇—) and fruit (–▼–) in section 1 (g and h), 2 (e and f), 3 (c and d) and 4 (a and b). The error bar represents +/- one standard error of the mean. ... 185
Figure 6.16 The mean Rb content (mg) (a) and concentration (mg g^{-1}) (b) of the leaf (—●—), stem (—◇—) and fruit (–▼–) in section 1 (g and h), 2 (e and f), 3 (c and d) and 4 (a and b). The error bar represents +/- one standard error of the mean. ... 190
Figure 7.1 (a) total biomass accumulation (g m^{-2}) and (b) total N accumulation (mg m^{-2}) at —●—F6, 0 kg N ha^{-1}, ···○···A3 50 kg N ha^{-1}, –▼–F6 125 kg N ha^{-1}, –△–F6 200 kg N ha^{-1} and –■–A3 200 kg N ha^{-1} with fitted logistic curves ... 207
Figure 7.2 (a) biomass (g m^{-2}) and (b) N accumulation (mg m^{-2}) at flowering, 4 NAWF and maturity at —●—F6, 0 kg N ha^{-1}, ···○···A3 50 kg N ha^{-1}, –▼–F6 125 kg N ha^{-1}, –△–F6 200 kg N ha^{-1} and –■–A3 200 kg N ha^{-1}, vertical bars represent the LSD at 0.05. ... 207
Figure 7.3 N concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity at —●—F6, 0 kg N ha^{-1}, ···○···A3 50 kg N ha^{-1}, –▼–F6 125 kg N ha^{-1}, –△–F6 200 kg N ha^{-1} and –■–A3 200 kg N ha^{-1}, vertical bars represent the LSD at 0.05. ... 208
Figure 7.4 N concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity in the (a) Whole plants, (b) Leaves, (c) Stems and (d) Fruit at —●—F6, 0 kg N ha^{-1}, ···○···A3 50 kg N ha-1, –▼–F6 125 kg N ha^{-1}, –△–F6 200 kg N ha^{-1} and –■–A3 200 kg N ha^{-1}, vertical bars represent the LSD at 0.05. ... 211
Figure 7.5 Derived N accumulation curves for the daily total plant accumulation of N (◆) and the daily fruit accumulation of N (◇) in plants from (a) nil plots, plots fertilised with (b) 50 kg N ha^{-1}, (c) 125 kg N ha^{-1}, (d) 200 kg N ha^{-1} (F6) and (e) 200 kg N ha^{-1} (A3). ... 212
Figure 7.6 (a) total biomass accumulation (g m^{-2}) in ●plus PK plots and ○ nil plots with fitted logistic curves; (b) total P accumulation (mg P m^{-2}) in ●PK plots and ○ nil plots with fitted logistic curves; and (c) total K accumulation (mg K m^{-2}) int ●PK plots and ○ nil plots with fitted logistic curves. ... 214
Figure 7.7 (a) biomass; (b) P and (c) K accumulation at flowering, 4 NAWF and maturity at —●—nil plots, ···○···PK plots. ... 215
Figure 7.8 The concentration of (a) P an (b) K in the whole plants at flowering, 4 NAWF and maturity in —●—nil plots, ···○···PK plots. Error bars represent the LSD at 0.05, no bars indicate n.s.d. ... 217
Figure 7.9 (a) leaf, (b) stem and (c) fruit dry weight at flowering, 4 NAWF and maturity in —●—nil plots and ···○···PK plots. ... 218
Figure 7.10 The leaf (a and b), stem (c and d) and fruit (e and f) P content (mg P m^{-2}) (a, c and e) and concentration (mg P g^{-1} m^{-2}) (b, d and f) in —●—nil plots and ···○···PK plots. Vertical bars show the LSD at 0.05, lack of vertical bars denotes no significant difference. 219
Figure 7.11 The leaf (a and b), stem (c and d) and fruit (e and f) K content (mg K m^{-2}) (a, c and e) and concentration (mg K g^{-1} m^{-2}) (b, d and f) in —●—nil plots and ···○···PK plots. Vertical bars show the LSD at 0.05, lack of vertical bars denotes no significant difference. 220

Figure 7.12 Derived P accumulation curves for the daily total plant accumulation of P (◆) and the daily fruit accumulation of P (◇) in plants from (a) nil plots and (b) plots fertilised with 60 kg P and 160 kg K ha^{-1}. 222
Figure 7.13 Derived K accumulation curves for the daily total plant accumulation of K (◆) and the daily fruit accumulation of K (◇) in plants from (a) nil plots and (b) plots fertilised with 60 kg P and 160 kg K ha^{-1}. 222
Figure 8.1 Mean (a) biomass, (b) N, (c) P and (d) K uptake in ● wet and ○ dry plots. Lines show fitted logistic curves. 238
Figure 8.2 Mean (a) biomass (g m^{-2}), (b) N, (c) P and (d) K uptake (mg m^{-2}) in ● wet and ○ dry plots at flowering, 4 NAWF and maturity. Error bars represent the L.S.D. at 0.05, and are not presented where there is no significant difference. 239
Figure 8.3 Mean N concentration (mg g^{-1} m^{-2}) in (a) whole plants, (b) leaf, (c) stem and (d) fruit at flowering, 4 NAWF and maturity in ● wet and ○ dry treatments. Error bar represents the LSD at 0.05. 243
Figure 8.4 Mean P concentration (mg g^{-1} m^{-2}) in (a) whole plants, (b) leaf, (c) stem and (d) fruit at flowering, 4 NAWF and maturity in ● wet and ○ dry plots. Error bar represents the LSD at 0.05. 244
Figure 8.5 Mean K concentration (mg g^{-1} m^{-2}) in (a) whole plants, (b) leaf, (c) stem and (d) fruit at flowering, 4 NAWF and maturity in ● wet and ○ dry plots. Error bar represents the LSD at a 0.05 significance. 245
Figure 8.6 Mean daily N uptake in ● the whole plant and ○ the fruit (mg N day^{-1} m^{-2}), calculated as the derivative of the logistic cure fitted to the accumulation data in Figure 8.1. 246
Figure 8.7 Mean daily P uptake in ● the whole plant and ○ the fruit (mg P day^{-1} m^{-2}), calculated as the derivative of the logistic cure fitted to the accumulation data in Figure 8.1. 248
Figure 8.8 Mean daily K uptake in ● the whole plant and ○ the fruit (mg K day^{-1} m^{-2}), calculated as the derivative of the logistic cure fitted to the accumulation data in Figure 8.1. 249

List of Equations

Equation 1 Conversion of $\delta^{15}N$ to Atom % 42

Abbreviations

A3	Field A3, at ACRI
ABA	Abscisic Acid
ACRI	Australian Cotton Research Institute
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of Variance
ANRA	Australian Natural Resources Atlas
AR	Absolute Ratio
ATP	Adenosine triphosphate
BRF	Bollgard Roundup Ready Flex
B3	Field B3, at ACRI
CHN Analyser	Carbon, Hydrogen and Nitrogen Analyser
CRDC	Cotton Research and Development Corporation
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAF	Days after flowering
DAS	Days after sowing
DNA	Deoxyribonucleic acid
EEF	End of Effective Flowering
F6	Field F6, at ACRI
IAA	Indole-3-acetic acid
ICAC	International Cotton Advisory Committee
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IRMS	Isotope-Ratio Mass Spectrometry
mRNA	Messenger Ribonucleic acid
NAM	Neutron Attenuation Meter
NSW	New South Wales
RCBD	Randomised Complete Block Design
RNA	Ribonucleic acid
R:V	Reproductive : Vegetative
4 NAWF	Four nodes above white flower

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CHAPTER 1

General Introduction

Cotton is a perennial plant produced throughout the world primarily for fibre and also for oil. In common with other terrestrial plants, cotton requires the adequate supply of nutrients for growth and development. Most (90%) cotton produced worldwide is *Gossypium hirsutum* (upland cotton) while *Gossypium barbadense* (pima cotton) accounts for 5% of world cotton production. Two other species are produced commercially on a smaller scale (*Gossypium herbaceum* and *Gossypium aboreum*). Most cotton grown in Australia is *G. hirsutum* (Cotton Australia 2003).

Commercial cotton production in Australia extends from Emerald in Queensland through to Hay in Southern NSW (Fitt 1994), in variable climatic regions and on different soil types (Figure 1.1). Since then, some cotton has been grown further north and south, outside the primary growing regions.

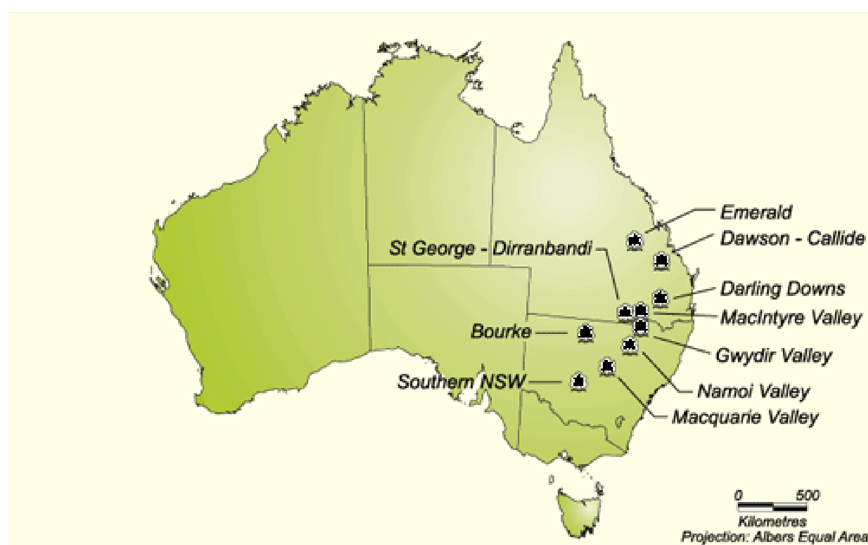

Figure 1.1 Map of cotton production areas in Australia (Australian Natural Resources Atlas (ANRA) 2002)

The Australian cotton industry is characterised by high input management, technological sophistication and highly mechanised farming and ginning operations (Fitt 1994; Cotton Australia 2003). Along with highly specialised and intensively managed farming systems, Australian cotton is grown mostly under furrow-irrigation and Australian bred cultivars, adapted to the climate and soil conditions, are the most

common. This intensive management has meant that over the past 25 years lint yields have steadily increased, to the point at which the average Australian cotton yield (1792 kg lint ha^{-1}) was 2.5 times the world average of 747 kg lint ha^{-1} in 2007 (ICAC 2007). In Australia, crops in excess of 2500 kg lint ha^{-1} or more are now being produced.

1.1 Nutrition in Australian cotton farming systems

The use of fertilisers on Australian cotton farms reflects both the demand for nutrients from cotton plants, and the inherently fertile soils on which cotton is predominantly grown. While P, K, S, Mg and Ca are required in large amounts for the production of cotton lint and seed, not all growers apply these nutrients as fertilisers. Table 2 shows the amount of nutrients removed in seed cotton at various yield levels, and the proportion of growers who apply these nutrients to their crops as fertilisers (Australian cotton cooperative research centre 2001).

Table 1.1 Nutrient uptake in cotton crops yielding between 6 and 16 bales (227 kg) ha^{-1}, and the proportion of Australian growers applying different nutrients as fertilisers each year (adapted from data presented in Australian cotton cooperative research centre (2001).

Nutrient	**Lint Yield (227 kg bales ha^{-1})**						**Proportion of growers applying fertiliser**
(Kg ha^{-1})	**6**	**8**	**10**	**12**	**14**	**16**	
N	43	68	116	155	185	215	100
P	15	20	26	28	33	37	50
K	25	34	37	44	52	59	10
S	4	6	10	12	14	17	*20**
Mg	9	12	16	17	20	23	*20**
Ca	3	4	6	7	8	9	*20**
(g ha^{-1})							
Fe	150	220	330	191	217	244	*20**
Mn	28	36	44	18	21	24	*20**
B	29	37	45	70	85	99	*20**
Zn	110	130	150	129	148	166	30
Cu	16	20	24	28	32	36	*20**

* 20% of growers apply some combination of S, Mg, Ca, Fe, Mn, B and Cu fertilisers depending on seasonal and environmental conditions

Nutrient use-efficiency is an important concept for cotton farmers, particularly as they seek to reduce input costs and minimise their dependence on non-renewable fertiliser resources. Fertilisers represent a small proportion of the total operating costs of a commercial cotton farm. They do, however, represent a large proportion of the greenhouse gas emissions from such facilities, and a significant environmental cost. This issue will become more important to the industry in the future. A better understanding of cotton nutrient use and demands, and the drivers for nutrient use-efficiency and for changes in demands, is important for the industry as a pre-emptive move towards improving nutrient use-efficiency and minimising the environmental impact of fertiliser use.

1.2 Nutrient distribution and redistribution in cotton plants

In the past 50 years, many studies have examined the nutrient uptake and partitioning in cotton plants. These have detailed the timing of uptake, the distribution of nutrients between tissues and within bolls and examined the effect of management strategies on nutrient use patterns (Bassett *et al.* 1970; Jones *et al.* 1974; Halevy 1976; Hearn 1976b; Leffler and Tubertini 1976; Leffler and Hunter 1985; Cassman *et al.* 1989a; Unruh and Silvertooth 1996; Pervez *et al.* 2004; Geng *et al.* 2005). In Australia, research has focussed on N, K and P nutrition, particularly the response of cotton to fertilisation and fertiliser rates, the enhancement of nutrient use efficiencies in high-yielding cropping systems including cotton, and the maintenance of soil fertility and condition for long term production sustainability (Constable and Rochester 1988; Constable *et al.* 1988; Constable *et al.* 1991; Freney *et al.* 1992; Rochester *et al.* 2001; McKenzie *et al.* 2003; Dorahy *et al.* 2004; Rochester and Constable 2006; Rochester 2007). There has been little research in Australia and the world linking nutrition and nutrient inputs to physiological processes, particularly the contribution of leaf nutrients from senescing leaves to the developing bolls.

Most studies relating to the uptake and partitioning of nutrients in cotton plants refer to the redistribution of nutrients from vegetative organs to reproductive structures, though very few quantify total redistribution or attempt to estimate the proportion of boll nutrients sourced from surrounding leaves and stems. As cotton plants grow indeterminately, they must continue to grow vegetatively to produce new reproductive

sites. Growth and partitioning models for cotton plants assume that some level of intra-plant competition for assimilates, nutrients and other growth resources takes place after flowering and through boll setting (Hearn 1976a). These models assume that;

1) cotton plants preferentially allocate resources to developing bolls at the expense of vegetative tissue
2) remobilisation and redistribution of assimilates and nutrients from vegetative tissue is a supplementary physiological mechanism to supply the preferred sinks (bolls) with resources for growth, and,
3) the accumulation of carbohydrates (and possibly nutrients) in bolls, feeds-back negatively to slow, and eventually stop the production of new vegetative and reproductive growth.

Several factors have been identified across many studies indicating that the redistribution of nutrients from leaves supplies the rapidly developing bolls with the nutrients that they require. These factors include;

1) coinciding with the period of peak nutrient demand from the seeds (Bassett *et al.* 1970; Leffler and Tubertini 1976), leaf activity (photosynthesis, protein and chlorophyll concentrations) and nutrient content declines (Constable and Rawson 1980c; Constable *et al.* 1988; Schwab *et al.* 2000).
2) the decline in root nutrient uptake, respiration and growth (Pettigrew *et al.* 2000)
3) the appearance of nutrient deficiency symptoms on leaves during the boll-filling period (Wright 1999; Pettigrew *et al.* 2000)
4) the relationship of boll nutrient accumulation and concentration to that of the subtending leaf (Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999; Wahid *et al.* 2004), and,
5) A comparison of the sink size and strength (indicated by the preferential allocation of nutrients to one tissue over another) of leaves and bolls (Hall and Brady 1977; Guitman *et al.* 1991; Oosterhuis and Bondada 2001).

The distribution, transport and allocation of carbon assimilates synthesized in leaves is well documented (Constable and Rawson 1980a; Wullschleger and Oosterhuis

1990b; Wullschleger and Oosterhuis 1991). There have been far fewer studies which have quantified or estimated the gross amount or proportion of nutrients redistributed from leaves to the developing bolls, or accounting for redistribution when describing the partitioning of nutrients. Several researchers have, examined the export of nutrients from a single leaf, or along a single branch. Wahid *et al.* (2004) published an analysis comparing the ratio of the nutrient content of the subtending leaf to that of the reproductive organ (boll) as a means of estimating redistribution. ^{15}N isotopes have also been used to measure the movement of N from a leaf into a subtending boll (Rosolem and Mikkelsen 1989; Bondada *et al.* 1996; Bondada *et al.* 1997), but similar data for P and K has not been published. These reports suggest a high degree of variability between the proportion of nutrients exported from the leaves, and that leaf export may not be capable of supplying developing bolls with all the nutrients required for growth. These reports conclude that continued root uptake must be required to support high yields (Bassett *et al.* 1970).

Despite, there being many studies outlining the uptake, distribution and redistribution of mineral nutrients in cotton crops, there are several key questions that remain unanswered in the field of physiological nutrient use and distribution. Firstly, it remains unclear if modern, high-yielding, transgenic cultivars accumulate nutrients at the same rate and distribute them in the same proportion as older, conventional, lower-yielding cultivars. Secondly, the proportion of nutrients in the reproductive structures supplied through the remobilisation of leaf and stem nutrients needs to be quantified, and, thirdly the effect of the source and sink ratio on redistribution of leaf nutrients, needs to be examined and quantified. The effect of environmental factors and agronomic management on the nutrient distribution and redistribution in a cotton plant also should be quantified and described. The efficient use of nutrients by cotton crops is possibly linked to the ability of the crop to redistribute nutrients, but this process is not well understood.

1.3 Objectives

The primary aim of this study was to describe and quantify the N, P and K dynamics in a cotton plant, particularly through the fruiting period. Understanding the distribution and redistribution of nutrients within and between plant structures will

help to inform management decisions and better understand the link between the plant's physiological processes and the need for fertiliser inputs. Increasing the understanding of how a plant and cotton growing system might become more efficient will assist the Australian cotton industry in its aim to increase nutrient use-efficiency, and reduce unnecessary environmental impacts and the cost of cotton production.

The specific objectives of this study were to;

1) Quantify the redistribution of N, P and K from vegetative tissues to reproductive tissues in high-yielding cotton crops representative of the high-input, high boll load, modern production systems used by the Australian cotton industry (Chapter 4).
2) Describe the accumulation pattern of N, P and K in a mature boll, and in boll components (Chapter 5).
3) Identify the source of N, P and K in a mature boll and the relative contribution of each source (Chapters 5 and 6).
4) Evaluate the effects water and nutrient supply on the redistribution of N, P and K within a cotton plant (Chapters 7 and 8).
5) Evaluate the effects of the boll load, or ratio of reproductive to vegetative tissue on the redistribution of N, P and K from sources to sinks.

CHAPTER 2

Literature Review

2.1 Introduction

Over the past century, research into plant nutrition has increased our understanding of nutrient uptake and functioning and the relationship between plant development, metabolic processes, reproduction and nutrient availability. Managing the supply and monitoring the status of the nutrients supplied to growing plants is an integral part of commercial agriculture. The allocation of mineral nutrients within a plant is influenced by a range of both direct and indirect factors, controlling nutrient uptake, storage, partitioning and transport. Managing this allocation is a crucial part of agricultural systems across the world, and the concept of nutrient use efficiency has been used to calculate the proportion of nutrients contained in economically valuable plant parts. In many cases, improvements in productivity have been associated not with increased total production, but with an increase in the allocation of resources to the organs to be harvested (Wardlaw 1990).

The Australian cotton industry, like many of Australia's agricultural industries, has identified increasing nutrient use efficiency as a priority. Cotton research in Australia has been focussed on improving plant productivity through breeding, agronomic management and water use efficiency. Few studies have focussed on integrating nutritional inputs with physiological processes, particularly on understanding the link between nutrient remobilisation from leaves and stems and its transport to developing bolls.

This review will examine the role of Nitrogen (N), Phosphorus (P) and Potassium (K) in plants, their role in cotton growth and development, and summarise previous studies reporting both a gross and proportional amount of N, P and K redistributed from one plant organ to another.

2.2 The role of N, P and K

There are many comprehensive reviews of nutrient function, uptake mechanism and metabolic role (e.g. Britto and Kronzucker 2006; Dreccer 2006; Tischner 2006), therefore these will not be discussed in detail in this review. A simpler overview will be presented as a framework in which to view these nutrients as plant constituents and to discuss their relation to cotton growth and development and their redistribution in cotton plants.

2.2.1 Nitrogen

2.2.1.1 Uptake and Assimilation

Nitrate (NO_3^-) and ammonium (NH_4^+) are the major sources of inorganic N taken up by plant roots. Nitrate is a highly mobile ion (in the xylem) and as such is transported directly from roots to above ground plant parts through xylem vessels, with bulk flow of water in the transpiration stream. Since the assimilation of NH_4^+ releases protons (to ensure charge compensation), and leaves have a limited capacity to dispose of excess protons, nearly all ammonium taken up is assimilated in the roots. Ammonium is incorporated in the roots and transported as organic compounds such as amino acids, amides or related amino acid compounds. The ratio of nitrate to ammonium uptake varies according to plant species, environmental conditions and N supply, but commonly is in the order of 10 – 20:1 (Epstein and Bloom 2005).

Cotton plants generally reduce and assimilate nitrate in leaves. This process has lead to the widespread use of petiole nitrate testing as a means of determining N uptake and supply to developing plant parts, and to identify deficiencies where they occur. Since nitrate levels in the petiole are a good indication of the access that a cotton plant has to N in the soil (Constable *et al.* 1991; McConnell and Mozaffari 2004).

2.2.1.2 Function

As much as 5% of a plant's dry weight is made up of N, and in some organs the proportion of N may be higher. Unlike lower plants, animals and people, plants cannot re-oxidise organically bound N to nitrate, so once the reduction and assimilation has taken place, it is a permanent change (Marschner 2002). The majority

of the N found in a plant at any time, therefore, will be in a reduced form, primarily as a low molecular weight organic compounds, which are intermediaries between structural and functional proteins and between the inorganic ions taken up from the soil.

Nitrate ions play an important role in maintaining the charge balance in cells, intracellular pH regulation and in osmoregulation (Mengel and Kirkby 2001; Marschner 2002; Epstein and Bloom 2005). N is an important elemental constituent of all proteins and as a structural component of cell structure, organelles and membranes. Due to their role in proteins N is found in every plant tissue to some degree. As much as 5% of a plant's dry weight is made up of N, and in some organs the proportion of N may be higher.

2.2.1.3 Relation of N supply to plant growth and development

N supply has been directly correlated with yield (Read *et al.* 2006), growth rate (Novoa and Loomis 1981), timing of senescence (Guitman *et al.* 1991; Hortensteiner and Feller 2002), photosynthesis (due to it's role in chlorophyll synthesis and function) (Wullschleger and Oosterhuis 1990a), root growth and shoot: root ratio (Levin *et al.* 1989), leaf dry weight (Yoshida *et al.* 1969; Grechi *et al.* 2007), mineral nutrient uptake, water uptake (Radin and Mathews 1989), lignin content (Koefoed 1993), plant size (Thompson *et al.* 1976), fruit retention and reproductive growth rate (McConnell and Mozaffari 2004; Read *et al.* 2006).

When N supply is sub-optimal, N is remobilised from mature leaves and redistributed to areas of new growth. This causes a yellowing of leaves (due to chlorophyll breakdown) and enhances leaf senescence, which is a typical symptom of N deficiency. Typically, over supply of N will cause an increase in shoot: root dry weight ratio, particularly when extra N is supplied in the rooting zone. Changes in leaf morphology can also be seen, often increasing leaf area and decreasing leaf thickness (Yoshida *et al.* 1969). In some plants, over supply of N slows reproductive development and reduces the ratio of reproductive growth to vegetative growth, resulting in yield penalties and reduced profitability (Thompson *et al.* 1976; Hearn 1981; Leffler and Hunter 1985).

2.2.2 Phosphorus

2.2.2.1 Uptake and assimilation

Unlike N, P is not reduced in plants; instead it remains in the oxidised form in which it is taken up by roots. There are three main forms of P, or methods of assimilation in higher plants, inorganic phosphate ions (P_i), phosphate esters (in which a P molecule is bound to a carbon chain, for example sugar phosphate) and attached to other phosphate molecules forming an energy rich phosphate bond (for example in ATP). Usually, inorganic phosphate absorbed by plant roots is esterified within minutes, however the inorganic P is released, and transported in xylem vessels (Mengel and Kirkby 2001; Marschner 2002; Epstein and Bloom 2005).

2.2.2.2 Function

The most prominent function of P is as a constituent of nucleic acids (DNA, RNA and mRNA) (Amtmann *et al.* 2006). In both DNA and RNA P is important in the formation of macromolecules, joining ribonucleoside units. The other major function of P in plants is as an energy supply and store. The energy required (or liberated) for metabolic processes, respiration, photosynthesis, biosynthesis and degradation of proteins is supplied by energy rich phosphates. Adenosine monophosphate (AMP), Adenosine diphosphate (ADP) and Adenosine triphosphate (ATP – the principal energy supplying coenzyme for many metabolic processes), are three of the most common energy supplying compounds in cells.

2.2.2.3 Relation of P supply to growth and development

On average, between 0.3 and 0.5% of a plant's dry weight is made up of P. The lack of, or sub-optimal supply of P has wide ranging consequences due to its key role in plant metabolism and as a protein constituent. Stunted growth, particularly a reduction in leaf number, surface area and expansion are the most obvious deficiency symptoms (Fredeen *et al.* 1989; Lynch *et al.* 1991). Photosynthesis is also decreased in P deficient plants (Lauer *et al.* 1989), despite the fact that chlorophyll and protein contents of deficient leaves often increase (Rao and Terry 1989), and leaves appear darker in colour since leaf expansion and the extension of epidermal cells are impaired (Hecht-Buchholz 1967; Treeby *et al.* 1987). Root hydraulic conductivity and

plant water relations are also affected by P deficiency (Radin and Mathews 1989; Skinner and Radin 1994).

Under P deficiency root growth is not inhibited as much as shoot growth, as typically more carbohydrate is partitioned to the roots to assist in root exploration of the soil and expansion to take up more P (Anuradha and Narayanan 1991; de Groot *et al.* 2001). In some plants available P and other mineral nutrients are also allocated to roots (Brouder and Cassman 1994). This allocation of resources leads to a delay in flower formation, a reduction in seed number and in fruit size in many plants (Cakmak *et al.* 1994; Singh *et al.* 2006a).

2.2.3 Potassium

2.2.3.1 Uptake and assimilation

Uptake of K is highly selective and is tightly controlled by the action of K^+ transporters (or carriers) and channels active in roots (Kochian and Lucas 1988; Luan *et al.* 2009). K^+ ions are highly mobile in the plant, both within cells and between organs. Most K in the plant is as a free ion in solution, as it is not readily metabolised and it forms only weak complexes with other organic molecules, from which it is readily exchanged (Marschner 2002; Maathuis 2009).

2.2.3.2 Function

In most cells throughout a plant the K^+ concentration in the cytosol and chloroplasts are maintained at around 100 – 200 mM (Schroppelmeier and Kaiser 1988; Maathuis and Sanders 1996; Smart *et al.* 1996; Thiel and Wolf 1997). K^+ ions play a central role in many plant functions, most importantly in maintaining turgor pressure, cell elongation and in stomatal opening and closing. It also has a key role in plant water relations, being the inorganic solute of highest concentrations in plant cells. Turgor pressure driven solute transport in the xylem, and water transport through actively changing the osmotic potential of cells, is mainly attributed to K^+ concentration and movement. Likewise the maintenance of cell (and subsequently tissue and plant) turgor, cell elongation and expansion and stomatal movement is driven by the increase or decrease in osmotic potential of organelles, cells and tissues through K^+ transport and accumulation (Marschner 2002).

Other key functions of K are in enzyme activation, protein synthesis, in photosynthesis and phloem transport (Amtmann *et al.* 2006). Potassium affects photosynthesis in various ways, through the opening and closing of stomata, through establishing the transmembrane pH gradient necessary for phosphorylation (and the synthesis of ATP) and through its role in the light activated H+ flux across the thylakoid membrane (Marschner 2002). It also plays a direct role in CO_2 fixation in chloroplasts. Under drought stress, chloroplasts lose much of their K^+, and CO_2 fixation decreases. The decline in photosynthesis under drought stress is reduced at high K^+ supply – explaining the high requirement for K by water stressed plants or plants exposed to highly saline conditions (Mengel and Kirkby 2001).

2.2.3.3 Relation of K supply to growth and development

Next to N, K is the mineral nutrient required in the highest amount by plants for growth and development, accounting for 2 – 5% of the plant dry weight, and found in high concentrations in most plant tissues. When K^+ supply is low, growth is retarded, cell expansion decreases and smaller cell sizes are common. Net redistribution of K^+ from mature tissues to young tissues is increased, and under severe deficiency mature leaves become chlorotic and die (Marschner 2002; Epstein and Bloom 2005).

K deficient plants are more susceptible to lodging (through inhibited lignification of stems) (Marschner 2002), drought stress (through the role of K^+ in stomatal opening and consequently the transpiration stream and through its role in maintaining cell turgor and osmotic potential) (Ashley *et al.* 2006), frost damage (Eastham *et al.* 1984) and fungal attack (DeVay *et al.* 1997).

2.3 N, P and K remobilisation and redistribution

After the initial uptake, transport and incorporation of nutrients into a plant organ, nutrients can be remobilised through the degradation of molecules, or the release of ions or low-molecular weight compounds from storage organs such as the vacuoles.
Remobilisation, and redistribution of nutrients, has several functions in the plant;

1) As a feedback mechanism to convey information about the nutritional status of the shoots, and thereby regulate nutrient uptake and root growth (Jackson 1997; Amtmann *et al.* 2006)

2) To control the accumulation of harmful substances or salts in the leaf and fruit tissues (Martinez and Lauchli 1994)
3) To provide reduced forms of certain nutrients, particularly N, for metabolism and growth in non-green tissues such as roots and stems, where nitrate reduction does not take place (Andrews 1986)
4) To maintain the charge balance and osmotic potential of the shoots and roots through maintaining fairly constant concentrations of osmotically active, or charges ions such as sucrose and K (as K^+) (Hellmann *et al.* 2000; Komor 2000)
5) To smooth fluctuations in supply to match a constant internal demand
6) To compensate for heterogeneous distribution of nutrients in the rooting zone (Anuradha and Narayanan 1991; Brouder and Cassman 1994)

In an agricultural system it contributes to the efficiency of nutrient inputs and is a mechanism of plant resilience to variations in water and nutrient supply.

2.3.1 Quantification of redistribution

Across many studies there have been several, well documented, factors used to indicate that the redistribution of nutrients from leaves supplies the rapidly developing fruit with the nutrients that they require, both indirect and direct measurements (or estimations of the extent) of the process.

Of all the methods used to estimate redistribution, the simplest (and by far most commonly used) is to compare the content of the leaf at peak nutrient content, and at senescence, assuming that the difference in the two is equivalent to the amount of the nutrient remobilised and redistributed. This method has been used to estimate the movement of many nutrients (Zhu and Oosterhuis 1992; Yang *et al.* 2009).

Killingbeck (1996) published an extensive review of data from 89 species of woody perennials to estimate the maximum potential remobilisation – a figure difficult to establish by looking at the variety of studies on the subject. He found that, while the potential for remobilisation was highly influenced by environmental and seasonal factors, there was some consistency across the many species in the lowest

concentration left in leaves at senescence of N and P, a measurement he used to establish potentials for remobilisation. In woody perennials, he finds that a concentration of 0.3%N, and 0.01%P in senesced leaves represent the ultimate potential for recycling of these nutrients, and he classifies plants as having demonstrated “highly proficient remobilisation” as those with <0.7% N and <0.04% P leaf in senesced leaves, and “incomplete remobilisation” as those with >1% N and > 0.05% P. Similar data is not available for K.

Physiological measurements and plant observations have been used successfully in many cases to estimate redistribution, or indicate that it is taking place. Leaf activity measurements, including photosynthesis, protein concentrations and chlorophyll concentrations have been used descriptively (Constable *et al.* 1988; Schwab *et al.* 2000) as have the decline in root nutrient uptake, respiration and growth (Pettigrew *et al.* 2000), and the appearance of deficiency symptoms on leaves (Wright 1999; Pettigrew *et al.* 2000).

The use of labelled radioisotopes or tracer molecules has been used successfully by many authors to establish patterns of nutrient movement and proportional remobilisation and redistribution, a method ideal for the quantification of the process (Marshall and Whiteway 1985; Kuhn *et al.* 1995; Buhler *et al.* 2003; Gessler *et al.* 2004; Gottlein *et al.* 2005; Peuke *et al.* 2006; Becker *et al.* 2008; Gotz *et al.* 2008; Wichern *et al.* 2009)

Guérin *et al.* (2007) used a mathematical combination of the sap composition with flow velocity provided the transported quantities of N and C, an accurate, but time consuming and costly method of measuring redistribution.

2.3.2 Remobilisation efficiencies of leaf nutrients

While studies quantifying the remobilisation of nutrients from leaves are uncommon, there is a wide range in the measured efficiencies, as shown in Table 2.1. This variation in reported remobilisation is linked in some studies to various environmental and physiological factors.

Table 2.1 Reported proportional remobilisation of N, P and K from leaves in a variety of crop species

N	P	K	Crop	Reference
20 – 61%	31 – 65%	25 – 84%	Switchgrass (*Panicum virgatum)*	(Yang *et al.* 2009)
0 – 70%			Sunflower (*Helianthus annus*)	(Hocking and Steer 1995)
50 – 75%			Wheat	(Guitman *et al.* 1991)
53%	51 – 63%	5 – 14%	Corn	(Pan *et al.* 1986)
78.5%	88.4%		Canola	(Hocking and Mason 1993)
14 – 64%			Cotton	(Rosolem and Mikkelsen 1989)

2.4 Mineral nutrition and cotton growth and development

2.4.1 Cotton growth and development

Cotton is a tropical, xerophytic perennial shrub, grown commercially as an annual plant. Unlike most other broadacre crops grown in Australia, cotton has an indeterminate growth habit, simultaneously producing reproductive and vegetative structures. As cotton is indeterminate, there is no morphological limit to its size, development or biomass production while conditions are favourable, and theoretically the production of fruiting branches and main-stem nodes could continue indefinitely (Hearn and Constable 1984).

A cotton plant, grown under cultivation as an annual, has a predictable growth habit and structure, shown schematically in Figure 2.1. The plant consists of a main stem (monopodia), from which sympodial (fruiting) branches grow. Depending on the temperature, cotton plants produce a new mainstem node every 2 – 4 days (Hearn and Constable 1984), at which a leaf (the “main-stem leaf”) and a sympodial branch grow, all sympodial branches arising from the axil of a main-stem leaf (Mauney 1986). There have been several extensive reviews and discussion of cotton growth from

physiological and morphological viewpoints (e.g. Hearn and Constable 1984; Mauney 1986; Cothren 1999).

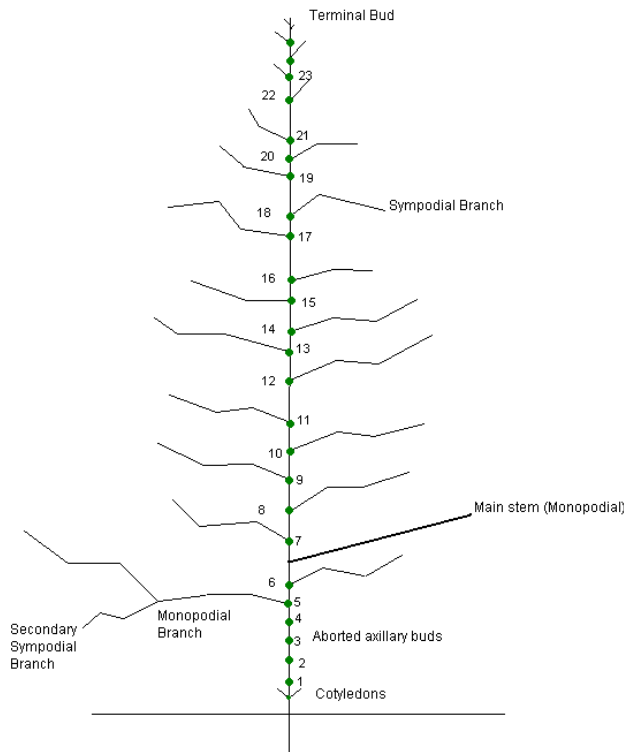

Figure 2.1 Schematic diagram of a cotton plant with 23 main stem nodes, showing the main stem, monopodial and sympodial branches.

Reproductive growth in cotton begins with the formation of flower buds (squares), with boll development beginning after fertilisation (Thompson *et al.* 1976; Bednarz and Roberts 2001; Wahid *et al.* 2004). The first viable boll is produced from node 4 – 10, depending on environmental conditions, on average the first boll appearing at node 6.3 (Kerby *et al.* 1987). Each sympodial branch, and each boll along the branch (as shown in Figure 2.2) is subtended by a leaf, which is proposed to be the primary source of assimilates to the fruit (Brown 1968), expanding before anthesis and senescing before boll maturity. Leaf expansion is complete 16 – 20 days after the leaf unfurls, after which photosynthesis begins to decline and assimilate export begins. Boll nutrient demand begins to increase after 20 days, precisely when the leaf export passes its peak. Hearn (1976) describes these leaves and bolls as being “out of phase”, an observation confirmed by many other authors (Constable and Rawson 1980a).

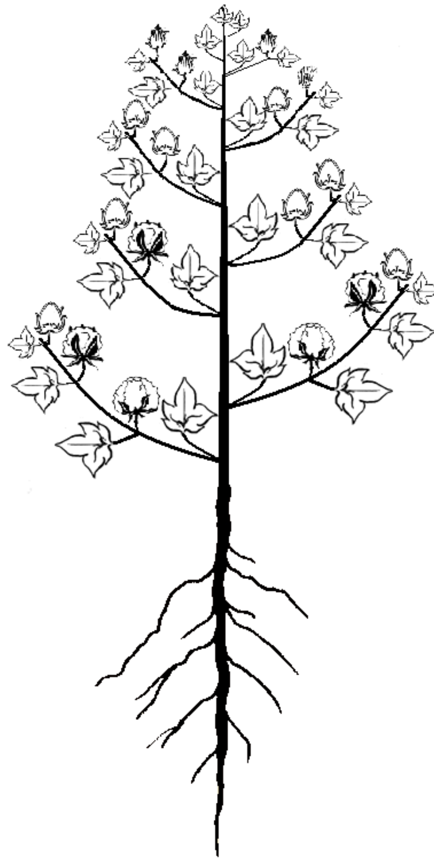

Figure 2.2 Cotton plant diagram showing the positioning of bolls along a fruiting branch, and up the plant, with open bolls, green bolls, flowers and squares at different stages of development.

The shedding of squares at an early stage of development, and the observed slowing and eventual stopping of root uptake, vegetative growth and development which occurs in cotton plants ("cutout") has been attributed to an imbalance between assimilate supply and boll demand, although there is also evidence that a complex interaction of hormonal factors also contributes to boll shedding (Mason 1922; Mauney 1986; Guinn and Brummett 1989). Sadras *et al.* (1997) found that, within a narrow range, the harvest index of cotton was fairly stable when analysed allometrically, indicating that a plant of a particular size can support a fairly stable number of bolls across a wide range of treatments and conditions, except where plant or environmental factors reduced the length of time for reproductive growth. The extent to which this ratio is nutritionally controlled has not been investigated.

Biotic and abiotic factors influence and regulate the growth rate and development pattern of a cotton plant. These factors have been examined separately and in combination in a number of studies over many years, leading to the current understanding of cotton growth and development and the improvement of cotton growth models and simulations, of which there are many (e.g. Hearn and Constable 1984; Mutsaers 1984). Some of the key factors affecting growth and development are radiation, water supply, temperature, pest and disease management and cultivar differences. These will not all be discussed in detail in this review, except as they affect nutrient uptake and distribution.

2.4.2 Nutrient uptake and distribution

The uptake and distribution of mineral nutrients by cotton crops has been the subject of several extensive studies over the past century, revisited and revised as crop management practices, cultivars and growing environments changed (e.g. Crowther 1938b; Olson and Bledsoe 1942; Crowther 1947; Bassett *et al.* 1970; Halevy 1976; Hearn 1981; Mullins and Burmester 1990; Unruh and Silvertooth 1996; Boquet and Breitenbeck 2000; Fritschi *et al.* 2004b; Janat 2004; Rochester 2007). Most of these studies have focussed on N, and to a lesser extent P and K, as shown in Table 2.2.

Over time, there has been a significant increase in the yield attainable by cotton growers, through better soil and fertiliser management, irrigation, cultivar improvements and weed and pest management improvements (Rochester 2007). The introduction and improvement of transgenic technology to the cotton industry in the last decade resulted in further supporting yield gains, and plants with a higher boll retention due to insect pest management and the reduction of boll losses to *Helicoverpa sp*. It has been reported that transgenic plants have a higher boll retention rate and higher yields than conventional cotton (Moser *et al.* 2000; Blanche *et al.* 2006) although others have reported no difference in total yield attributable to the gene technology, but rather to management and environmental factors (McCall and Robinson 2000). It is clear that modern systems of cotton production, particularly the highly managed, high input systems in Australia have resulted in significant yield increases and the production of large plants with a higher boll retention rate than was previously attainable. It is generally assumed that higher boll numbers place additional demands on leaf and stem sources of nutrients (Oosterhuis *et al.* 1997;

Oosterhuis and Steger 1998; Lopez *et al.* 2008). Wright (1999) attributed the rapid development of K deficiency symptoms and premature senescence of leaves to this increased demand on leaf nutrient resources from a heavy boll load. There are, however, few detailed studies linking leaf nutrient export to the ratio of reproductive organs to vegetative tissue, or quantifying the export rates and amounts of nutrients from senescing cotton leaves.

Table 2.2 gives a summary of the results of many of the cotton nutrient uptake and distribution studies from around the world in the last 100 years. Most of the work has focussed on N, P and K.

Table 2.2 Published nutrient uptake, nutrient efficiencies (kg uptake / 100kg lint ha^{-1}), nutrient removal in seed cotton, fertiliser and irrigation treatments and uptake rates and proportions at different growth stages (Planting, Flowering, End of Effective Flowering (that is, the last flower produced that will reach maturity, EEF) and Maturity). A = Total uptake (kg ha^{-1}), B = Uptake rate (kg ha^{-1} day^{-1}) and C = Proportion of total taken up in this period.

	At maturity					Planting - Flowering			Flowering - EEF			EEF - Maturity			Reference
Nutrient	Total Uptake (kg ha^{-1})	Removal in seed cotton (kg ha^{-1})	Efficiency (kg uptake / 100kg lint)	Fertiliser Rates	Cultivar	A	B	C	A	B	C	A	B	C	
N	142	63 – 83	10	134 kg N ha^{-1}	Acala 4-42			< 15%		1.5 - 2	67%				Bassett *et al.* (1970)
P	19	9 – 12	1.3	134 kg N ha^{-1}	Acala 4-42			< 15%		0.34	67%				Bassett *et al.* (1970)
K	127	16 – 24	9	134 kg N ha^{-1}	Acala 4-42			< 15%		2.1 - 3.4					Bassett *et al.* (1970)
Ca	90 - 160	6.2 - 13.1		134 kg N ha^{-1}	Acala 4-42										Bassett *et al.* (1970)
Mg	35			134 kg N ha^{-1}	Acala 4-42										Bassett *et al.* (1970)
N	103.6	40		0 kg N ha^{-1}	Deltapine 41		0.18			-0.3			1.2		Boquet and Breitenbeck (2000)
N	208.5	80.7		84 kg N ha^{-1}	Deltapine 41		2.9			2.1			1.7		Boquet and Breitenbeck (2000)
N	245.1	94.4		168 kg N ha^{-1}	Deltapine 41		0.29 - 4.3			2.7			1.1		Boquet and Breitenbeck (2000)
K	69.2														Coker *et al.* (2000)
N			29												Olson and Bledsoe (1942)
N			10												Maples *et al.* (1977)

N			20.3												Oosterhuis *et al.* (1983)
P	30	15 – 26			Siokra-324i				19.8 - 22.2	2.6 - 3.4					Dorahy *et al.* (2004)
Fe	600 g		29.34 g		Deltapine 61										Constable *et al.* (1988)
Mn	450 g		5.34 g		Deltapine 61										Constable *et al.* (1988)
B	200 g		9.34 g		Deltapine 61										Constable *et al.* (1988)
Zn	60 g		5.78 g		Deltapine 61										Constable *et al.* (1988)
Cu	20 g		1.3 g		Deltapine 61										Constable *et al.* (1988)
N	223.7	98	13.2		Acala 1517-C	19.9	0.35	8.9	155.2	3.78	69.3	48.6	1.17	21.7	Halevy (1976)
N	235.4	109	13.8		Acala 4-42	13.8	0.59	2.4	154.3	3.77	65.5	67.3	2.31	28.6	Halevy (1976)
P	43.9	19	2.6		Acala 1517-C	2.3	0.04	5.2	26.8	0.66	61.1	14.8	0.31	33.7	Halevy (1976)
P	46.2	21	2.7		Acala 4-42	1.8	0.03	3.9	24.9	0.61	53.9	19.5	0.59	42.2	Halevy (1976)
K	164.1	47	10		Acala 1517-C	16.5	0.29	10	122.4	3.06	74.6	25.2	1.8	15.4	Halevy (1976)
K	184.5	43	11		Acala 4-42	11.7	0.21	6.3	141.2	3.09	76.6	31.6	2.26	17.1	Halevy (1976)
N	127 - 155		19.9		Deltapine 90; Stoneville 825; Coker 315; Paymaster 145										Mullins and Burmester (1990)
P	16.3 - 18.2	9.1	2.5		Deltapine 90;					0.62	40				Mullins and Burmester (1990)

					Stoneville 825; Coker 315; Paymaster 145										
K	99 - 112		15.3		Deltapine 90; Stoneville 825; Coker 315; Paymaster 145					2.2	31				Mullins and Burmester (1990)
N	145.1		9.6	0 kg N ha^{-1}											Janat (2004)
N	333.7		16.2	120 kg N ha^{-1}											Janat (2004)
N	417.2		18.8	240 kg N ha^{-1}											Janat (2004)

A good summary of the historical development of methods and management changes over time the time period represented in Table 2.2 is given by Mullins and Burmester (2010). Prior to 1945, dry matter uptake and nutrient accumulation patterns received a great deal of attention, mostly in non-irrigated production systems in the USA (Mason 1922; Crowther 1938a; b; Olson and Bledsoe 1942; Crowther 1947). The yield and size of these plants was relatively small compared to modern Australian cotton production systems. In one of the key studies from this period Olson and Bledsoe (1942) measured an "unusually high-yielding" crop of 747 kg lint ha^{-1}, which is well below the average Australian yield of 2120 kg ha^{-1} .

In the mid 1970's and 1980's there was renewed interest in cotton nutrient uptake and partitioning, particularly in irrigated and more intensively managed cotton systems across the world. Forty years ago Bassett *et al.* (1970) who studied plants yielding an average of 1178 – 1628kg lint ha^{-1} commented that; *"nearly all the results dealing with dry matter production and nutrient uptake were published prior to 1942, when yields were low compared with those presently obtainable with more productive varieties, improved management and irrigation."*

Just as the practices, cultivars and technology had changed in the 40 years between these sets of studies, in the past four decades there have been major advances in cotton production in Australia leading to increases in yield, nutrient and water management and pest control. The average Australian cotton yield is 2120 kg lint ha^{-1}, more than two and a half times the world average yield, and far higher than that reported in previous studies. In addition to higher lint yields, modern transgenic varieties grown in Australia may retain more fruit on lower branches (Mills *et al.* 2008), and retain a higher proportion of the developing fruit throughout the fruiting branches (Moser *et al.* 2000; Blanche *et al.* 2006; Bange *et al.* 2008). The effect of these changes on the nutrient uptake and distribution patterns in high-yielding plants has not been described.

Recent studies investigating the dry matter and nutrient partitioning in cotton have focused on N nutrition, as the twofold pressure of minimizing the environmental impact, and increasing the profit margin of cotton production have lead to an interest in increasing the efficiency of N fertiliser use (e.g. Boquet and Breitenbeck 2000; Fritschi *et al.* 2004a; Fritschi *et al.* 2004b; Janat 2004; Wiedenfeld *et al.* 2009). These recent studies have reported some differences to

prior studies in terms of the distribution of dry matter and N, however they have been inconsistent. There are few studies with which to compare these values and determine if the partitioning of nutrients has changed with improvements in cultivars, technologies and management techniques, or if modern cotton production produces cotton plants with a similar partitioning and accumulation pattern to those produced 30-40 years ago. Similarly the timing of uptake pre and post flowering has not been widely reported for modern cultivars and production systems. Data is particularly lacking for transgenic Australian cultivars. There has been little research in Australia and the world linking nutrition and nutrient inputs to physiological processes, particularly the contribution of leaf nutrients to the developing bolls.

2.4.2.1 Nitrogen

Nitrogen is the nutrient accumulated in the highest amount by developing cotton crops and the N uptake by a crop is directly correlated with flower bud production, leaf production and expansion, and fruit retention (Marcus-Wyner and Rains 1982; Zhu and Oosterhuis 1992). Total uptake is highly dependent on the growing conditions, as shown by the wide variation (from 103 – 417 kg ha^{-1}) in total uptake given in Table 2.2. If available, cotton can absorb more N than it needs to support boll production and growth (Hearn 1981), although high N and water supply can lead to rank vegetative growth, the shedding of squares and a delay in cutout and boll maturity (Thompson *et al.* 1976).

Nitrogen removal in seed cotton ranges from 40 – 109 kg ha^{-1} (Halevy 1976; Boquet and Breitenbeck 2000), a statistic that seems to be linked to fertiliser rates and growing conditions. The efficiency of uptake, in terms of N uptake per 100 kg lint gives some comparison between studies, although older, lower yielding varieties (e.g. Olson and Bledsoe 1942) have a higher "efficiency" of uptake, through a lower lint % in the bolls and a higher vegetative to reproductive ratio (Mullins and Burmester 2010). For irrigated cotton the reported uptake efficiency is between 8 – 21 kg N per 100 kg lint (Bassett *et al.* 1970; Halevy 1976; Halevy *et al.* 1987; Unruh and Silvertooth 1996; Rochester and Constable 2006), a figure affected by water stress, nutrient supply, radiation and the reproductive to vegetative ration of the plant (Hearn 1975a; Guitman *et al.* 1991; Milroy and Bange 2003; Hou *et al.* 2007).

The uptake of N typically peaks during the early – mid flowering period (Mullins and Burmester 2010). As shown in Table 2.2, uptake during this period can account for up to 69%

of total N uptake, although reported uptake rates vary. Historically, with irrigation and increased fertilisation, the reported rate of uptake has increased. Jones *et al.* (1974) found a peak uptake rate of 0.0516 g N $plant^{-1}$ day^{-1} in a low yielding system with 4.9 plants m^{-2}. Only twenty years later, with irrigation and improvements in cultivars and management Halevy (1976) reported an uptake of 0.077 g N $plant^{-1}$ day^{-1} in a crop yielding 1700 kg lint ha^{-1} with 6 plants m^{-2}. More recent uptake rates of 3.6 kg N ha^{-1} day^{-1} (Fritschi *et al.* 2004b) and 4.4 kg N ha^{-1} day^{-1} (Boquet and Breitenbeck 2000) have been reported, although neither of these two studies examined transgenic cultivars and both observed an increase in the accumulation rate with an increase of fertilisation.

The distribution of N varies with the growth stage of the plant, with the leaves being the major sink for N before flowering, after which time the bolls become the site of the most N accumulation and the leaves begin exporting N (Zhu and Oosterhuis 1992). After flowering, Halevy (1976) observed a decrease in the N concentration of leaves from 4% to 2.5%, in stems from 1.5 – 0.9% and an increase in the fruit concentration to 3.8%. Other authors have recorded a similar reduction in leaf and stem N concentration during boll filling (Thompson *et al.* 1976; Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999). A typical distribution of N at maturity, based on a number of studies is; 21% in leaves, 11% in stems, 9% in boll walls and bracts, 55% in seeds and 4% in lint (Bassett *et al.* 1970; Halevy *et al.* 1987; Mullins and Burmester 1990; Boquet and Breitenbeck 2000; Fritschi *et al.* 2004b). Fritschi *et al.* (2004a) observed that more modern cultivars developed a greater reproductive: vegetative ratio (R:V) than older cultivars, as did Meredith and Wells (1989). They cite this higher R:V as the reason for a lower reported leaf N concentration, in some more recent studies, than those pre-1940 and from the 1970's, however, the effect of other management and environmental factors was not quantified, which may have also influenced the leaf N concentration in these studies.

2.4.2.2 Phosphorus

As given in Table 2.2, the reported total uptake of P by a cotton crop varies from 16.3 to 43.2 kg ha^{-1}. There are far less studies examining the response of cotton to P fertiliser, due in part to the infrequency of P fertilisation in commercial systems, and to the variable response of cotton to P fertilisation, as outlined by Dorahy *et al.* (2004). Singh *et al.* (2006a) reported an interaction between P uptake and plant P status with plant water content and interacting with

water supply, but there are few other studies examining the effect of plant physiological, environmental or management factors on P uptake in cotton.

Reported amounts of P removed in seed cotton ranges from 9 to 30 kg ha^{-1}, with an efficiency of 1.3 to 2.7 kg P per 100 kg lint (Table 2.2). The peak uptake period for P is from early to mid flowering, the rate ranging from 0.17 to 0.72 kg P ha^{-1} day^{-1} (Bassett *et al.* 1970; Halevy 1976). As with N, the uptake rate varies with P supply and environmental conditions (Dorahy *et al.* 2008).

A typical distribution of P at maturity, based on a number of studies is; 20% in leaves, 11% in stems, 16% in boll walls and bracts, 53% in seeds and lint (Bassett *et al.* 1970; Halevy *et al.* 1987; Mullins and Burmester 1990). Halevy (1976) observed that the rate, uptake and distribution pattern of P is closely related to that of N, although there is much less decline in the P content of vegetative plant parts post-cutout than that observed for N.

2.4.2.3 Potassium

Cotton is considered to be less efficient that many plant species at obtaining K from the soil, and in some parts of the world K deficiency symptoms appear more frequently than in other crops (Mullins and Burmester 1994; Wright 1999; Mullins and Burmester 2010). As such, there has been significant interest in the application of K as a foliar fertiliser around the world, with variable results (Halevy and Markovitz 1988; Miley and Oosterhuis 1994; Mullins and Burmester 1994; Oosterhuis *et al.* 1994; Chang and Oosterhuis 1995; Roberts and Howard 1995; Snyder *et al.* 1995; Howard *et al.* 1998; Bednarz *et al.* 1999; Howard *et al.* 2000; Howard *et al.* 2001a). The uptake of K, and partitioning to developing fruits is essential to support lint development and for bolls to reach maturity (Leffler and Tubertini 1976).

The total reported K accumulation of a cotton crop ranges from 69 – 276 kg ha^{-1} (Table 2.2). As with P, the uptake of K closely follows that of N, and varies with water and nutrient supply, environmental conditions and between cultivars (Combrink and Davies 1987; Cassman *et al.* 1989a; Cassman *et al.* 1990; Bednarz and Oosterhuis 1999; Egilla *et al.* 2001; Lopez *et al.* 2008). The efficiency of K uptake and partitioning ranges from 7.6 – 27 kg K per 100 kg lint (Table 2.2). As with N, the crops reported to have the highest efficiency of uptake

are older, lower yielding varieties with a lower lint % than modern varieties, and grown at low planting densities.

The uptake of K peaks during early boll filling, shortly after flowering. Bassett *et al.* (1970) reported that accumulation of K occurred later than for N and P, and that peak accumulation occurred when the accumulation of N and P began to decline. The total accumulation curves were similar in most other studies in Table 5. Peak accumulation rates of K can exceed that of N uptake during the period of rapid uptake at the onset of flowering, and can be as high as 3 – 5 kg K ha^{-1} day^{-1} in high-yielding, irrigated systems (Cassman *et al.* 1989a). This observation was also observed by other authors (Bassett *et al.* 1970; Halevy 1976; Halevy *et al.* 1987).

The typical distribution of K between plant parts varies with irrigation, plant K supply and cultivar (Halevy 1976; Halevy and Markovitz 1988; Kochian and Lucas 1988; Mullins and Burmester 1990; Oosterhuis *et al.* 1997; Howard *et al.* 2001a). As with N and P, most K is found in the seeds at maturity, and the leaves and stems show a pronounced decrease in content, similar to or exceeding that of N. Distribution of 25% in stems, 20% in leaves, 36% in boll walls and bracts and 19% in seeds have been reported (Mullins and Burmester 1990), with a much higher amount of K in the boll walls than N or P, and proportionally lower in the seeds. Similar distribution data for transgenic, high-yielding crops in Australia has not been recently reported.

2.4.3 Nutrient distribution and cotton plant development

As an indeterminate plant, cotton does not exhibit distinct vegetative and reproductive phases. This growth habit means that unlike cereals and other determinate crops, cotton plants require a continuous supply of nutrients throughout the growing season, to support the simultaneous development of vegetative and reproductive structures (Rosolem and Mikkelsen 1989; Makhdum *et al.* 2007). The nutrient uptake, partitioning between tissues and rate of accumulation varies across different growth stages. Although these stages do overlap to a certain extent, they can be broken down into pre-flowering, peak flowering, boll-filling, first open boll, and maturity (60% open bolls). These stages have been used to describe and compare nutrient accumulation and uptake in several studies (Halevy 1976; Halevy *et al.* 1987; Cassman *et al.* 1989a; Mullins and Burmester 1990). Since these stages are not fixed points in a plant's growth and development, they will here be discussed as *pre-flowering* (the period until the appearance of the first white flower, including squaring), *flowering – end of*

effective flowering (the period during which bolls are "filling" until the last effective boll is produced), *end of effective flowering – maturity* (60% open bolls) (also described in Table 2.2).

Figure 2.3 shows an example of the dry matter and N accumulation in different plant parts with time. Dry matter accumulation and N, P and K uptake generally follow sigmoidal curves with a period of slow growth and nutrient uptake followed by an almost exponential period of growth, reaching a point and tapering (and slightly declining in some instances) in the characteristic "S shape".

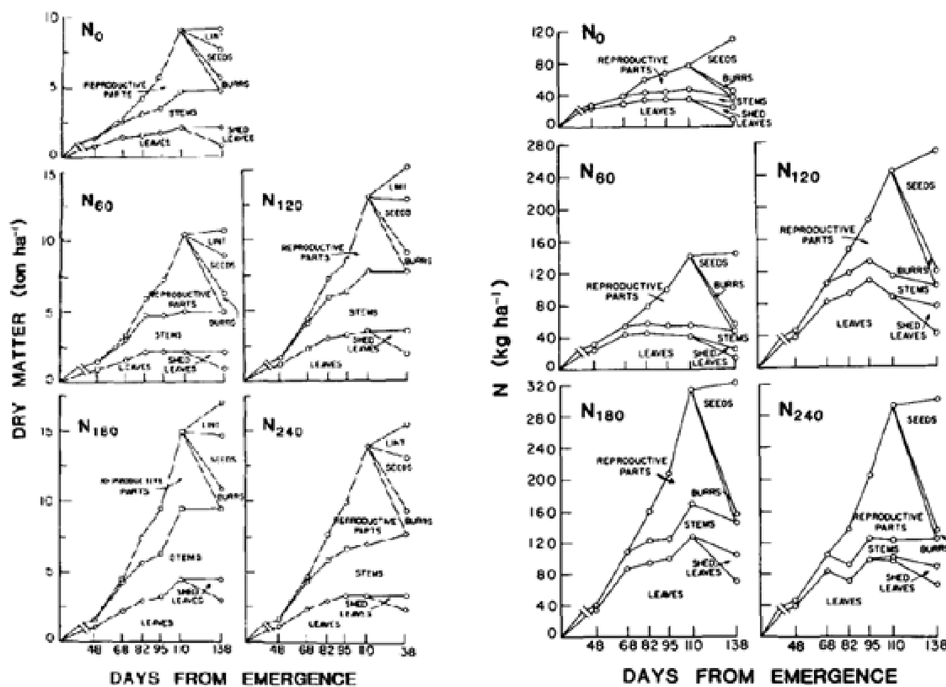

Figure 2.3 Example of dry matter and N accumulation and partitioning over time in a cotton plant, fertilised with 0 – 240 kg N ha^{-1} from Halevy *et al.* (1976).

While many studies have described the nutrient uptake and distribution in these growth stages and compared uptake rates between cultivars, water supply and nutrient supply, there is a lack of data describing the effects of nutrient supply at each of these stages on final yield, plant morphology or nutrient status. Similarly linking the descriptive information about plant nutrient uptake with physiological measurements and observations is not common, especially not for cotton plants.

2.4.3.1 Pre-Flowering

Before the appearance of the first flower, dry matter and nutrients are partitioned between leaves, stems and roots. Leaves act as sinks for nutrients until expansion is complete (16 – 20 days after the leaf unfurls) after which they become a source of carbon assimilates and nutrients for the development of new vegetative growth and squares (Oosterhuis and Urwiler 1988). Typically stems and branches account for 60 – 70% of above ground dry matter and leaves for 30 – 40%. After the onset of squaring, reproductive structures account for around 12% plant dry matter and N (Boquet and Breitenbeck 2000). Bassett (1970) reported that there were 40 – 80 days of slow N, P and K uptake until first square, followed by a period of rapid uptake till first flower, which is shown in the data from Halevy *et al,* (1976) in Figure 2.3.

An examination of the dry matter and nutrient accumulation curves of developing cotton plants from many studies shows that the acquisition and accumulation of nutrients precedes the production of dry matter. Cotton plants are characterised by a period of slow growth and nutrient uptake prior to flowering, and nutrient uptake increasing slightly before that of dry matter (Figure 2.3). As shown in Table 2.2, a few studies have quantified the nutrient uptake pre-flowering, more modern studies reporting generally < 15% of all nutrients accumulated at this time with around 95% of nutrients allocated to vegetative structures. This figure has declined over time. White (1914) reported more than 60% of total plant N, 60% of K and 80% of P had been accumulated by first flower. Bassett *et al.* (1970); Oosterhuis *et al.* (1983) and Halevy *et al.* (1987) all noted that less than 30% of plant dry matter, and between 20 – 30% of N, P and K had been accumulated by first flower in high-yielding systems from the 1970's and 1980's. It is unclear in the literature if this trend has continued and if new, very high-yielding varieties in highly managed systems rapidly take even more nutrients up after flowering.

The factors affecting the uptake of nutrients and accumulation of biomass pre-flowering are the same as those affecting growth and development through the entire season. Water and nutrient supply, temperature and light have been shown to change the germination, growth rate, and development of cotton plants from planting to flowering (Hearn 1976a; Hearn and Constable 1984; Reekie and Bazzaz 1987; Constable *et al.* 1988; Heitholt and Meredith 1998; Zhang *et al.* 2008).

2.4.3.2 Flowering – End of effective flowering

After flowering, cotton plants are characterised by a period of rapid vegetative and reproductive growth, accompanied by fast accumulation of N, P and K. In a very short period (30-40 days) up to 72% of dry matter accumulation and nutrient uptake has been reported (Bassett *et al.*, 1970; Halevy, 1976). The maximum uptake rates of N, P and K have all been recorded after flowering (section 2.4.2), as cotton plants develop reproductive structures with a high concentration of nutrients, corresponding to an increased demand (Mullins and Burmester 2010), as well as being the period when root growth is maximum (Schwab *et al.* 2000). In this growth phase, the rate of uptake is correlated with nutrient supply. Boquet and Breitenbeck (2000) observed an increase from an accumulation rate of 2.9 kg ha^{-1} day^{-1} to 4.3 kg ha^{-1} day^{-1} when N fertilisation was doubled from 84 kg ha^{-1} to 168 kg ha^{-1}. Water supply has also been shown to significantly impact the accumulation rate of nutrients at this stage, particularly when it is lacking, through restricting root growth and the transport of nutrients in soil solution to the root surface (especially for P, which has a low soil mobility) (Krieg and Sung 1986; Ball *et al.* 1994; Skinner and Radin 1994; Coker *et al.* 2000; Raats 2007).

Towards the end of this period, the functioning of some leaves begins to decline, and depending on environmental and management factors such as radiation, temperature, water and nutrient supply sink-sink competition between the roots and developing bolls, and between older and younger bolls will occur (Krieg and Sung 1986; Rosolem and Mikkelsen 1989; Wright 1999; Baker and Baker 2010).

2.4.3.3 End of effective flowering – Maturity

This growth phase is characterised by the cessation of production of new reproductive sites and the slowing or stopping of root- uptake and vegetative growth, which causes the "end of effective flowering" and the plant reaches cutout. It is most likely a combination of sink: sink competition for carbon assimilates and nutrients between roots and bolls, the production of hormones signalling the shortage of growth substrates, and environmental constraints on further vegetative growth (Mason 1922; Mauney 1986; Reekie and Bazzaz 1987; Halevy and Markovitz 1988; Guinn and Brummett 1989). Cotton plants generally produce more squares than it can support until maturity, and the shedding of squares is a natural process regulated internally and by environmental stresses (Constable 1991).

Nutrient stress, particularly N stress, can result in earlier maturity and cutout, and can reduce yield (Hearn 1975a; b; Leffler and Hunter 1985; Bondada *et al.* 1996), conversely over supply of N can delay maturity through increasing the period of vegetative growth at the beginning of the growing season, or through re-growth at the end of the season (Bondada *et al.* 1996; Rochester *et al.* 2001; McConnell and Mozaffari 2004).

2.4.4 Nutrient redistribution in cotton plants

As shown in Figure 2.3, and similarly reported in many of the studies discussed in the preceding section, the N, P and K content of vegetative tissue declines after cutout is reached, between the end of effective flowering (at around 4 NAWF) and maturity. Most studies describing nutrient uptake and distribution in cotton plants determine the redistribution of nutrients begins after the initiation of flowering. This conclusion is based primarily on the observed decline in leaf nutrient concentrations and content, and assumes that vegetative nutrient export supplies developing bolls. This assumption is well founded, as in many studies the accumulation of nutrients in developing bolls exceeds the total plant accumulation at this period, indicating that redistribution must be occurring (Halevy 1976; Halevy *et al.* 1987). There is a substantial amount of evidence to suggest that the process of carbon export from leaves is correlated with boll retention and boll size (Benedict *et al.* 1973; Patterson *et al.* 1978; Constable and Rawson 1980a; Krieg and Sung 1986; Lieth *et al.* 1986; Pline *et al.* 2003; Li *et al.* 2009). The export of N (and to a lesser extent P and K) is linked to carbon export and the physiological decline of carbon producing leaves(Pate and Atkins 1983; Kavakli *et al.* 2000; Gotz *et al.* 2007; Grechi *et al.* 2007; Yasumura 2009). It is, therefore, a valid assumption that the decline in leaf nutrient content is caused by the remobilisation and the subsequent redistribution of leaf nutrients.

Rosolem and Mikkelsen (1989) studied the source of N in mature bolls at different nodes up the main stem and concluded that after the first square stage the leaves become the major source of N in the plant. Other authors have similarly concluded that the leaves are the main source of assimilate and nutrient supply to developing bolls (Thompson *et al.* 1976; Wullschleger and Oosterhuis 1990b; Venkatakrishnan 1994; Wahid *et al.* 2004) although many have stated that the subtending leaf nutrient export is inadequate to supply boll demands, and that nutrients from other plant parts or continued root uptake both play an important role in boll nutrition (Constable *et al.* 1988; Halevy and Markovitz 1988; Heitholt and Schmidt 1994; Boquet and Breitenbeck 2000; Pervez *et al.* 2004).

While frequently referred to, there is a lack of studies specifically measuring the proportion of nutrients exported from leaves, particularly on a single leaf or branch scale. Several studies have been published describing the accumulation of nutrients within an individual boll, and along a fruiting branch (Leffler and Hunter 1985; Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999; Li *et al.* 2009). Redistribution of N has been measured by Zhu and Oosterhuis through the sampling of a sympodial branch at regular intervals during leaf, square and boll development. They concluded that a main stem leaf can export 60% of its total N content within 42 days of reaching maximum leaf area. Similar studies for P and K have not been published, although the pattern of boll nutrient accumulation has been examined by Leffler and Tubertini (1976) and Zhao and Oosterhuis (1999).

Estimations of the redistribution of leaf nutrients can be made from published data on cotton nutrient uptake and distribution, through the calculation of leaf nutrient decline from cutout to maturity; these are given in Table 2.3. Many studies, particularly older ones do not present raw data, and so no calculations can be made, similarly there are few published leaf P and K contents; however several N redistributions could be calculated.

Table 2.3 Calculated amounts of N redistributed from leaves (k ha^{-1}) and as a proportion of total leaf nutrients at the peak content (%) from published nutrient uptake studies

Reference	Redistributed N (%)
Boquet and Breitenbeck (2000)	55 kg ha^{-1} (55%)
Fritschi *et al.* (2004b)	50 kg ha^{-1} (71.4%)
Oosterhuis *et al.* (1983)	24 kg ha^{-1} (27.3%) (high fertiliser rate)
Oosterhuis *et al.* (1983)	33 kg ha^{-1} (56%) (low fertiliser rate)

These calculations, as well as published data, show that redistribution in cotton is highly variable, as shown for other crops in Table 2.1. In some studies the leaf nutrient decline is increases with nutrient stress, as with Oosterhuis *et al.* (1983) in Table 2.3.

An estimation of the highest potential redistribution, or the lowest concentration left in senesced leaves, as well as an examination of how nutrient supply, water management and plant morphology affect the proportion of leaf nutrients exported and redistributed to

developing bolls is lacking, and would be valuable in determining the nutrient use efficiency of developing plants and maximising the potential use of nutrients applied.

2.4.5 Factors affecting cotton nutrient uptake, distribution and redistribution

2.4.5.1 Nutrient supply

There have been many studies examining the effect of N, P and K supply on their uptake and partitioning within the plant (e.g. Hearn 1975b; Constable and Hearn 1981; Halevy and Markovitz 1988; Rochester *et al.* 1993; Pate *et al.* 1994; Sawan *et al.* 1998; Boquet and Breitenbeck 2000; Howard *et al.* 2001b; Oosterhuis 2003; Pervez *et al.* 2004; Dorahy *et al.* 2007). An increase in plant size, yield, seed size and number of fruiting sites has been broadly attributed to the application of fertilisers. Many studies have shown that increasing the nutrient supply results in a higher concentration of N, P or K in the plant, particularly in the leaves, without always causing an increase in yield or boll number (Halevy *et al.* 1987; Pettigrew *et al.* 1996; Read *et al.* 2006). Excess nutrient supply, particularly of N, promotes increased vegetative growth and may reduce gin turnout (Boquet *et al.* 1994; Boquet and Breitenbeck 2000; Fritschi *et al.* 2004b; McConnell and Mozaffari 2004; Girma *et al.* 2007).

While the effect of increasing the N, P and K supply on cotton growth and development is clear, and has been extensively reported, the effect of the application of nutrients on the redistribution of those nutrients from one plant organ to another has not been widely studied in cotton. The deficiency of many essential plant nutrients can cause leaf senescence or the degradation of leaf pigments causing leaf discolouration. Typically a deficiency in a highly mobile nutrient will show initially through the yellowing and senescence of older leaves, as the nutrients are remobilised and redistributed to young leaves, which will remain green. This kind of senescence response to a limited supply of a nutrient may vary in rate to the programmed natural senescence described in x (Pettigrew *et al.* 2000; Djanaguiraman *et al.* 2009). A deficiency in a non-mobile element will cause the senescence of young tissue, occurring through a limitation to normal growth rather than the breakdown of mature cells (Hendry 1988; Smart 1994).

Several studies have highlighted the relationship between the concentration of nutrients in a mature leaf prior to the onset of senescence and the lifespan of the leaf and rate of senescence

of the leaf (Thompson *et al.* 1976; Christensen *et al.* 1981; Smart 1994; Pettigrew *et al.* 2000; Andersson and Johansson 2006; Djanaguiraman *et al.* 2009). Leaves with a higher concentration of N senesce more slowly, and maintain a higher respiration rate than those grown in N limited conditions Makino *et al.* (1984), and a low N level could result in a reduced cytokinin level in some plants, linking the nutritional status of the plant to the functioning of plant hormones (Singh *et al.* 1992). Unlike with N, the abundance or lack of P in the soil has not been shown to affect the rate or timing of leaf senescence (Wahid *et al.* 2004; Amtmann *et al.* 2006). K transport out of the leaves has been linked to K supply, particularly early in the season (Wright 1999). While there have been links between the N, P and K concentration in the leaf, and leaf senescence, this relationship has not been previously quantified.

2.4.5.2 Water

Pre-flowering the dominant sink for carbohydrates is the roots, with 85% of the total root system developed by flowering. Under water stress cotton is adapted to prioritise root growth and allocation to roots continues; delaying the initiation of flowering and limiting the number of squares retained on the plant. While allocation continues, root growth may also be restricted and extension limited through very dry or waterlogged soil (Hake and Grimes 2010). Fruit shedding occurs 10-14 days after squaring, and unshed fruit may be smaller under water stress. Under early season water stress, fruit lower in the crop is retained due to reduced growth and shorter plants with fewer nodes.

Water deficit stress increases shedding of squares firstly through altering the normal hormonal balance of the abscission zone through increasing the activity of cellulose and pectinase by altering the levels of IAA, ABA and ethylene produced by the plant (Guinn and Brummett 1989; Bahrun *et al.* 2002; Hake and Grimes 2010), and secondly by reducing photosynthesis (Constable and Rawson 1982). Excess water has also been shown to increase fruit shedding, attributed primarily through an increase in ABA in the anoxic roots, which is converted to ethylene in the leaves and fruit (Hake and Grimes 2010). Bange *et al.* (2004) showed a reduction in biomass of 32% due to waterlogging, and a reduction in yield related to reduced boll numbers.

Both waterlogging (Belford 1981) and drought stress (Rosenthal *et al.* 1987; Sharabi-Schwager *et al.* 2009) have been shown to trigger leaf senescence in several plant species, although through different mechanisms. Prolonged drought causes an increase in the rate of protein degradation, probably as a response to the limited uptake of nutrients from the soil, which are generally taken up from the soil solution (Neumann 2005). Waterlogging may cause root anoxia and prevent nutrient uptake, stimulating the same response as drought stress through the mobilisation of leaf nutrients through cell and tissue senescence and the mobilisation of these nutrients to supply young leaves and fruit. There is limited data about the effect of water stress or waterlogging on the redistribution of nutrients from cotton leaves.

2.4.5.3 Source: sink ratio

The largest sink for N, P and K in cotton plants is the bolls, containing over half the N, P and K in the whole plant at maturity. In cotton plants most tissues act as both sources and sinks of nutrients at various stages of their lifecycle (Brown 1973; Krieg and Sung 1986; Rosolem and Mikkelsen 1989; Howard *et al.* 1998; Baker and Baker 2010). The transition from sink to source occurs in leaves shortly after flowering (Zhang *et al.* 2007), and in some boll components during boll maturation (Leffler and Tubertini 1976; Thompson *et al.* 1976). The roots, while acting both as a source and sink, have been shown to be relatively neutral throughout the plant development (Rosolem and Mikkelsen 1989). The seed acts as a sink from initiation till maturity for N, P and K (Leffler and Tubertini 1976).

Agronomic management and environmental conditions change the rooting depth and size (Vandeleur *et al.* 2005), the leaf size and number (Singh *et al.* 2006a), the number of bolls initiated and the boll retention (Heitholt 1994; Jones *et al.* 1996; Wahid *et al.* 2004; Bange *et al.* 2008), therefore changing the ratio of sources to sinks.

Competition between the sinks and sources for the same resources can limit the growth and final yield of a plant. This is particularly evident in indeterminate plants, such as cotton, in which the vegetative and reproductive phases of growth overlap, and both source and sink tissues grow simultaneously. Leaves of many species function and live longer if flowering and fruit growth is prevented through removal of the fruit (Guitman *et al.* 1991).

It is well documented in cotton crops and other indeterminate plants that vegetative growth is reduced with an increasing fruit load. 'Cutout', when vegetative growth slows and eventually stops, is generally attributed to competition for assimilates from reproductive growth (Pettigrew *et al.* 2000). At this stage of development, root absorption of nutrients can decline (Wright 1999) and it is assumed that the nutrient demands of the fruit is supplemented through translocation of nutrients from vegetative structures (Pettigrew *et al.* 2000). Transgenic cotton varieties have higher boll retention rates than conventional varieties (Moser *et al.* 2000; Blanche *et al.* 2006; Bange *et al.* 2008) leading to potentially higher demands for nutrients particularly during the boll development stage, although there is only limited circumstantial data to support this assumption.

It is generally assumed that higher boll numbers place additional demands on leaf and stem sources of nutrients (Oosterhuis *et al.* 1997; Oosterhuis and Steger 1998; Lopez *et al.* 2008), and that the increase in the R:V increases redistribution, although this relationship has not been quantified. In addition to changes in fruit retention, higher yields (>2000 kg lint ha^{-1}) that are being attained by Australian growers may have also have changed nutrient redistribution from source to sink. The influence that variation in boll nutrient demand has on the partitioning of nutrients in the whole plant, and the flows of nutrients between vegetative and reproductive structures is not well documented for high retention/yielding cotton crops.

2.4.5.4 Other factors affecting nutrient distribution and redistribution

As well as water, nutrient supply and plant morphology (in terms of the source to sink ratio), other factors affect the uptake, distribution and redistribution of N, P and K in cotton plants. As shown in Table 2.2, there are some differences in the uptake of nutrients, between different cultivars. Bange and Milroy (2004) studied eight genotypes with varying season lengths, and showed that there were differences in the timing of the start of the reproductive stage between cultivars, and between cotton planted on different dates. The rate of biomass accumulation and the partitioning of biomass between reproductive and vegetative structures did not change with plant size through the season. Different cultivars have been shown to take up nutrients at a different rate and partition them slightly differently (Cassman *et al.* 1989a). The different rates of, or proportional redistribution between cotton genotypes has not been evaluated.

One of the most significant factors affecting the uptake and distribution of nutrients in cotton plants is the growth rate of the root system and its efficiency in terms of nutrient acquisition and absorption. Considering its importance, studies of cotton root systems are rare, particularly their role in nutrient uptake. Schwab *et al.* (2000) compared the tap root length and the size of the root biomass, and linked it to the above-ground accumulation of nutrients. Analysis of the nutrient contents of the roots, their role as sinks or sources, and their relative functioning through the development of the cotton plant is lacking. The role of the root system in nutrient uptake is influenced by soil type, genotype and agronomic management (Brouder and Cassman 1994; Skinner and Radin 1994; Schwab *et al.* 2000), but the effect of root functioning on the partitioning of nutrients in the above ground plant parts, and in stimulating the redistribution of nutrients in cotton plants has not been investigated.

Climatic conditions, temperature and light also affect the growth and development of cotton plants, and as such have an impact on the accumulation of nutrients, and potentially on the redistribution of nutrients from one plant to another (Reddy *et al.* 1991; Huang and Grunes 1992; Aslam *et al.* 2001; Loka and Oosterhuis 2010). Exposure of above or below ground plant parts to extremes of temperature, either hot or cold, may trigger leaf yellowing and senescence, and promote the export of nutrients from leaves. Heat shock to either the roots or the leaves may reduce the cytokinin level to a point at which senescence of the tissue begins in leaves (Harding *et al.* 1990), as well as increasing the permeability of the thylakoid membrane (Galiba *et al.* 1997). Cold temperatures similarly increase the rate and onset of senescence in leaf tissue through a loss of chlorophyll and membrane function, although the cold temperatures have been shown to slow the rate of senescence once it has begun (Thomas *et al.* 1980).

2.5 Summary

Plant nutrient acquisition, assimilation and metabolism, as well as allocation to one tissue or process over another is regulated by a complex interaction of many factors. While some processes and mechanisms have been studied frequently and in great detail, there are others for which there is little or no quantitative data, or for which the mechanisms and processes elude researchers.

Cotton nutrient use and allocation has been the subject of many studies over time, as management and cultivars have changed. The current cotton production systems in Australia differ from many of those studies in 1) the cultivars grown, 2) the prevalence of transgenic technology, which has changed the morphology and potentially physiological processes in the cotton plants, 3) the yields attainable are greater than those examined in the benchmark studies of nutrient partitioning and uptake and 4) the management of agronomic inputs, pests and weeds are more highly controlled. The cultivar, agronomic management, environmental variables and source: sink ratios in plants, are significant factors affecting the uptake and distribution of nutrients in cotton plants.

There has been limited research about the process of N, P and K redistribution from cotton leaves, although it is clear that it is a fundamental process in the plant for sustaining high yields and supporting reproductive growth. The extent to which redistribution supports the high yields attainable by Australian cotton growers, the potential maximum redistribution of N, P and K from leaves, and the way that very high-yielding crops have changed the nutrient partitioning and distribution of nutrients is unclear.

There is a need for quantitative data on the nutrient partitioning of very high-yielding cotton crops, on the proportional redistribution of nutrients in these plants and the extent to which internal and external factors influence the redistribution of N, P and K. This information will contribute to a better understanding of cotton nutrient use, and to the aim of producing cotton in highly efficient, sustainable and profitable cropping systems into the future.

CHAPTER 3

General Materials and Methods

In the 2007-08, 2008-09 and 2009-10 cotton seasons (approximately October – May), nine field experiments were carried out to investigate the uptake, distribution and re-distribution of mineral nutrients in high-yielding cotton. Field experiments were carried out at three locations in north-west NSW (Table 3.1).

Table 3.1 Location and year of each field experiment

Experiment	Cotton Season	Location	Chapter referring to experiment
1	2007-08	ACRI, 'Keytah' and 'Cardale'	4
2	2008-09	ACRI	4 and 7
3	2007-08	'Cardale'	7
4	2008-09	ACRI	4 and 8
5	2009-10	ACRI	4 and 7
6	2009-10	ACRI	6
7	2008-09	ACRI	5
8	2009 - 10	ACRI	5

3.1 Site descriptions

As shown in Figure 3.1, the three sites were relatively close to one another geographically, with two near Narrabri and one close to Moree.

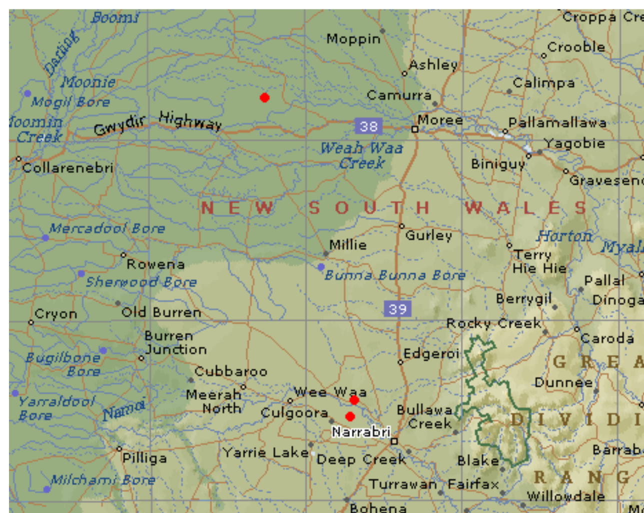

Figure 3.1 Location of the three experimental sites in north-west NSW, at ACRI, Narrabri, 'Cardale', Narrabri and 'Keytah', Moree. The sites are marked in red on the map.

3.1.1 Australian Cotton Research Institute (ACRI), Narrabri

Experiments 1, 2 and 4 – 9 were carried out at the Australian Cotton Research Institute (ACRI), “Myall Vale”, located approximately 30 km east of Narrabri in north-west NSW, Australia (30°12’S, 149°59’E).

The region is characterised by hot summers with high daily maximum (34.5^{o}C average over December, January and February) and minimum (18.6^{o}C) temperatures, and cool winters, with an average daily maximum of 18°C, and minimum of 4.3^{o}C. Average annual rainfall is 644 mm, with 220 mm falling in the summer months. Rainfall is highly variable, ranging from 422 – 861 mm annually (Bureau of Meteorology 2011).

The soil at this site was a fertile alkaline dark grey-brown cracking medium clay, classified as a fine, thermic, montmorillonitic Typic Haplustert, a grey vertosol under the Australian classification system (Isbell 1996).

3.1.2 ‘Cardale’, Narrabri

Experiments 1 and 3 were carried out on ‘Cardale’, Narrabri; a commercial cotton property located approximately 20 km west of Narrabri, in north-west NSW, Australia (149°67’E, 30°26’S). The climate is the same as described above. The soil at this site was a self-mulching, alkaline medium grey vertosol in the Australian classification system (Isbell 1996).

3.1.3 ‘Keytah’, Moree

Experiment 1 was carried out on the commercial cotton property ‘Keytah’, located approximately 45 km west of Moree in north-west NSW, Australia (149°31’E, 29°29’S).

The climate in Moree is similar to that of Narrabri, with hot summers, with high daily maximum (32.9^{o}C average over December, January and February) and minimum (19.2^{o}C) temperatures, and cool winters, with an average daily maximum of 19.1^{o}C, and minimum of 5.1^{o}C. Average annual rainfall is 613 mm, with 230 mm falling in the summer months. Rainfall is highly variable as in Narrabri, ranging from 498.3 – 825 mm annually (Bureau of Meteorology 2011). The soil at ‘Keytah’ is a self-mulching black vertosol (Isbell 1996).

3.2 Cultivar

In all experiments the CSIRO cultivar 'Sicot71BRF' was used, except where otherwise specified. Sicot71BRF is a full season cultivar with a compact growth habit, very high yield potential, good disease resistance and high fibre quality throughout the Australian cotton growing regions (C.S.D 2009). It has been one of the most popular varieties grown in Australia since its release in 2009 (C.S.D 2009).

Sicot71BRF is a transgenic cultivar containing the Bollgard II ® *Bacillus thuringiensis* (*Bt*) insecticide protein stack Cry 1Ac and Cry 2Ab, for the control of lepidopteron pests and the 'Roundup Ready Flex' ® technology for tolerance of glyphosate application throughout the growth stages in both vegetative and reproductive plant parts, through the manipulation of the CP4-EPSPS protein sequence. Both these technologies are owned and licensed by Monsanto® (Monsanto Australia 2011).

3.3 Standard procedures

3.3.1 Biomass sampling

Sampling of above ground biomass of whole plants and small subsections of plants was used to calculate shoot growth rate, total biomass, total nutrient uptake and biomass and nutrient partitioning in all experiments. Whole plants were sampled by cutting the mainstem at the soil, and separating plants into the relevant subsections, described for each experiment.
Fresh tissue was dried at 70°C for at least 72 hours, weighed, and ground using a Foss Tecator Cyclotec 1093 sample mill fitted with a 1 mm screen and stored in air tight containers to prevent moisture absorption before analysis.

3.3.2 Nutrient analysis

3.3.2.1 Nitrogen

Oven dried plant tissue was analysed for N concentration using a Leco TruSpec® CHN analyser. In the Leco TruSpec® analyser a sample is burnt in a tin capsule at 950°C, in pure (99.9%) oxygen, resulting in the production of N_2 and N-oxides. The reduction of the oxides occurs through passing the gas through hot Cu scrubbers. The N content of the sample is determined using a thermal conductivity detector (Leco Corporation 2008).

3.3.2.2 Other nutrients

Oven dried plant tissue was analysed for P, K, Mg, S, Cu, Zn, B, Fe, Na, Mn and other micronutrients using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Samples were analysed according to the method of Wheal *et al.* (2011). Oven dried samples were digested using a nitric acid digestion and finished with hydrochloric acid in an open glass tube. Duplicate samples were run every 15 samples to test for homogeneity and accepted with a less than 2% variation between the duplicates.

3.3.2.3 Rubidium

The concentration of Rb in the oven dried plant tissue was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), as described by Isaac and Johnson (1985), using a hydrochloric acid and nitric acid digestion. Duplicate samples were run to test for homogeneity, as for other nutrients.

3.3.2.4 N Isotopes (^{15}N)

The total N concentration and the ^{15}N concentration of oven dried samples from experiments 6 and 8 were determined by Isotope-Ratio Mass Spectrometry (IRMS), using the method described by Jensen (1991). The ratio of ^{15}N to ^{14}N was calculated as $\delta^{15}N$. Delta values were converted to atom % using Equation 1.

Equation 1 Conversion of $\delta^{15}N$ to Atom %

$$\text{Atom \%} = \frac{100 \text{ x AR x } (\delta^{15}\text{N value} / 1000 + 1)}{1 + \text{AR x } (\delta^{15}\text{N value} / 1000 + 1)}$$

(where AR is the absolute ration of mole fractions = 0.0036764)

Atom % was converted to atom% excess using 0.3663 as the natural abundance of ^{15}N, as reported by Hauck (1982).

3.3.3 Lint yield

Lint yield was calculated by handpicking all open bolls from a 2 m^2 subsection of each plot (or area, see individual experiment descriptions). The lint was ginned in a 10 saw gin (Continental Eagle Corp, Prattville, AL, USA) and total yield extrapolated from the ginned lint weight.

3.4 Experiment descriptions

The detailed procedures used in the field experiments listed in Table 3.1 are detailed below.

3.4.1 Field experiment 1 – 2007-08, ACRI, 'Keytah' and 'Cardale'.

A field experiment was carried out at ACRI, 'Cardale and 'Keytah', in the 2007-08 cotton season. Sicot71BRF cotton was sown on the 4th October, 2007 at a rate of 15 plants m^{-2} at ACRI, Narrabri. N was applied at a rate of 150 kg ha^{-1} as urea, zinc applied as zinc sulfate heptahydrate at 1 kg ha^{-1}. No other fertilizers were applied.

Sicot71BRF cotton was sown on the 15th October, 2007 at a rate of 20 plants m^{-2} (10 plants m^{-1} in 80 cm rows) a 'Keytah', Moree. 100 kg ha^{-1} anhydrous ammonium was applied pre-planting, and a further 250 kg ha^{-1} urea applied after sowing, equating to a total of 197 kg N ha^{-1}. No other fertilizers were applied.

Sicot71BRF cotton was sown on the 1st October, 2007 at a rate of 10 plants m^{-2} at 'Cardale', Narrabri. N was applied at a rate of 150 kg ha^{-1} as urea, zinc applied as zinc sulfate heptahydrate at 1 kg ha^{-1}. No other fertilizers were applied.
All crops were furrow irrigated and pest and weed management was applied as required to maintain vigorous crop growth and reduce variation within the sampling area.

3.4.1.1 Plant sampling and analysis

At approximately 10 day intervals between flowering and defoliation (given in Table 3.1), three replicate samples of 1 m^2 of plants were taken and dried, ground and analysed for N, P, K and other nutrients as described in section 3.3.

Table 3.2 The dates, DAS and day degrees after sowing of each whole plant sample taken from ACRI, Cardale and Keytah in 2007-08. Day Degrees calculated using 12°C as base temperature.

Site	Date	Days after Sowing	Day Degrees from Sowing
ACRI	3 Jan	91	1056
	14 Jan	102	1222
	22 Jan	110	1318
	2 Feb	121	1469
	13 Feb	132	1589
	25 Feb	144	1734
	6 Mar	154	1824
	17 Mar	165	1957
	26 Mar	174	2066
CARDALE	8 Jan	99	1132
	23 Jan	114	1334
	11 Feb	133	1570
	21 Feb	143	1680
	3 Mar	154	1794
	15 Mar	166	1926
	25 Mar	176	2047
	8 Apr	190	2163
KEYTAH	1 Feb	109	1331
	19 Feb	127	1527
	29 Feb	137	1637
	10 Mar	147	1741
	27 Mar	164	1939
	4 Apr	172	2002

3.4.1.2 Data analysis

Data was analysed using Genstat® 14th edition. Data was combined with data from other experiments as described in section 4.2.

3.4.2 Experiment 2 – N fertiliser rates

To compare the total nutrient uptake and distribution of high-yielding cotton supplied with varying amounts of N, and to quantify the impact of N-supply on the redistribution of nutrients from vegetative to reproductive plant parts a field experiment was carried out at ACRI in the 2008-09 cotton season. Sicot71BRF cotton was sown on the 13th October, 2008, at a rate of 15 plants m^{-2}.

3.4.2.1 Experimental design and treatments

The experiment was carried out within a larger Randomised Complete Block Design. The whole experiment combined crop rotation treatments with N rate applications. In this experiment, whole plots with an identical crop rotation history, which excluded legumes and other N-fixing crops were selected. All plots had a previous wheat crop in the area. Plots where 0 (treatment 0), 125 (treatment 5) and 200 kg (treatment 8) N ha^{-1} were used, highlighted and in bold in Figure 3.2, which shows the plot layout of the larger experiment in the wheat rotation areas. Plots were 8 x 16 m.

Figure 3.2 Experimental design, showing all 9 levels of N-application in the larger experiment, and highlighted are the 0 (0 kg N ha^{-1}), 5 (125 kg N ha^{-1}) and 8 (200 kg N ha^{-1}) plots sampled in experiment 2

Block 1	Block 2	Block 3	Block 4
1	1	**0**	4
4	**8**	**5**	3
2	7	1	**0**
0	3	**8**	2
7	**0**	2	6
6	**5**	6	7
5	0	0	1
3	4	7	**5**
0	2	4	0
8	6	3	**8**

3.4.2.2 Plant sampling and analysis

Whole plants were sampled, partitioned, dried and ground (as described in section 3.3) from randomly selected 1 m^2 sections from the centre 4 rows of each plot at flowering, mid-flowering, cutout, boll filling and maturity, the dates and day degrees of which are given in Table 3.3.

Table 3.3 Sampling dates, DAS and day degrees after sowing for experiment 2

Date	Days from Sowing	Day Degrees from Sowing
23 Dec 2008	71	808
13 Jan 2009	92	1117
3 Feb 2009	113	1462
24 Feb 2009	134	1764
17 Mar 2009	155	2022

Partitioned samples were analysed for N, P, K and other nutrients as described in section 3.2.

3.4.2.3 Data analysis

Data was analysed using Genstat® 14th edition. Total plant biomass and uptake of N, P and K, partitioning of biomass and N, P and K, yield, and the reproductive to vegetative ratio of the plants were compared using ANOVA. Redistribution was calculated by fitting logistic curves to the total plant uptake and fruit nutrient accumulation, taking the derivative of the logistic curve to give a parabolic equation, which was plotted using SigmaPlot®. The area between the two curves was calculated, giving total redistribution. Calculated redistribution of N, P and K were compared using ANOVA.

3.4.3 Experiment 3 – P and K fertiliser rates

To compare the total nutrient uptake and distribution of high-yielding cotton supplied with varying amounts of P and K, and to quantify the impact of P and K supply on the redistribution of nutrients from vegetative to reproductive plant parts a field experiment was carried out at 'Cardale' in the 2007-08 cotton season. Sicot71BRF cotton was sown on the 1st October, 2007.

3.4.3.1 Experimental design and treatments

The experiment was a Randomised Complete Block Design, with 4 blocks and PK treatments randomised in each block. Fertiliser treatments were combined, instead of applied as a factorial design since there is no interaction between P or K, either as fertilisers, in the soil, or within the plant which would limit the uptake of one or the other in a combined treatment. To save space and the number of samples, the treatments were combined. Plots were 8 x 16 m. The experiment design is given in Figure 3.3. Treatments were a either no P and K fertiliser,

or plus P and K, with the application of 60 kg P ha^{-1}, and 160 kg K ha^{-1} applied as a side dressing. Two adjacent fields were used, N2 and N3, with 2 blocks in each (Figure 3.3).

Figure 3.3 Experimental design experiment 3 showing plots with P and K fertiliser or no P and K added.

	Block 1	Block 2	Block 3	Block 4
Field name	N2	N2	N3	N3
Plot 1	Plus PK	Plus PK	Nil PK	Plus PK
Plot 2	Nil PK	Nil PK	Plus PK	Nil PK

3.4.3.2 Plant sampling and analysis

Whole plants were sampled, partitioned, dried and ground (as described in section 3.3) from randomly selected $1m^2$ sections of each plot at flowering, cutout, boll filling and maturity, the dates and day degrees of which are given in Table 3.4. Plants were sampled from the centre rows of each plot to minimise interactions between plots.

Table 3.4 Sampling dates, DAS and day degrees after sowing for experiment 3

Date	Days from Sowing	Day Degrees from Sowing
8 Jan 2008	99	1132
11 Feb 2008	133	1570
3 March 2008	154	1794
8 April 2008	190	2163

Partitioned samples were analysed for N, P, K and other nutrients, and yield calculated as described in section 3.2.

3.4.3.3 Data analysis

Data was analysed using Genstat® 14th edition. Total plant biomass and uptake of N, P and K, partitioning of biomass and N, P and K, yield and the reproductive to vegetative ratio of the plants were compared using ANOVA. Redistribution was calculated by fitting logistic curves to the total plant uptake and fruit nutrient accumulation, taking the derivative of the logistic curve to give a parabolic equation, which was plotted using SigmaPlot®. The area between the two curves was calculated, giving total redistribution. Calculated redistribution of N, P and K were compared using ANOVA.

3.4.4 Experiment 4 – Deficit irrigation rates

To compare the total nutrient uptake and distribution of high-yielding cotton grown in conditions with varying degrees of water stress, and to quantify the impact of water supply on the redistribution of nutrients from vegetative to reproductive plant parts a field experiment was carried out at ACRI in the 2008-09 cotton season. Sicot71BRF cotton was sown on the 15th October, 2008.

3.4.4.1 Experimental design and treatments

Experiment 4 was carried out within a larger experiment involving the application of six different irrigation schedules across the 2008-09 growing season. In this experiment the nutrient uptake and distribution from two of the irrigation treatments applied, "frequent" irrigation, watered at a 40 mm soil water deficit, and "extended" irrigation, watered at a 120 mm soil water deficit, were investigated. Deficit irrigation involves refilling the soil water profile once a designated water deficit is reached.

The experiment was a RCBD with four blocks, with six randomly allocated 164 m long plots under different irrigation treatments in each block. Only three of the six treatments applied were sampled in experiment 4, treatments 1, 3 and 4 shown in Figure 3.4. The width of each plot varied under different irrigation treatments to minimise lateral movement of water through the soil from one block to another (drier plots being wider than more frequently irrigated plots).

Figure 3.4 Experimental design of experiment 4, showing the six applied irrigation treatments, of which treatments 1 (frequent irrigation at a 40 mm deficit) and 3 (extended irrigation at a 120 mm deficit) were investigated for nutrient uptake, distribution and redistribution (highlighted in grey).

Block	Irrigation Treatment	Number of rows
1	5	16
	4	16
	6	16
	2	16
	3	20
	1	12
2	**1**	12
	2	16
	6	16
	4	16
	5	16
	3	20
3	**3**	20
	2	16
	1	12
	6	16
	4	16
	5	16
4	2	16
	1	12
	6	16
	4	16
	5	16
	3	20

Soil water deficits were measured weekly to a 120 mm depth in 15 cm intervals, using a CPN Corpotation Hydroprobe®, model 503DR, neutron attenuation meter (NAM). The NAM was calibrated using the methodology of Tennakoon and Hulugalle (2006). Since soil water was monitored frequently, rainfall was accounted for in determining deficits and irrigation scheduled accordingly. Total rainfall throughout the growing season totalled 327 mm. Irrigation treatments are given in Table 3.5.

Table 3.5 Dates, DAS and day degrees after sowing of irrigation treatments applied in experiment 4

Treatment	Irrigation Date	DAS	Day degrees after sowing
Frequent (40 mm deficit)	9 Dec, 2008	55	550
	22 Dec, 2008	68	708
	2 Jan, 2009	79	866
	9 Jan, 2009	86	976
	15 Jan, 2009	92	1068
	23 Jan, 2009	100	1189
	30 Jan, 2009	107	1309
	5 Feb, 2009	113	1414
	11 Feb, 2009	119	1526
	27 Feb, 2009	135	1721
	13 Mar, 2009	149	1957
Extended (120 mm deficit)	16 Jan, 2009	93	1087
	6 Feb, 2009	114	1434

3.4.4.2 Plant sampling and analysis

Whole plants were sampled, partitioned, dried and ground (as described in section 3.3) from randomly selected 1 m^2 sections of each plot at flowering, mid-flowering, cutout, boll filling and maturity, the dates and day degrees of which are given in Table 3.6. Plants were sampled from the centre rows of each plot to minimise interactions between plots.

Table 3.6 Sampling dates, DAS and day degrees after sowing for experiment 4

Date	Days after Sowing	Day Degrees from Sowing
31 Dec, 2008	77	900
14 Jan, 2009	91	1113
9 Feb, 2009	117	1575
2 Mar, 2009	138	1845
30 Mar, 2009	166	2179

Partitioned samples were analysed for N, P, K and other nutrients, and yield calculated as described in section 3.2.

3.4.4.3 Data analysis

Data was analysed using Genstat® 14th edition. Total plant biomass and uptake of N, P and K, partitioning of biomass and N, P and K, yield and the reproductive to vegetative ratio of the plants were compared using ANOVA. Redistribution was calculate by fitting logistic curves to the total plant uptake and fruit nutrient accumulation, taking the derivative of the logistic

curve to give a parabolic equation, which was plotted using SigmaPlot®. The area between the two curves was calculated, giving total redistribution. Calculated redistribution of N, P and K were compared using ANOVA.

3.4.5 Experiment 5 – Nitrogen fertiliser rates

To investigate the effect of N supply on the nutrient uptake, partitioning and redistribution from vegetative to reproductive plant parts, a field experiment was carried out at ACRI, Narrabri in the 2009-10 cotton season. Sicot71BRF cotton was planted at a rate of 15 plants m^{-2}, on the 15th October, 2009.

3.4.5.1 Experimental design and treatments

Two N treatments ("Low", 50kg N ha^{-1}; and "High", 200kg N ha^{-1}) were applied in a RCBD, with four blocks and 8, 4 x 35 m plots.

3.4.5.1.1 Nitrogen Treatment

Two N treatments were applied, "High" and "Low". Pre-planting all plots were applied Anhydrous Ammonium at a rate of 61 kg ha^{-1}, an equivalent of 50 kg N ha^{-1}. Low plots were given no extra N. High plots were additionally side-dressed with 326 kg ha^{-1} urea, an equivalent of 150 kg ha^{-1}, bringing the total N supply to 200 kg ha^{-1}. Granular urea was applied using an offset disc at a depth of 15 cm, and plants were watered following urea application.

3.4.5.2 Plant sampling and analysis

Whole plants were sampled, partitioned, dried and ground (as described in section 3.3) from randomly selected 1 m^2 sections of each plot at flowering, mid-flowering, cutout, boll filling and maturity, the dates and day degrees of which are given in Table 3.7. Plants were sampled from the centre two rows of each plot to minimise interactions between plots.

Table 3.7 Sampling dates, DAS and day degrees after sowing for experiment 5

Date	Days from Sowing	Day Degrees from Sowing
7 Jan, 2010	84	1185
27 Jan, 2010	104	1503
23 Feb, 2010	131	1900
18 March, 2010	154	2163
4 April, 2010	175	2400

Partitioned samples were analysed for N, P, K and other nutrients, and yield calculated as described in section 3.2.

3.4.5.3 Data analysis

Data was analysed using Genstat® 14th edition. Total plant biomass and uptake of N, P and K, partitioning of biomass and N, P and K, yield and the reproductive to vegetative ratio of the plants were compared using ANOVA. Redistribution was calculate by fitting logistic curves to the total plant uptake and fruit nutrient accumulation, taking the derivative of the logistic curve to give a parabolic equation, which was plotted using SigmaPlot®. The area between the two curves was calculated, giving total redistribution. Calculated redistribution of N, P and K were compared using ANOVA.

3.4.6 Experiment 6 – Application of ^{15}N isotope and RbCl to soil

3.4.6.1 Experimental design and treatments

2 x 2 m sections of the centre two rows of cotton in the "high N" plots of experiment 5 were marked off before flowering, the experimental design, planting and fertiliser details given in section 3.4.5.1. The 2 rows were randomly divided into 1) labelled and 2) non labelled (control) treatment sub-plots of 1 x 2 m.

3.4.6.1.1 Plant density and spacing

Plants were thinned by hand to a density of 10 plants m^{-2} before flowering.

3.4.6.1.2 ^{15}N and Rb application

^{15}N and Rb solutions were applied once, pre-flowering on December 18th, 2009 (725 day degrees from sowing) directly to the soil adjacent to the growing cotton crop. Beside both the control and the labelled plots, a 30 cm deep trench was dug on the side of the bed, 15 cm from the base of the cotton plant, as shown in Figure 3.5 and Figure 3.6 (b).

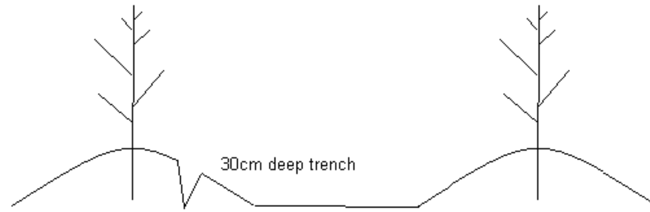

Figure 3.5 Schematic diagram of ^{15}N and Rb solution placement

Rb was applied as a 0.02 M solution of RbCl applied at a rate of 2.4184 g L^{-1} (equivalent to 0.1795 g Rb per plot). ^{15}N was applied as a solution of 98.47% ^{15}N urea applied at a rate of 0.4432 g per plot (0.1 g ^{15}N excess per plot). Into both trenches a total of 105 mL of solution was applied, in 21 aliquots of 5 mL applied at 10 cm intervals along the 2 m subsection of the plot (Figure 3.6a and c). Control plots received only deionised water and labelled plots received a solution of ^{15}N and RbCl.

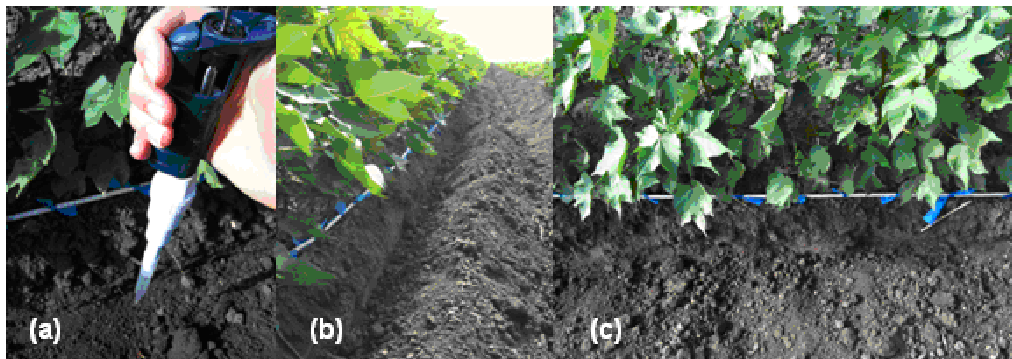

Figure 3.6 (a) pipette used to apply an equal volume of ^{15}N urea and Rb solution to the soil, (b) the 30cm deep trench dug 15cm from the base of the cotton plants and (c) top view of the application trench showing the marker tape used to mark 10cm intervals for fertiliser solution application

After the application of the fertiliser solution, trenches were covered with moist soil and packed down by hand. Plants were irrigated 4 days after the application of the solution, on the 22nd Dec, 2009.

3.4.6.2 Plant sampling and analysis

Plants were sampled at five dates between application of ^{15}N and RbCl and maturity (Table 3.8). Sampling dates coincided with major plant development phases, flowering, cutout, mid-boll filling, first open boll and maturity.

Table 3.8 Sampling dates experiment 6

Sampling Date	Days from sowing / days from ^{15}N and Rb application	Day degrees from sowing / day degrees from ^{15}N and Rb application
7/1/2010	84 / 20	1185 / 460
26/1/2010	103 / 39	1484 / 759
22/2/2010	130 / 66	1687 / 962
22/3/2010	158 / 94	2042 / 1317
6/4/2010	173 / 109	2198 / 1473

Two replicate plants were sampled from each plot at each sampling time (2 plants from 4 plots, making 8 replicate plants). Plants were partitioned into 5 sub sections based on the mainstem node, from the base to node 6, from node 7 – 11, from node 12 – 16, from node 17 – 21 and 21+. Monopodial (vegetative) branches were divided according to the node sections also and combined with the higher samples; for example, a monopodial branch arising from node 4, and being 7 nodes long, would be partitioned into 2 sections, the bottom 2 nodes (equivalent to nodes 5 and 6 on the main stem) would be combined into section 1, and the following 5 nodes, equivalent to the mainstem nodes 7-11 would be combined with section 2. Each sub-section, except section 5, was then partitioned into leaf, stem (including petiole) and fruit (including bracts, capsule walls, seed and lint) fractions. Section 5 was analysed as a whole, due to its late development and small biomass. There were up to 13 sub-samples per plant.

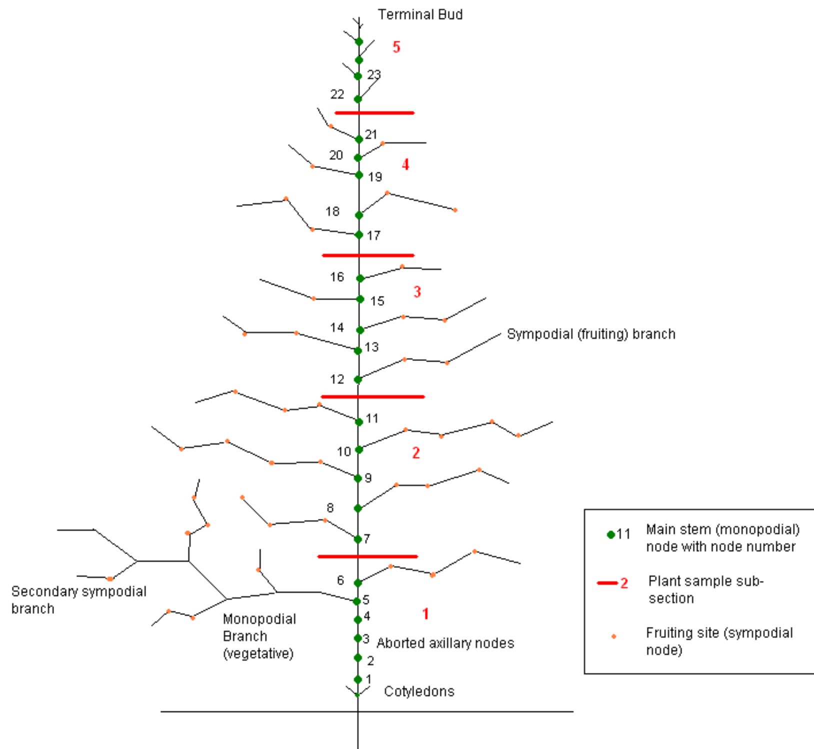

Figure 3.7 Schematic diagram of plant sub-sampling sections for experiment 8

Each sub-sample was dried, ground and analysed for N, P and K as described in section 3.3.2. Isotope analysis and Rb analysis were carried out separately to nutrient analysis as described in sections 3.3.2.3 and 3.3.2.4. ^{15}N excess content was calculated as described in section 3.3.2.4.

3.4.6.3 Data analysis

Data was analysed using Genstat® 14th edition. The biomass and total accumulation of N, P and K in each section was compared using ANOVA, and the partitioning of N, P and K between leaf, stem and fruit fractions compared between plants to eliminate differences in plant size. Redistribution was calculated by the change in the total vegetative and reproductive concentration of ^{15}N between sampling dates and comparisons between subsections made using ANOVA. The R:V ratio of each section was used as a factor in the analysis to determine if the boll load in the sections influenced the redistribution of N, P or K from the vegetative to the reproductive tissue.

3.4.7 Experiment 7 - Nutrient partitioning along a sympodial branch

A field experiment was carried out at ACRI, Narrabri in the 2008-09 cotton season to examine the N, P and K accumulation in the leaves, stems, petioles, bracts, boll walls, seed and lint developed at one mainstem node position in cotton plants not exposed to water or nutrient stress. Node 11 was chosen as the sampled node, being referred to in other studies as representative of the whole plant. While node 10 has previously been referred to as a "representative node" for the whole plant (Oosterhuis and Wullschleger 1988; Zhu and Oosterhuis 1992), Thompson *et al.* (1976) found that node 11 was the most likely to retain fruit at positions one and two. As such, node 11, being likely to retain fruit, and being slightly higher in the plants, which were larger and more vigorous than those in other studies (such as Oosterhuis and Wullschleger 1988; Zhu and Oosterhuis 1992), was used as the representative node.

3.4.7.1 Experimental design

The experiment was designed as a Time Series Design, with random sampling from four blocks. Blocks were 2m x 30m areas of Sicot71BRF cotton sown with 15 plants m^{-2}, and thinned by hand to 10 plants m^{-2} before flowering. All branches in each block with a white flower in the first fruiting position on the 11th node were tagged using plastic tape, as shown in Figure 3.8. Tagging the branches with a white flower ensured that the fruit at position 1 was of an identical age between all branches. Plants were tagged on the 12th January, 2009.

Figure 3.8 Tagging method of branched with white flowers in first position.

3.4.7.2 Plant sampling and analysis

At intervals between 3 and 7 days, one whole plant with a tagged branch was sampled from each plot, giving four replicate samples of branches at each sampling date. Only branches with 2 fruit, at position 1 and position 2 on the branch were sampled (Figure 3.9).

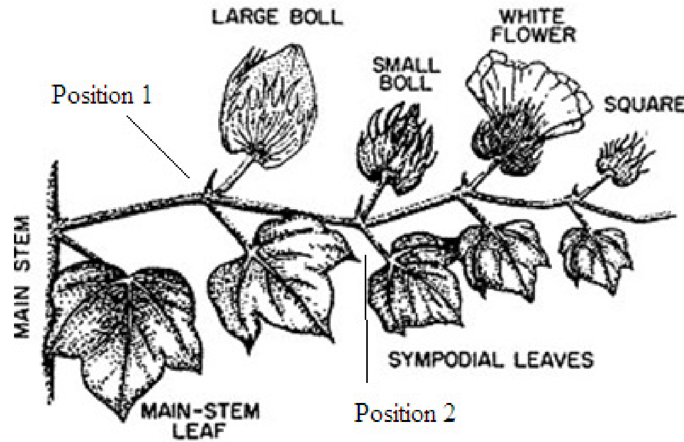

Figure 3.9 Diagram of a sympodial branch of a cotton plant, showing the fruiting positions and various tissues sampled in experiment 7. Diagram from (Zhu and Oosterhuis 1992)

Table 3.9 Sampling dates, and days from sowing, day degrees from sowing, and days from tagging and day degrees from tagging for experiment 7 in the 2008-09 cotton season.

Sampling date	Days from sowing	Growing day degrees	Days from tagging	Day degrees from tagging
15 Jan, 2009	94	1152	3	51
21 Jan, 2009	100	1245	9	144
28 Jan, 2009	107	1359	16	258
2 Feb, 2009	112	1445	21	344
6 Feb, 2009	116	1518	25	417
10 Feb, 2009	120	1596	29	495
13 Feb, 2009	123	1633	32	532
17 Feb, 2009	127	1668	36	567
21 Feb, 2009	131	1721	40	620
24 Feb, 2009	134	1764	43	663
27 Feb, 2009	137	1805	46	704
2 March, 2009	140	1845	49	744
5 March, 2009	143	1885	52	784
9 March, 2009	147	1927	56	826
12 March, 2009	150	1965	59	864
16 March, 2009	154	2014	63	913
19 March, 2009	157	2045	66	944
26 March, 2009	164	2133	73	1032

Data recorded for each plant included;

- Nodes above and below the tagged branch
- Fruit on the node above and node below the tagged branch
- Number of leaves on the branch
- Number and type (square, flower, green boll or open boll) of fruit on the tagged branch
- Number of fruiting positions on the tagged branch
- The dry weight, N, P and K concentration of the leaves, stems, petioles, and partitioned fruit (boll walls, seed, bracts and lint) (by the method described in section 3.3). The leaf, petiole, stem, boll wall, bracts, seed and lint from each position (1, 2 and 3), as well as the main stem leaf, main stem leaf petiole and mainstem node segment were ground and analysed separately.

3.4.7.3 Data analysis

The pattern of accumulation of biomass, N, P and K for each partitioned section of the sympodial branch was graphed as a function of plant age (in days from sowing and day degrees from sowing), and in days from flowering at position 1 and day degrees from flowering at position 1. A paired t-test statistic method was used to compare the concentrations of N, P and K in partitioned sections at different fruiting positions, and the calculated redistribution of N, P and K from leaves.

Total redistribution was calculated by the difference in N, P or K content from the peak content to the content at maturity (mg), where there was a decline.

Accumulation at similar growth stages (in days from flowering at position 1) were compared using ANOVAs.

3.4.8 Experiment 8 – ^{15}N and RbCl application and distribution along a sympodial branch

A similar field experiment to experiment 8 was carried out in the 2009-10 cotton season to specifically quantify the contribution N and K from single leaves to subtending bolls on a sympodial branch, through the use of an ^{15}N isotope solution and an Rb solution applied to the main stem and 1st position leaves of branches at the 11th node in an unstressed, high-

yielding Sicot71BRF crop. The crop was sown on the 15th October 2009 at a rate of 15 plants m^{-2}, and thinned by hand to a density of 10 plants m^{-2} before flowering.

3.4.8.1 Experimental design

Branches in a 16 x 5 m area with a white flower at position 1 on the 4th February, 2010 were tagged with a plastic marker (as shown in Figure 3.8). The experiment was a RCBD, with four replicate blocks of 4 x 5 m with four randomised treatments, in 1 x 5 m plots. Each plot was one row of cotton x 5 m (50 plants).

3.4.8.2 ^{15}N and Rb application

Two treatments, a labelling treatment and a control treatment were applied to either the mainstem or 1st position leaves on the 11th node of each plant in each block (the four treatments are given in Table 3.10). Rb and ^{15}N were applied an approximate rate of 1% of the total content of the leaf, the equivalent of 0.4 mg Rb per leaf and 0.7 mg N per leaf. This was the equivalent of 0.5659 mg RbCl (an equivalent of 0.3999 mg), and 1.5217 g Urea (98.47% ^{15}N excess, in solution the equivalent of 0.68929 mg ^{15}N excess per leaf) per leaf.

Table 3.10 Treatments applied to leaves on the tagged branches in experiment 9

Treatment	Solution	Application point
1	0.68929 mg ^{15}N excess and 0.5659 RbCl in 0.6 mL deionised water	Main stem leaf
2	0.68929 mg ^{15}N excess and 0.5659 RbCl in 0.6 mL deionised water	1st position leaf
3	Deionised water	Main stem leaf
4	Deionised water	1st position leaf

The solution of either water or ^{15}N and RbCl was applied through the direct injection of 0.6 mL into the point of attachment of the petiole to the treatment leaf, as shown in Figure 3.10.

Figure 3.10 Injection of ^{15}N and RbCl solution into the Main stem and 1st position leaves

3.4.8.3 Plant sampling and analysis

Due to the cost of analysis of plant material and isotope analysis, fewer samples were taken from experiment 8 as in experiment 7. At five growth dates, approximately every 10 days (although actual dates varied due to rainfall delaying the collection of the samples), given in Table 3.11, two whole plants were removed from each plot, making the sample 8 replicate plants at each sampling stage.

Table 3.11 Sampling dates, days from sowing, day degrees from sowing and days and day degrees from the application of the treatment solutions in experiment 8

Sample date	Days from sowing	Day degrees from sowing	Days from solution application / flowering	Day degrees from solution application / flowering
18th Feb, 2010	126	1828	14	205
25th Feb, 2010	133	1930	21	307
10th March, 2010	146	2079	34	456
22nd March, 2010	154	2212	46	589
6th April, 2010	173	2381	61	758

The following data was collected from each collected plant;

- Nodes above and below the tagged branch
- Number of leaves on the tagged branch
- Number and type (square, flower, green boll or open boll) of fruit on the tagged branch
- Number of fruiting positions on the tagged branch
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the leaves, stems, petioles, at position 1, 2 and 3+, and the main stem leaf and node segment of the main stem (by the method described in section 3.3) from the tagged branch (18th Feb, 10th March and 6th April samples only).
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the boll walls, bracts, seed and lint of the boll at position 1 from the tagged branch (by the method described in section 3.3) (18th Feb, 10th March and 6th April samples only).
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the pooled dried and ground leaf, stem and fruit samples from the node above (number 12) and node below (number 10) the tagged branch (18th Feb, 10th March and 6th April samples only).

- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the dried and ground leaf, stem and fruit samples from nodes 13+ and nodes 1-9 (18th Feb, 10th March and 6th April samples only).

^{15}N excess content was calculated as described in section 3.3.2.4. The redistribution of ^{15}N and Rb from the treated leaf to the parts of node 11 and to nodes above and below was then calculated as the difference in the ^{15}N or Rb content between sampling times.

3.4.8.4 Data analysis

Data was analysed using Genstat® 14th edition. The dry weights and N, P and K content of the leaves at the main stem and 1st position were analysed using ANOVA to establish that the injection method did not hinder leaf growth or functioning.

The dry weight, N, P and K accumulation in the partitioned sections and nodes above and below the treated leaves were compared to the controls, using a paired t-test and ANOVA.

Redistribution was quantified through measuring the concentration of ^{15}N and Rb in each leaf along the 11th node, and in the leaves and fruit of the branches above and below the treated leaf.

CHAPTER 4

Nutrient uptake and distribution in non-stressed high-yielding cotton

4.1 Introduction

Over the past century cotton nutrient uptake and partitioning has been extensively documented, and revisited periodically as management practices, cultivars and production conditions have changed. In the years since many of the major studies describing cotton nutrient uptake and partitioning were carried out (Bassett *et al.* 1970; Halevy 1976; Halevy *et al.* 1987; Mullins and Burmester 1990), cultivars, technologies and management techniques have improved. Recent studies investigating the dry matter and nutrient partitioning in cotton have focused on N nutrition (e.g. Boquet and Breitenbeck 2000; Fritschi *et al.* 2004a; Fritschi *et al.* 2004b; Janat 2004; Rochester and Constable 2006; Wiedenfeld *et al.* 2009). These recent studies have reported some differences to prior studies in terms of the distribution of dry matter and N, however differences have been inconsistent.

In addition to higher lint yields, modern transgenic varieties grown in Australia may retain more fruit on lower branches (Mills *et al.* 2008), and retain a higher proportion of the developing fruit throughout the fruiting branches (Moser *et al.* 2000; Blanche *et al.* 2006; Bange *et al.* 2008). While this may not necessarily in itself lead to higher yields, since cotton has a tendency to compensate for a lower boll number by increasing the size of the bolls (Mills *et al.* 2008), it may alter the distribution pattern of nutrients in the plant, and place additional demands on leaf nutrient resources (Cassman *et al.* 1989a; Cassman *et al.* 1989b; Wright 1999; Rochester 2007). Similarly the timing of uptake pre and post flowering has not been widely reported for modern cultivars and production systems. Data is particularly lacking for transgenic Australian cultivars.

The amount of nutrients remobilised and redistributed from one tissue to another in cotton cultivars has never been explicitly quantified at a whole plant scale. As described in section 2.3.1, there have been several methods in other species used to quantify redistribution, including calculating the balance between peak nutrient content and content at maturity, determining the minimum concentration in a mature leaf which represents complete or incomplete remobilisation, using physiological measurements such as photosynthesis or root

respiration, describing the appearance of deficiency symptoms on leaves or using isotope tracers. Determining the amount of nutrients redistributed in high-yielding cotton crops will help to describe the efficiency with which they use nutrients, evaluate differences in NUE between sites, seasons and treatments and describe the way high-yielding cotton plants allocate nutrients.

The interaction between growth, nutrient uptake and the redistribution of nutrients from leaves and stems to bolls has also not been previously described. The redistribution of N, P and K from cotton leaves has been linked to a high source: sink ratio, particularly at 4 NAWF (Wright 1999; Pettigrew *et al.* 2000), and also to the inability of the roots to take up nutrients late in the season, due to the allocation of carbon and nutrients to the developing bolls preventing root growth and functioning. Since the actual redistribution of N, P and K has not been quantified, nor has its contribution to the developing bolls, these assertions are supported by circumstantial evidence only.

This chapter aims to;

1) Determine the uptake and partitioning of N, P and K by a range of high-yielding cotton crops in Australia, and comparing them to historical data;
2) Establish a method for quantifying nutrient redistribution in a whole plant;
3) Quantify the redistributed fraction of vegetative N, P and K in the bolls at a whole plant scale.

4.2 Materials and methods

To compare the nutrient uptake, distribution and redistribution in non-stressed, high-yielding plants six different crops were grown in the 2007-08, 2008-09 and 2009-10 cotton seasons. For the purposes of this analysis all plots from experiment 1 (section 3.4.1), the high N plots (with no N stress) from experiments 2 (section 3.4.2) and 5 (section 3.4.5) and the control plots from experiment 4 (section 3.4.4) were used. The experimental design and crop management is described in these respective sections. A description of each site and the environmental conditions can be found in section 3.1.

In this chapter, since all experiments involved a crop grown at ACRI, Narrabri, the sites will be referred to the names given in Table 4.1.

Table 4.1 Details of the location and names of each site in chapter 4, and the references to the detailed materials and methods references from chapter 3

Experiment Number and method reference	Experimental Site	Name
1, section 3.4.1	ACRI, Field F6	ACRI
1, section 3.4.1	Cardale	Cardale
1, section 3.4.1	Keytah	Keytah
2, section 3.4.2	ACRI, Field F6	F6
4, section 3.4.4	ACRI, Field B3	B3
5, section 3.4.5	ACRI, Field A3	A3

4.2.1 Plant Sampling and analysis

Replicate samples of a 1 m^2 area of plants were taken from each site at regular intervals between flowering and defoliation of the crop. Four replicate samples were taken from experiments 1, 2, 4 and 5 at each sampling time. These sampling dates, and days from sowing are shown in Table 3.2, Table 3.3, Table 3.5, Table 3.6.

Whole plants were partitioned into leaves, stems (including petioles) and fruit (squares, flowers and bolls including seed, lint, boll walls and bracts). Samples were dried, ground and analysed for N, P and K as described in 3.3.2. After defoliation yield was determined by handpicking as described in 3.3.3.

4.2.2 Data Analysis

Data was analysed using Genstat® 14th edition. Total biomass, N, P and K were analysed using ANOVAs. Bernacchi *et al.* (2007) and Gedroc *et al.* (1996) found that accounting for growth stages, more differences in dry matter accumulation and partitioning between plants as they develop can be demonstrated. Analysis of plants at a specific growth stage accounts for some of the differences in growth rate and seasonal environmental effects. To account for these differences between the crops, three separate ANOVAs were carried out to compare the dry matter accumulation and nutrient uptake and partitioning at flowering, 4 NAWF and maturity, rather than using plant age or thermal time as a factor. Correlation coefficients were calculated using Genstat® 14th edition.

4.3 Results

4.3.1 Crop growth and development

The timing of growth and development is given in Table 4.1. There were few differences in the time to flowering, open boll or maturity.

Table 4.2 Development and timing of key growth stages at the six sites in experiments 1, 2, 4 and 5.

Growth Stage	ACRI	Keytah	Cardale
Sowing	4th Oct, 2007	15th Oct, 2007	1st Oct, 2007
Emergence	12th Oct, 2007	24th Oct, 2007	10th Oct, 2007
Squaring	23rd Nov, 2007	1st Dec, 2007	22nd Nov, 2007
First Flower	9th Dec, 2007	16th Dec, 2007	9th Dec, 2007
Open Boll	11th Feb, 2008	21st Feb, 2008	12th Feb, 2008
4 NAWF	25th Feb, 2008	19th Feb, 2008	3rd Mar, 2008
Maturity	26th Mar, 2008	8th Apr, 2008	4th Apr, 2008

Growth Stage	F6	B3	A3
Sowing	13th Oct, 2008	15th Oct, 2008	15th Oct, 2009
Emergence	21st Oct, 2008	25th Oct, 2008	23rd Oct, 2009
Squaring	29th Nov, 2008	2nd Dec, 2008	22nd Nov, 2009
First Flower	21st Dec, 2008	23rd Dec, 2008	10th Dec, 2009
Open Boll	7th Feb, 2009	8th Feb, 2009	29th Jan, 2010
4 NAWF	3rd Feb, 2009	9th Feb, 2009	27th Jan, 2010
Maturity	17th Mar, 2009	30th Mar, 2009	8th April, 2010

4.3.2 Yield, boll size and boll number

There was a difference in the yield, boll number and average boll size between the six crops (Table 4.3). The bolls of the highest yielding crop (ACRI, 07-08) were the smallest, and largest in number. The lowest yielding crop (Cardale, 07-08) had the largest bolls (P = 0.033), although the fewest in number. All crops were above or equal to the average Australian cotton crop yield (2120 kg lint ha^{-1}) and the yields used in nutrient uptake studies in the past ($P < 0.05$).

Table 4.3 Yield, boll number and average boll weight. Means followed by the same letter are not significantly different at *P* = 0.05

Site	Yield (kg lint ha^{-1})	Number of bolls m^{-2}	Average Boll Weight (g)	Gin turnout (% lint of seed cotton)
ACRI 07-08	3270.7^{a}	169^{a}	5.1^{ac}	45.2^{c}
Cardale 07-08	2133.8^{b}	125^{b}	6.5^{b}	41.9^{b}
Keytah 07-08	3085.6^{ad}	149^{ab}	5.7^{ab}	39.6^{a}
F6 08-09	2489.8^{c}	164^{a}	5.1^{ac}	42.2^{b}
B3 08-09	2656.8^{cd}	152^{ab}	5.7^{ab}	40.1^{a}
A3 09-10	2983.3^{ad}	191^{a}	4.6^{c}	41.6^{b}
LSD	360.7	34.74	1.073	1.107
P	<0.001	0.021	0.033	<0.001

4.3.3 Total biomass and nutrient uptake

The accumulation of biomass, N, P and K followed a sigmoidal curve (Figure 4.1). While little variation in biomass accumulation was observed, the lowest yielding crop at Cardale had a lower biomass accumulation ($P = 0.015$), while the highest yielding crop at ACRI had the highest biomass accumulation (Figure 4.1a).

There was no difference in the accumulation of N between the sites, except for a lower N content at Cardale at flowering ($P < 0.05$) (Figure 4.1b). The total P uptake at A3 was higher than other crops at maturity, and the P uptake at Cardale consistently lower ($P = 0.004$) (Figure 4.1c). The uptake and accumulation of K was also lower at Cardale throughout the growing season ($P < 0.001$) (Figure 4.1d).

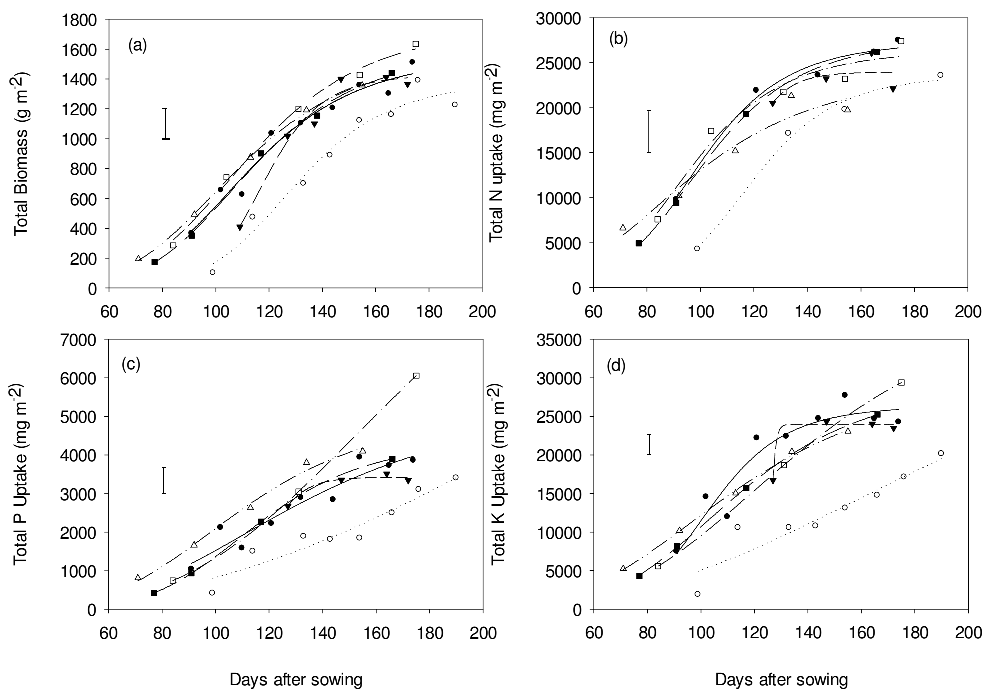

Figure 4.1 (a) Total biomass accumulation (g m^{-2}), (b) N accumulation (mg m^{-2}), (c) P accumulation (mg m^{-2}) and (d) K accumulation (mg m^{-2}) and fitted sigmoidal curves at ACRI (——●——), Cardale (·····○·····), Keytah (— —▼— —), F6 (···—△—···), B3 (— —■— —) and A3 (··—□—·—). Vertical bar represents the least significant difference *P* < 0.05.

The analysis of total dry weight and nutrient uptake using separate ANOVAs for flowering, 4 NAWF and maturity highlighted more differences than when analysed as a function of DAS, especially in the case of N accumulation (Table 4.4). There was no difference in total N uptake at 4 NAWF (P = 0.147), or at maturity (P = 0.096), however at flowering the crop at Keytah had a higher total N uptake than all other sites (P < 0.001), accumulating at least 2009 mg m^{-2} more N than all other crops.

Variation in total P uptake decreased over the growing season. Considerable variation occurred at flowering and 4 NAWF (Table 4.4). At maturity, the total P uptake was the same for all sites except for at A3, where the uptake was higher by 1963 mg than the next highest uptake (at F6) (P = 0.035). Variation in K uptake followed a similar trend, with far more variation at the beginning of the season than at the end.

Table 4.4 Dry weight, N, P and K content m^{-2} at the six sites at flowering, 4 NAWF and maturity. * = $P < 0.05$, ** = $P < 0.001$. Means followed by the same letter are not significantly different at $P = 0.05$.

	Total Amount			Proportion of total accumulated by each growth stage (%)		
Site	**Flowering**	**4 NAWF**	**Maturity**	**Flowering**	**4 NAWF**	**Maturity**
	Dry Weight (Total amount presented in g m^{-2})					
ACRI	365^{d}	1034^{b}	1510^{bc}	24.2	68.5	100
Keytah	419^{d}	1019^{b}	1366^{ab}	30.7	74.6	100
Cardale	101^{a}	699^{a}	1225^{a}	8.2	57.1	100
F6	195^{b}	875^{ab}	1358^{ab}	14.3	64.4	100
B3	176^{b}	902^{ab}	1440^{abc}	12.2	62.7	100
A3	286^{c}	742^{ab}	1635^{c}	17.5	45.4	100
Average	257	878	1422	17.8	62.1	100
	**	*	*			
LSD	74.8	208	245.8			
	N (Total amount presented in mg m^{-2})					
ACRI	9761^{c}	21905	27505	35.5	79.6	100
Keytah	11770^{d}	20522	22127	53.2	92.7	100
Cardale	4279^{a}	17126	23594	18.1	72.6	100
F6	6614^{b}	15186	19716	33.5	77.0	100
B3	4932^{a}	19323	26190	18.8	73.8	100
A3	7596^{b}	17426	26409	28.8	66.0	100
Average	7492	18581	24257	31.3	77.0	100
	**	n.s.	n.s.			
LSD	1432					
	P (Total amount presented in mg m^{-2})					
ACRI	1040^{c}	2218^{ab}	3859^{a}	26.9	57.5	100
Keytah	1551^{d}	2679^{bc}	3346^{a}	46.3	80.1	100
Cardale	414^{a}	1886^{a}	3405^{a}	12.1	55.4	100
F6	807^{b}	2625^{b}	4095^{a}	19.7	64.1	100
B3	426^{a}	2275^{ab}	3902^{a}	10.9	58.3	100
A3	743^{b}	3052^{c}	6058^{b}	12.3	50.4	100
Average	830	2456	4111	21.4	60.9	100
	*	*	*			
LSD	262	520	1355			
	K (Total amount presented in mg m^{-2})					
ACRI	7473^{c}	22175^{c}	24258^{b}	30.8	91.4	100
Keytah	7524^{c}	16731^{b}	23505^{ab}	32.0	71.2	100
Cardale	1888^{a}	10560^{a}	20124^{a}	9.4	52.5	100
F6	5250^{b}	15029^{b}	23038^{ab}	22.8	65.2	100
B3	4293^{b}	15726^{b}	25239^{b}	17.0	62.3	100
A3	5583^{b}	18682^{bc}	29402^{c}	19	63.5	100
Average	5335	16484	24261	21.8	67.7	100
	**	**	**			
LSD	1403	4220	3742			

4.3.4 Biomass and nutrient partitioning

4.3.4.1 Biomass

The partitioning of biomass between leaf, stem and fruit is given in Figure 4.2. Analysis of the leaf, stem and fruit dry weigh fractions show that variation in total dry weight (given in Table 4.4) is accounted for by differences in the partitioning of dry weight between leaves, stems and fruit. Variation in the total dry weight at maturity is mainly accounted for by differences in stem dry weight (P = 0.001), with no variation in fruit dry weight (P = 0.826) and little variation in leaf dry weight (P = 0.035). Variation in yield, despite the lack of variation in fruit dry weight at maturity can be attributed to a difference in lint % (Table 4.3). All sites except for ACRI and F6 showed a decline in leaf dry weight between 4 NAWF and maturity, at Cardale and Keytah the leaf dry weight declined by a half over this time. The highest yielding sites (ACRI, Keytah and A3) had a higher stem dry weight at maturity (P = 0.001) than the lower yielding sites.

There was significant variation between the proportional allocation of biomass and nutrients, particularly to the fruit as the plants developed (Table 4.5). The lowest yielding site had the highest proportional allocation to the fruit at maturity (Cardale) while the highest yielding sites (ACRI, A3 and Keytah) had a lower allocation of biomass to the fruit. The proportional allocation of N, P and K was higher than the allocation of biomass to fruit.

The peak rate of biomass accumulation occurred between flowering and 4 NAWF, with the total biomass accumulation ranging from 16.19 g dry matter m^{-2} day^{-1} at F6 to 33.34 g dry matter m^{-2} day^{-1} at Keytah (derived from Figure 4.2). During this period most accumulation occurred in the fruit at all sites (Figure 4.2c), while the leaf and stem accumulation rate slowed or declined, except the stem accumulation rate at A3 and ACRI. The highest fruit growth rates were measured at sites with the lowest fruit dry weight at 4 NAWF (A3, Cardale and B3), which accumulated 9.99, 9.34 and 9.74 g dry weight m^{-2} day^{-1} respectively.

Table 4.5 The proportional accumulation (%) of biomass, N, P and K at flowering, 4 NAWF, and maturity for the six sites, and the mean of the pooled data from all sites.

		Biomass			N			P			K		
Site		**Fl.**	**4 NAWF**	**Mat.**	**Fl.**	**4 NAWF**	**Mat.**	**Fl.**	**4 NAWF**	**Mat.**	**Fl.**	**4 NAWF**	**Mat.**
ACRI	Leaf	30.0	21.0	15.3	47.5	37.3	22.4	31.3	20.7	14.7	26.7	19.8	14.5
	Stem	35.3	23.8	26.7	18.5	10.9	12.7	18.9	9.3	14.9	44.8	28.3	19.1
	Fruit	34.7	55.2	58.0	34.0	51.7	64.9	49.9	70.1	70.4	28.5	51.8	66.4
Cardale	Leaf	83.56	31.14	12.56	85.40	50.10	17.17	69.66	35.42	7.33	76.09	26.58	6.06
	Stem		32.49	23.21		15.65	10.52		16.68	8.32		31.48	9.27
	Fruit	16.44	36.37	64.23	14.60	34.25	72.31	30.34	47.90	84.35	23.91	41.94	84.67
Keytah	Leaf	43.67	21.77	11.65	56.96	38.44	18.93	33.84	23.07	10.92	35.26	18.57	8.56
	Stem		33.54	27.53		12.75	12.90		13.29	12.77		30.11	16.27
	Fruit	56.33	44.69	60.82	43.04	48.81	68.18	66.16	63.64	76.31	64.74	51.32	75.18
F6	Leaf	50.87	21.79	15.48	68.14	41.08	21.79	57.02	17.92	12.59	34.13	21.04	9.36
	Stem	44.60	26.68	23.04	26.54	10.15	9.74	35.47	21.08	10.57	62.21	27.03	14.89
	Fruit	4.53	51.54	61.48	5.32	48.77	68.47	7.52	61.00	76.84	3.66	51.93	75.75
B3	Leaf	44.73	27.54	14.52	60.51	48.56	25.75	50.25	27.04	17.48	30.28	21.42	12.87
	Stem	48.41	30.43	26.00	30.24	11.85	13.65	35.07	10.05	16.76	63.29	30.25	20.39
	Fruit	6.86	42.04	59.48	9.26	39.59	60.60	14.67	62.92	65.77	6.44	48.34	66.74
A3	Leaf	41.90	31.93	13.78	62.36	57.07	21.43	53.56	30.43	11.58	30.06	16.41	7.02
	Stem	51.28	44.29	30.92	28.73	17.46	10.47	31.56	15.93	8.71	62.14	34.79	17.75
	Fruit	6.83	23.78	55.30	8.91	25.47	68.10	14.88	53.64	79.72	7.81	48.80	75.23
MEAN	Leaf	49.12	25.85	13.88	63.47	45.43	21.25	49.27	25.76	12.42	38.75	20.64	9.73
	Stem	44.89	31.88	26.24	26.01	13.13	11.67	30.24	14.38	12.01	58.10	30.33	16.27
	Fruit	20.95	42.27	59.88	19.19	41.44	67.09	30.57	59.86	75.56	22.52	49.03	73.99

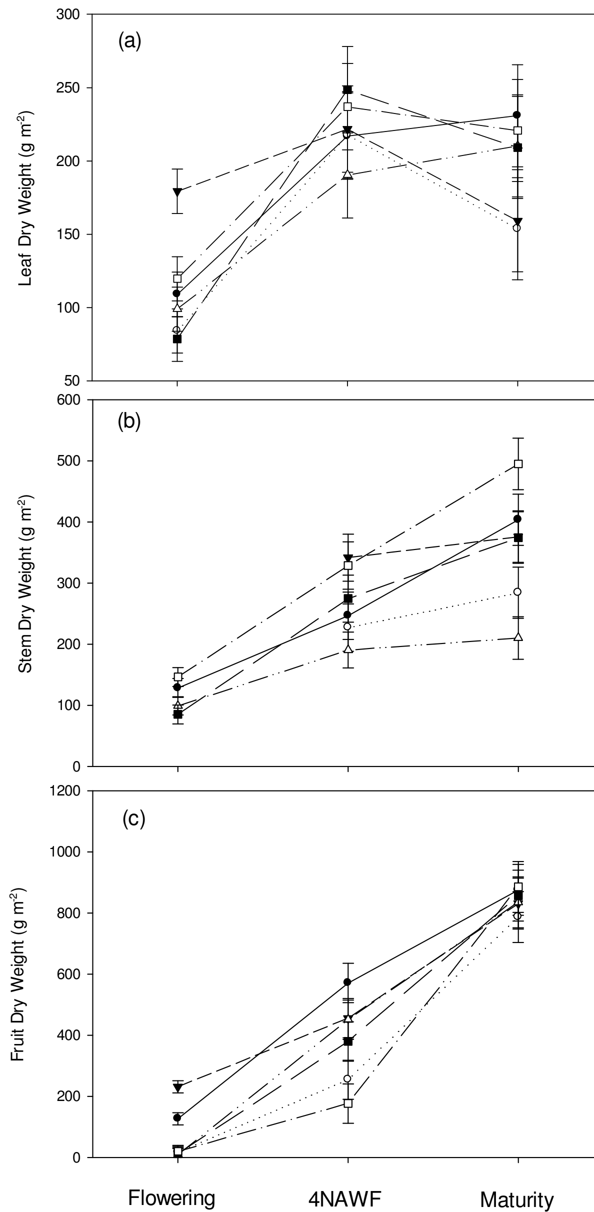

Figure 4.2 Leaf (a), stem (b) and fruit (c) dry matter at ACRI (—●—), Cardale (···○···), Keytah (— ▼ —), F6 (···△—··), B3 (— ■ —) and A3 (··—□—·—) at flowering, 4 NAWF and maturity.

4.3.4.2 N content and concentration

The N content of the leaf, stem and fruit tissues (Figure 4.3) was similar to the dry weight of each tissue, except that the stem and leaf N content declined to a larger extent than the dry weight between 4 NAWF and maturity. The N content of the fruit increased from flowering

to 4 NAWF and again from 4 NAWF to maturity at all sites ($P < 0.001$), and the N content of the leaves increased until 4 NAWF then decreased until maturity.

The N concentration of the leaves, stems and fruit followed the reverse trend to that of the N content. At sites with a high tissue dry weight the N concentration in each of the three tissue groups was lowest, and in those with a low dry weight the N concentration was the highest. This trend occurred at all three growth stages, in each tissue except for in the stems at 4 NAWF where there was no difference in the N concentration ($P = 0.093$) and in the fruit at maturity ($P = 0.125$) (Figure 4.3).

The decline in the N content and concentration of the leaves and stems was higher than the decline in the biomass between 4 NAWF and maturity at all sites, indicating that the export of N was occurring at this time.

There was some variability in the rate of increase in the N content of the fruit between flowering and 4 NAWF, and 4 NAWF and maturity between the six sites. The fruit at Cardale and A3 had the lowest rate of fruit N accumulation before 4 NAWF, and then the highest rate after 4 NAWF. The other four sites maintained a similar rate of N accumulation from flowering until maturity (Figure 4.3e).

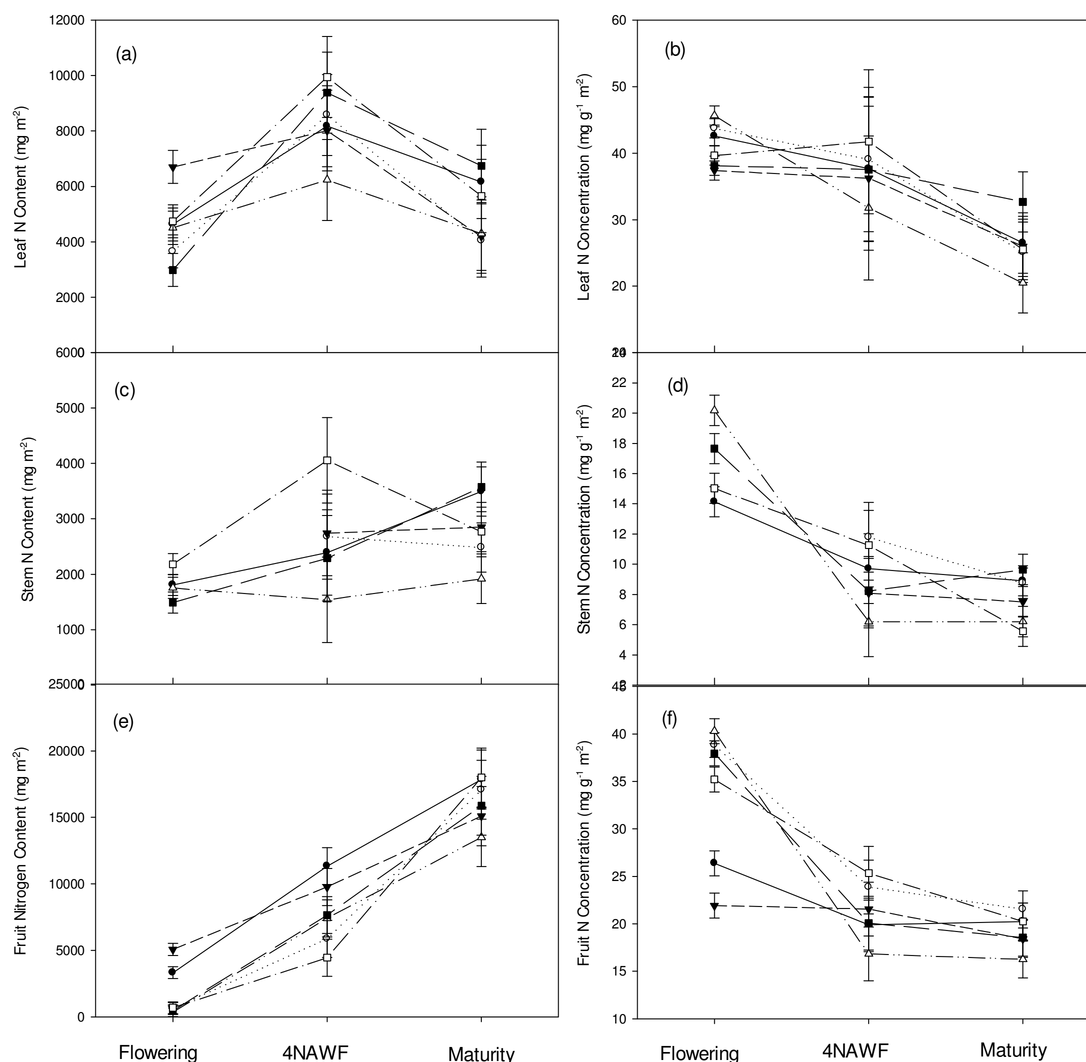

Figure 4.3 Leaf (a and b), stem (c and d) and fruit (e and f) N content (mg m^{-2}) (a, c and e) and concentration (mg g^{-1} m^{-2}) (b, d and f) at flowering, 4 NAWF and maturity at ACRI (—●—), Cardale (····○····), Keytah (— ▼ —), F6 (··· △ ···), B3 (— ■ —) and A3 (··—□—·—). Error bars represent the LSD at *P* = 0.05.

4.3.4.3 P content and concentration

Broadly the pattern of P accumulation and partitioning followed that of N uptake and partitioning, especially in the leaf and stem tissues (Figure 4.4).

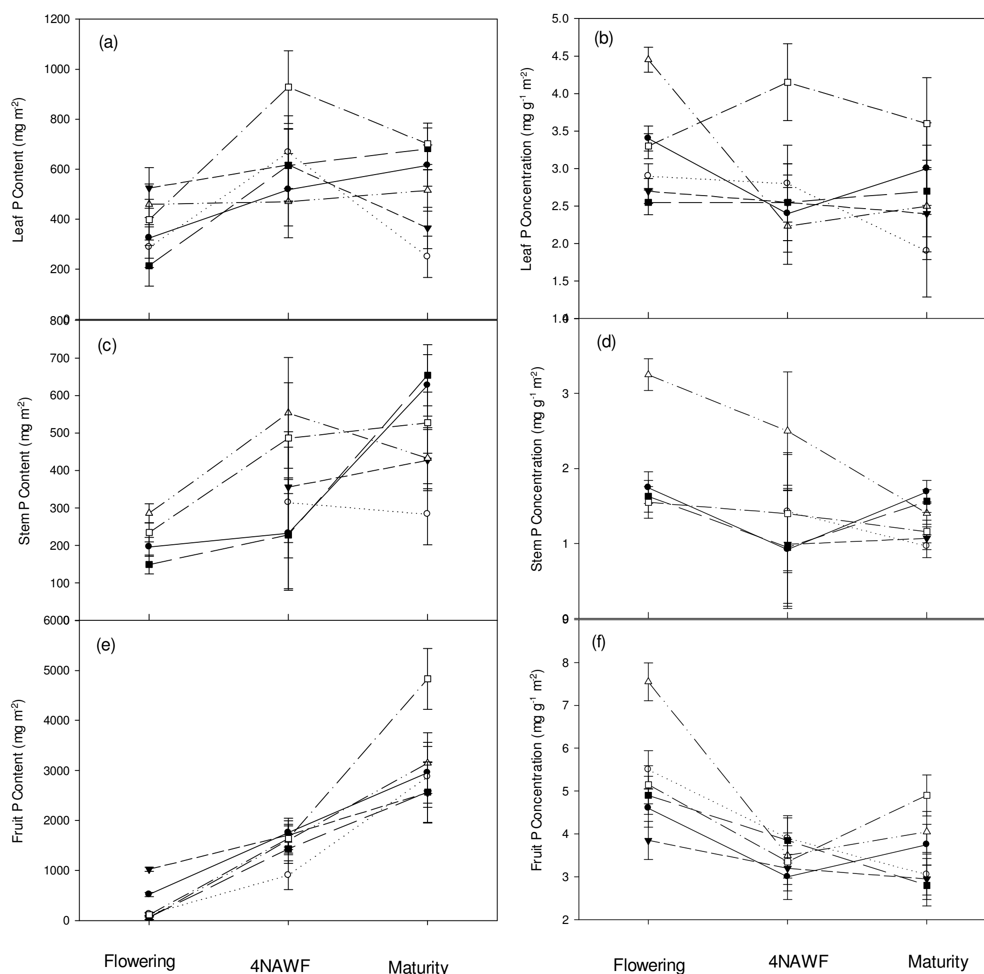

Figure 4.4 Leaf (a and b), stem (c and d) and fruit (e and f) P content (mg m^{-2}) and concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity at ACRI (——●——), Cardale (····○·····), Keytah (— —▼— —), F6 (···—△·—··), B3 (— —■ —) and A3 (··—□—·—).Error bars represent the LSD at *P* = 0.05.

Plants with a higher P content at flowering (Keytah and ACRI, P <0.001) did not maintain a higher P content until maturity. The difference in P concentration at flowering corresponded to the differences in leaf and stem concentrations, with F6 showing a much higher stem concentration (P = 0.003), and A3 maintaining a higher leaf P concentration than all other sites (P < 0.05). At maturity the fruit at A3 had a higher content and concentration than at other sites (Figure 4.4f), indicating that the extra P taken up by this crop did not equate to more fruit, but to fruit with more P in the tissue than at other sites.

There was no clear pattern linking the content and concentration of P in any tissue. The leaves at F6 had the highest P content and concentration (4.45 mg g^{-1} m^{-2}, much higher than

the next highest concentration of 3.4 mg g^{-1} m^{-2}). The crop at Keytah had a high content, but lower P concentration in the leaves at flowering. At maturity crops with a high P content had a higher P concentration that those with a low content ($P = 0.018$). There was no difference in the concentration of P in the leaves at maturity ($P = 0.171$). This indicates that larger plants distributed roughly the same amount of P to each leaf as smaller plants.

As with N, the P content and concentration of vegetative plant parts (leaves and stems) remained constant or declined from 4 NAWF to maturity. The only exception to this was the stems at B3 and ACRI, where the P content increased three fold (from around 230 mg m^{-2} to around 650 mg m^{-2}). At half the sites the P content of the leaf tissue declined by half to two thirds, while at the other three sites, the content remained fairly constant, even though the leaf biomass declined in this period (Figure 4.2).

As with dry matter and N, the peak daily uptake rate of P occurred between flowering and 4 NAWF. Some sites showed a higher rate of P export from the leaves after 4 NAWF than others, Cardale, Keytah and A3 showing the highest proportional export. The import of P into the fruit remained at a similar rate from flowering to maturity in all sites, except ACRI, Keytah and A3 where the rate decreased by up to 50%, indicating that P import preceded dry matter accumulation.

4.3.4.4 K content and concentration

The pattern of K uptake and the changes in tissue K concentration over the growing season varied from the similar pattern observed for N and P. The decline in leaf K content was far more variable than the decline in N or P content in leaves from 4 NAWF to maturity (Figure 4.5a). The decline in leaf K ranged from 120 mg m^{-2} at B3 to 1588 mg m^{-2} at Cardale, equating to 3.5% and 56.5% of the leaf K at 4 NAWF respectively. The concentration of K in the leaf tissue also varied between sites (Figure 4.5b). ACRI maintained a higher leaf K concentration at all growth stages, ($P < 0.05$). F6 had a much steeper decline in leaf K concentration than the other sites ($P < 0.001$), most likely related to the increase in leaf dry weight over this period (Figure 4.2a) while the total content was declining (Figure 4.5a).

The decline in stem K between 4 NAWF and maturity was more consistent that for N or P (Figure 4.5c), at the same time the dry weight increased indicating that there was a net export of K from the stems.

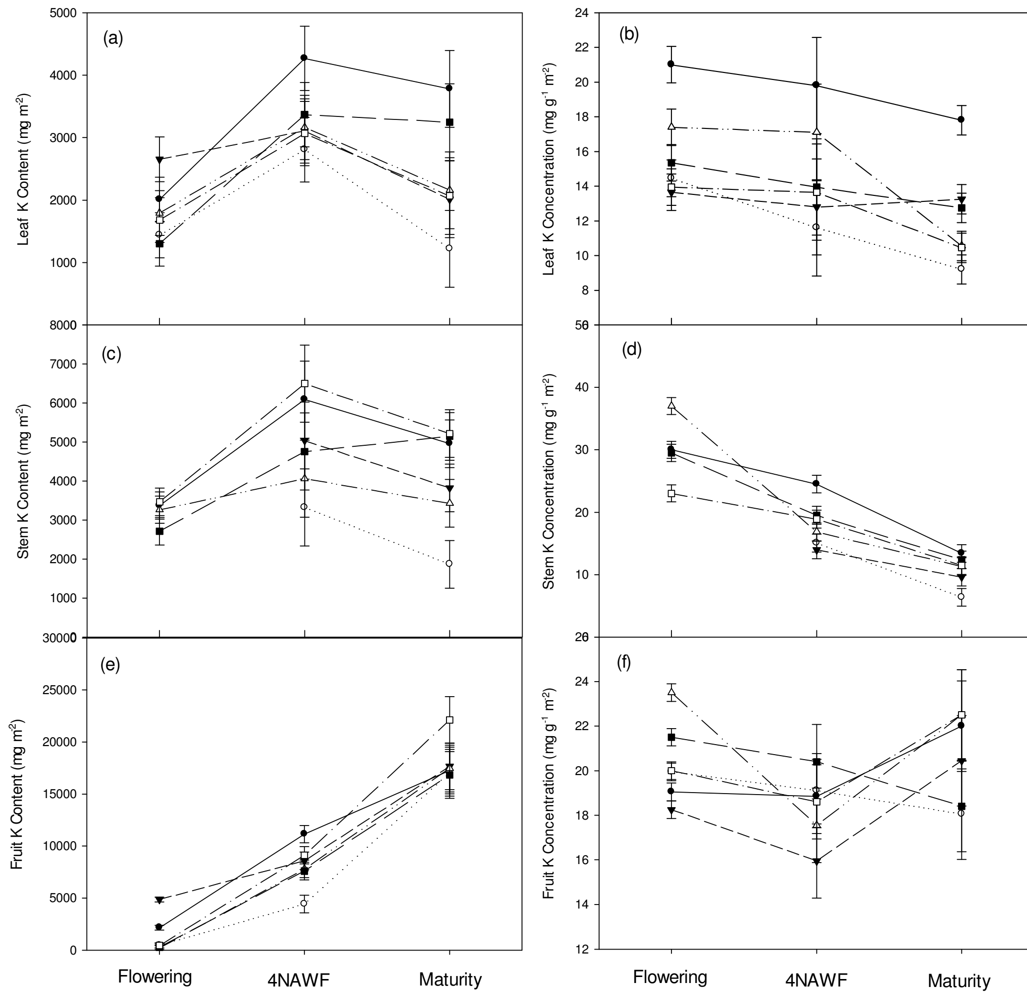

Figure 4.5 Leaf (a and b), stem (c and d) and fruit (e and f) K content (mg m^{-2}) and concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity at ACRI ( ), Cardale ( ), Keytah ( ), F6 ( ), B3 ( ) and A3 ( ).Error bars represent the LSD at *P* = 0.05.

Fruit K content increased at all sites throughout the growing season, and there was no difference in fruit K content at maturity (*P* = 0.132). There were different rates of uptake between flowering and 4 NAWF and between 4 NAWF and maturity, the crop at Cardale accumulating 12611 mg K m^{-2} after 4 NAWF, equating to 74% of the total fruit K. The crop at ACRI however accumulated only 6123 mg K m^{-2} after 4 NAWF, which equated to only 35% of the total fruit K.

Only crops at B3 and Cardale showed a decline in fruit K concentration between 4 NAWF and maturity, while the other four showed an increase by between 3.15 mg g^{-1} m^{2} at ACRI to 4.95 mg g^{-1} m^{-2} at F6. This increase in concentration, during a time of rapid growth (Figure 4.2c) indicates that the import of K exceeded the fruit growth. The decline in fruit K concentration at B3 and Cardale indicates that import may not have been as fast, due to either a deficiency in supply or a limitation in the transport of K from the roots or leaves.

4.3.5 Nutrient redistribution

Three methods for estimating N, P and K remobilisation were used.

Firstly, assuming that the leaves were the primary source of mobile nutrients prior to remobilisation and translocation to developing sinks, the decline in leaf nutrient content between the peak content and maturity was calculated (Figure 4.3, Figure 4.4 and Figure 4.5). The amount leaf nutrient decline is given in Table 4.6.

Secondly, the decline in total vegetative biomass nutrient decline between peak nutrient content and maturity was calculated, by the addition of the leaf and stem nutrient contents. The amount of vegetative nutrient decline is given in Table 4.6.

Thirdly, the method successfully used by Bange and Milroy (2004) to estimate the point at which carbon assimilate production was equal to the fruit demand was used to calculate the total nutrient accumulation rate, the fruit nutrient accumulation rate, and to estimate redistributed nutrients as a fraction of the fruit accumulation. Logistic curves were fitted to the total N, P and K uptake data and to the fruit N, P and K accumulation data (shown in Figure 4.3, Figure 4.4 and Figure 4.5). The derivative of these curves was calculated to give the daily uptake rate of each nutrient for N (Figure 4.6), P (Figure 4.7), and K (Figure 4.8). The area between the fruit accumulation curve and the total uptake curve after the point at which total uptake rate = fruit uptake rate was calculated until maturity to estimate the total amount of N, P or K supplied by redistribution (Table 4.6).

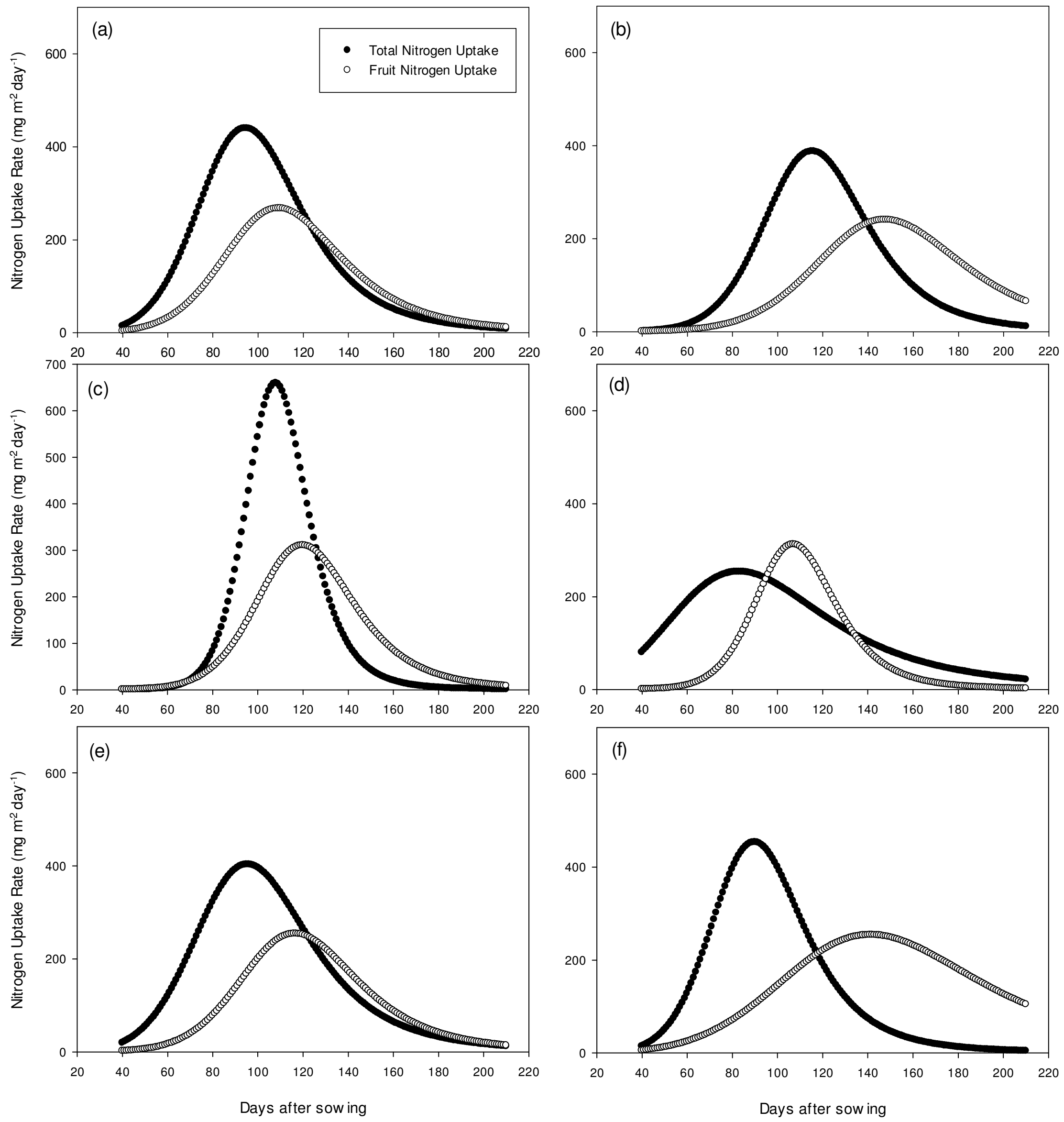

Figure 4.6 Derived N accumulation curves for total N accumulation (mg m^{-2}) and fruit N accumulation (mg m^{-2}) for control plots at (a) ACRI 07-08, (b) Cardale 07-08, (c) Keytah 07-08, (d) F6 08-09, (e) B3 08-09 and (f) A3 08-09

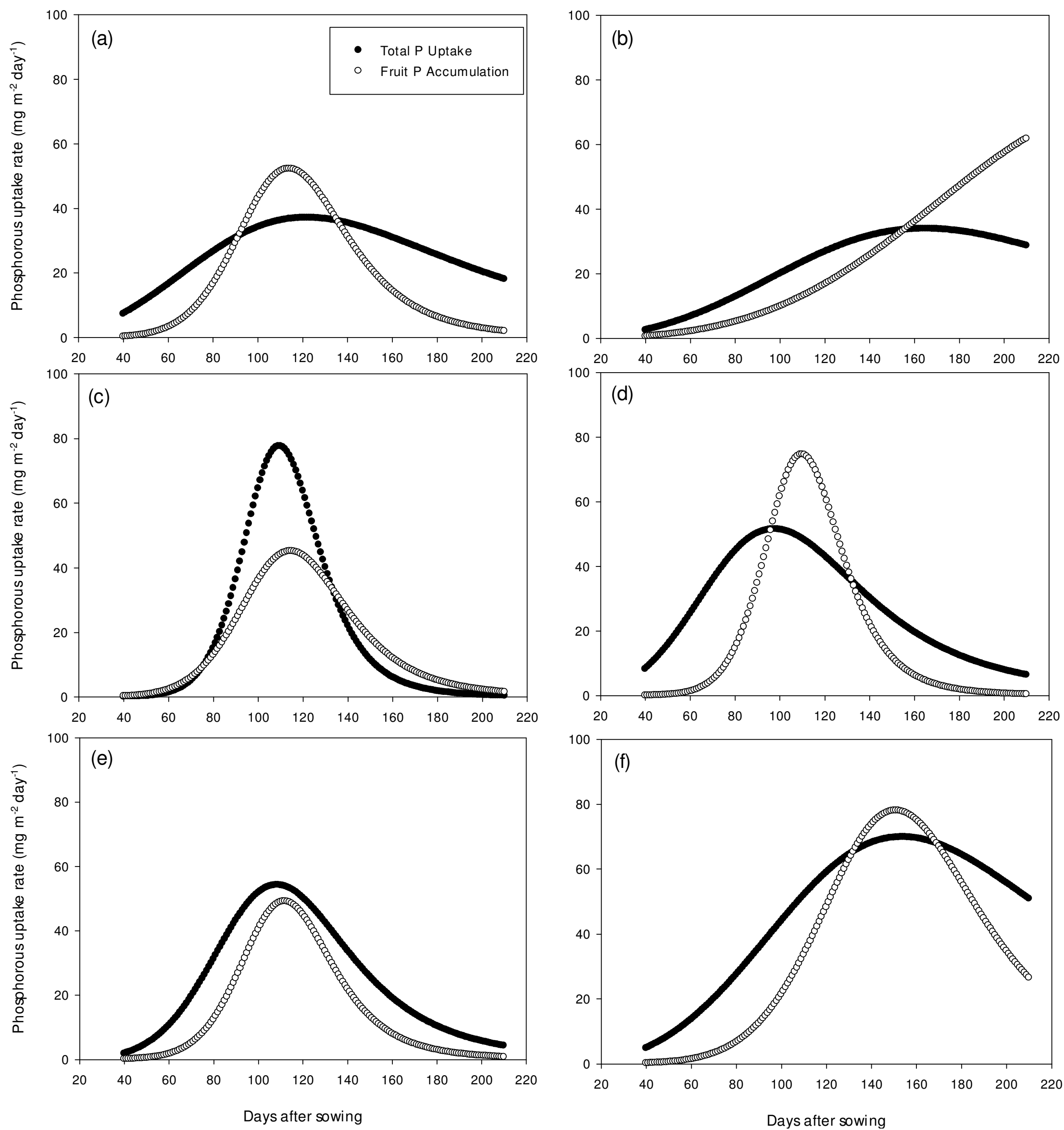

Figure 4.7 Derived P accumulation curves for total P accumulation (mg m^{-2}) and fruit P accumulation (mg m^{-2}) for control plots at (a) ACRI 07-08, (b) Cardale 07-08, (c) Keytah 07-08, (d) F6 08-09, (e) B3 08-09 and (f) A3 08-09

The crop at Cardale continued to accumulate P at a high rate in the fruit until maturity, resulting in the sigmoidal fruit P accumulation curve not reaching a peak, despite an R^2 of 0.95. This lead to the difference in the shape of the derivative curve given in Figure 4.7b.

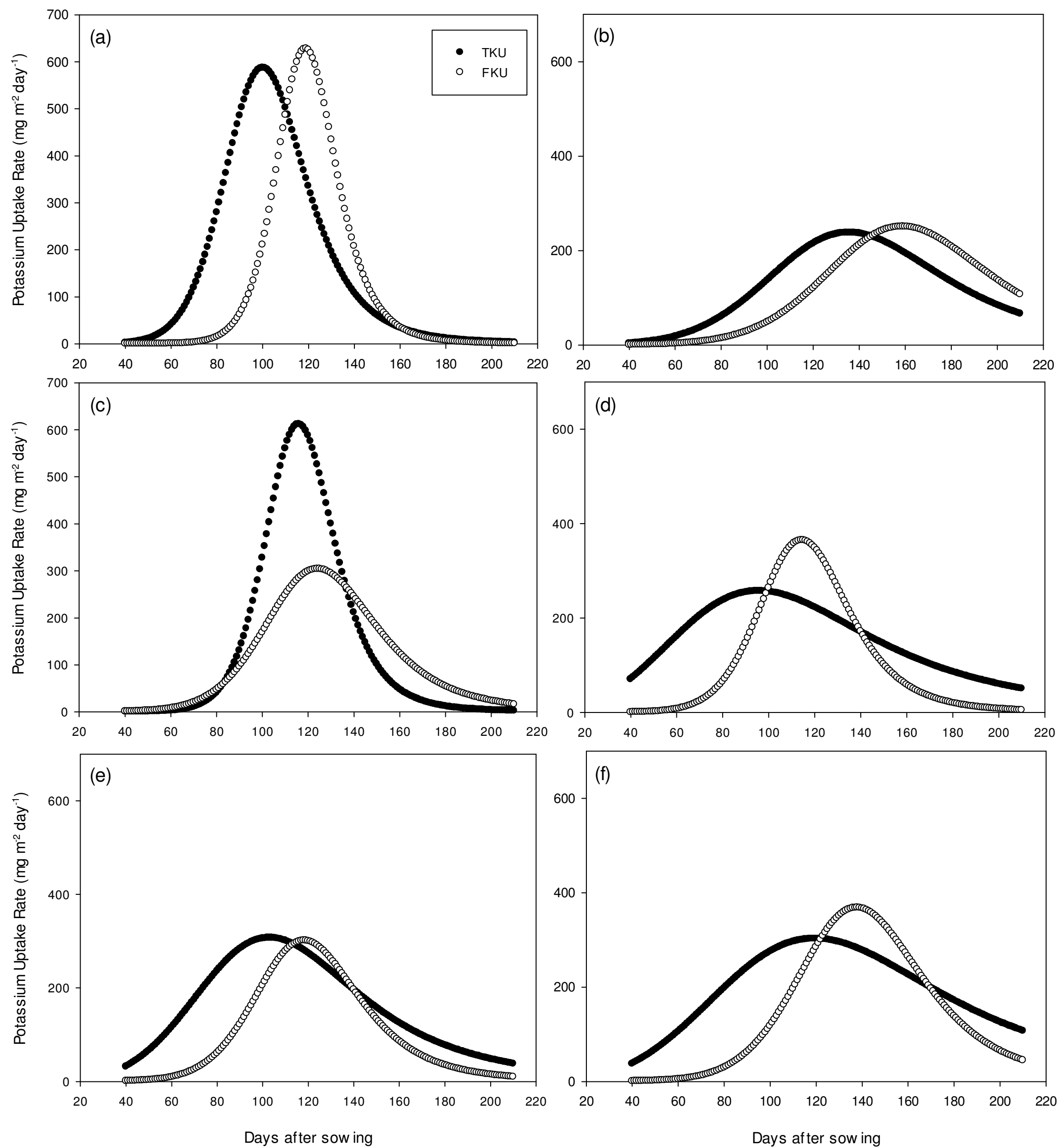

Figure 4.8 Derived K accumulation curves for total K accumulation (mg m^{-2}) and fruit K accumulation (mg m^{-2}) for control plots at (a) ACRI 07-08, (b) Cardale 07-08, (c) Keytah 07-08, (d) F6 08-09, (e) B3 08-09 and (f) A3 08-09

A comparison of the results calculated using the three methods described to calculate redistribution of N, P and K is given in Table 4.6. For reasons presented in the discussion, method 3 was chosen as the most accurate estimation of redistribution of N, P and K, and was used in the remainder of this chapter to compare redistribution between sites.

Table 4.6 Estimation of redistributed N, P and K by three methods, leaf nutrient decline, vegetative nutrient decline and the comparison of total nutrient accumulation and fruit nutrient accumulation

	ACRI	Cardale	Keytah	F6	B3	A3
	N (mg m^{-2})					
Leaf content decline	2012	4529	3829	1941	2640	5301
Vegetative content decline	912	4727	3721	1563	1355	5679
Derived measurement of fruit accumulation rate vs total accumulation rate	1120	5113	3454	2078	1267	9291
	P (mg m^{-2})					
Leaf content decline	0	418	253	128	0	227
Vegetative content decline	0	449	181	126	0	186
Derived measurement of fruit uptake rate vs total uptake rate.	0	340	207	365	0	184
	K (mg m^{-2})					
Leaf content decline	485	1588	1414	1005	120	1001
Vegetative content decline	1617	3047	3061	2567	0	2282
Derived measurement of fruit uptake rate vs total uptake rate	6786	2607	2342	2745	0	2559

4.3.5.1 N redistribution

Using the results from method 3, the proportion of fruit N supplied by redistribution was calculated (Table 4.7), which showed a considerable range.

Table 4.7 Mean amount of redistributed N (mg N m^{-2}) and the proportion of fruit N supplied by redistribution at the six different sites. Means followed by the same letter are not significantly different at *P* < 0.05.

	ACRI	Cardale	Keytah	F6	B3	A3	
Redistributed N (mg m^{-2})	1120[a]	5113[d]	3454[c]	2078[b]	1267[a]	9291[e]	P < 0.001 L.S.D. = 848
Proportion of fruit N from redistribution (%)	6.3	30.0	22.9	15.4	8.0	51.7	

Table 4.8 shows a summary of the correlation coefficients between redistributed N (mg m^{-2}) and other plant growth, N uptake and N partitioning measurements. There were only three significant relationships, shown as bold numbers, between the ratio of fruit N: vegetative N at cutout, fruit dry weight: vegetative dry weight at cutout and to the fruit dry weight at 4 NAWF. A low fruit dry weight at 4 NAWF (and therefore a rapid increase in fruit dry weight until maturity) was correlated with high rates of redistribution. Similarly a low R:V ratio at 4 NAWF was correlated with high redistribution. The proportional allocation of biomass to fruit at maturity was not related to redistribution.

Table 4.8 Correlation *P* values between redistributed N (mg m^{-2}), other growth parameters and N uptake data. A significant correlation (P < 0.05) is highlighted in bold text.

Parameter	P value of correlation with Redistributed N (mg m^{-2})
Number of bolls m^{-2}	0.52
Average boll weight (g)	0.66
Yield	0.74
Fruit dry weight at 4 NAWF (g m^{-2})	**0.03**
Fruit dry weight at maturity (g m^{-2})	0.85
Plant dry weight at 4 NAWF (g m^{-2})	0.12
Plant dry weight at maturity (g m^{-2})	0.5
Ratio of fruit N : total plant N at 4 NAWF	**0.03**
Ratio of fruit N : total plant N at maturity	0.29
Ratio of Fruit dry weight : total plant dry weight at 4 NAWF	**0.01**
Ratio of Fruit dry weight : total plant dry weight at maturity	0.44
Total N uptake	0.81
N uptake at 4 NAWF	0.48

4.3.5.2 P redistribution

Using the results from method 3, the proportion of fruit P supplied by redistribution was calculated (Table 4.9), which showed a smaller range than the redistribution of N. There was substantial variation in the gross amount of P redistributed and in the proportion of fruit P supplied by redistribution at the different sites.

Table 4.9 Mean amount of redistributed P (mg P m^{-2}) and the proportion of fruit P supplied by redistribution at the six different sites. Means followed by the same letter are not significantly different at *P* < 0.05

	ACRI	Cardale	Keytah	F6	B3	A3	
Redistributed P (mg m^{-2})	0[a]	340[c]	207[b]	365[c]	0[a]	184[b]	$P < 0.05$ L.S.D = 45
Proportion of fruit P from redistribution	0	17.7	8.1	11.6	0	3.8	

There were no significant correlations between P redistribution and yield, dry weight or P accumulation patterns (Table 4.10).

Table 4.10 Correlation *P* values between redistributed P (mg m^{-2}), other growth parameters and P uptake data. A significant correlation ($P < 0.05$) is highlighted in bold text.

Parameter	Correlation coefficient with Redistributed P (mg m^{-2})
Number of bolls m^{-2}	0.57
Average boll weight (g)	0.66
Yield	0.17
Fruit dry weight at 4 NAWF (g m^{-2})	0.50
Fruit dry weight at maturity (g m^{-2})	0.14
Plant dry weight at 4 NAWF (g m^{-2})	0.27
Plant dry weight at maturity (g m^{-2})	0.24
Ratio of fruit P : total plant P at 4 NAWF	0.13
Ratio of fruit P : total plant P at maturity	0.15
Ratio of Fruit dry weight : total plant dry weight at 4 NAWF	0.26
Ratio of Fruit dry weight : total plant dry weight at maturity	0.72
Total P uptake	0.91
P uptake at 4 NAWF	0.88

4.3.5.3 K redistribution

Using the results from method 3, the proportion of fruit K supplied by redistribution was calculated (Table 4.11), which showed a considerable range, and did not occur in the same crops as redistributed large amounts in N and P.

Table 4.11 Mean amount of redistributed K (mg m^{-2}) and the proportion of fruit K supplied by redistribution at the six different sites. Means followed by the same letter are not significantly different at *P* < 0.05.

	ACRI	Cardale	Keytah	F6	B3	A3	
Redistributed K (mg m^{-2})	6786^{c}	2607^{b}	2342^{b}	2745^{b}	0^{a}	2559^{b}	P < 0.05 L.S.D = 1806
Proportion of fruit K from redistribution	39.3	15.3	13.3	15.7	0	11.6	

As shown in Table 4.12, there was no correlation between yield, boll number, boll size, plant dry weight or fruit dry weight and K redistribution.

Table 4.12 Correlation *P* values between redistributed K (mg m^{-2}), other growth parameters and K uptake data. A significant correlation (P < 0.05) is highlighted in bold text.

Parameter	Correlation coefficient with Redistributed K (mg m^{-2})
Number of bolls m^{-2}	0.62
Average boll weight (g)	0.65
Yield	0.64
Fruit dry weight at 4 NAWF (g m^{-2})	0.31
Fruit dry weight at maturity (g m^{-2})	0.63
Plant dry weight at 4 NAWF (g m^{-2})	0.47
Plant dry weight at maturity (g m^{-2})	0.70
Ratio of fruit K : total plant K at 4 NAWF	0.73
Ratio of fruit K : total plant K at maturity	0.08
Ratio of Fruit dry weight : total plant dry weight at 4 NAWF	0.63
Ratio of Fruit dry weight : total plant dry weight at maturity	0.37
Total K uptake	0.88
K uptake at 4 NAWF	0.21

4.3.5.4 Relationship between N, P and K redistribution

There was no relationship between the redistribution of N, P and K, or between the proportion of the mature fruit N, P or K supplied by redistribution (Table 4.13). The

redistribution of each occurred independently, and plants redistributing a large amount of one nutrient were not more likely to redistribute a large amount of either of the other two.

Table 4.13 Correlation *P* values between redistributed K (mg m^{-2}), other growth parameters and P uptake data

		1	2	3	4	5	6
Proportion of Fruit K supplied by redistribution	**1**	-					
Proportion of Fruit N supplied by redistribution	**2**	0.62	-				
Proportion of Fruit P supplied by redistribution	**3**	0.84	0.58	-			
K redistribution (mg m^{-2})	**4**	**<0.001**	0.74	0.80	-		
N redistribution (mg m^{-2})	**5**	0.66	**<0.001**	0.67	0.78	-	
P redistribution (mg m^{-2})	**6**	0.77	0.42	**0.009**	0.77	0.51	-
		1	2	3	4	5	6

4.4 Discussion

The six crops examined in this study can be regarded as "high-yielding" cotton crops, both when compared to those described in previous nutrient uptake and partitioning studies (between 747 – 1700 kg ha^{-1}), and to the Australian average yield (2040 kg ha^{-1}) (Table 4.3). As well as being higher yielding than the benchmark studies from pre-1945 (Olson and Bledsoe, 1943), and from the 1970s and 80s (Bassett *et al.* 1970; Halevy 1976; Halevy *et al.* 1987), the cotton crops studied were larger plants with a higher nutrient uptake than those previously studied.

4.4.1 Total biomass and nutrient uptake

A frequently reported trend in biomass and nutrient accumulation is that modern cultivars grown in high input systems with good pest control accumulate more biomass and nutrients after flowering than the older parent varieties (Mullins and Burmester 2010). In early, pre-1945 studies 12.2% of dry matter uptake occurred before squaring, 28.8% before first flower and 48.5% before the first open boll. Bassett *et al.* (1970) recorded 2 – 4% of total dry matter uptake prior to squaring, and only 7 – 10% at first flower. Similarly low values were recorded by Mullins and Burmester (1990) and Halevy (1976) indicating that modern cultivars accumulated far more dry matter and nutrients after flowering than the older varieties. This was attributed to varietal improvements, being less determinate and with a prolonged boll setting period, and to better management, insect control and irrigation practices. This

assumption was supported by previous research, Meredith and Wells (1989) demonstrated that modern cultivars partition more of their dry matter (and nutrients) into reproductive tissue. Similarly Oosterhuis *et al.* (1983) demonstrated that one modern cultivar took up a higher proportion of accumulated nutrients after flowering than their older parent varieties; reporting 40% of total N, and 60% of total biomass was taken up between 10 and 16weeks after sowing.

Examining the mean of the six crops examined in this analysis, the crops took up proportionally 17.8% of biomass, 31.3% of N, 21.4% of P and 32.8% of total K pre-flowering, at much higher proportions than those previously reported, and not following the trend of a lower pre-flowering biomass and nutrient accumulation in high-yielding modern cultivars.

There was no clear pattern of dry matter and nutrient accumulation for Sicot71BRF, with a large range of biomass (8-30%), N (18 – 53%), P (10 – 46%) and K (9 – 32%) accumulation pre-flowering. There is some indication that this pre-flowering uptake of nutrients is related to supporting high yields. There were strong correlations between N, P, K and biomass accumulation pre-flowering and yield. Since the uptake of N, P and K were related to biomass accumulation; a simpler explanation is that bigger plants at flowering supported more bolls and therefore produced higher yields. The size and nutrient status of the cotton plants pre-flowering may, therefore, be a more significant factor in the production of very high-yielding crops than previously reported. Even within these six crops analysed, the larger crops were the highest yielding (Table 4.3 and Table 4.4).

The rate of nutrient uptake in the whole plants showed considerable variation throughout different phases of growth. Pre-flowering, accumulation rates were relatively low, although were higher in all crops than those reported by Halevy (1976). Halevy reported a tenfold increase in the uptake rate of N after flowering for a period of around 30 days, however in these high-yielding crops, the rate increased only by a factor of 3.9 for N, by 5.5 for P, and by 5.6 for K. This shows that these high-yielding modern cultivars had a higher uptake rate, and maintained a higher rate of nutrient uptake throughout their lifecycle than cultivars previously studied. This rapid early season uptake could be due to management factors, but could also

indicate that high-yielding cultivars had a faster rate of root growth, providing the physical means of taking up nutrients faster than older varieties.

4.4.2 Biomass and Nutrient Partitioning

In early studies of cotton biomass partitioning, the average distribution of dry matter at maturity was 25 – 41% in stems, 13 – 30% in leaves, 12 – 16% in boll walls and bracts, 19 – 25% in seed and 9 – 12% in lint (e.g. Olson and Bledsoe 1942). The accumulation of nutrients in these plant parts was found to follow a similar pattern to the dry matter uptake, with nutrient accumulation preceding dry matter production. Halevy (1976) and Bassett *et al.* (1970) reported similar results, finding the distribution of dry matter to be 23.1% in stems, 17.4% in leaves, 16.3% in bolls walls and bracts, 25.3% in seed and 17.9% in lint. In a more recent study Fritschi *et al.* (2004b) found the distribution of N to be 21% in leaves, 11.1% in stems, 8.8% in boll walls and bracts, 55.4% in seeds and 3.7% in lint. The proportion of N in the seed and lint was higher than that reported by Mullins and Burmester (1990) and Boquet and Breitenbeck (2000), but not much higher than that reported by Basset in 1970.

The average partitioning of biomass at maturity across these transgenic high-yielding crops was 13.9% in leaves (range 11.6 – 15.5%), 26.2% in stems (range 23 – 31%) and 59.9% in fruit (range 55.3 – 64.2%). These results are not widely different from those reported by Olson and Bledsoe (1942), or by Basset in 1970 and Halevy in 1976, although it the proportion of biomass in leaves at maturity is at the lowest end of the reported range in these studies (13 – 30%), and the proportion of biomass in the fruit is higher than the reported range (40 – 53% in 1945, and 59.5% in 1976). The partitioning of biomass in these crops supports the findings of Meredith and Wells (1989) that, at least in the case of Sicot71BRF, this trend has continued.

The efficiency of biomass production and nutrient use in crops is often measured as a harvest index, or as a ratio of reproductive biomass or nutrients to the rest of the plant. Within the six crops analysed, there was a difference in the reproductive: vegetative ratio of tissue at 4 NAWF and at maturity (calculated from Figure 4.2). It is interesting to note that despite there being no difference in the fruit dry weight between sites, some crops developed the fruit load on a smaller plant, with less vegetative growth. The three highest yielding crops at ACRI, A3 and Keytah had the lowest R:V ratio at maturity (1.37, 1.26 and 1.5 respectively), while the lower yielding crops had a higher R:V, the highest being at Cardale (1.84), the site with a

lower yield ($P<0.001$). Traditionally crop production and nutrient use efficiency has been measured in terms of the R:V ratio, or harvest index of the crop at maturity, and so the Cardale crop, while being the lowest yielding would be classed as the most efficient. This raises the question of if further efficiency gains could be made, and if very high-yielding crops are reaching their maximum potential yield.

The higher yielding sites also maintained the vegetative growth rate and nutrient uptake rate longer than the lower yielding site, contributing to the lower R:V ratio at maturity. This indicates that, as well as having faster early season root growth and development, very high-yielding cultivars may maintain root growth and uptake throughout the season, and did not reach the point of traditional cutout where vegetative growth and root uptake declines. The consequences of this for nutrient allocation and redistribution should be investigated further.

Since then, the crops described in this chapter are higher yielding than those previously described, and partition more biomass to reproductive structures, the question of whether this shift in biomass allocation has impacted nutrient partitioning, and nutrient redistribution from leaves to bolls can be raised.

4.4.3 Quantifying redistribution

In this analysis, three methods for estimating redistribution were carried out. Firstly, to calculate the decline in leaf nutrient content between its peak and maturity, secondly to calculate the decline in vegetative nutrient content between its peak and maturity, and thirdly to plot daily total plant nutrient accumulation and daily fruit nutrient accumulation, and to calculate the amount of fruit nutrient accumulation that exceeded plant uptake. Each method had its own limitations and advantages.

Many studies referring to, or estimating redistribution of nutrients from vegetative to reproductive plants use the first method carried out in this chapter to quantify redistribution. There are several limitations to this method, firstly it does not account for leaf nutrient loss through shedding of leaves, and may, therefore, over estimate redistribution at a whole plant scale. Secondly, it does not account for redistribution of nutrients stored, or cycling through the stems and petioles of developing plants, which may be more significant for some nutrients than others, for example K, which is found in higher concentrations in stems than other nutrients. Thirdly, it assumes that all exported nutrients from leaves are distributed to

bolls and not to the production of other vegetative matter, such as younger leaves, stems or roots. Despite these drawbacks, it remains one of the simplest and most widely used estimates of redistribution, and accurate for making comparisons between treatments or plants.

The second method is similar to the first, and has similar limitations in its exclusion of shed senesced material and the assumption of non-allocation to roots; however, it improves the method through the inclusion of stems and petiole nutrients in the calculation. The stem nutrient content varied considerably between the six crops studied, with increases in the content of the stems in some cases, and decreases in others after flowering. The contribution of the stems in terms of supplying nutrients to developing bolls seems to be highest at A3 and Cardale, crops which recorded the highest proportional redistribution of N and a relatively high redistribution of P and K. This method, therefore, increases the accuracy of the redistribution estimate from method 1.

The third method used overcomes some of the limitations of the first two methods, in that the rate of uptake is calculated for the whole plant, and for the fruit fraction only, encompassing the uptake of nutrients in all plant parts, and excluding any nutrients lost through senescence or shedding. However, this measurement is a derived measurement based on fitted curves, and relies therefore on the goodness of fit of the logistic curves to the uptake of nutrients. This method may not be suitable therefore, for estimating redistribution in all circumstances. In this analysis, however, it provided a more accurate estimation of redistribution than the other methods, accounting for the continued root uptake and vegetative growth in some of the highest yielding sites. For this reason, provided that total and fruit uptake follow a sigmoidal curve, method three should be used as a method to estimate redistribution in preference to the balance methods (1 and 2), and will be used in the remainder of this study.

4.4.4 N distribution and redistribution

There was no difference in the total N uptake, the leaf N content or the stem N content between the six sites at 4 NAWF. While some plants maintained a higher N content in the stems than others there was no difference in the total N uptake, leaf N content or fruit N content at maturity between the six sites. The redistribution calculations, however, highlight differences in the movement of N into the fruit, and the proportion of the total fruit N that was supplied by redistribution of plant N.

Redistributed N accounted for between 6.3 and 51.7% of total fruit N, indicating that at each site, the physiological mechanisms employed to allocate the same amount of N to developing bolls were very different. The highest yielding crop, at ACRI, redistributed the lowest amount of N (1120 mg m^{-2}), while the lowest yielding crop at Cardale redistributed almost five times this amount (5113 mg N m^{-2}). There was, however, no consistent relationship between yield, boll number, boll size, total N uptake, plant dry weight and N redistribution.

The two crops with the highest redistribution of N from 4 NAWF to maturity were the crops with the largest increase in fruit dry weight and fruit N between 4 NAWF and maturity. There was a strong correlation between N redistribution and plant size, fruit dry weight, the ratio of fruit dry weight to plant dry weight (the reproductive to vegetative ratio), and the partitioning of N at 4 NAWF, but not at maturity. These results indicate that the redistribution of N is, at least in part, driven by the rapid development of bolls. The size of the source did not seem to affect the redistribution of N (Table 4.8), indicating that efficiency of redistribution is not driven by N uptake but potentially by the rate of increase in sink demand. This confirms the hypothesis that redistribution is a supplementary mechanism of N supply during times when sink demand exceeds supply from root uptake. The lack of correlation with yield, boll number or boll size shows that in non-stressed situations, plant reliance on redistribution for N supply to developing bolls is non-limiting to yield and boll retention.

4.4.5 P distribution and redistribution

The redistribution of P in the six crops varied from 0 – 365 mg m^{-2}, equating to between 0 and 17.7% of the fruit P accumulation at maturity. At ACRI and B3 there was no evidence of P redistribution within the plants. At these two sites there was continuous uptake of P in the leaf, stem and fruit tissues throughout the growing season, with a three fold increase in the stem P content between 4 NAWF and maturity. The concentration of P in the leaf and stem tissues also increased at these two sites from 4 NAWF to maturity. While the fruit P accumulation rate exceeded the total crop uptake rate for a period, the crop P uptake rate remained high at ACRI and B3, probably reducing the need for redistribution to occur. These two crops also had the lowest rate of N redistribution, raising the possibility that P redistribution is linked to N redistribution. Since many of the storage molecules for P, and nucleic acids and membranes containing P also contain N, the redistribution of these could be linked, however without molecular analysis this theory remains speculative.

Redistribution was recorded to a lesser degree at the other four sites, with Cardale and F6 redistributing more P than Keytah and A3. The total P content of the fruit was highest at A3, with no difference between the other sites. Since the higher content in the A3 bolls did not rely on redistributed vegetative P, this indicates that redistribution was a supplementary mechanism for P supply to developing bolls, not the primary means of supporting a high boll load.

As with N there was no correlation between boll size, boll number, plant P uptake and P redistribution. There was a non-significant trend linking yield and P redistribution, with lower yielding plants redistributing more P. Similarly there was some evidence for a negative correlation between the proportion of plant P in fruit and P redistribution, with plants partitioning more P to the fruit at 4 NAWF and at maturity showing a lower redistribution. At ACRI and Keytah the proportion of plant P in the fruit was almost the same at 4 NAWF and maturity, and both crops recorded no redistribution of P, whereas all other crops showed an increase in the proportion of plant P partitioned to the fruit (by 36.5% at Cardale, 13.7% at Keytah, 15.84% at F6 and 26.08% at A3) and subsequently relied on redistribution in part to supply the extra P needed.

4.4.6 K distribution and redistribution

Similar redistribution of K was measured at four of the six sites (Cardale, Keytah, F6 and A3), accounting for between 11.6 and 15.7 of the total fruit K. At B3 no redistribution of K was recorded, as crop K uptake continued and fruit K accumulation declined before the end of the season. At ACRI redistribution was around three times as high as at the other sites, accounting for 39.3% of the total fruit K. At ACRI the increase in fruit K between 4 NAWF and maturity was lower than at all other sites, indicating that sink pressure did not play a major role in driving the movement of K from the source to the developing sink (bolls). Instead this extra redistribution could be attributed to the fact that the crop at ACRI took up only 2082 mg K m^{-2} after 4 NAWF, at which time it had accumulated 91% of the total K. Only 35% of the total fruit accumulation after 4 NAWF was supplied by root uptake and therefore the crop must have relied on vegetative K to supply the additional demand. The total plant accumulation rate after 4 NAWF at ACRI of 39.29 mg m^{-2} day^{-1} was less than a third of that recorded in the other crops, confirming this theory. The reason that the K uptake in this crop declined so much after 4 NAWF, while N and P uptake continued, is difficult to

establish. Soil deficiencies seem unlikely, as the crop grown in the same location the next year (F6) showed a continued higher rate of accumulation of K post 4 NAWF (190.69 mg m^{-2} day^{-1}), without the addition of K fertilisers.

4.4.7 Conclusions

This analysis showed that these crops were higher yielding than those described in previous studies of nutrient uptake and distribution, and that these high-yielding crops partition more biomass and nutrients to reproductive structures than older varieties.

Despite the six crops examined in this analysis being high-yielding and having several similarities of nutrient uptake and distribution, there was significant variation in the gross amount of N, P and K redistributed from vegetative tissue to the developing bolls, and the proportion of the boll nutrients supplied though redistribution. There are several key conclusions that can be made from this data. Firstly, it is clear that redistribution occurs in some conditions and not others, and where it does take place, to a highly variable degree. When root uptake continues past 4 NAWF, it seems that redistribution is minimal. Therefore, redistribution is a supplementary process, occurring when the plant cannot access the nutrients it requires, or when root growth is limited. Secondly, redistribution does not seem to be primarily related to the source: sink ratio in the case of P and K, although there was some correlation in the case of N. This contradicts the findings of Wright (1999) and Pettigrew *et al.* (2000) that rapid K redistribution from leaves causing premature senescence is primarily driven by an imbalance of the source and sink tissue, and suggests rather that redistribution will occur because of environmental and management conditions, or due to cultivar growth habits, or due to an interaction of these factors. This hypothesis, however, needs further investigation, particularly to establish why N redistribution seems to be related to fruit growth rate and the ratio between source and sink tissue, and not other nutrients.

As discussed in the literature review, the redistribution of nutrients is affected by many factors, several of which may have influenced these results and require further investigation and explanation.

1) The validity of measuring redistribution at a whole plant scale, when there may be significant variation in the redistribution of nutrients from leaves in the lower portion of the canopy to the upper, creating the artificial result of very little or no redistribution because different leaf ages and positions were not taken into account

2) While some very high amounts of redistribution were measured the maximum potential redistribution was not quantified, and the variation between plants makes it difficult to establish a benchmark figure representing efficient redistribution.
3) The effect of the source: sink ratio on the redistribution of nutrients showed a significant correlation in the redistribution of N (although not P or K), and should be investigated further.
4) The influence of water and nutrient management and environmental conditions was not measured. All crops were grown with nutrient and water supply technically adequate for non-stressed cotton production; however variations in climate and stresses through the season may have impacted growth and development, and the distribution and redistribution of nutrients.

CHAPTER 5

Redistribution of N, P and K along a single sympodial branch

5.1 Introduction

In the previous chapter, a method for quantifying redistribution of N, P and K from vegetative to reproductive tissue was established, and a high degree of variability in both the gross and proportional amount of these nutrients cycled from one tissue to another was measured. Even between plants of similar size, yield, boll number and vegetative biomass the amount of N, P and K redistributed was widely variable when measured at a whole plant scale. As highlighted in the conclusions of that chapter, the method did not account for the age or growth stage of the vegetative or reproductive tissue, or for differences in nutrient content and growth rate in different parts of the canopy. Importantly, the method does not account for vegetative to vegetative redistribution, nor did it define a maximum potential redistribution against which to measure efficiency. In this chapter, the redistribution of N, P and K between different tissues will be quantified within a single branch, examining nutrient transfer between tissues during the reproductive phase of growth along a single node, allowing redistribution to be calculated with more accuracy.

Understanding the nutrient accumulation, and the source of nutrients supplied to bolls, as well as the pattern of export and reallocation of leaf nutrients will help to explain and predict the nutrient requirements of bolls, and to validate the whole plant methodology for measuring nutrient redistribution. The key questions that need to be addressed to quantify the boll nutrient contents and the contribution of redistribution to the mature boll are;

1) What are the source / sources of N, P and K in a mature boll?
2) What is the potential redistribution of N, P and K from leaves?
3) What factors change the source of N, P and K to a developing boll?

While there have been many studies measuring the accumulation and partitioning of nutrients to bolls within a whole plant (e.g. Krieg and Sung 1986; Halevy and Markovitz 1988; Bondada *et al.* 1996; Jones *et al.* 1996; Boquet and Breitenbeck 2000; Pervez *et al.* 2004; Wahid *et al.* 2004; Read *et al.* 2006; Singh *et al.* 2006a), or defining the accumulation of

nutrients within bolls as they develop (Leffler and Tubertini 1976; Thompson *et al.* 1976; Leffler and Hunter 1985; Zhao and Oosterhuis 1999; Wahid *et al.* 2004), there have been relatively few defining the source of the boll nutrients, or examining the supply from individual leaves to bolls.

It is generally assumed that the main source of N, P, K and other nutrients in a developing boll is the subtending or adjacent leaf on the sympodial branch. Li *et al.* (2009) published a model predicting the biomass, oil and protein content of a cotton boll based on the subtending leaf N content, asserting that; "*The subtending leaf of the cotton boll is the main source organ for boll growth and the N concentration in the subtending leaf directly influence the cotton seed growth and development*". Wahid *et al.* (2004) directly linked the concentration of macronutrients in the bolls with that of the subtending leaf – by comparing the ratio of nutrients between the two and calculating redistribution as the change in that ratio. Many other authors have linked the surrounding leaves with developing bolls, arguing that the subtending leaf is principally involved in partitioning nutrients to the developing fruit, due to its direct vascular connections and its proximity (Hellmann *et al.* 2000; Offler *et al.* 2000; Ruan *et al.* 2000; Turgeon 2000).

Constable and Rawson (1980b) examined the carbon production and allocation dynamics from single leaves at nodes 5, 7 and 9 from the unfurling of the mainstem leaf for 70 days. In related studies they established a carbon budget for the cotton plant through the examination of the allocation and redistribution of labelled ^{14}C from a single node segment. They concluded that the leaves at a single node were incapable of supplying all of the bolls' demand during filling, and carbon imported into bolls was sourced from removed sites, *not the adjacent leaves* (Constable and Rawson 1980a; Constable and Rawson 1980c; b; 1982). Whilst this evidence that the carbon required for bolls to develop and fill is transported from leaves removed from the fruiting site, not only those adjacent to it, is generally accepted (Reekie and Bazzaz 1987; Wullschleger and Oosterhuis 1990b; Heitholt 1994; Geiger *et al.* 2000; Pline *et al.* 2003; De la Barrera and Nobel 2004), there is a lack of clear data about the source of other nutrients required for boll growth and development, particularly for P and K.

The assumption that the subtending leaf is the major source of nutrients for the adjacent developing boll seems to be widely accepted, though there is some evidence that a significant

proportion of the nutrients in mature bolls comes from other leaves (that is on different nodes, or at other positions on the same node), from remobilisation of stem nutrients or from continued root uptake (Zhu and Oosterhuis 1992). The data presented in the Chapter 4 suggests that, on balance, all the high-yielding crops studied could not have supplied the demand from the developing boll load from redistribution of vegetative nutrients alone. In their detailed study of the N partitioning and allocation of a single branch, Zhu and Oosterhuis (1992) concluded that, even assuming that all the N in the subtending leaves *could* be remobilised, the individual sympodial leaves may not have been capable of supplying all the N required for maximum development of the subtending boll, that is the content of the mature bolls was higher than the maximum content of the mature leaves. They suggest that leaves from different nodes, the mainstem leaf, or sympodial leaves further along the branch may supplement the adjacent sympodial leaf supply, to which continued root uptake could be added. Similar studies for P and K have not been carried out.

The source of boll nutrients and the importance of the subtending leaf in N, P and K supply to developing bolls need to be further defined and investigated. It is well established that carbon is imported into bolls from leaves on nodes physically removed from the sympodial branch, although the contribution of upper or lower leaf nutrients to developing bolls in unclear. A better understanding of the source of nutrients for developing bolls, and the pattern of accumulation and transport between tissues along a sympodial branch will be useful in defining both a maximum and optimum level of redistribution, and contribute to the development of models to maximise nutrient use efficiency and optimise boll development and yields.

The main aims of this chapter are to;

1) quantify the demand for nutrients from a single boll in terms of accumulation in various tissues and the timing of this demand, by examining the N, P and K accumulation in the leaves, stems, petioles, bracts, boll walls, seed and lint developed at node 11
2) quantify the redistribution of N and K from the main stem leaf and first position leaf to other plant parts through the use of ^{15}N and Rb tracers
3) establish the importance of redistribution from a subtending leaf in supplying boll N, P and K

4) establish a potential and an average standard "redistributed fraction" of boll N, P and K accumulation and leaf nutrient export for comparison with whole plant studies.

5.2 Materials and methods

Detailed descriptions of the experiments described in this chapter are given in sections 3.4.7 (experiment 7) and 3.4.8 (experiment 8). These two experiments examined the N, P and K accumulation along a sympodial branch, a summary of which is given here. Both experiments sampled a single node (node 11), as a group of tissues representative of the whole plant, and reflecting the average accumulation and growth pattern of the whole plant.

5.2.1 Experiment 8 – N, P and K partitioning along a sympodial branch

A field experiment was carried out at ACRI, Narrabri in the 2007-08, and 2008-09 cotton seasons to examine the N, P and K accumulation in the leaves, stems, petioles, bracts, boll walls, seed and lint developed at node 11. Node 11 was chosen as the sampled node, being referred to in other studies as representative of the whole plant, while node 10 has previously been referred to as a "representative node" for the whole plant (Oosterhuis and Wullschleger 1988; Zhu and Oosterhuis 1992), Thompson *et al.* (1976) found that node 11 was the most likely to retain fruit at positions one and two. As such, node 11, being likely to retain fruit, and being slightly higher in the plants, which were larger and more vigorous than those in other studies (such as Oosterhuis and Wullschleger 1988; Zhu and Oosterhuis 1992), was used as the representative node.

Plants with a white flower at position 1 were tagged on one day the 12th January, 2009. At intervals between 3 and 7 days, one whole plant with a tagged branch was sampled from each plot, giving four replicate samples of branches at each sampling date. Only branches with 2 fruit, at position 1 and position 2 on the branch were sampled. Sampling dates are given in Table 3.9. Data recorded for each plant included;

- Nodes above and below the tagged branch
- Fruit on the node above and node below the tagged branch
- Number of leaves on the branch
- Number and type (square, flower, green boll or open boll) of fruit on the tagged branch
- Number of fruiting positions on the tagged branch

- The dry weight, N, P and K concentration of the leaves, stems, petioles, and partitioned fruit (boll walls, seed, bracts and lint) (by the method described in section 3.3). The leaf, petiole, stem, boll wall, bracts, seed and lint from each position (1, 2 and 3), as well as the main stem leaf, main stem leaf petiole and mainstem node segment were ground and analysed separately.

Data was analysed using Genstat 14th edition as described in section 3.4.7.3.

5.2.2 Experiment 9 - ^{15}N and RbCl application and distribution along a sympodial branch

A similar field experiment to experiment 8 was carried out in the 2009-10 cotton season to specifically quantify the contribution N and K from single leaves to subtending bolls on a sympodial branch, through the use of an ^{15}N isotope solution and an Rb solution applied to the main stem and 1st position leaves of branches at the 11th node in an unstressed, high-yielding Sicot71BRF crop. Branches in a 16 x 5m area with a white flower at position 1 on the 4th February, 2010 were tagged with a plastic marker. The experimental design, crop development and treatments are described in detail section 3.4.8.

Two treatments, a labelling treatment and a control treatment were applied to either the mainstem or 1st position leaves on the 11th node of each plant in each block (Table 5.1). Rb and ^{15}N were applied an approximate rate of 1% of the total content of the leaf, the equivalent of 0.4 mg Rb per leaf and 0.7 mg N per leaf. This was the equivalent of 0.5659 mg RbCl (an equivalent of 0.3999 mg), and 1.5217 g Urea (98.47% ^{15}N excess, in solution the equivalent of 0.68929 mg ^{15}N excess per leaf) per leaf.

Table 5.1 Treatments applied to leaves on the tagged branches in experiment 8

Treatment	Solution	Application point
1	0.68929 mg ^{15}N excess and 0.5659 RbCl in 0.6 mL deionised water	Main stem leaf
2	0.68929 mg ^{15}N excess and 0.5659 RbCl in 0.6 mL deionised water	1st position leaf
3	Deionised water	Main stem leaf
4	Deionised water	1st position leaf

At five growth dates, approximately every 10 days, two whole plants were removed from each plot (Table 3.11).

The following data was collected from each collected plant;

- Nodes above and below the tagged branch
- Number of leaves on the tagged branch
- Number and type (square, flower, green boll or open boll) of fruit on the tagged branch
- Number of fruiting positions on the tagged branch
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the leaves, stems, petioles, at position 1, 2 and 3+, and the main stem leaf and node segment of the main stem (by the method described in section 3.3) from the tagged branch (18th Feb, 10th March and 6th April samples only).
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the boll walls, bracts, seed and lint of the boll at position 1 from the tagged branch (by the method described in section 3.3) (18th Feb, 10th March and 6th April samples only).
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the pooled dried and ground leaf, stem and fruit samples from the node above (number 12) and node below (number 10) the tagged branch (18th Feb, 10th March and 6th April samples only).
- The dry weight, N, P, K, Rb and ^{15}N excess concentration of the dried and ground leaf, stem and fruit samples from nodes 13+ and nodes 1-9 (18th Feb, 10th March and 6th April samples only).

Data was analysed using Genstat 14th edition as described in section 3.4.8.4.

5.3 Results

5.3.1 Dry weight

From the day of flowering at position 1 at the node 11 branch, until maturity of the boll at position 2, the total dry weight of the combined tissues (leaf, stem, petiole and boll) at position 1 and 2 increased, while the total dry weight of the mainstem node, leaf and petiole remained relatively constant Figure 5.1. The dry weight of position 1 was higher than at position 2 throughout the growth and development of each.

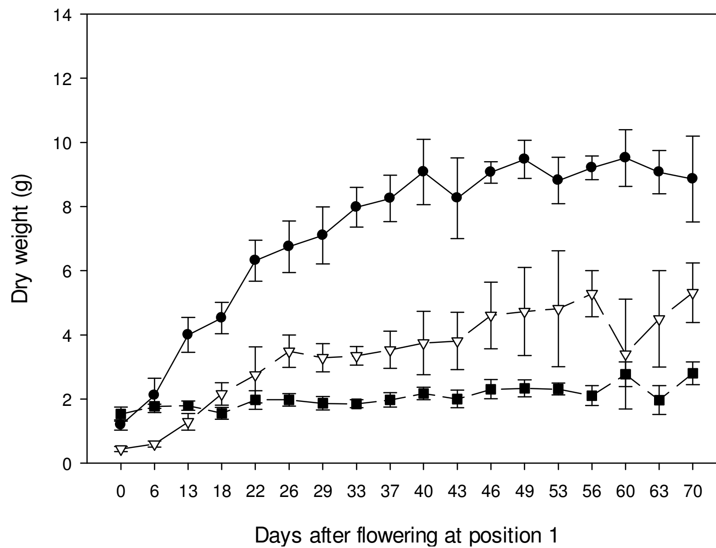

Figure 5.1 The accumulation of dry weight in the mainstem node and leaf (- —■— -), combined position 1 tissues (—●—) and combined position 2 tissues (—▽ —) from flowering at position 1 (day 0) to maturity at position 2 (day 70). Error bars represent +/- one standard error of the mean.

The relatively small change in the dry weight of the mainstem node segment after flowering saw its proportion of the total branch decrease from 48% at flowering to 16% at maturity. The combined position 1 tissue contained the bulk of the branch dry weight, containing 37% at flowering, peaking at 60%, and declining to 52% as the position 2 boll filled. The tissue at position 2 contained 31% of the total branch dry matter at maturity.

The fruit, leaf, stem and petiole dry weight declined with distance from the mainstem (Figure 5.2). The mainstem leaf and petiole had the highest dry weight, followed by the 1st position leaf, then the second ($P < 0.05$). Similarly the petiole and stem dry weight decreased along the branch ($P < 0.05$).

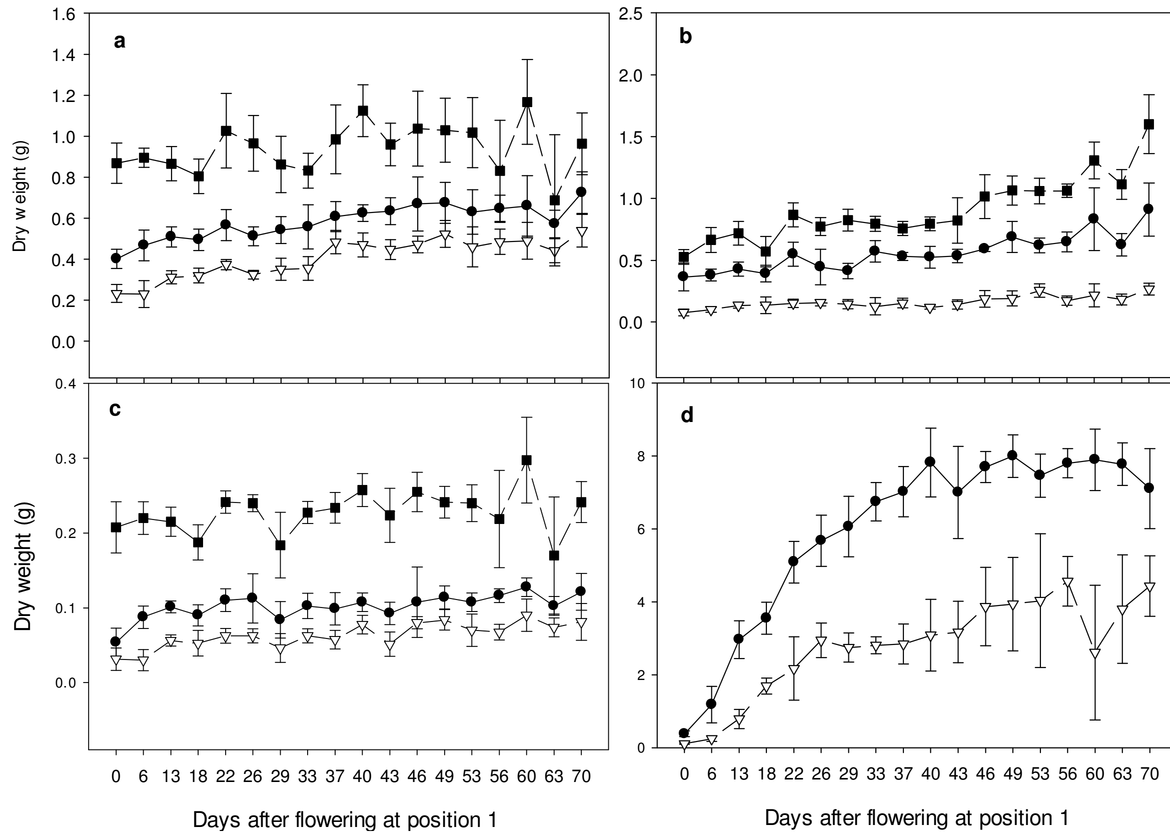

Figure 5.2 The (a) leaf, (b) stem, (c) petiole and (d) fruit dry weight in the mainstem section ( ), at position 1 ( ) and position 2( ) from flowering at position 1 to maturity at position 2. Error bars represent +/- one standard error of the mean.

As well as the dry weight of the various tissues, the rate of change in dry weight varied between the tissues at different positions. The peak biomass accumulation rate in the position 1 fruit was 0.39 g day^{-1} at 22 DAF, although the peak dry weight of 8.2 g occurred at 49 DAF, after which it did not decline ($P < 0.001$). The peak biomass accumulation rate in the position 2 fruit was 0.19 g day^{-1} at 46 days after position 1 flowering, although the peak dry weight of 4.46 g occurred at 56 DAF, after which it did not decline ($P < 0.001$). The rate of dry weight accumulation in the vegetative tissues (the leaf, stem and petiole) was low at each position, little growth was measured after first position flowering. Within the bolls, the seed and lint accumulated dry weight at the greatest rate, and accounted for the highest proportion of the total fruit weight (Figure 5.3).

The seed and lint at position 1 accumulated dry weight at a rapid rate for around 40 DAF, while the seed and lint at position 2 accumulated dry weight for a similar period (from flowering at 6 days after 1st position flowering) although at a much slower rate. The bract, wall, seed and lint dry weight of the position 1 boll was higher than that of the position 2 boll ($P < 0.05$) (Figure 5.3). The difference in boll dry weight between the two was due to the lower seed dry weight, which was 2.82 g at maturity at position 1, accounting for a total of 39% of the boll weight, and 1.76 g at position 2, and accounting for the same proportion of the boll. The lint dry weight was 1.8 times the weight at position 1 than at position 2 (2.53 g and 1.39 g respectively). The bracts ($P = 0.03$) and boll wall ($P < 0.001$) reached a higher biomass at position 1 than position 2.

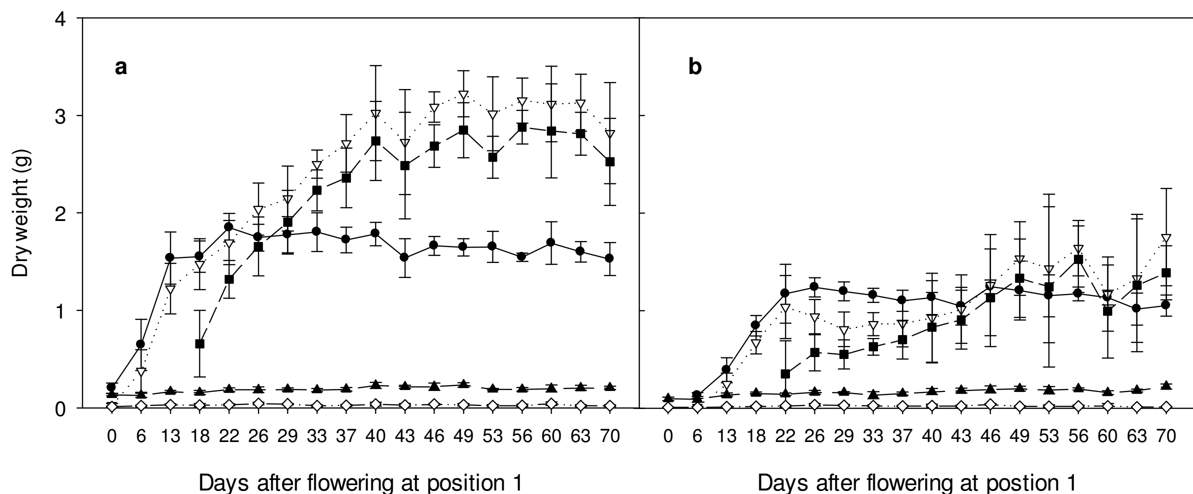

Figure 5.3 (a) Position 1 and (b) position 2 boll component dry weights; boll wall (——●——), seed (······▽······), lint (— —■— —), petiolule (···—◇—···) and bract (‾ —▲— ‾). Error bars represent +/- one standard error of the mean.

The difference in the allocation patterns along the branch are highlighted by examining the ratio of reproductive tissue to vegetative tissue (Figure 5.4 a and c), and the equivalent of the harvest index – that is the ratio of the seed and lint to other tissues at each position (Figure 5.4 b and d). Figure 5.4 shows the ratio of seed and lint, or total reproductive structures as a proportion of the total dry weight (a and b), or to the vegetative tissue dry weight (c and d). The smaller lint and seed dry weight at position 2 lead to a lower ratio of seed and lint: vegetative tissue (leaf, stem, petiole, boll wall and bract) and a lower proportional allocation to seed and lint at position 2 than at position 1 during the peak growth period of the boll,

although there was no difference between the position 1 and 2 at maturity ($P < 0.05$). There was no difference in the ratio of reproductive to vegetative tissues at each position, nor in the proportional allocation of biomass at each position to the total reproductive structure (including the boll wall and the bract).

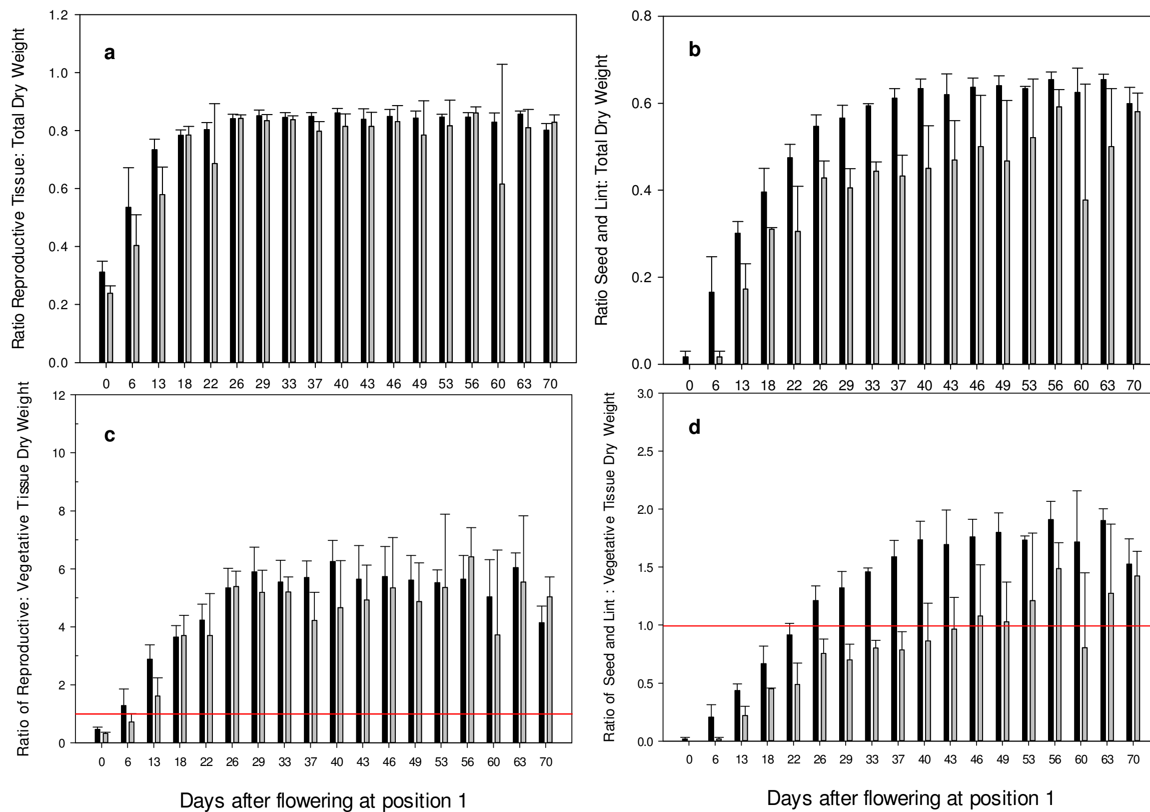

Figure 5.4 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total dry weight, (b) seed and lint to total dry weight, (c) reproductive tissue to vegetative tissue (leaf, petiole and stem), and (d) seed and lint to vegetative dry weight (including the bract and boll wall) at position 1 (▬) and position 2 (▭). The red line in (c) and (d) indicates the 1:1 ratio. Error bars represent the L.S.D at $P = 0.05$.

5.3.2 Nitrogen

The N content of the combined tissues at each position along the branch reflected the total dry weight, with the content at position 1 higher than position 2, and both fruiting positions having a higher N content at maturity than the mainstem section (Figure 5.5a). The N content of position 1 and 2 increased rapidly with the development of the fruit (Figure 5.5a). Despite differences in the content of N at each position, there was little variation in the concentration of N between the positions (Figure 5.5b), with an increase in the concentration of N was

observed at positions 1 and 2 during the rapid development of the fruit, and then a decline, with no difference in the N concentration between the positions at maturity (P<0.05).

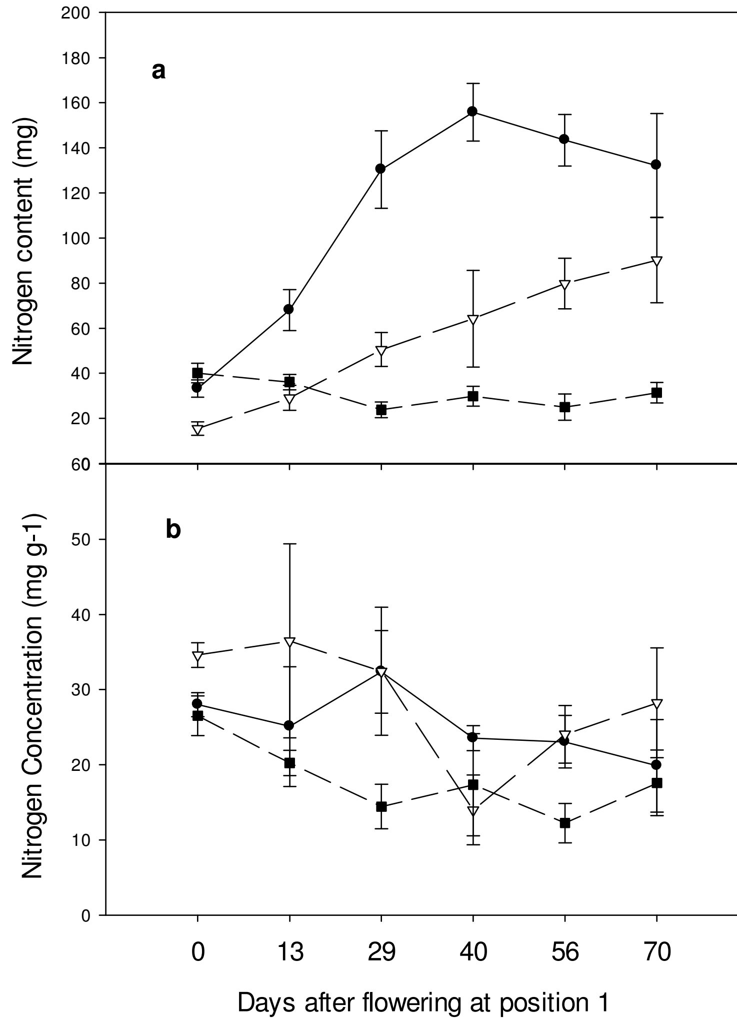

Figure 5.5 The (a) N content (mg) and (b) concentration (mg g^{-1}) in the mainstem node and leaf (—■—), position 1 tissue (—●—) and position 2 tissue (– –▽– –) from flowering at position 1 to maturity at position 2. Error bars represent +/- one standard error of the mean.

The proportional allocation of N between the positions was nearly identical to the allocation of dry matter, with each segment containing a similar proportion of the total branch N and dry matter at flowering and at maturity.

The content of N along the branch (Figure 5.6) did not follow the same pattern as the allocation of dry weight (Figure 5.2). At flowering the N content was highest in the tissues with the highest dry weight, though the concentration followed the opposite trend. At

maturity there was very little variation in the leaf or petiole content between the three sections. The stem N content increased in the node segment and position 1, while there was very little increase at position 2. The concentration of N in all tissues declined with growth and development, and the export of N from some tissues. The only increase in N concentration was in the stems (Figure 5.6d), which was accompanied by an increase in the N content, and the fruit at position 2, which had a corresponding increase in the N content (Figure 5.6h).

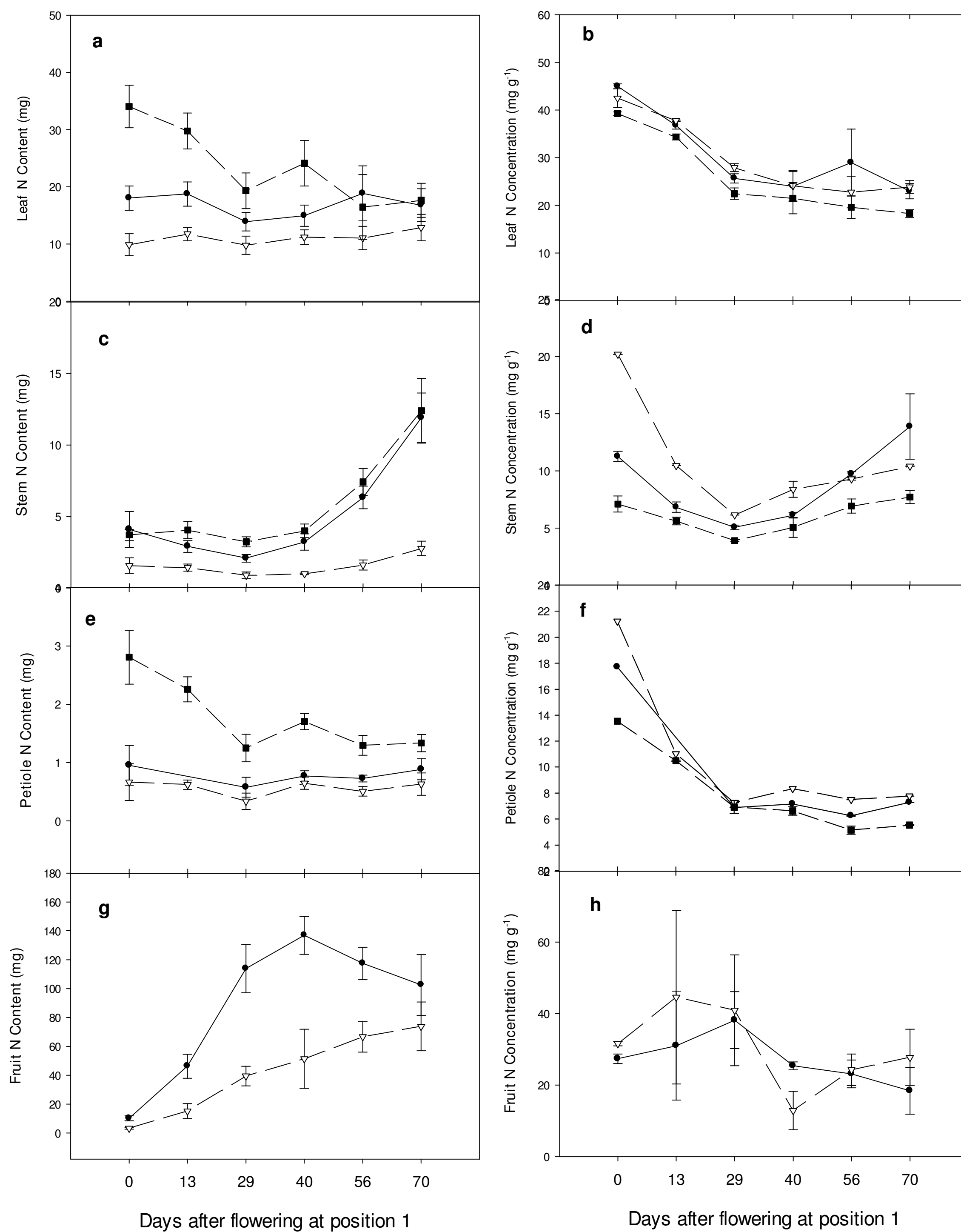

Figure 5.6 The N content (mg) (a), (c), (e) and (g) and concentration (mg g^{-1}) (b), (d), (f) and (h) of the leaves (a and b), stems (c and d), petiole (e and f) and fruit (g and h) in the mainstem (–■–), position 1 tissue (–●–) and position 2 tissue (– ▽ –). Error bars represent +/- one standard error of the mean.

While the seed N content increased from flowering, the variation in the partitioning of N between the boll components varied considerably from flowering to maturity – following a similar pattern in the bolls at both position 1 and 2 (Figure 5.7). The bract and wall content peaked around 13 DAF (13DAF at position 1 and 29DAF at position 2), and the lint content peaked at 29DAF. The seed content continued to rise, reaching a peak at position 1 but not at position 2. The concentration of N in the seeds also rose until maturity, while it declined in all other tissues, as their dry weight increased (the lint and walls), and the content declined (walls, lint and bracts).

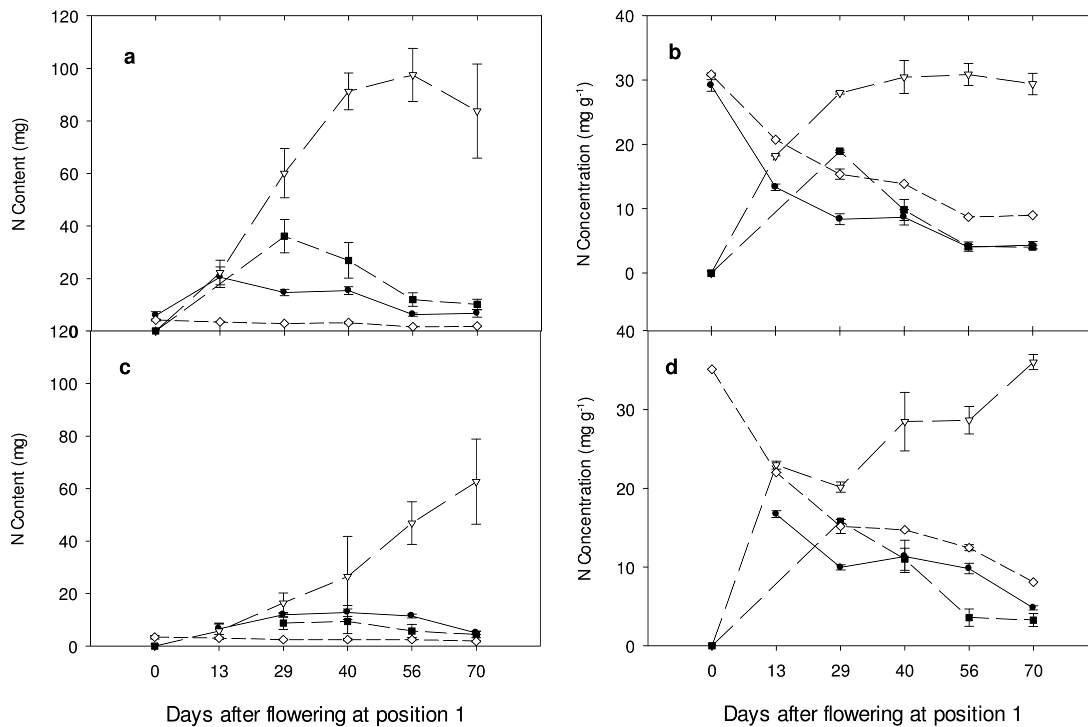

Figure 5.7 The N content (mg) (a) and (c) and concentration (mg g^{-1}) (b) and (d) in the wall (—■—), seed (- -▽- -), lint (- —■— -), bract (- -◇- - -) at position 1 (a and b) and position 2 (c and d). Error bars represent +/- one standard error of the mean.

There was more variation in the ratio of N distribution between vegetative and reproductive parts, and in the ratio of seed and lint N to vegetative N, as well as in the proportion of total N allocated to reproductive parts, and to the seed and lint, than in the equivalent comparisons of dry weight (Figure 5.8). The proportional allocation of N to reproductive parts in position 1 was higher until 56 DAF, while both positions had the same allocation of dry weight by

18DAF ($P < 0.05$). The seed and lint at both positions accounted for a higher percentage of the total N at position 1 and 2 than of the total dry weight, indicating it was a more highly concentrated tissue than others (Figure 5.8b). The ratio of reproductive N to vegetative N peaked in both positions before declining slightly at position 2, and to less than half the peak at position 1 (Figure 5.8c). The ratio of seed and lint N to vegetative N was much higher than the equivalent ratio of the dry weight, emphasising the strength of the seed and lint sink and the movement of N from vegetative tissues during boll development. The R:V and seed and lint: V ratio was higher at position 1 than position 2 at equivalent ages, indicating that the position 1 bolls, and seed and lint, were a stronger sink than those at position 2.

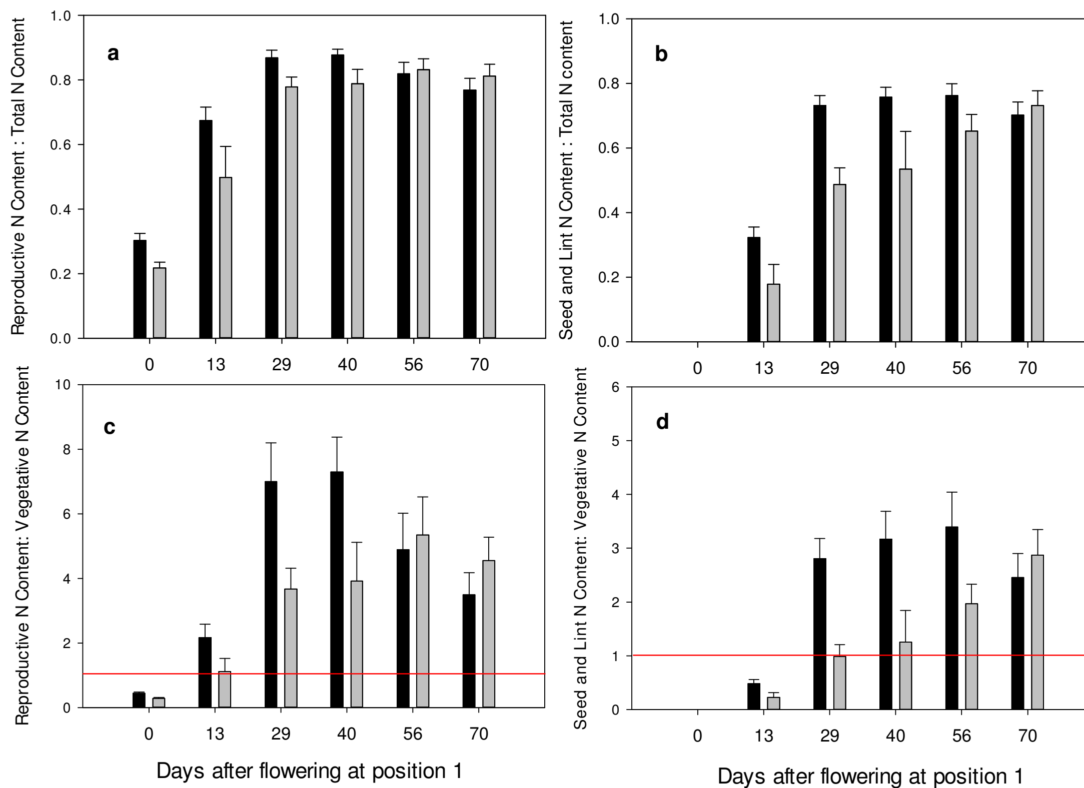

Figure 5.8 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total N content, (b) seed and lint to total N content, (c) reproductive tissue to vegetative tissue (leaf, petiole and stem), and (d) seed and lint to vegetative N content (including the bract and boll wall) at position 1 (▬) and position 2 (▭). The red line in (c) and (d) indicates the 1:1 ratio. Error bars represent the L.S.D. at $P = 0.05$.

5.3.2.1 N Redistribution

In the whole branch there were tissues which behaved as a sink (import and accumulation of N), as a source (export of N), which changed from a sink to a source and from a source to a sink of N (Table 5.2). The total export of N from the source tissue did not account for the

import of N into the sinks, on balance there was an import of 123 mg N into the branch. The total seed import was 83.8 mg at position 1, and 62.7 mg at position 2, which was the major sink along the whole branch, accounting for 58% of the total N in the branch at maturity. Even were all the exported N at each position to have been imported into the seed, exported N at each position would account for 53% at position 1 (44.2 mg) and 23% at position 2 (14.4 mg), with the export from the mainstem equating to a further 17.9 mg available.

Table 5.2 Tissues from the main stem, position 1 (1) and position 2 (2) classified as a source or a sink, and the total amount of N imported or exported from each group from flowering at position 1 to maturity at position 2

	Source	Sink	Sink then Source	Source then Sink
	Bract 1	Seed 1	Wall 1	Stem 1
	Bract 2	Seed 2	Wall 2	Stem 2
	Main Stem Leaf	Leaf 2	Lint 1	Node Segment
	Main Stem Leaf Petiole		Lint 2	Petiole 1
	Leaf 1			Petiole 2
Export (mg N)	23.9		73.0	3.95
Import (mg N)		149	52.6	22.0
Total Export	101			
Total Import	224			
Balance	Import of 123 (48.5% of total branch content at maturity)			

5.3.2.1.1 ^{15}N import and export from the mainstem leaf

There was no difference between the dry weight (g), N content (mg) or N concentration (mg g^{-1}) of any of the tissues along the sympodial branch at node 11, or to the leaf and fruit tissues above and below node 11 of the plants injected with the ^{15}N and RbCl solution (hereafter referred to as "mainstem leaf treatment") and those injected with water (the "control" treatment) ($P > 0.05$).

Between 14 days and 61 days after the application of the labeled solution (at flowering at position 1) there was a decrease in the total N content in mg in the labeled mainstem leaves of 7 mg, or 26% of its total content. There was a much greater export of the ^{15}N applied to the leaves (Figure 5.9). The mean ^{15}N excess content of the control leaf during this time increased from 0 mg to 0.01mg – possibly due to contamination of one of the samples. The ^{15}N excess content of the treated mainstem leaf decreased from 0.08 mg to 0.01 mg, a decline

of 81% (Figure 5.9). Assuming that the ^{15}N export is representative of the export of total N from the leaf (although import may also have occurred); the total export between 14 and 61 days after position 1 flowering would have been 21.7 mg, or three times the amount indicated by the balance in the content alone.

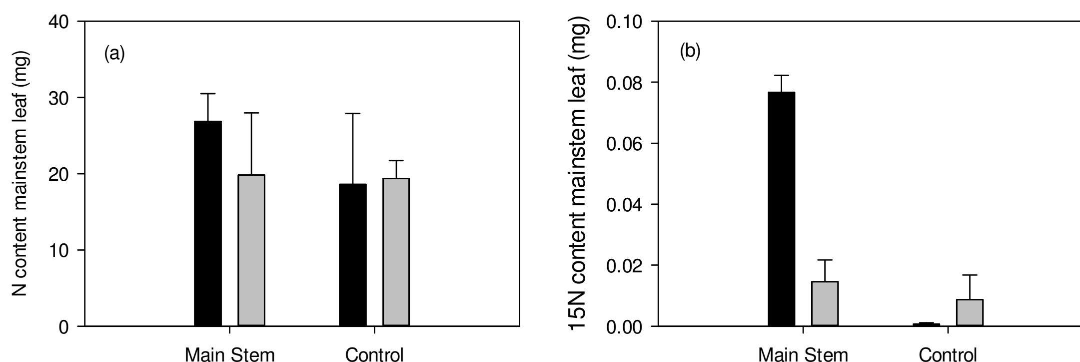

Figure 5.9 The mean N (a) and ^{15}N (b) content (mg) of the mainstem leaf in the branches to which ^{15}N was applied to the mainstem leaf, and the control branches at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean. Error bars represent the L.S.D at P = 0.05.

Since there was a considerable amount of ^{15}N exported from the mainstem leaf, the fate of the applied solution can be measured by examining the accumulation of ^{15}N in the surrounding tissue within the node, and in the tissues above and below the labeled leaf.

There was some ^{15}N excess found in the control tissues at both dates, as shown in Figures 5.12, 13, 14 and 15. This could be due to contamination of the samples during processing, or due to background ^{15}N at the site being higher than the 0.3663% used as the average terrestrial abundance. By only including samples with a higher ^{15}N excess content than the control in any calculations of redistribution, any difference in the background ^{15}N is accounted for.

There was no difference in the N content of any analysed tissues. Of the tissues on the labeled node (node 11), the ^{15}N content was higher in the position 1 stem, position 2 leaf, position 1 seed, and in the position 1 boll walls and leaf at 14 days after labeling (Figure 5.10 and Figure 5.11).

The increase in the ^{15}N in the vegetative tissues at node 11 is given in Figure 5.10. The decrease in the N and ^{15}N content of leaf 1 between 14 and 61 days corresponds to the export

of N from this tissue found in experiment 8. The increase in the ^{15}N content of leaf 2 and of the stems, shows that vegetative – vegetative redistribution occurred along the sympodial branch from the mainstem leaf to other leaves, and through connective tissues (the stems).

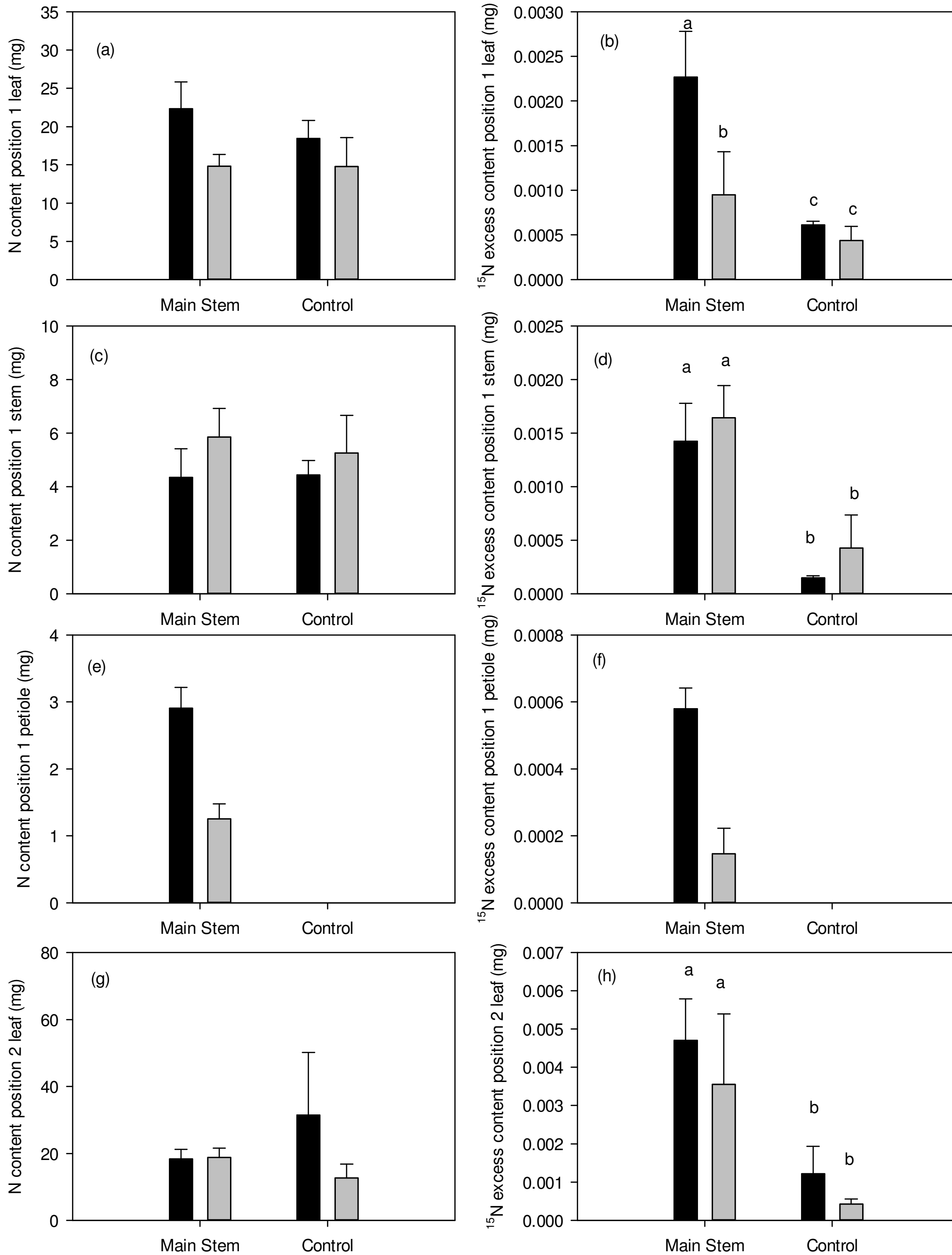

Figure 5.10 The N content (mg) (a, c, e and g) and ^{15}N excess content (mg) (b, d, f and h) of the position 1 leaf (a and b), stem (c and d), petiole (e and f) and position 2 leaf (g and h) in the branch to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, letters above the bars represent a significant difference at *P* < 0.05.

Figure 5.11 shows the change in N and ^{15}N excess content of the reproductive plant parts, excluding the lint. Unlike in experiment 8, the bracts were analysed with the boll walls. As with experiment 8, there was significant export of N from the boll walls after 14 days – and there was no difference in the ^{15}N content of the control and treated boll walls and bracts at 61 days after labeling (Figure 5.11 c and d). The seeds accumulated N and ^{15}N throughout the development of the boll (Figure 5.11 a and b).

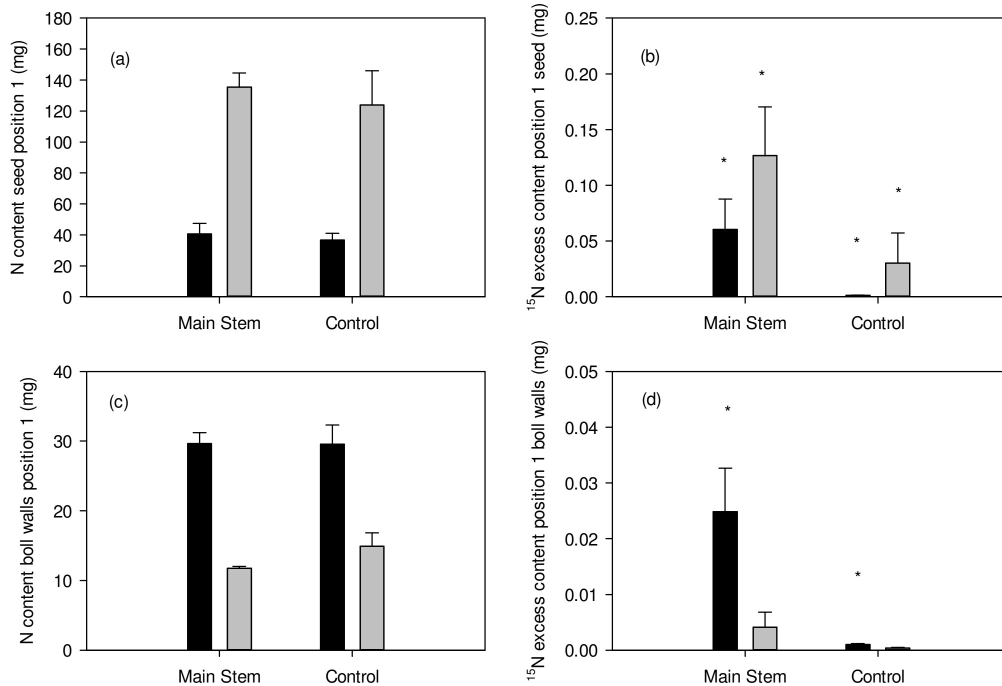

Figure 5.11 The N content (mg) (a and c) and ^{15}N excess content (mg) (b and d) of the position 1 seed (a and b) and boll walls (including the petiole and bracts) (c and d) in the branch to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments.

Export of ^{15}N from the mainstem leaf also occurred to other nodes, both above and below the labeled leaf, indicating that there was extensive cycling of N throughout the whole cotton plant, not just between adjacent tissues. Figure 5.12 shows the N and ^{15}N content of the leaves in the nodes immediately above (12) and below (10) the labeled node, and the pooled tissues above (13+) and below (1-9) these. There was an increase in the ^{15}N content of the leaves in nodes 1-9 at 14 days after labeling, but no difference in the ^{15}N content of the leaves at the other positions, or in the lowest leaves at 61 days.

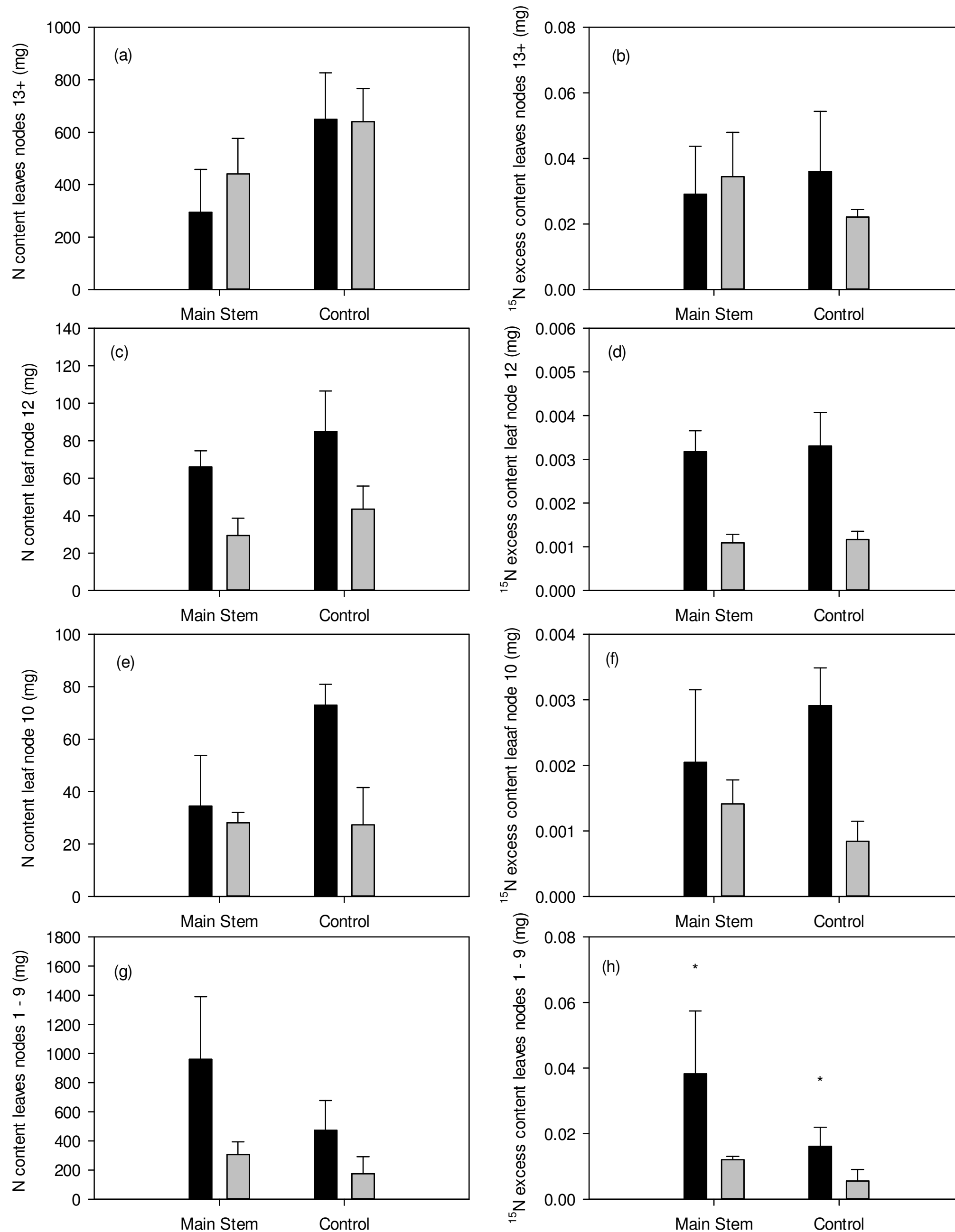

Figure 5.12 The N content (mg) (a, c, e and g) and ^{15}N excess content (mg) (b, d, f and h) in the leaves at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments.

Figure 5.13 shows the N and ^{15}N excess content of the fruit (seed, boll wall, lint and bract) in the nodes immediately above (12) and below (10) the labeled node, and the pooled tissues above (13+) and below (1-9) these. The ^{15}N content of the fruit was higher than the control (*P*

< 0.05) at all nodes and at both sampling times, except for at node 12, 14 days after labeling (Figure 5.13d).

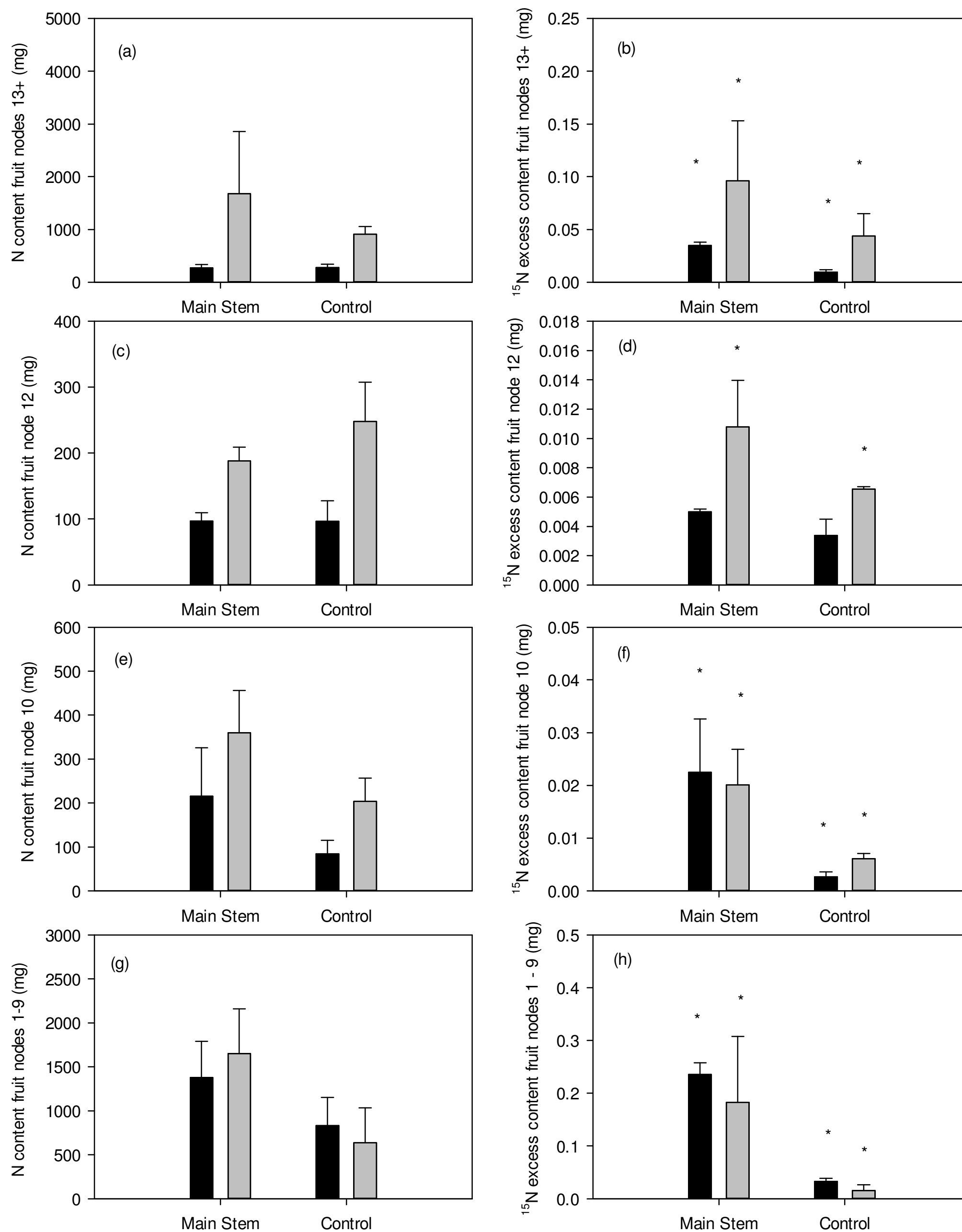

Figure 5.13 The N content (mg) (a, c, e and g) and ^{15}N excess content (mg) (b, d, f and h) in the fruit at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments.

Table 5.3 shows the recovery of the ^{15}N, and its proportional distribution between the plant parts analysed. In total 0.689 mg ^{15}N excess was applied to the plants, and 78% recovered 14 days after label application, and 71% at 61 days after application. The remaining ^{15}N may have been translocated to tissues not analysed (the stems of the nodes above and below the labeled branch, the petioles and fruit at position 2 of node 11), or may have been redistributed to the roots, which were not recovered.

Of the ^{15}N recovered, 31.5% was still in the tissues of node 11 14 days after application, and 29.6% was present after 61 days. Of the ^{15}N in the sympodial branch, most (35% after 14 days and 84% after 61 days) was found in the seed at position 1.

Since not all tissues had significantly higher ^{15}N excess content than the corresponding controls, these tissues can be excluded from the analysis of redistribution of the mainstem leaf N and are recorded as 0 in Table 5.3. Of the total amount of ^{15}N in the plant, excluding those tissues where no significant difference from the control was found, most was distributed between plant parts in the 14 days after application. 85.8% of the total (0.46 mg) was found in parts other than the labeled leaf after 14 days, compared with 97.2% (0.49 mg) after 61days. Assuming that all the change in ^{15}N represented the export of total N from the mainstem leaf, this equates to an export of 26.05 mg N exported from the leaf (97.2% of its content at 14 days after labeling, which, based on experiment 8 can be assumed to be the same as at the time of labeling – day 0).

Table 5.3 The mean ^{15}N excess content (mg) in each tissue of the mainstem leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the total ^{15}N excess in each.

Tissue	^{15}N excess 14 DAF (mg)	^{15}N excess 61 DAF (mg)	^{15}N 14 DAF (% of total)	^{15}N 61 DAF (% of total)
MS Leaf	0.077	0.015	11.1	2.1
Leaf 1	0	0	0	0
Stem 1	0.001	0.002	0.2	0.2
Petiole 1	0	0	0	0
Leaf 2	0.005	0.004	0.7	0.5
Seed 1	0.060	0.127	8.8	18.4
Walls 1	0.025	0	3.6	0
Leaf 13+	0.029	0.034	4.2	5.0
Fruit 13+	0.035	0.096	5.1	13.9
Leaf 12	0.003	0	0.5	0
Fruit 12	0.005	0.011	0.7	1.6
Leaf 10	0	0	0	0
Fruit 10	0.022	0.020	3.3	2.9
Leaf 1-9	0.038	0	5.5	0
Fruit 1-9	0.235	0.183	34.2	26.5
Other plant parts (^{15}N not recovered)	0.148	0.179	22.1	28.9

If 26.05 mg of N was exported from the mainstem leaf, its distribution would be proportionally the same as the distribution of ^{15}N. The total mg of N that this represents is given in Table 5.4. The proportion of the total N supplied by the mainstem leaf is less than 1% for most tissues. Almost 5% of the position 1 seed N was supplied by the mainstem leaf, 2% of the walls, and more of the N in the leaf at position 2 than position 1.

Table 5.4 The equivalent supply and relative contribution of redistribution from the mainstem leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 26.05 mg

Tissue	mg N supplied from MS Leaf at node 11, position 1 maturity	% of total N supplied from redistribution of mainstem leaf N
Leaf 1	0.05	0.34
Stem 1	0.09	1.48
Petiole 1	0.01	0.61
Leaf 2	0.19	0.99
Seed 1	6.65	4.91
Walls 1	0.22	1.84
Leaf 13	1.81	0.41
Fruit 13	5.05	0.30
Leaf 12	0.06	0.20
Fruit 12	0.57	0.30
Leaf 10	0.07	0.26
Fruit 10	1.06	0.29
Leaf 9	0.63	0.21
Fruit 9	9.61	0.58

5.3.2.1.2 ^{15}N import and export from 1st position leaf

There was no difference between the dry weight (g), N content (mg) or N concentration (mg g^{-1}) of any of the tissues along the sympodial branch at node 11, or to the leaf and fruit tissues above and below node 11 of the plants injected with the ^{15}N and RbCl solution (hereafter referred to as "1st position treatment") and those injected with water (the "control" treatment) ($P < 0.05$).

Between 14 days and 61 days after the application of the labeled solution (at flowering at position 1) there was a decrease in the total N content in mg in the labeled 1st leaves of 6.3 mg, or 32.9% of its total content. As with the mainstem leaf treatment there was a much greater export of the ^{15}N applied to the leaves (Figure 5.14). The ^{15}N content of the treated 1st position leaf decreased from 0.06 mg to 0.018 mg, a decline of 69.3% (Figure 5.14b). Assuming that the ^{15}N export is representative of the export of total N from the leaf (although

import may also have occurred); the total export between 14 and 61 days after position 1 flowering would have been 13.3 mg.

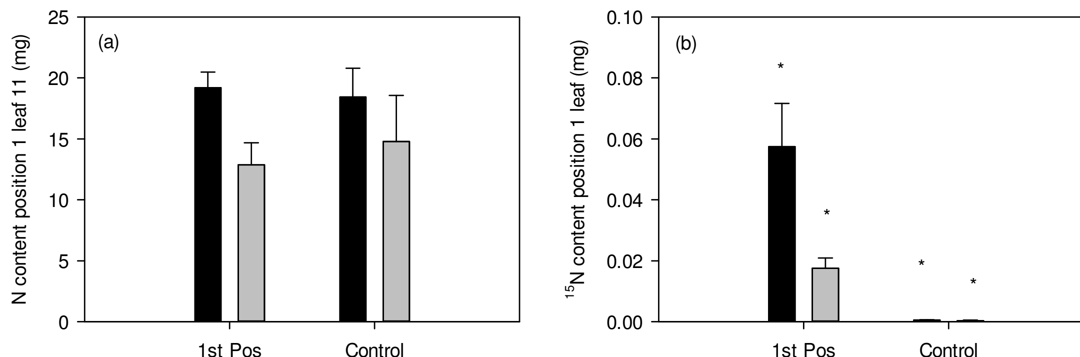

Figure 5.14 The mean N (a) and ^{15}N excess (b) content (mg) of the 1st position leaf in the branches to which ^{15}N was applied to the 1st position leaf, and the control branches at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments.

As with the mainstem treatment was some ^{15}N found in the control tissues at both dates when samples were analysed, as shown in Figure 5.15 and Figure 5.16. As with the mainstem treatment, this was probably due to contamination or a higher background ^{15}N at the site than the 0.3663% used as the average terrestrial abundance. Again, only samples with a higher ^{15}N excess content than the control were included in any calculations of redistribution, to account for any difference in the background ^{15}N. There was no difference in the N content of any analysed tissues, except for the boll walls, where the control treatment had a higher N content at 61 days than the 1st position treatment.

The increase in the ^{15}N in the vegetative tissues at node 11 is given in Figure 5.10. There was no change in the ^{15}N content of the mainstem leaf, or the mainstem node segment, but there was an increase in the ^{15}N content of the stem at position 1, and the position 2 leaf at both dates measured.

Figure 5.16 shows the change in N and ^{15}N content of the reproductive tissue at position 1, excluding the lint. As with experiment 8, there was significant export of N from the boll walls after 14 days – although the treated branch maintained a higher ^{15}N content in the boll walls

until maturity (P = 0.04). The seeds accumulated N and ^{15}N throughout the development of the boll (Figure 5.16 a and b).

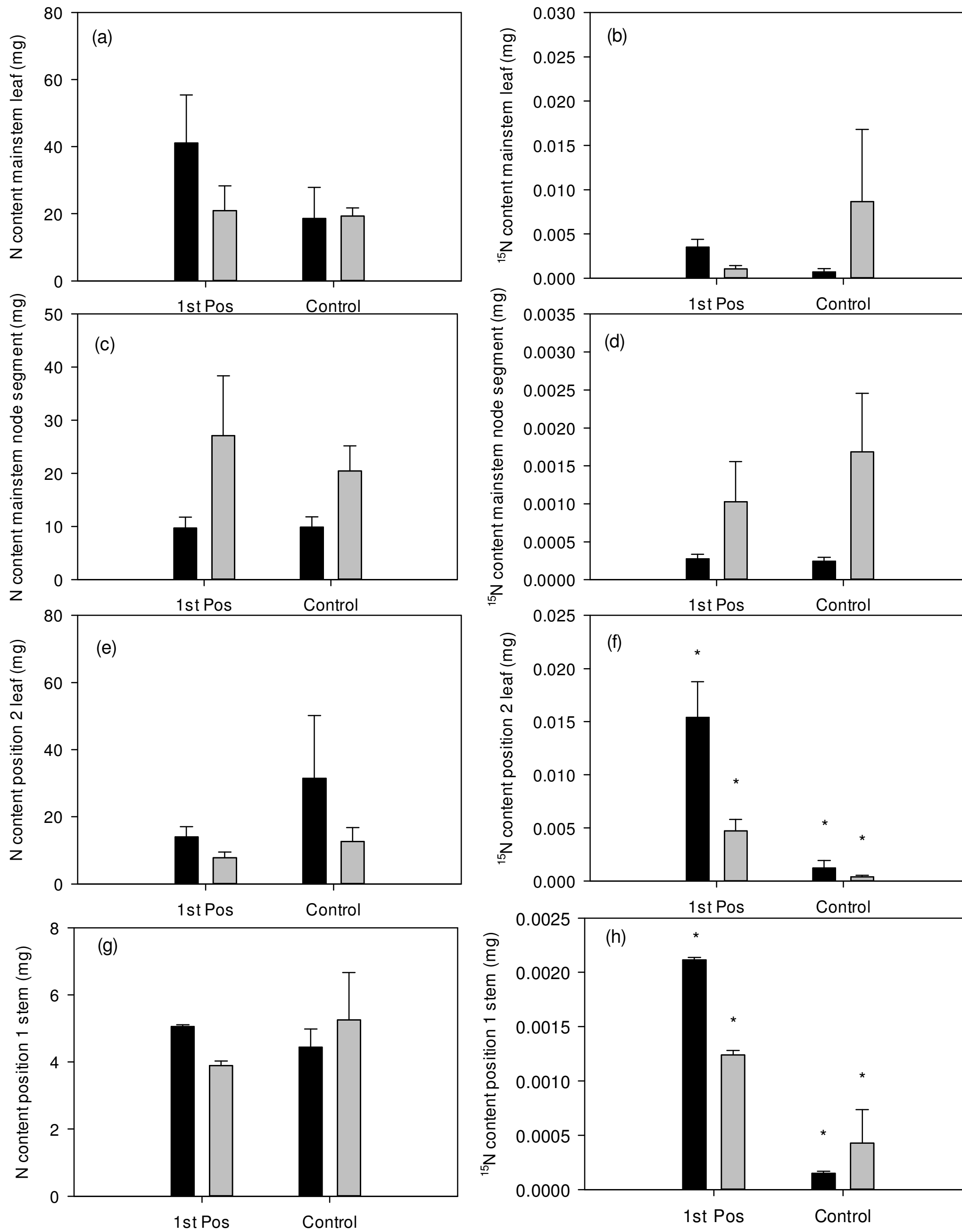

Figure 5.15 The N content (mg) (a, c, e and g) and ^{15}N content (mg) (b, d, f and h) of the mainstem leaf (a and b), mainstem node segment (c and d), position 2 leaf (e and f) and position 1 stem (g and h) in the branch to which ^{15}N was applied to the 1st position leaf, and the control branch at 14 DAF ( ) and 61 DAF ( ) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at P < 0.05 between the treatments.

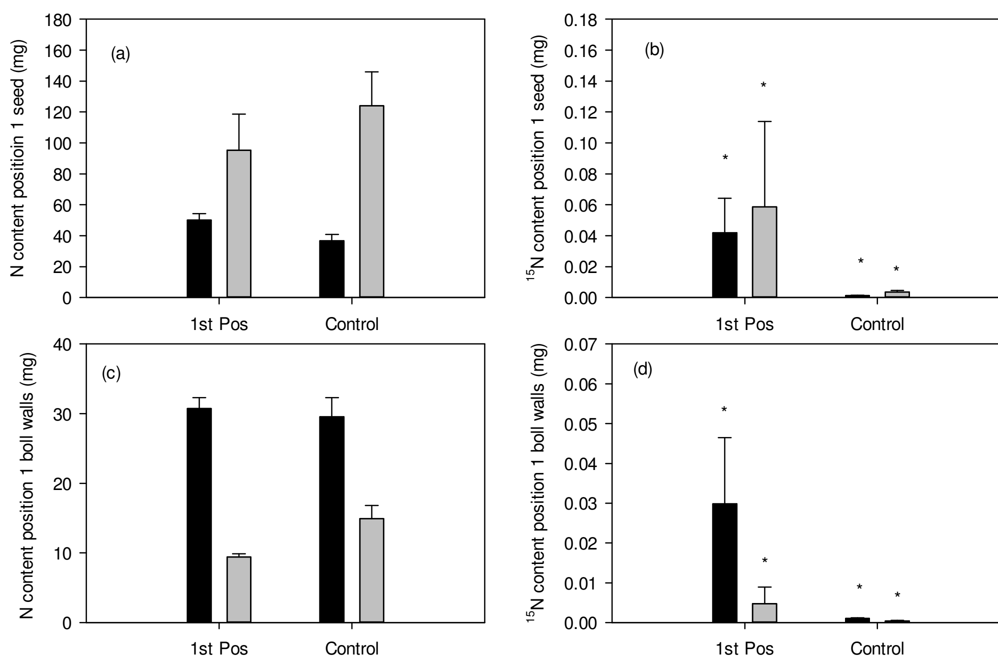

Figure 5.16 The N content (mg) (a and c) and ^{15}N content (mg) (b and d) of the position 1 seed (a and b) and boll walls (including the petiolule and bracts) (c and d) in the branch to which ^{15}N was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments.

Unlike from the mainstem leaf, there was no increase in the ^{15}N content of the leaves or the fruit of the nodes above and below node 11 ($P > 0.05$) (data not presented).

Table 5.5 shows the recovery of the ^{15}N, and its proportional distribution between the plant parts analysed. In total 0.689 mg ^{15}N excess was applied to the plants, and 38.5% recovered 14 days after label application, and 25.4% at 61 days after application. The remaining ^{15}N may have been found in tissues not analysed (the stems of the nodes above and below the labeled branch, the petioles and fruit at position 2 of node 11), or may have been redistributed to the roots, which were not recovered. The much lower recovery of the ^{15}N from this treatment suggests that a significant proportion of the ^{15}N may have been redistributed to the 2nd position boll or to unanalysed vegetative plant parts.

Of the ^{15}N recovered, 31.5% was still in the tissues of node 11, 14 days after application, and 29.6% was present after 61 days. Of the ^{15}N in the sympodial branch, a significant proportion

was found in the position 1 leaf, seed and boll walls – the leaf accounting for 20% of the total at maturity and the seed for 66%.

Since not all tissues had a significantly higher ^{15}N content than the corresponding controls, these tissues can be excluded from the analysis of redistribution of the mainstem leaf N and have a 0 mg content in Table 5.5. Of the total amount of ^{15}N recovered, excluding those tissues where no significant difference from the control was found, most was distributed between plant parts in the 14 days after application. 78.4% of the total was found in parts other than the labeled leaf after 14 days, compared with 90% after 61days. Assuming that all the change in ^{15}N represents the export of total N from the mainstem leaf, this equates to an export of 17.25 mg N exported from the leaf (90% of its content at 14 days after labeling, which, based on experiment 8 is assumed to be the same as at the time of labeling – day 0).

Table 5.5 The mean ^{15}N excess content (mg) in each tissue of the 1st position leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the total ^{15}N excess in each.

Tissue	^{15}N excess content 14 DAF (mg)	^{15}Nexcess content 61 DAF (mg)	% of total 14 DAF	% of total 61 DAF
MS Leaf	0	0	0	0
Leaf 1	0.057	0.018	8.3	2.6
Stem 1	0.002	0.001	0.3	0.2
MS Node	0	0	0	0
Leaf 2	0.015	0.005	2.2	0.7
Seed 1	0.042	0.059	6.1	8.5
Walls 1	0.030	0.005	4.3	0.7
Leaf 13	0	0	0	0
Fruit 13	0	0	0	0
Leaf 12	0	0	0	0
Fruit 12	0	0	0	0
Leaf 10	0	0	0	0
Fruit 10	0	0	0	0
Leaf 9	0	0	0	0
Fruit 9	0	0	0	0
Other plant parts (^{15}N not recovered)	0.424	0.514	78.8	87.3

If 17.25 mg was exported from the mainstem leaf, its distribution would be proportionally the same as the distribution of ^{15}N. The total mg of N that this represents is given in Table 5.6. The proportion of the total N supplied by the 1st position leaf was very low for most tissues analysed other than the reproductive tissue at position 1, and the leaf at position 2.

Table 5.6 The equivalent supply and relative contribution of redistribution from the 1st position leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 17.25 mg

Tissue	N supplied from MS Leaf at node 11, position 1 maturity (mg)	% of total N
MS Leaf	0.12	0.56
Stem 1	0.14	3.48
MS Node	0.11	0.41
Leaf 2	0.52	6.66
Seed 1	6.42	6.74
Walls 1	0.52	5.51
Leaf 13	1.10	0.25
Fruit 13	1.46	0.09
Leaf 12	0.14	0.46
Fruit 12	0.31	0.16
Leaf 10	0.19	0.67
Fruit 10	1.18	0.33
Leaf 9	0.51	0.17
Fruit 9	4.54	0.28

5.3.3 Phosphorus

The P content increased in the mainstem, position 1 and position 2 portions of the branches as they grew and developed (Figure 5.17). Position 1 had a faster rate of increase and accumulated more P than position 2, although the concentration of P was higher at position 2. The P concentration at position 1 peaked at 29 DAF, following the same pattern as the N concentration in the tissues. The increase in the concentration of P in position 1 from 40 days after position 1 flowering accompanied the similar increase in N concentration (Figure 5.5).

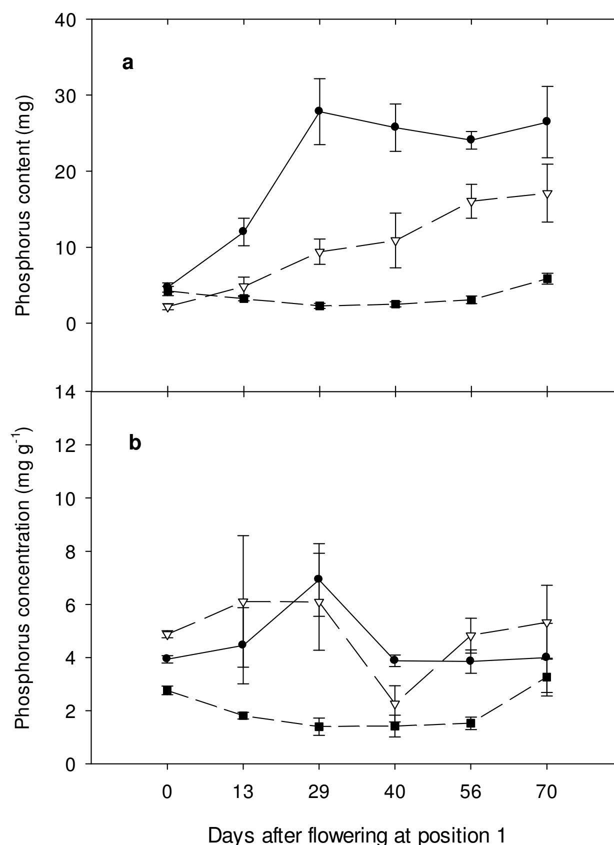

Figure 5.17 The (a) P content (mg) and (b) concentration (mg g^{-1}) in the mainstem node and leaf (—■—), combined position 1 tissue (—●—) and combined position 2 tissue (– –▽– –) from flowering at position 1 to maturity at position 2. The error bar represents +/- one standard error of the mean.

Proportionally, most of the total branch P was in the tissues at position 1, accounting for 53% of the total P at maturity. The proportional allocation of P was the same as the allocation of N, though there was an earlier accumulation of P in the fruiting positions than of N, position 1 and 2 containing 42% and 19% of the P at flowering respectively.

The pattern of accumulation and export of P from the leaves, stems, petioles and fruit was similar in all positions. There was an increase in the P content of all leaves, stems and petioles from 40 DAF at position 1 till maturity, except for the mainstem leaf, which continued to export P until 56 DAF. This increase in P content was accompanied by an increase in P concentration in the tissues. As with N and dry weight, the P content decreased with distance from the mainstem in all tissues, being highest in the mainstem section and lowest at position 2 ($P < 0.05$). The reverse was true of the concentration of P in the tissues,

although from 29 DAF there was no difference in the concentration of P in any of the leaves ($P = 0.001$), or stems ($P = 0.03$).

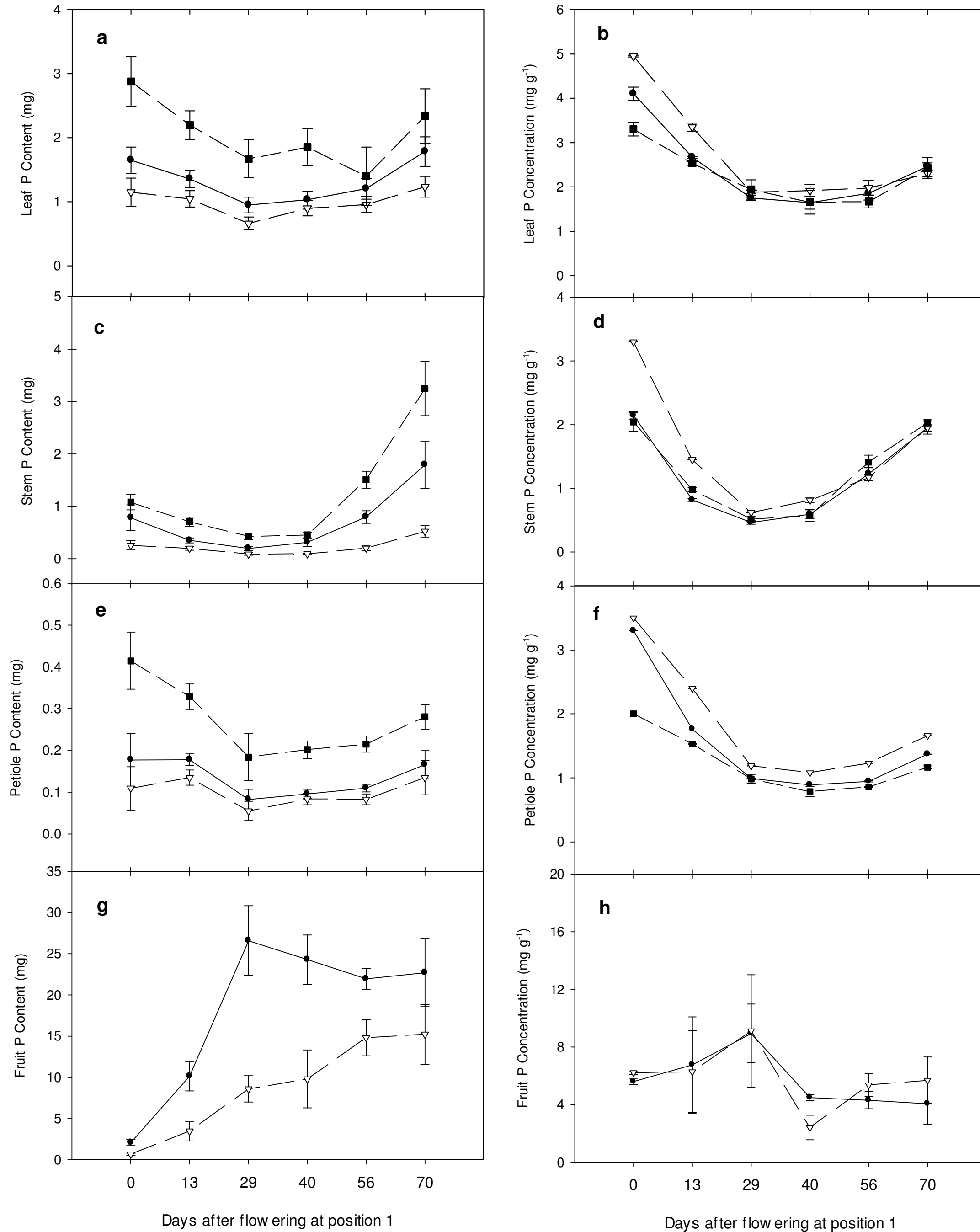

Figure 5.18 The P content (mg) (a), (c), (e) and (g) and concentration (mg g^{-1}) (b), (d), (f) and (h) of the leaves (a and b), stems (c and d), petiole (e and f) and fruit (g and h) in the mainstem (–■–), position 1 tissue (–●–) and position 2 tissue (– ▽ –). The error bar represents +/- one standard error of the mean.

The seed was the major sink for P in the bolls at position 1 and position 2 (Figure 5.19 a and c). The lint at both positions accumulated P at the same rate as the seed until 29 DAF at position 1, when its content declined by 92% at position 1 and 85% at position 2, to almost 0. There was little variation in the bract of the bolls, with the position 1 bract exporting 0.3 mg P (40% of its total) and the position 2 bract importing 0.3 mg P by maturity. The boll walls acted in a similar manner to the lint, accumulating P at both positions until 29 DAF at position 1 and then exporting over half of their P content, 2.54 mg (51%) at position 1 and 1.86 mg (55%) at position 2. Despite the rapid growth rate of the seed, the concentration of P continued to increase at position 1 until maturity (Figure 5.19 b), and decreased slightly at position 2, before increasing again after 29 DAF (Figure 5.19d).

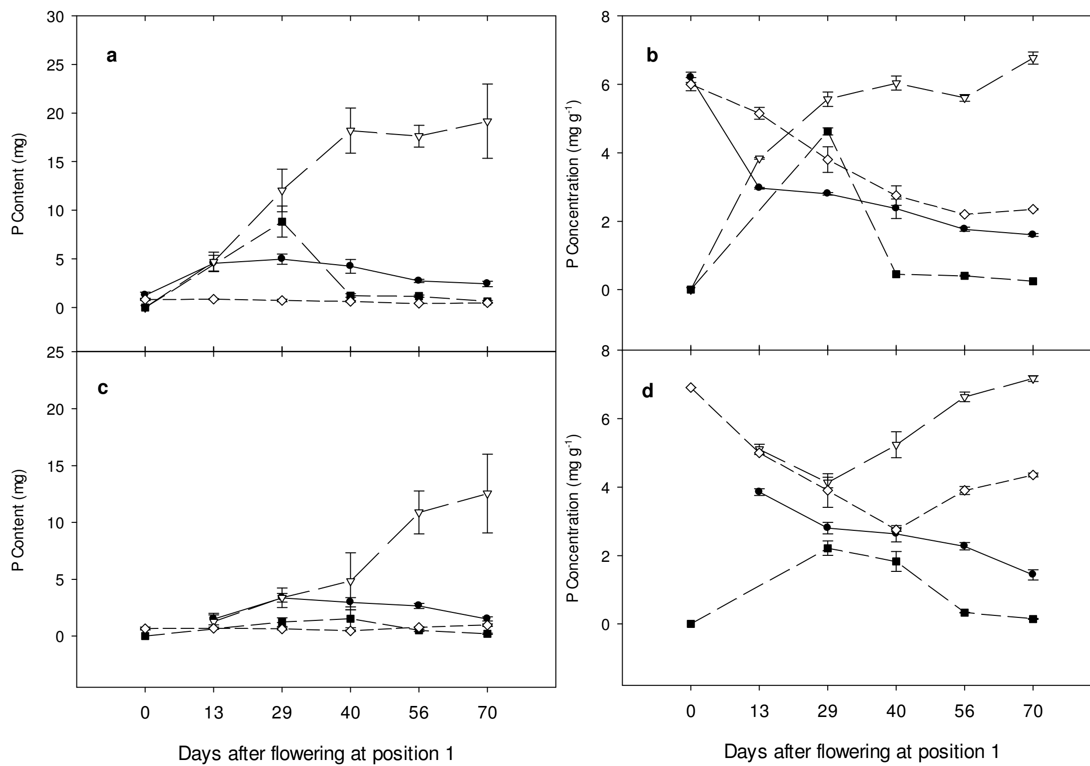

Figure 5.19 The P content (mg) (a) and (c) and concentration (mg g^{-1}) (b) and (d) in the wall (—●—), seed (- ▽ -), lint (- ■ -), bract (- ◇ -) at position 1 (a and b) and position 2 (c and d). The error bar represents +/- one standard error of the mean.

There was no difference in proportion of the total P at each position allocated to reproductive structures at maturity (Figure 5.20a). The seed and lint at position 1 accounted for a higher proportion of the total P in position 1 earlier in fruit development than at an equivalent age at

position 2 ($P < 0.001$ at 29 DAF at position 1 and position 2), but there was no difference in the proportional allocation of P to seed and lint at maturity in either position ($P < 0.05$) (Figure 5.20b).

The ratio of reproductive P to vegetative P (Figure 5.20c) was much higher at position 1 during boll filling, but higher at position 2 at maturity ($P < 0.05$), probably due to the increase in leaf and stem P at position 1 after 56 DAF (Figure 5.18 a and c). Both positions showed a much higher allocation of P to reproductive structures than vegetative, indicating that the bolls were a strong sink for P. The ratio of seed and lint P to vegetative P (counting the bract and boll wall as vegetative) was still high, although much lower than the ratio including the bract and boll wall. These two structures accumulated a great deal of P, particularly early in the boll filling phase, and the export of P from the lint led to a decline in the seed and lint total P content (Figure 5.20d). There was no difference in the ratio of seed and lint P to vegetative P between the positions at maturity ($P < 0.001$).

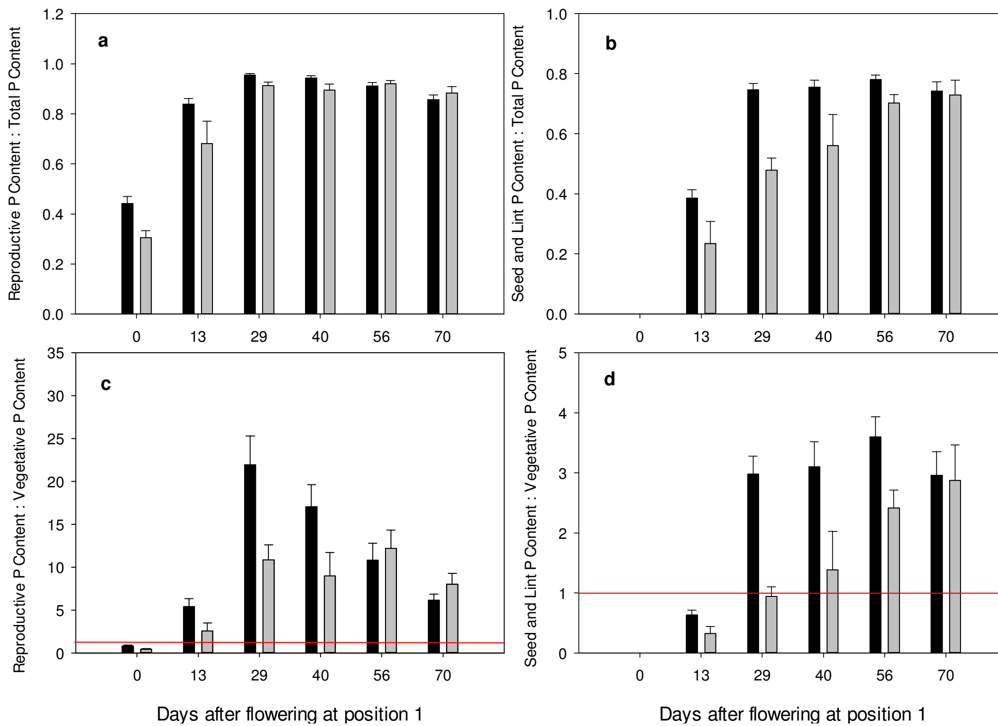

Figure 5.20 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total P content, (b) seed and lint to total P content, (c) reproductive tissue P to vegetative tissue (leaf, petiole and stem) P, and (d) seed and lint to vegetative P content (including the bract and boll wall) at position 1 (▬) and position 2 (▭). The red line in (c) and (d) indicates the 1:1 ratio. The error bar represents the standard error of the mean.

5.3.3.1 P redistribution

Unlike with N, there were very few tissues that acted solely as a sink or a source of P during the reproductive phase of the branch. As shown in Table 5.7 the only tissue not importing P was the bract at position 1. Likewise the only sinks of P which did not also export the nutrient were the seeds, which accumulated P from flowering to maturity. Overall, there was a significant increase in the amount of P in the branch, with a 445% increase in total P in the branch from flowering at position 1 to maturity. Most tissues in the branch acted as a source of P – exporting some of their P, and showing a decline in P content (Figure 5.18) for the first 40 DAF, during the period of rapid increase in the P content of the fruit. After this time, these tissues (the leaves, stems and petioles) acted as sinks, accumulating P and increasing the concentration of P in the tissue.

Table 5.7 Tissues classified as a source or a sink, and the total amount of P imported or exported from each group.

	Source	**Sink**	**Sink then Source**	**Source then Sink**
	Bract 1	Seed 1	Lint 1	Bract 2
		Seed 2	Lint 2	Leaf 1
			Wall 1	Leaf 2
			Wall 2	Main Stem Leaf
				Main Stem Node Segment
				Stem 1
				Stem 2
				Main Stem Leaf Petiole
				Petiole 1
				Petiole 2
Export (mg P)	0.33		13.9	4.36
Import (mg P)		31.7	15.9	7.65
Total Export	18.6			
Total Import	55.3			
Balance	Import of 36.7 mg (74% of total branch P content at maturity)			

On balance, the seed (the major sink for P) accounted for around 75% of the total P at each position (Figure 5.20b). The import of 19.2 mg P at position 1 and 12.6 mg at position 2 occurred in the first phase of boll filling – the seed at position 1 had accumulated 94% of its total by 40 DAF, and the seed at position 2 had accumulated 87% of its total P by 48 DAF (56 DAF at position 1). The total exported P from the mainstem section by 40 DAF at

position 1 was 2.07 mg, and from position 1 tissue 9.82 mg (although the wall tissue also accumulated 3.71 mg at this time so, on balance, the export was 6.11 mg). Together, this exported P from surrounding tissue could potentially account for 33% of the seed P, and including the P from the mainstem section, up to 45%.

At position 2, the total export from surrounding tissue was 6.32 mg (on balance 4.47 mg, accounting for the 1.85 mg increase in the wall content) by 56 DAF at position 1 (when the peak period of seed accumulation was over). This could potentially account for 41% of the accumulated P in the seed.

After the period of peak accumulation in the seeds, the P content of the leaves, stems and petioles rose, indicating either a sink (seed) driven demand for the export of P from these tissues, or an increase in the supply of P at this point in the season.

5.3.4 Potassium

As with P and N, the accumulation of K in the total tissue at each position increased through the boll filing period, and followed the pattern of dry weight accumulation. There was a greater rate of increase in the total K content of position 1 than position 2 (Figure 5.21). The total K content at position 1 peaked at 29 DAF, and then did not change ($P < 0.05$), while at position 2 the K content continued to rise until maturity. K content declined with distance from the mainstem ($P < 0.05$).

As shown in Figure 5.21b, the concentration of K in the position 1 tissue peaked at 29 DAF, after which it fell, probably due to growth dilution. The concentration of K fell substantially at 40 DAF at position 1, as it did with P, and then rose rapidly till maturity. The concentration of K in the mainstem section fell as export of K occurred (shown by a decline in the K content), however it rose again after 56 DAF at position 1, accompanied by a rise in the K content of these tissues.

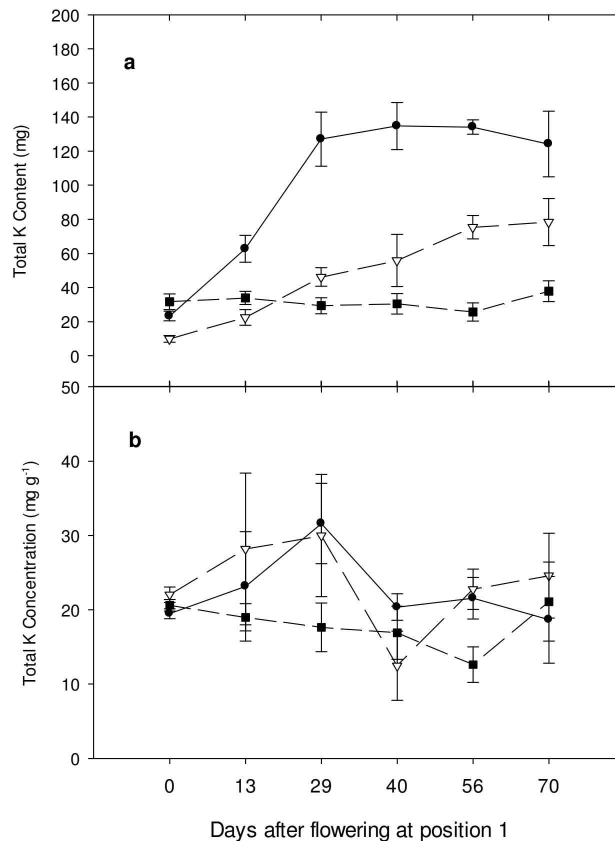

Figure 5.21 The (a) K content (mg) and (b) concentration (mg g^{-1}) in the mainstem node and leaf (—■—), position 1 tissue (—●—) and position 2 tissue (- —▽ —) from flowering at position 1 to maturity at position 2. The error bars represent +/- one standard error of the mean.

The proportional allocation of total branch K was similar to the distribution of N and dry weight. The majority of the total branch K (52%) was in position 1 tissue at maturity, with 32% in position 2 tissue and 16% in the mainstem tissue.

There was a distinct pattern in the K content of the various leaves, stems and petioles of the branch, with the mainstem maintaining a higher content than the position 1 tissue, which in turn held a higher content than the position 2 tissue ($P < 0.05$). There was less variation in the concentration of K in the tissues, with no difference in the leaf concentration throughout the measured period ($P < 0.05$) (Figure 5.22b), and a similar concentration in the K concentration in all stems (Figure 5.22d) and petioles (Figure 5.22f), particularly as the bolls reached maturity.

As with P there was an increase in the K content and concentration of the leaves, stems and petioles after 40 DAF at position 1, with most of the increase occurring after 56 DAF. Peak fruit accumulation occurred at position 1 before 29 DAF, after which time there was no import of K ($P < 0.05$).

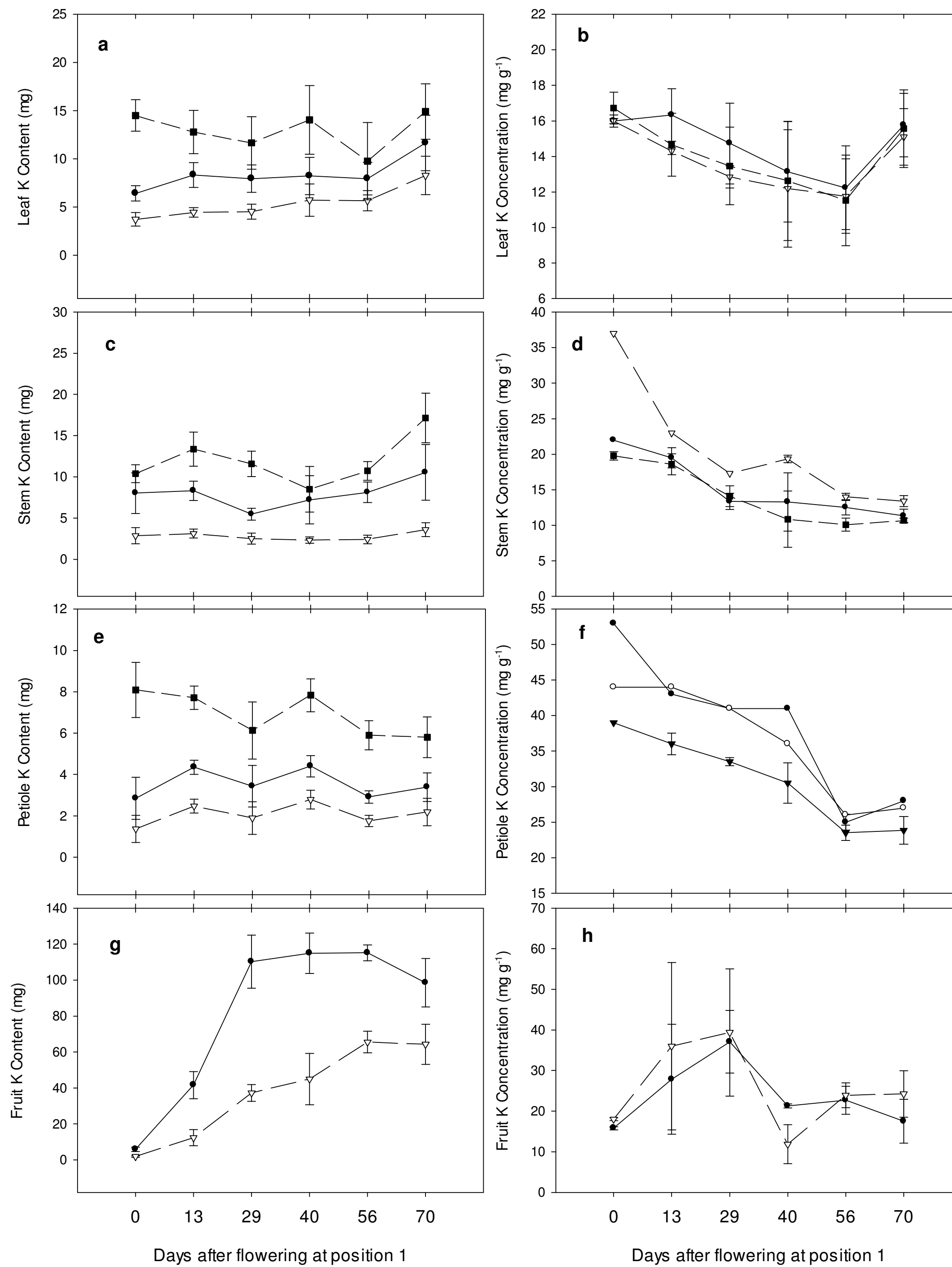

Figure 5.22 The K content (mg) (a), (c), (e) and (g) and concentration (mg g^{-1}) (b), (d), (f) and (h) of the leaves (a and b), stems (c and d), petiole (e and f) and fruit (g and h) in the mainstem (–■–), position 1 tissue (–●–) and position 2 tissue (–▽–). The error bar represents +/- one standard error of the mean.

Unlike for N and P, the boll wall contained the most K in the boll, with the highest concentration at both position 1 and 2 (Figure 5.23 a and c). The seed was also a sink for K, importing K and maintaining a high K concentration until maturity. The boll wall and seed K peaked at 40 DAF at position 1, and at 48 DAF at position 2 (56 DAF at position 1). The lint at position 1 accumulated far more K than in position 2, before exporting 73% of its K content (42.5 mg) from 29 DAF. At position 2 the lint accumulated far less K, but followed a similar pattern to that at position 1, exporting 30% of its total K (2.33 mg). While there was only a small amount of K in the bracts, they maintained a high K concentration until maturity at both positions and a steady K content from flowering. Both the bracts had a high K content before flowering at either position, with 78% of the final content accumulated prior to flowering at position 1, and 83% at position 2.

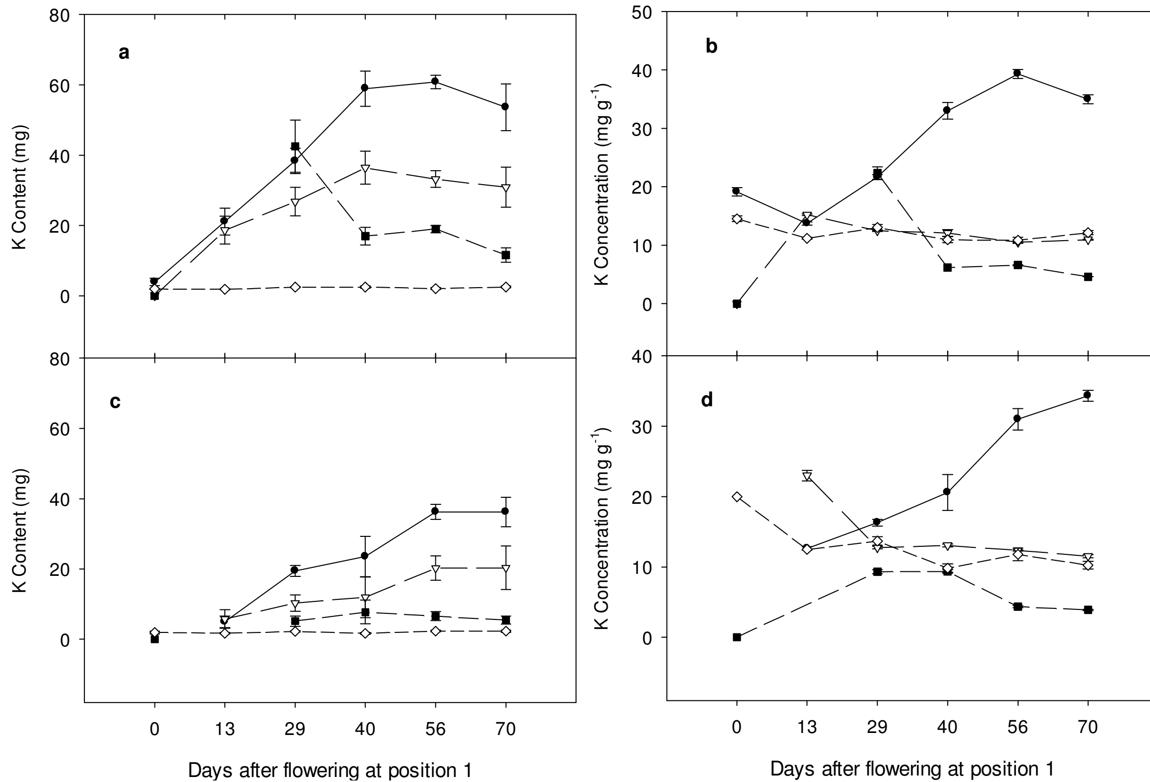

Figure 5.23 The K content (mg) (a) and (c) and concentration (mg g^{-1}) (b) and (d) in the wall (—●—), seed (- —▽- –), lint (- —■— -), bract (- –◇– – -) at position 1 (a and b) and position 2 (c and d). The error bar represents +/- one standard error of the mean.

Due to the large amount of K in the boll walls and bracts, the proportion of total K at position 1 and 2 in the seed and lint (Figure 5.24b) was much lower than the similar measurement of N and P distribution (Figure 5.8b and Figure 5.20b), reaching only 34% and 32% at positions 1 and 2 respectively at maturity. Likewise the ratio of seed and lint K to vegetative K was less than 1:1 at both positions, except for at position1 at 29 DAF, before the export of any of the lint K (Figure 5.23a). This was much lower than a similar measurement of the ratio of N and P, indicating a much higher concentration of K in vegetative parts than of N and P, and highlighting the difference in the movement and allocation of these nutrients within the same structures.

Conversely, the high wall and bract K content and concentration lead to a very high R:V and seed and lint: V ratio (Figure 5.24a and c) for K at both position 1 and 2, with no difference in the R: Total K ratio between positions ($P < 0.05$), although there was a slightly higher R:V ratio at position 2 at maturity.

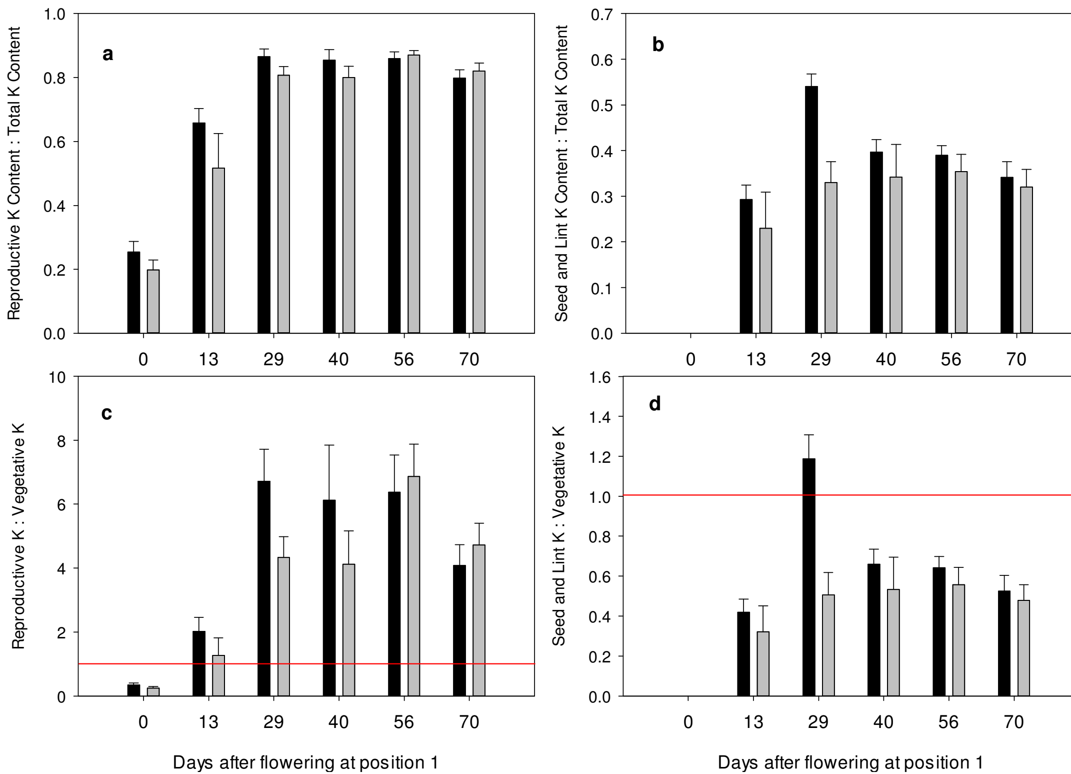

Figure 5.24 The ratio of (a) reproductive tissue (seed, lint, boll wall and bract) to total K content, (b) seed and lint to total K content, (c) reproductive tissue K to vegetative tissue (leaf, petiole and stem) P, and (d) seed and lint to vegetative K content (including the bract and boll wall) at position 1 (▬) and position 2 (▭). The red line in (c) and (d) indicates the 1:1 ratio. The error bar represents one standard error of the mean.

5.3.4.1 K redistribution

There were only a few tissues which behaved as a source of K. The leaves, bracts, stems and seed imported K until maturity. The lint, boll walls and seed at position 1, and the lint at position 2 exported some K after a significant amount of K had been accumulated.

As with P, the majority of the tissues acted as both a sink and a source of K between flowering and maturity, with the shift in behaviour mainly occurred after the accumulation in the major sinks (that is, the seed and the boll walls) was complete. The K export from the lint occurred early in the boll's development, from 29 DAF, which was well before peak accumulation in the seed and walls.

The behaviour of the leaves in terms of their K import and export was different from the other nutrients. The mainstem leaf behaved in a similar way in terms of the export of N and P. It exported 33% of the total K (4.73 mg) before 56 DAF at position 1 (coinciding with the period of peak seed and wall accumulation at position 2), and then imported a further 5.14 mg by 70 DAF. This meant that the K content of the leaf was higher at maturity than it was at flowering ($P < 0.001$). The leaves at position 1 and 2, however, behaved differently to one another. The leaf at position 1 had a fairly constant K content, though a declining K concentration, during the period of rapid K import into the seed, wall and lint at position 1. It then proceeded to import 3.17 mg, which equated to 32% of its total K at maturity between 56 and 70 DAF. Similarly leaf 2, showed only a slight increase in the K content before 56 DAF, before importing 2.61 mg K, or 32% of its total between 56 and 70 DAF. This import into the leaves, particularly during the period of peak import into the boll components suggests that the leaf was not a major source of the K into the boll.

While the petioles remained fairly neutral in terms of their total import and export of K, the stems behaved in a similar way with regards to their K content and concentration as they did with P. There was a decline in both the content and concentration in the stems until after the period of peak import into the bolls, that is 40 DAF at position 1, and 48 DAF at position 2 (56 DAF at position 1). After this point there was an increase in both the K content and concentration in the stems.

Table 5.8 Tissues classified as a source or a sink, and the total amount of K imported or exported from each group.

	Source	Sink	Sink then Source	Source then Sink
	Mainstem Leaf Petiole	Bract 1	Lint 1	Stem 1
		Bract 2	Lint 2	Stem 2
		Leaf 1	Seed 1	Mainstem Leaf
		Leaf 2	Wall 1	
		Mainstem Node segment		
		Petiole 1		
		Petiole 2		
		Seed 2		
		Wall 2		
Export (mg P)	2.29		46.01	8.09
Import (mg P)		74.53	143.6	11.49
Total Export	56.39			
Total Import	229.62			
Balance	Import of 173.23mg (72% of total branch content at maturity)			

The seed and boll walls (the major sinks of K), accounted for 35% of the total branch K at position 1 and 24% at position 2, a total of 59% of the total branch K. At position 1, the total export from all tissues by peak K content in the walls and seeds (at 40 DAF) was 28.1 mg – coming predominantly from the lint. Since the lint accumulated 42.52 mg by 29 DAF, during which time the only export from a position 1 tissue was 2 mg from the stem. Therefore, it can be reasonably concluded that the 84.5 mg K in the position 1 boll walls and seed at maturity (after the export of 12.77 mg) was not supplied by redistribution of leaf, stem, petiole or bract nutrients.

The total export from the lint, seed and walls after the peak period of seed and wall accumulation (40 DAF) was 5.37 mg from the lint, 5.56 mg from the seed and 5.33 mg from the walls. This 16.26 mg is more than the 6.87 mg imported by the leaf, stem and bracts during this time – indicating that there may be some exchange of K from the boll back to the vegetative structures, or to leaves, stems or bolls removed from the boll which initially accumulated the nutrients. There was no similar export of K from the seed and boll walls at position 2, though accumulation stopped after 56 DAF ($P = 0.003$) (Figure 5.23c).

Looking at the changes in the content of K in each tissue, there does not seem to be substantial evidence linking the export of K from vegetative structures with the import of K into the walls, seed and lint of the developing boll. This implies that the sink strength of the bolls for K is lower than for N or P, or that the very early import of substantial amounts of K into the bolls is supplied by remote sites, or through root uptake, and not by the surrounding tissue.

5.3.4.1.1 Rb import and export from the mainstem leaf

There was no difference between the dry weight (g) or K content (mg) of any of the tissues along the sympodial branch at node 11, or to the leaf and fruit tissues above and below node 11 of the plants injected with the ^{15}N and RbCl solution (hereafter referred to as "mainstem leaf treatment") and those injected with water (the "control" treatment) ($P > 0.05$).

Between 14 days and 61 days after the application of the labeled solution (at flowering at position 1) there was no change in the total K content in mg in the labeled mainstem leaves ($P < 0.05$). There was, however, a large export of Rb (0.43 mg, or 85.4%) from the labeled leaf (Figure 5.25). Assuming that the Rb export is representative of the export of total K from the leaf (although import may also have occurred); the total export between 14 and 61 days after position 1 flowering would have been 6.13 mg.

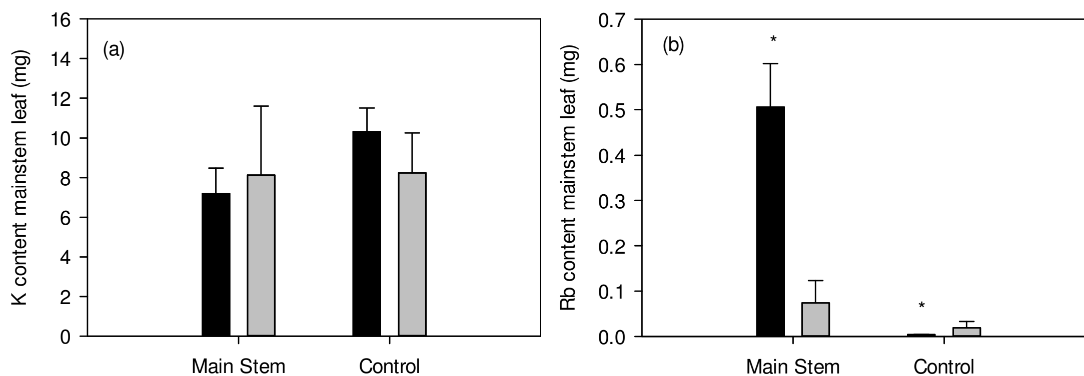

Figure 5.25 The mean K (a) and Rb (b) content (mg) of the mainstem leaf in the branches to which RbCl was applied to the mainstem leaf, and the control branches at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments.

As with the analysis of the ^{15}N data, the fate of the applied solution can be measured by examining the accumulation of Rb in the surrounding tissue within the node, and in the tissues above and below the labeled leaf.

There was some Rb found in the control tissues at both dates when samples were analysed, as shown in Figure 5.25, Figure 5.26, Figure 5.27 and Figure 5.28. As with the ^{15}N this may have been due to contamination, or background Rb in the soil. There was no difference in the K content in any of the analysed tissues. Of the tissues on the labeled node (node 11), the Rb content was higher than in the control plants in the vegetative tissues – that is the 1st and 2nd position leaves and the 1st position stem ($P < 0.05$), but not in the seed or boll walls at either 14 or 61 DAF ($P > 0.05$). There was no difference in the mainstem leaf Rb content of the mainstem at 61 days after labeling. The increased Rb in the vegetative tissues at node 11 is given in Figure 5.26. Since there is no difference in the Rb content of the reproductive tissue on node 11, the data is not shown.

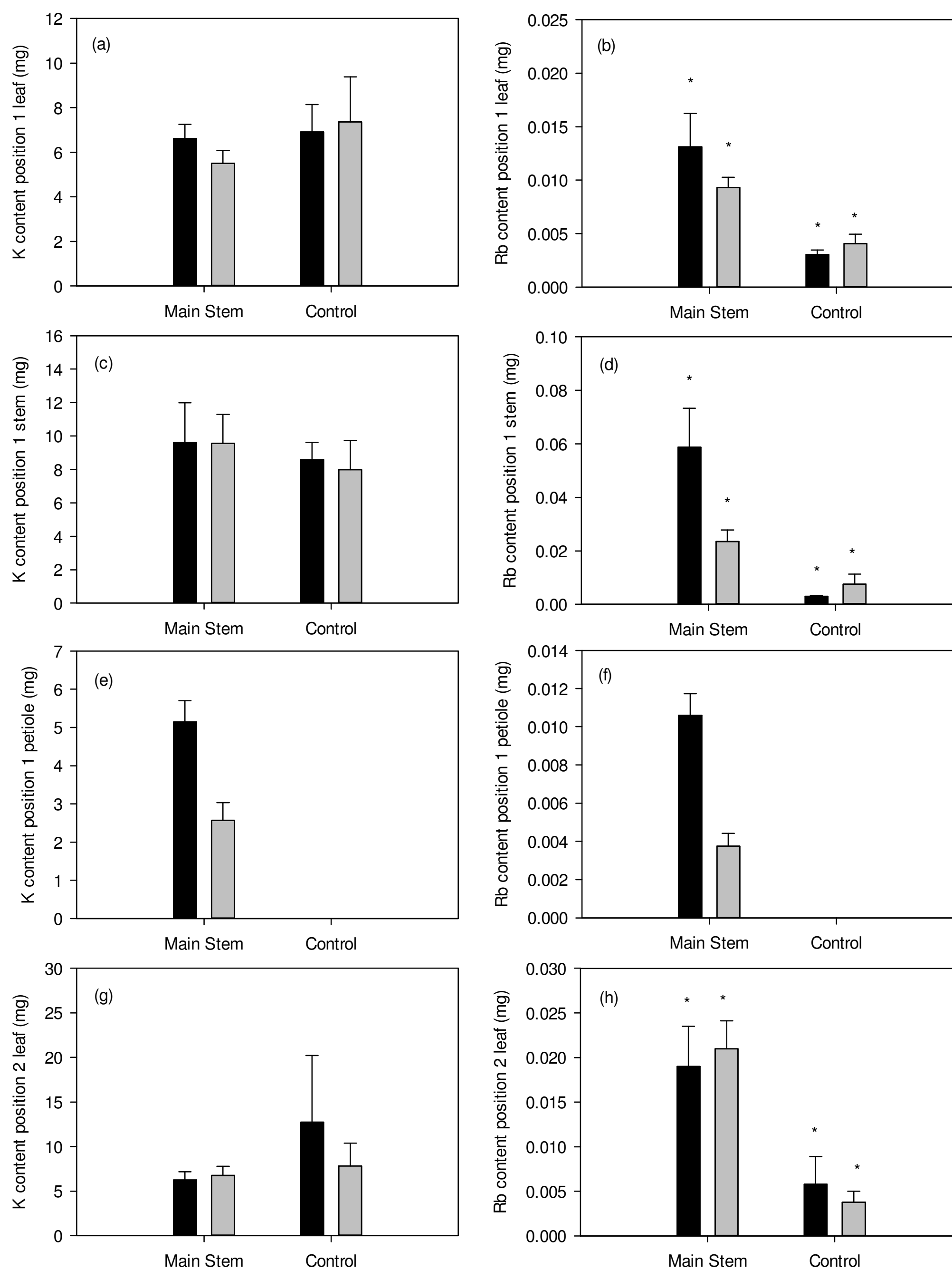

Figure 5.26 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) of the position 1 leaf (a and b), position 1 stem (c and d), position 1 petiole (e and f) and position 2 leaf (g and h) in the branch to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments.

While at node 11 there was no redistribution of the Rb from the mainstem leaf to the reproductive tissue, there was an increase in the Rb content of the fruit below the labeled

node, at nodes 1-9 (*P* = 0.008) and at node 10 (*P* = 0.019). There was no difference in the Rb content of the fruit at node 12, or 13 and above after 14 days, but after 61 days there was a significant increase in the Rb content (*P* = 0.027) (Figure 5.27). There was no difference in the Rb content compared with the control treatment in the leaves above or below the labeled node, except for the leaves of nodes 13 and above, which had a higher Rb content 14 days after labeling (*P* = 0.04) (Figure 5.28).

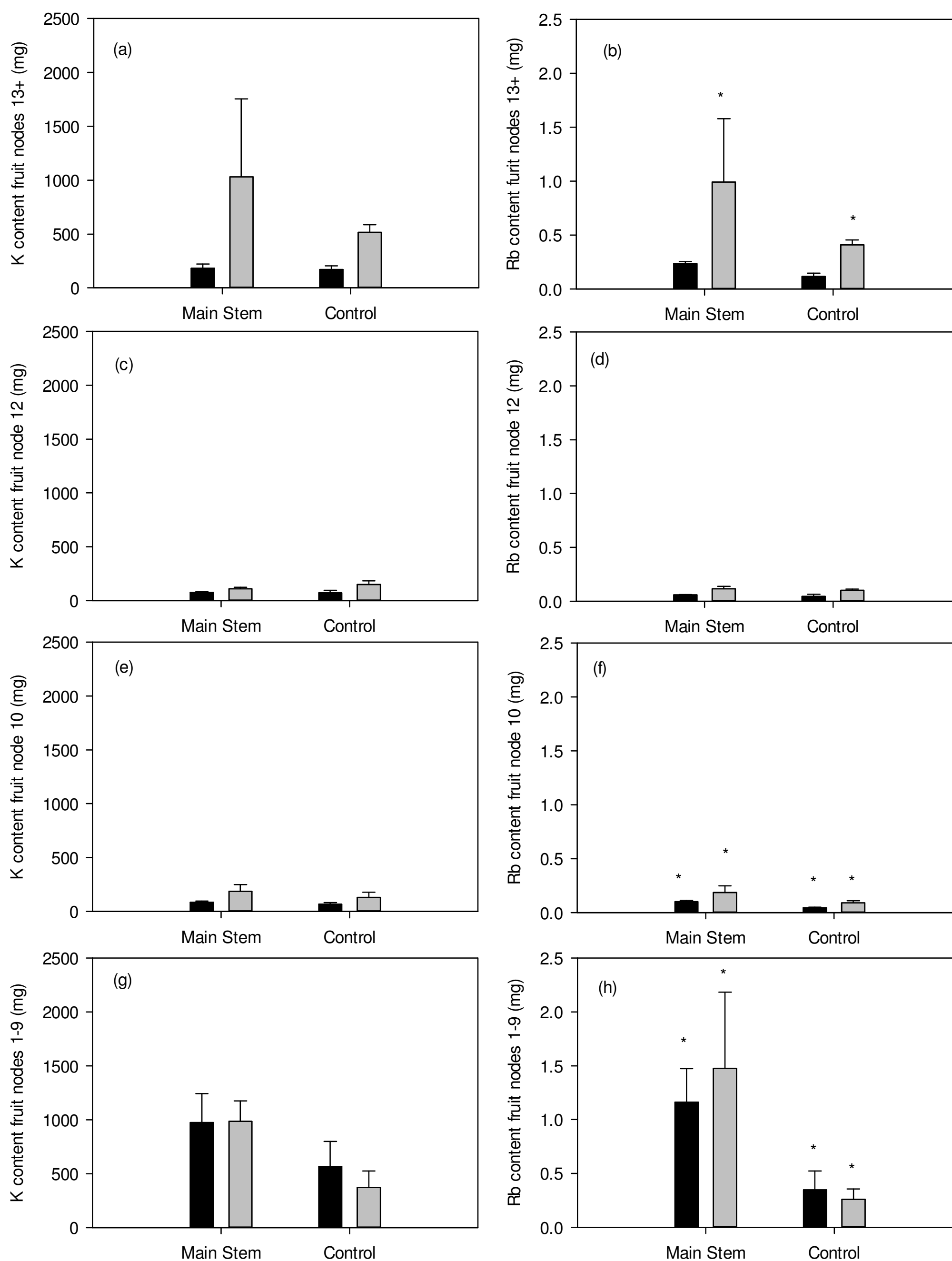

Figure 5.27 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the fruit at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments.

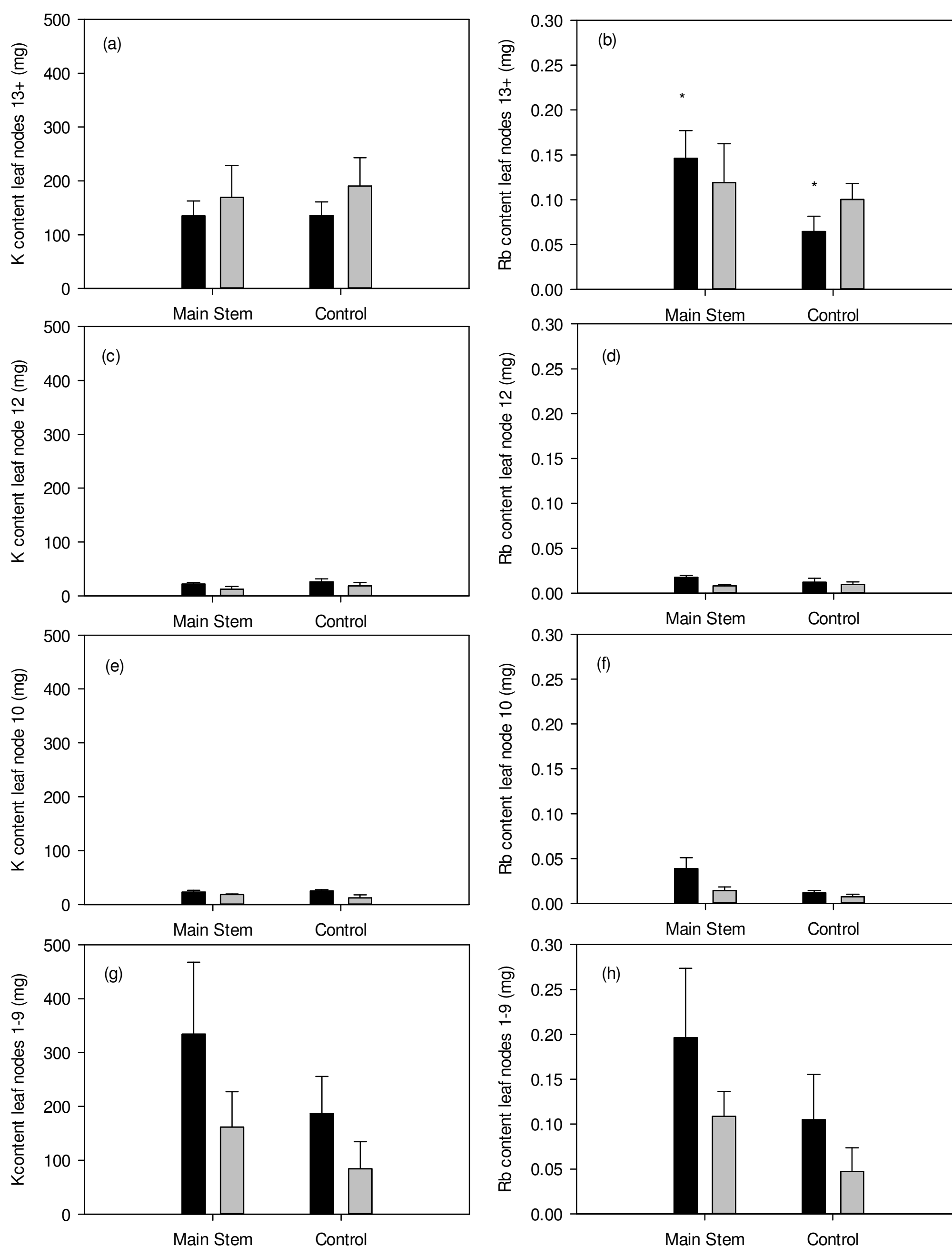

Figure 5.28 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the leaves at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the mainstem leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments.

Table 5.9 shows the total Rb content, adjusted for the mean control Rb content of each tissue and the proportional distribution of the Rb. Where there was no difference from the control tissue, the amount is recorded as 0. The majority of the Rb was distributed to the fruit below

the labeled branch, and at 61 days after labeling 29% was found in the fruit at the top of the plant (nodes 13+). The increase in the Rb content occurred only in the fruit at nodes removed from the mainstem leaf to which the label was applied.

Table 5.9 The mean adjusted Rb content (mg) in each tissue which had a higher Rb content than the control (at a significance of *P* < 0.05) of the mainstem leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the Rb in each.

Tissue	Rb content 14 DAF (mg)	Rb content 61 DAF (mg)	Rb content 14 DAF (% of total)	Rb content 61 DAF (% of total)
MS Leaf	0.502	0.055	30.1	2.8
Leaf 1	0.010	0.005	0.6	0.3
Stem 1	0.056	0.016	3.3	0.8
Leaf 2	0.013	0.017	0.8	0.9
Seed 1	0	0	0	0
Walls 1	0	0	0	0
Leaf 13	0.081	0	4.9	0
Fruit 13	0.120	0.581	7.2	29.0
Leaf 12	0	0	0	0
Fruit 12	0.013	0.017	0.8	0.8
Leaf 10	0	0	0	0
Fruit 10	0.057	0.098	3.4	4.9
Leaf 9	0	0	0	0
Fruit 9	0.814	1.214	48.9	60.6
Total Rb (mg)	1.665	2.003		

A high proportion of the total Rb was exported from the mainstem leaf within the 14 days from labeling, although between the two dates 85.4% of the Rb from the labeled leaf was exported. At maturity, 97% of the total Rb was contained in tissues other than the mainstem, labeled leaf, equating to an export of 7.89 mg of K (assuming that the K content of the leaf at 14 days was the same as at day 0, consistent with the results from experiment 8). The contribution of K from the mainstem leaf, in terms of proportional supply to the surrounding tissues was very low. Redistribution of K from the mainstem leaf accounted for between 0

and 1% of the total K found in the tissues of the labeled plant at maturity, the highest proportional contribution being to the 2nd position leaf on node 11, at 1.03%.

Table 5.10 The equivalent supply and relative contribution of K redistribution from the mainstem leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 7.89 mg

Tissue	mg K supplied from MS Leaf at node 11, position 1 maturity	% of total K
Leaf 1	0.02	0.39
Stem 1	0.06	0.68
Leaf 2	0.07	1.03
Seed 1	0	0
Walls 1	0	0
Leaf 13	0	0
Fruit 13	2.35	0.23
Leaf 12	0	0
Fruit 12	0.07	0.06
Leaf 10	0	0
Fruit 10	0.40	0.21
Leaf 9	0	0
Fruit 9	4.92	0.50

5.3.4.1.2 Rb import and export from the 1st Position Leaf

There was no difference between the dry weight (g) or K content (mg) of any of the tissues along the sympodial branch at node 11 between the plants injected with the ^{15}N and RbCl solution and the control plants. Nor was there a difference between the dry weight or K content of the leaf and fruit tissues above and below node 11 of the treated (hereafter referred to as "1st leaf treatment") and control plants (the "control" treatment) ($P > 0.05$).

Between 14 days and 61 days after the application of the labeled solution (at flowering at position 1) there was no change in the total K content in mg in the labeled 1st position leaves ($P < 0.05$). There was, however, a large export of Rb from the labeled leaf (0.42 mg or 86.4%) (Figure 5.29). There was no difference in the Rb content of the labeled or control leaf at maturity ($P = 0.135$). Assuming that the Rb export is representative of the export of total K

from the leaf (although import may also have occurred); the total export between 14 and 61 days after position 1 flowering would have been 5.44 mg.

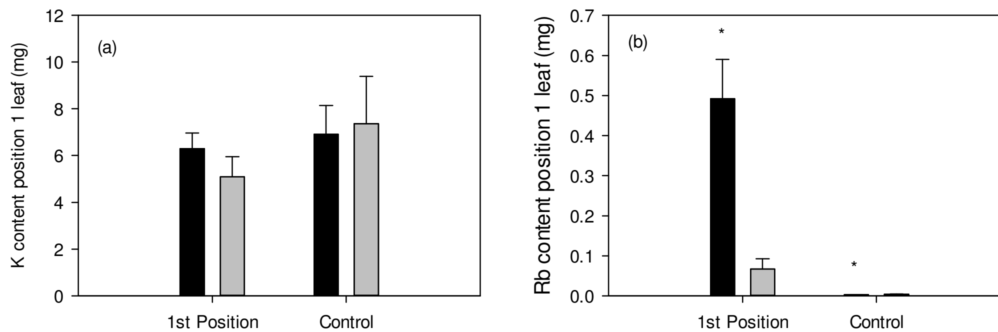

Figure 5.29 The mean K (a) and Rb (b) content (mg) of the 1st position leaf in the branches to which RbCl was applied to the 1st position leaf, and the control branches at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at $P < 0.05$ between the treatments.

There was an increase in the Rb content of both vegetative (Figure 5.30) and reproductive (Figure 5.31) tissues along the sympodial branch at node 11 to which RbCl was applied. The mainstem leaf had a higher Rb concentration at 14 days after labeling, after which it declined and was the same as the control leaf at 61 days after labeling – probably due to the export of this Rb to other organs (Figure 5.30b). The Rb content in the leaf at position 2 (Figure 5.30f), and in the stem at position 1 (Figure 5.30h) and the mainstem node segment (Figure 5.30d) was higher than the control at 14 days after labeling, and despite a decrease in the content of 0.05 mg, 0.05 mg and 0.06 mg respectively, remained higher at 61 days after labeling ($P < 0.05$).

The seed (Figure 5.31b) and boll walls (Figure 5.31d) of the position 1 fruit on the labeled branch had a higher Rb content at 14 and 61 days after labeling, with no extra import of Rb occurring after 14 days ($P < 0.05$).

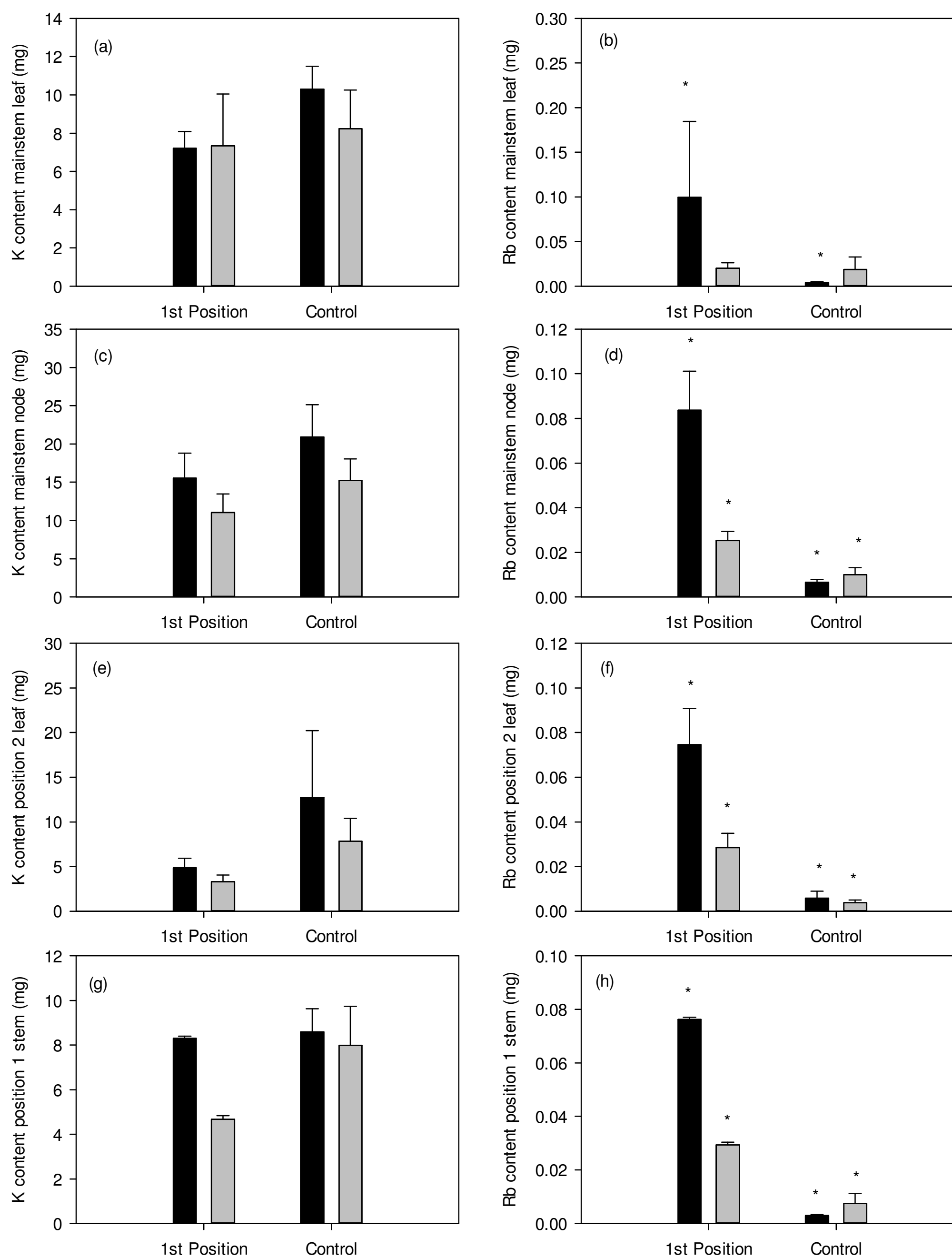

Figure 5.30 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) of the mainstem leaf (a and b), mainstem node segment (c and d), position 2 leaf (e and f) and position 1 stem (g and h) in the branch to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF (■) and 61 DAF (□) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at *P* < 0.05 between the treatments.

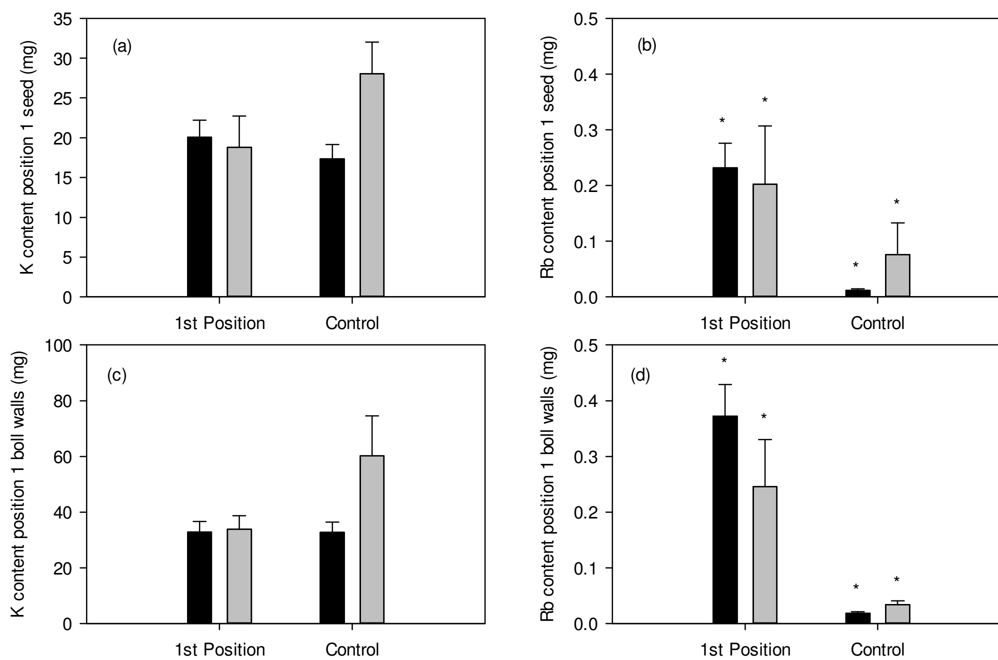

Figure 5.31 The K content (mg) (a and c) and Rb content (mg) (b and d) of the position 1 seed (a and b) and position 1 boll walls and bracts (c and d) in the branch to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments.

There was no increase in the Rb content ($P > 0.05$) of the leaves (Figure 5.32) or fruit (Figure 5.33) of the nodes above the labelled 1st position leaf. There was an increase in the Rb content of the leaves of the node immediately below the labelled leaf at both 14 and 61 days after labelling ($P > 0.05$) (Figure 5.32f), and in the fruit at 61 days after labelling ($P = 0.04$) (Figure 5.33f), and an increase in the leaves and fruit of nodes 1-9 at both 14 and 61 days after labelling ($P > 0.05$) (Figure 5.32h and Figure 5.33h).

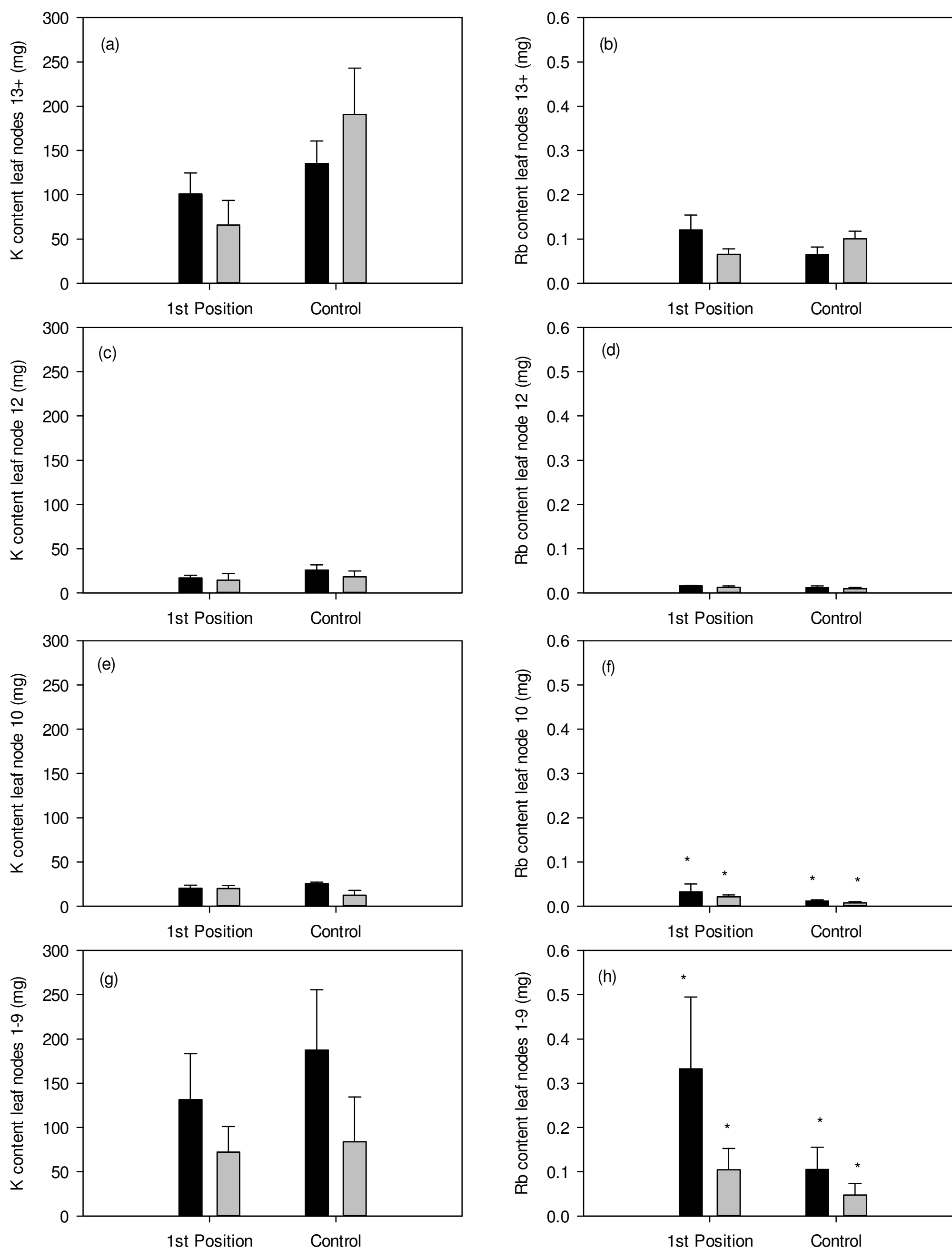

Figure 5.32 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the leaves at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments.

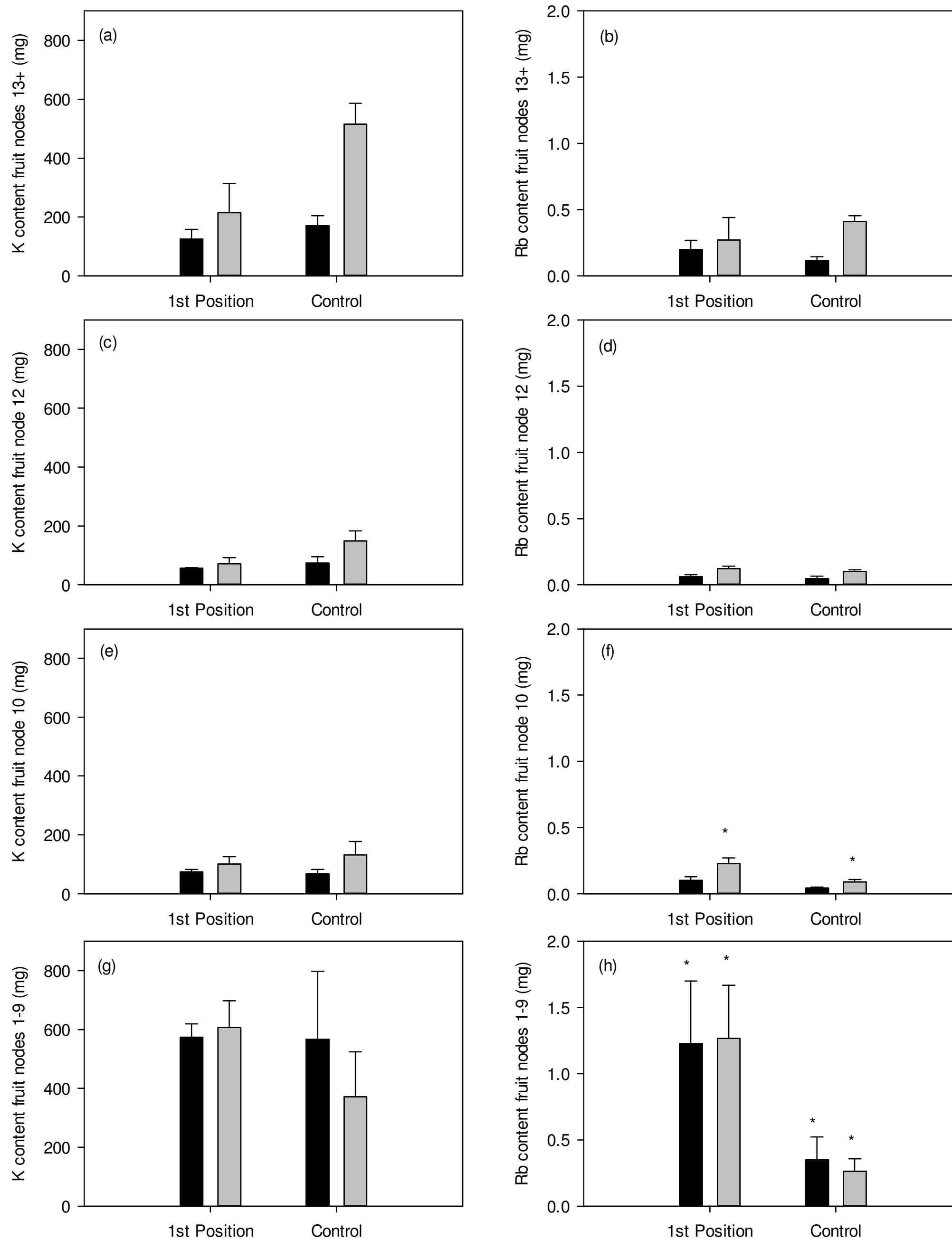

Figure 5.33 The K content (mg) (a, c, e and g) and Rb content (mg) (b, d, f and h) in the fruit at nodes 1 – 9 (g and h), 10 (e and f), 12 (c and d) and 13 – top (a and b) of the plants to which RbCl was applied to the 1st position leaf, and the control branch at 14 DAF (▬) and 61 DAF (▭) at position 1. The error bar represents the standard error of the mean, a * represents a significant difference at 0.05 between the treatments.

Table 5.11 shows the total Rb content, adjusted for the mean control Rb content of each tissue and the proportional distribution of the Rb. Where there was no difference from the control tissue, the amount is recorded as 0. The majority of the Rb was distributed between

the nodes below the labeled branch and the tissues along the sympodial node. After 14 days, 54% of the total Rb was still in the labeled branch, with 58% in vegetative tissues and 42% in the reproductive tissues. The mainstem node and stem tissues accounted for a significant proportion of the total Rb, following the same trend of high K content in the connective tissue recorded in experiment 8. After 61 days only 25% of the total Rb remained in the tissues at node 11, with 85% of the total found in the seed and boll walls at position 1. Of the remaining 75%, 95% was found in the fruit at nodes 1-10, below the labeled branch.

Table 5.11 The mean adjusted Rb content (mg) in each tissue which had a higher Rb content than the control (at $P < 0.05$) of the 1st position leaf treatment plants at 14 and 61 days after treatment, and the proportion of the total amount of the Rb in each.

Tissue	Rb content 14 days after labeling (mg)	Rb content 61 days after labeling (mg)	Rb content 14 days after labeling (% of total)	Rb content 61 days after labeling (% of total)
MS Leaf	0.095	0	3.7	0
MS Node	0.077	0.015	3.0	0.9
Leaf 1	0.488	0	19.1	0
Stem 1	0.073	0.022	2.9	1.4
Leaf 2	0.069	0.025	2.7	1.5
Seed 1	0.220	0.126	8.6	7.8
Walls 1	0.354	0.212	13.8	13.1
Leaf 13	0	0	0	0
Fruit 13	0	0	0	0
Leaf 12	0	0	0	0
Fruit 12	0	0	0	0
Leaf 10	0.020	0.014	0.8	0.9
Fruit 10	0.058	0.138	2.3	8.5
Leaf 9	0.228	0.057	8.9	3.5
Fruit 9	0.879	1.005	34.3	62.3
Total Rb (mg)	2.562	1.613		

At maturity, there was no difference in Rb content between the labeled and control leaf, equating to an export of 6.3 mg of K (assuming that the K content of the leaf at 14 days was the same as at day 0, in line with the results from experiment 8). Redistribution of K from the 1st position leaf accounted for between 0 and 1% of the total K found below the labeled node

at maturity. Redistribution accounted for between 1 and 2% of the total K in the reproductive tissues at position1, and for 1.42% of the total K in the position 2 leaf.

Table 5.12 The equivalent supply and relative contribution of K redistribution from the 1st position leaf to each tissue along the sympodial branch and the leaf and fruit above and below the mainstem leaf, assuming a redistribution of 7.89 mg

Tissue	K supplied from 1st position Leaf at node 11, position 1 maturity (mg)	K from 1st position leaf (% of total)
MS Leaf	0	0
MS Node	0.06	0.54
Stem 1	0.09	0.89
Leaf 2	0.10	1.42
Seed 1	0.49	1.94
Walls 1	0.83	1.54
Leaf 13	0	0
Fruit 13	0	0
Leaf 12	0	0
Fruit 12	0	0
Leaf 10	0.05	0.30
Fruit 10	0.54	0.29
Leaf 9	0.22	0.14
Fruit 9	3.92	0.40

5.4 Discussion

In this chapter the nutrient distribution and redistribution were examined on a smaller scale than the previous chapter – focusing on one sympodial branch at the 11th node. This chapter aimed to describe the behaviour of specific tissues as a source or a sink, to quantify the demand for nutrients from a single boll, and to determine the potential redistribution from vegetative and reproductive tissues for supplying adjacent sinks. This information is helpful in terms of explaining and predicting the demand for nutrients from developing bolls, and in relating this demand to potential supply from redistribution or root uptake.

5.4.1 Nutrient distribution and growth patterns along the sympodial branch

The first aim of this chapter was to describe the pattern of N, P and K accumulation and redistribution along a single sympodial branch, in order to explain and predict the N, P and K requirements of developing bolls and describe the behaviour of different tissues as a source or a sink. The timing of the changes in the dry weight, N, P and K at each position along the branch was similar to that previously reported for nodes 8, 10, 11 and 12 (Thompson *et al.* 1976; Constable and Rawson 1980a; Constable and Rawson 1980b; 1982; Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999; Zhang *et al.* 2007). The majority of the dry weight, N, P and K was found in the position 1 tissue, with nutrient concentration declining with distance from the mainstem. The mainstem leaf was the largest, followed by the sympodial branches in order along the branch, and the 1st position boll was larger than the second. The fruit was the major sink for N, P and K along the branch.

In experiment 8, the dry weight and nutrient content was measured only from flowering at position 1, meaning that much of the expansion and development phase of the mainstem and 1st position leaves were not recorded. This is in contrast to previous studies examining the development of the sympodial branch from the unfurling of the mainstem leaf (Zhu and Oosterhuis 1992), the unfurling of the 1st position leaf (Mutsaers 1984), or the development of a square at position 1 (Zhao and Oosterhuis 1999), which found three common development patterns. Firstly that the mainstem leaf dry weight and N content peaks at flowering at position 1 (around 21 days after unfurling), secondly that the sympodial leaf peak dry weight and N content both occur *after* flowering at the corresponding fruiting position, the peak N content occurring around 7 days after peak dry weight, and thirdly, that the period after flowering is the time when export of nutrients from leaves is occurring (Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999; Zhang *et al.* 2007; Mullins and Burmester 2010). Since the dry weight and N content at flowering was similar to those reported in these published studies, it is assumed that the pre-flowering nutrient dynamics of the branch are similar to those previously reported, and therefore that the peak mainstem leaf dry weight and N content occurred at day 0 (at flowering at position 1).

The timing of the peak nutrient content and export of nutrients is an important feature of the nutrient dynamics of the branch. Circumstantially, a link between the demand for nutrients from the fruit and the export from the leaves has been made to explain the redistribution of

nutrients from the leaves based on the timing of export from the leaves and import into the bolls (e.g. Thompson *et al.* 1976; Patterson *et al.* 1978; Leffler and Hunter 1985; Krieg and Sung 1986; Boquet *et al.* 1994; Boquet and Breitenbeck 2000). The accumulation of dry weight, N, P and K each showed different patterns, and varied between the tissues studied and their position along the branch. The N, P and K content of the mainstem leaf was assumed to peak at flowering, while sympodial leaves did not appear to reach a definite peak in N content, and accumulated P and K through the boll filling period. The stems all along the branch continued to accumulate nutrients throughout the fruiting period, and while the N, P and K content of the petioles at position 1 and 2 did not change through the fruiting period, the mainstem petiole N, P and K content peaked at position 1 flowering. The timing of N, P and K demand from the fruit varied between the nutrients. The lack of change in the nutrient content of the sympodial leaves compared to the mainstem leaf confirms previous research, Zhu and Oosterhuis (1992) commented that "the sympodial leaves appeared to export only a small amount of their total N". Although similar results are not available for K, Zhao and Oosterhuis (1999) found a similar accumulation pattern for N and P in leaves.

The largest sink for N along the branch was the seed, which continued to import N at a higher rate than it accrued dry weight until 40 DAF. The lint and boll walls accumulated N initially and then exported a significant proportion of the total, after 13 days (boll walls) and 29 days (lint). Rosolem and Mikkelsen (1989) found that there was considerable remobilisation of N from the boll walls and lint, suggesting that these were sources of the seed N, concluding that up to 10% of the seed N could come from the boll walls. This assertion, as with many relating to the redistribution of N and other nutrients, assumes that the difference in nutrient content from the peak to that at maturity is equal to redistribution, and that the allocation of redistributed nutrients is to adjacent sinks. While it seems logical that the nutrients within the boll would be allocated to the same boll there is no quantitative data to prove this theory. In my experiment the increase in seed N from 13 to 56 DAF was 75.2 mg, and the total export from the boll walls was 13.8 mg, or 18% of the increase in the seed content (assuming all the N was allocated to the seed). This is higher than that suggested by Rosolem and Mikkelsen (1989). A further 26 mg could have been provided by export from the lint, equating to a total of 34.5% of the total seed N accumulated after this time. This means that redistribution between boll structures may account for a significant proportion of the total N in the seed, while the total N content of the fruit as a whole appears unchanged. This suggests that early

supply of N to the fruit may be of more significance that later, that is the first 2-3 weeks after flowering may be the most significant time for N supply to developing bolls.

The boll at position 2 seemed to lack access to the same amount of N in the first few weeks after flowering. During this time the boll wall, lint and seed content were all approximately half of the content in the position 1 boll. The rate of increase in seed N content was very low until 40 DAF at position 1 – the timing coinciding with the cessation of import into the seed at position 1. This correlation in timing suggests that there is a hierarchy in sinks, with the N being allocated to the position 1 boll at the expense of the position 2 boll, which accumulated N once the position 1 boll had reached peak content and maturity. Many authors have recorded the lower boll weight and nutrient content of position 2 bolls (and those further along the branch) (Benedict *et al.* 1973; Jones *et al.* 1974; Thompson *et al.* 1976; Patterson *et al.* 1978; Krieg and Sung 1986; Wullschleger and Oosterhuis 1990b; Boquet *et al.* 1994; Heitholt 1994; Heitholt and Schmidt 1994; Read *et al.* 2006; Li *et al.* 2009). This is confirmed by this data, although it is supported in a new way, explaining the observation through the timing of demand from the two bolls, and the preferential allocation to the position 1 fruit at the expense of that at position 2. Theoretically then, the size of the position 2 boll is limited by the supply of N and, if extra N fertiliser was applied the N content of the position 2 boll, and its size and lint yield, may be increased. Further research examining the application of N during flowering and its impact on the size of position 2 bolls could confirm this.

The pattern of P accumulation and content changes was similar to that of N, the seed being the major sink along the branch and continuing to accumulate P until 40 DAF at position 1 and reaching a peak at 70 DAF in the position 2 boll. Similarly to the N, the rate of accumulation in the position 2 seeds increased after the accumulation stopped in the position 1 tissue, confirming the same conclusions about the priorities of sink supply as described for N. As with N, the boll walls and lint exported a large proportion of their P after reaching a peak at 29 DAF, exporting a total of 4.4 mg of P. This P, if accumulated by the seed, would account for 62% of the P accumulated in the seed between 29 and 40 DAF.

The peak period of K demand from the fruit occurred earlier than for N and P. The peak K content of the fruit at position 1 occurred at 29 DAF at position 1, and in the position 2 fruit

at 56 DAF. The position 1 fruit accumulated more K than the position 2 fruit, and at a much higher rate, particularly in the first 29 DAF. At both positions, the boll wall was the major sink for K, which continued to accumulate K until 56 DAF. The lint at position 1 accumulated the same amount of K as the boll walls, and more than the seed at 29 DAF, after which the content and concentration declined by 73%. At position 2, the K content of the lint was much lower than position 1, although it also declined after 40 days. This high concentration of K in the boll walls and lint confirms previous analysis of boll tissues by Leffler and Tubertini (1976), who also found a significant decline in the K content of the fibre. The early import of K into the fibre has been linked to the osmotic regulation of fibre elongation (Dhindsa *et al.* 1975), and the lower lint dry weight in boll 2 may be linked to the lower lint K content. Looking at the changes in the content of K in each tissue, there does not seem to be substantial evidence linking the export of K from vegetative structures with the import of K into the walls, seed and lint of the developing boll.

The timing of boll demand for K is earlier than for N or P, and yet there was no significant drop in the K content of the leaves or stems of the surrounding tissues, except for a minimal change in the K content of the mainstem leaf. The Rb tracing experiment explored the proportion of boll K supplied by the mainstem and 1st position leaves, but circumstantially there does not seem to be any synchronicity in the timing of demand and supply. The major source of K was the lint, confirming the theory proposed by Leffler and Tubertini (1976) that there seems to be a "physiological continuum" between the boll tissues, recycling the nutrients within the boll depending on function and timing of development. This equally implies that the very early import of substantial amounts of K into the bolls is supplied by remote sites, or through root uptake, not by the surrounding tissue. As with N and P the rate of increase of K in the seed and boll walls increased after the peak content was reached at position 1 (at 40 DAF), indicating that the available K was allocated to the first position boll preferentially over the second.

When analysed by position, there was no difference in the ratio of seed and lint, or total boll mass to vegetative dry weight, N, P or K at maturity between the two positions. This indicates that, at both fruiting positions, the boll and leaf structures were proportioned according to each other and that some relationship between the size and nutrient content of each probably exists. A key question raised by this relationship, is whether the leaf and boll

sizes are related to one another by a limitation in supply or demand, that is, does the demand for carbon assimilates, nutrients or water from the developing boll limit the growth of the subtending leaf, or does the leaf size (which is smaller at position 2) limit the potential size of the subtending boll. There was a clear hierarchy of sinks, the position 2 boll accumulating N, P and K at a much higher rate after the accumulation in the position 1 boll had stopped. This relationship suggests that boll size and nutrient content is limited by the source of nutrients. The proportion of boll nutrients sourced from the subtending leaf was examined in experiment 9, however the relatively small change in the N, P and K content of the subtending leaves to the bolls suggest that the source limitation to boll growth is not the subtending leaf. The similarity in the ratio of the leaf and fruit size at each position therefore is possibly due to carbon supply or a limitation in the transport rate of nutrients and water to the positions further along the branch.

5.4.2 Nutrient redistribution from individual leaves

The use of stable isotopes or of a tracer molecule in defining and quantifying nutrient redistribution from one tissue to another has been successfully used to measure the redistribution of carbon, (Benedict *et al.* 1973; Benedict *et al.* 1976; Isebrands *et al.* 1976; Stephenson and Wilson 1977; Constable and Rawson 1982), N (Hocking and Steer 1995; Bondada *et al.* 1996; Andersson *et al.* 2005; Britto and Kronzucker 2006; Dreccer 2006; Tischner 2006), P (Dorahy *et al.* 2007; Dorahy *et al.* 2008) and K (Richards 1941; 1944; Hafez and Rains 1972; Schenk and Feller 1990; Ho *et al.* 1996) between plant parts. In this study a novel method of ^{15}N and Rb application was used to add the tracer to the plant tissue, and to calculate the redistribution of N and K from individual leaves to other plant parts. All assessment of redistribution using stable isotopes or tracers depends on the incorporation of the applied molecule into the plant tissue in question. A key question, therefore, is whether the direct injection of the solution to the vascular tissue at the base of the leaf would result in the incorporation of the tracer into the leaf tissue (and therefore mimic redistribution of the nutrients) or would enter the xylem and phloem solution and be moved by diffusion through the vascular system, rather than the active incorporation or transport of the tracers from one tissue to the other. There are several factors which indicate that the injection of the solution directly into the base of the leaf resulted in the incorporation of the solution into the leaf tissue, therefore mimicking the movement of N and K from the leaf;

1) There was a significant increase in the ^{15}N and Rb content of some tissues and not others, indicating that the movement of the solution from the labelled leaf was not ubiquitous, but showed some active process was at work.
2) Had the solution been moved by diffusion through the vascular solution in the xylem and phloem, the concentration of Rb and ^{15}N (or the relative concentration) in individual tissues would be similar or the same, since both molecules were applied on the same solution. The distribution pattern of the two minerals was different, for example there was no increase in the ^{15}N content of any tissue below the labelled 1st position leaf, but there was an increase in the Rb content of the leaves and fruit at node 10, and the pooled leaves and fruit from nodes 1-9. This indicates that there was some differentiation in the movement of the solution from one point to another. (Although it does not necessarily follow that the differentiation came from active transport from the leaf, it does indicate that the distribution of the ^{15}N and the Rb was not merely through bulk flow in the xylem or diffusion through the phloem sap).
3) While much of the distribution of the ^{15}N and Rb through the plant (in terms of the increase in ^{15}N or Rb content compared to the control tissue) occurred in the first 14 days after application, there was evidence of active transport from the leaves after this point. After 14 days it could be reasonably concluded that the ^{15}N or Rb in the leaf had been incorporated into the tissue itself, rather than diffused through the xylem and phloem sap. By calculating redistribution only as the proportion of ^{15}N or Rb transported out of the labelled leaf between 14 and 60 DAF the amount of N and K redistributed from the leaf in this time period is accurately represented. An earlier date than 14 days could have been chosen, which may have improved measurement of the K redistribution in particular, especially since much of the K accumulation in the boll walls, lint and seeds occurred in the first two weeks after flowering. The original protocol aimed to take the samples at 10 days, but rainfall prevented access to the site till 14 DAF. A further replicated experiment should be carried out, with sampling starting at 5 days after labelling to capture this early redistribution, and with frequent samples taken between flowering and maturity.

Since there was no difference in dry weight or nutrient content between the treated and control leaves, and their surrounding tissues during the 70 DAF, it can be assumed that the injection of the leaves did not make a difference to the leaf functioning, or to the cycling of

water and nutrients out of the leaves. This has been a criticism of direct application of an isotope solution into the phloem via the wick method, which involves the plant absorbing the solution via a cotton wick inserted into a hole in the stem, and has been used successfully on softer stems such as the petiole of the cotton plant (Wichern *et al.* 2009). The method used in experiment 9, through the injection of the plant with the sample rather than the absorption of the solution by the plant would potentially cause more damage to the plant parts injected, however no change in dry weight or nutrient changes were observed. Microscopic imagery to examine cell damage could further support the applicability of this method and the functioning of the vascular tissue in the leaf and petiole.

Since not all tissues were analysed for ^{15}N, the recovery rate cannot be calculated, the stems, roots and 3rd and 4th position sympodial tissues were not accounted for. The relatively high concentration of ^{15}N in the position 2 leaf indicates that potential redistribution to the other position 2 tissues may also have been high, and the position 2 fruit and stem may have contained a significant amount of the ^{15}N redistributed from the labelled leaves, particularly the 1st position leaf. The cost of analysis and limited resources for this experiment meant that not all tissues could be analysed. A future experiment, analysing more tissues and with a greater number of replications should be carried out to investigate the hypothesis that the position 1 leaf redistributed ^{15}N to the position 2 fruit, and also could eliminate some of the variation in uptake of ^{15}N.

The recovery of the Rb was low, although, in contrast to the ^{15}N recovery, there was more Rb recovered in the 1st position leaf treatment to the mainstem leaf treatment. This adds further weight to the argument that the non-recovered ^{15}N could be found in the position 2 fruit or the other unanalysed tissues, since the recovery of Rb indicates that there was not a difference in application method or structural damage to the leaf which could account for the lack of recovery. Unlike with the ^{15}N treatment, total Rb was not measured as a means of calculation, instead the difference from the control tissue was used as a means of estimating the amount of elevated Rb in the tissue, which has also been used in previous studies (Schenk and Feller 1990; Ho *et al.* 1996). This method, using a relative increase and then relative changes to measure import or export of the same Rb pool means that the total recovery becomes a meaningless measurement and differences in recovery between treatments do not preclude comparisons being made.

5.4.2.1 N and K redistribution from the mainstem leaf

Using the "balance method" which has been used by many authors to quantify redistribution (e.g. Zhu and Oosterhuis 1992; Wahid *et al.* 2004), the change from the peak N concentration to the end of the sampling period was 17.5mg (52%) in experiment 8 (section 5.3.2.1), and 7.02 mg (26%) in experiment 9 (section 5.3.2.1.1). Previous research quantifying export from a mainstem leaf is limited, Zhu and Oosterhuis (1992) reporting 60% N export for the mainstem leaves, and no other studies referring to redistribution from individual leaves throughout their development. This range in data (26 – 60%) shows that this figure is not consistent between leaves, or between experiments. The ^{15}N data presented in this chapter quantifies redistribution of N from a single point in time, at 14 DAF at position 1. Since peak N content of the mainstem leaves occurs shortly before flowering at position 1 (Zhu and Oosterhuis 1992), some export may have been unaccounted for. However, using the change in ^{15}N as a measurement of actual redistribution, a decline of 81% was found, equating to an export of 21.7 mg N from the mainstem leaf. This shows that in the mainstem leaf, import and export of N may have been occurring simultaneously, since the decline in ^{15}N was much greater than the decline in total N.

This hypothesis, that the mainstem leaf may be importing and exporting N simultaneously is logical based on the leaf functioning and its role in N storage and assimilation. In cotton plants the leaf is the primary site of N reduction, assimilating the nitrate taken up by the roots into the low molecular weight organic nitrogenous compounds (e.g. amino acids) for storage, long distance transport in the phloem or assimilation into high molecular weight compounds in the plant cells. Since these amino acids and other organic low molecular weight compounds are transported from the leaf to other tissues for assimilation, the leaf is an important site for N supply. N export from the leaf comes from both the assimilated N in the proteins which functionally and structurally support the leaf growth, development and photosynthesis, and also from cycling of N through the chloroplasts (the site of reduction). This experiment shows that while structural protein degradation during leaf senescence may have resulted in the redistribution of between 26 and 40% of the mainstem leaf N, the incorporation of new nitrate sources meant that the total N analysis of the leaf tissue remained high, despite the export of N occurring. The mainstem leaf is therefore a significant site of N recycling and reduction of N for translocation to the developing bolls, as well as

exporting previously functional N compounds through subsequent redistribution associated with senescence.

Analysing the export of N after 14 days from the point of labelling, allows the assumption that the ^{15}N in the leaf at 14 days had been incorporated into the leaf structures or stored in the vacuoles. Immediate analysis of the leaf may have overestimated the amount of ^{15}N incorporated into the leaf, since some may have been initially transported directly into the phloem, reflecting the passive distribution of ^{15}N from the point of labelling, rather than the active transport of N from the leaf. Analysis of the change in ^{15}N content from 14 to 60 days allows the data to be interpreted as the distribution of N which was incorporated into the leaf structure, and released from *either* storage in the vacuoles or degradation of proteins. It can be asserted therefore that the 81% of ^{15}N exported from the leaf between 14 and 60 days after labelling is a true reflection of the export of N from the mainstem leaf. This figure is higher than that reported previously, although the methodology used in previous studies would not account for the cycling of nitrate through the leaf, and therefore may underestimate the redistribution of leaf N.

Potential mainstem leaf export can be defined as 81%. The variability in the single leaf data shows that, as with the whole plants described in Chapter 5, there is inherently a great deal of variation in the movement of nutrients through a cotton plant. The source of this variability could be due to localised changes in nutrient, water or light supply, or differences in plant morphology. The length of the vascular connections, the source-sink ratio, the root morphology and functioning and the plant node or location of the leaf may all impact this figure. More experiments, with a higher number of replicate samples, and potentially carried out in a glasshouse or environmentally controlled conditions could eliminate some of these factors.

K redistribution measured by comparing the peak content with the content at maturity was 4.73mg (33%) in experiment 8 (after which it began to import large quantities of K) and zero in experiment 9 (section 5.3.4.1.1), indicating that there was little evidence for a significant export in K due to the breakdown of organic compounds and senescence, which may have occurred after the measured period. There was, however, significant export of the Rb incorporated into the leaf tissue between 14 and 60 days after labelling, with a decline of

84.5% of the total Rb during this time. This export would be representative of K export, although simultaneous import must have occurred, as there was no change in the total K content of the labelled leaf over this time.

Simultaneous K import and export may be representative of the significant flows of K from one plant part to another. Most K in the cotton plant is found as a free K^+ ions in solution, and it is not readily metabolised and it forms only weak complexes with other organic molecules, from which it is readily exchanged (Marschner 2002; Maathuis 2009). The role of these free K^+ ions is primarily in plant water relations, but also in enzyme activation, phloem transport, protein synthesis and photosynthesis. As such, K may have been cycling through the leaf in solution and moved with phloem and xylem sap in and out of the leaf at this time to places of increasing demand. This exchange of K through the leaf therefore probably represents normal functioning of the leaf, but can be classed as "redistribution" because the Rb was clearly released from the inter-cellular solution in the leaf and transported to sites of high K demand, even if there was subsequent replacement of the redistributed K. The maximum potential K redistributed from a mainstem leaf at node 11 is therefore 85%. Further replication of this study in a controlled environment and field context should be carried out to confirm this figure.

5.4.2.2 N and K redistribution from the 1st position leaf

As with the mainstem leaf, there was a difference in the peak N content and N content of the 1st position leaf at maturity. The change from the peak N concentration to the end of the sampling period was 2.1 mg (11%) in experiment 8 and 6.3 mg (33%) in experiment 9. Previous research quantifying export from a 1st position leaf is rare, with no studies quantifying the export either as a gross or net figure. Zhu and Oosterhuis (1992) found that the sympodial leaves exported a relatively small amount of N by using the balance method. The range in the change in N content during the flowering period shows that individual leaves behaved differently in terms of export of N, and that the variability found in other experiments is similarly found in the first position leaf.

Using the ^{15}N export to estimate the export of total N from the leaf 13.3 mg, or 69%, of its total occurred, indicating that, as with the mainstem leaf, import and export were simultaneously occurring in the 1st position leaf. Potential export from the mainstem leaf

could be defined as 69% - far higher than any experiment using the balance method has calculated, but lower than the mainstem leaf.

K redistribution from the first position leaf was similar to the mainstem leaf, in that there was little change in the total K content during the period measured, but a large proportion (86%) of the Rb incorporated into the leaf at 14 days after labelling was redistributed out of the leaf. As with the mainstem leaf, this probably represented K being recycled through the leaf, rather that leaf senescence and export of K through cell death or collapse. Both the mainstem and 1st position leaf therefore, have the same potential redistribution with 85 and 86% being transported out of the leaf between 14 and 60 days after labelling.

5.4.2.3 Distribution of remobilised nutrients from the mainstem and 1st position leaves

The distribution of the ^{15}N and Rb at 60 days after labelling represents the sinks to which the redistributed N from each leaf is allocated within the plant. There were distinct differences in the allocation patterns of the ^{15}N and Rb from each leaf, and between the two tracer molecules.

The mainstem leaf supplied N to the position 1 seed, boll walls and stem, the fruit in the nodes above and below it and the leaves at the bottom of the plant. The mainstem leaf also supplied some N (0.3-0.6% of the total) to the rest of the fruit on the plant. Proportionally most of the redistributed N was allocated to the seed at position 1 on the 11th node, and the fruit below the labelled leaf, indicating that the developing sinks (fruit) below the mainstem leaf were given priority over those above it. The fruit immediately below the labelled node contained twice as much ^{15}N than that above it, confirming this hypothesis. At the point of flowering at position 1, node 11 there was developing fruit from nodes 6 – 10, many of which would have been less than 40 days old, and therefore still rapidly accumulating N, which may explain the translocation of ^{15}N to the fruit lower in the canopy.

K redistributed from the mainstem leaf was allocated to different sites than the N redistributed from the same leaf. While the mainstem leaf provided a significant amount of N to the developing seed, there was no redistribution of K from the mainstem leaf to any of the fruit tissues at position 1, despite the large amount of K in the boll walls, lint and seed. There was an increase of Rb in the vegetative tissues at position 1, indicating that potentially K

from the mainstem leaf was exchanged for K in the first position leaf, since there was a larger proportional increase in Rb than in K during this time. Similarly the leaf at position 2 accumulated Rb from the mainstem leaf. As with ^{15}N, there was an increase in the Rb content of the fruit on the node immediately below the labelled leaf and that on the nodes at the bottom of the plant. There was also an increase in the Rb content of the fruit at the top of the plant, though export to the fruit immediately above the labelled leaf. This means that the mainstem redistributed K all over the plant, and to most of the fruit developing in this period. Since K is a very mobile ion in the plant, this long distance transport is to be expected, although the preference of the plant to use K from the mainstem leaf to supply fruit removed from the labelled node rather than that on the subtending sympodial branch has not been previously reported, the general assumption being that leaves would supply the bolls in close proximity to them. Since most of the vegetative tissues along the branch continued to import K until maturity, there was evidently no shortage of K supply and it was not limiting to growth. A replication of this experiment should be carried out under different rates of K supply to determine if K deficiency is a trigger for K redistribution, and if a reduction in new growth after labelling would mean that K from mainstem leaves may be allocated to fruit loser to them.

The pattern of allocation from the 1st position leaf was distinctly different to that of the mainstem leaf, with no redistribution of N from the first position leaf to the leaves or fruit on any other node above and below the labelled branch. This confirms previous research suggesting that the sympodial leaves are likely to supply N to adjacent tissues and the subtending boll. The total amount of N supplied to the boll, and the proportion of the total boll N was, however, relatively low with only 6.75% of the seed N and 5.5% of the boll wall N being sourced from the subtending leaf. This amount can hardly qualify it as the major supply organ for the boll. Contrary to the N redistribution from the 1st position leaf, most of the redistributed K was in the fruit below node 11, 62% of the excess Rb in the plant was found in the fruit from nodes 1-9 at 60 days after labelling, and 8.5% in the fruit on node 10. A small amount of K was redistributed from the 1st position leaf to the seed, boll walls and vegetative tissues along the branch – including the mainstem leaf. This redistribution to other vegetative tissues demonstrates the mobility of the K through the plant, and adds further weight to the argument that the leaves continuously exchange their K throughout their growth and development. As for N, the subtending leaf could hardly be classified as the major source

organ for K for the developing boll, supplying only 1.6% of the K in the boll walls and 1.9% in the seed.

This data calls into question two widely held assumptions. Firstly that the sympodial leaf supplies most of the nutrients in the mature boll, and secondly, that any N and K remobilised from the sympodial leaves will be allocated to the boll adjacent to the leaf. It is clear from this data that up to 7% of the seed and boll wall N were provided from the leaf subtending the boll, and that a further 5% was provided by the mainstem leaf. This combined total of 13% of the N in the mature boll is very different from the statements made by other authors in previous research, who refer to the subtending leaf as the "main source organ for boll growth" (Hellmann *et al.* 2000; Offler *et al.* 2000; Ruan *et al.* 2000; Turgeon 2000; Wahid *et al.* 2004; Li *et al.* 2009). Similarly the 2% of boll K supplied by the subtending leaf is very low, and there was no redistribution of K from the mainstem leaf to the 1st position leaf on node 11. It seems more likely that the necessary nutrients for boll development are supplied by a combination of root uptake and redistribution from leaves removed from the fruit. There seems to be far more interconnection, and plasticity in the allocation of nutrients than previous research suggests, with N and K being transported both up and down the plant to supply sinks at all nodes. There were definite differences between the mainstem and sympodial leaves, particularly for K, with the mainstem leaf supplying K to the whole plant, and the sympodial leaf to the branch on which it is situated.

The hypothesis that it is demand for N from the developing boll which results in the decline in photosynthesis, due to the degradation of the photosynthetic proteins as a source of N (which was proposed by Constable and Rawson (1980b)), is contradicted by this data. Most of the remobilised N and K from both leaves were found in tissues removed from the labelled node. Demand from bolls developing beneath the leaves at node 11 may have been the driver for N remobilisation from these leaves, rather than the development of the subtending boll. This theory would account for the seeming difference in timing of peak N, N export, photosynthesis and dry weight between the leaves and developing bolls.

5.4.3 Conclusions

This data suggests that in terms of the remobilisation of nutrients and the plants distribution of these, the whole cotton plant is far more inter-connected and inter-dependent than previous

research suggests. The subtending boll is *not* the driver for N or K, or by extrapolation, P movement out of the leaves, but rather it is the development of bolls all over the plant which is the sink for redistributed nutrients from mainstem and 1st position leaves.

Key questions raised by these experiments are;

1) What is the proportional supply of N, P and K from mainstem nodes to the 2nd position boll, and is this significantly different from the supply to the 1st position boll?
2) What is the source of the remaining N, P and K in mature bolls not accounted for by redistribution of mainstem and subtending leaf resources?
3) Does the potential redistribution vary at different nodes up and down the mainstem, or do all leaves function in a similar way?
4) How much does the sink size (in terms of numbers of bolls, their position and the ratio of bolls to leaves) in different parts of the canopy drive redistribution?
5) What is the effect of agronomic management on the potential redistribution of N, P and K from leaves?

Some of these questions will be answered in the proceeding chapters. The following chapter will examine the redistribution of N and K between different sections of the canopy and test the potential rates of redistribution defined in this chapter. Chapters 7 and 8 will examine the effect of nutrient and water supply on redistribution.

CHAPTER 6

N, P and K accumulation and redistribution in relation to position in the canopy of high-yielding cotton plants

6.1 Introduction

In the previous chapter, a detailed analysis of the distribution and redistribution of nutrients along a single sympodial branch was calculated, the potential redistribution from the mainstem, and a sympodial leaf was estimated, and the contribution of single leaves to the supply of N and K to developing bolls was quantified. It was concluded that the demand for N, P and K by the major sinks could not be provided primarily by export from the subtending and mainstem leaves; that demand for nutrients is largely met by redistribution of nutrients from remote sites within the plant, or from continued root uptake. This followed on from the conclusions of the previous chapter that root uptake must provide the some of the resources required for boll development, and is supplemented at some level by nutrient redistribution. In this chapter these conclusions will be more closely investigated by the measuring the ^{15}N and Rb uptake from the soil after flowering, and comparing their redistribution to determine if some parts of the canopy either rely on redistributed nutrients or root uptake more than others, or supply more nutrients via redistribution to the rest of the plant.

Modern cultivars grown in high input systems with good pest control accumulate more biomass and nutrients after flowering than the older parental varieties (Mullins and Burmester 2010). This may indicate that root uptake accounts for more of the nutrients in mature bolls of modern, high-yielding cotton than in previous studies using older cultivars. In earlier studies (pre-1945), 28.8% of dry matter accumulation occurred before first flower and 48.5% before the first open boll (Mason 1922; Crowther 1938b; Richards 1941), while Bassett *et al.* (1970) recorded only 7 – 10% at first flower. This increase in uptake after flowering has been attributed to varietal improvements, specifically, decreased determinacy and a prolonged boll-setting period, and to better management, insect control and irrigation practices. It may also indicate that the functioning of roots continues until later in the season, providing more nutrients and water for growth and development and providing a greater proportion of the nutrients in mature bolls. The relative contribution of root-acquired nutrients directly supplying developing bolls has not been quantified, nor has any measure of the differential contribution of root-acquired nutrients to bolls in different parts of the canopy.

There have been few previous studies comparing redistribution at different levels in the canopy. Zhao and Oosterhuis (1999) compared the accumulation of nutrients in the leaves and squares at nodes 8, 10 and 12 and found a similar nutrient content and accumulation pattern with age between tissues on the different nodes, except for a lower P content in the squares at node 8. Constable and Rawson (1980b) found that leaf area varied between nodes, the largest leaves being at nodes 7-9 and smaller leaves above and below this middle section. A higher nutrient content would follow this larger leaf size, as nutrient content and dry weight of leaves are generally closely related. Constable and Rawson (1980b) found that there was no difference in photosynthetic rate per unit area, the gas exchange of the leaves or C storage in leaves with age in different parts of the canopy, despite a difference in leaf size. Since these leaves behaved in a similar way in terms of their carbon production and storage, they may also function according to the same pattern with age in terms of their nutrient storage and redistribution. This has not been investigated on a single leaf basis beyond the middle section of the canopy (nodes 8 – 12) – where this hypothesis was confirmed (Zhao and Oosterhuis 1999).

Rosolem and Mikkelsen (1989) examined the N distribution and redistribution in plants grown in a controlled environment glasshouse throughout the fruiting period. They supplied the plants with ^{15}N labelled fertiliser in pulses every 30 days from squaring until first open boll, at 150 days. They described the cotton plant in terms of each tissue's behaviour as a source or a sink in three sections, from nodes 0 – 5, 6 – 10 and 11 – 15. They also included the roots in their analysis, although concluded that they were neither a net source or sink for N. The bottom of the plant mobilised N from the leaves to the rest of the plant, although there was a significant amount of N which was simultaneously imported from the roots into the bottom and middle leaves to replace that which was exported (a similar finding to that of chapter 5). They also quantified the contribution of redistributed N to the seeds and boll walls of each section, calculating that over half the N in the mature seeds was provided by redistribution of N from other plant parts. The top of the plant behaved as a sink for N throughout the whole growth period. These plants were much smaller than those used in the experiments described in chapters 4 and 5, and so may not behave in the same way. Measurements of nutrient redistribution and accumulation also were not carried out until all

bolls had reached maturity, and so some of the redistribution of leaf and stem nutrients from the top of the plant may not have been accounted for.

Brown (1968); Thompson *et al.* (1976); Constable and Rawson (1980a); and Constable (1991) all described the different growth, development and timing of dry matter accumulation, development and in the case of Thompson *et al.* (1976), N accumulation in developing bolls in different parts of the canopy. They found similar results, showing that the biggest leaves and fruit were in the middle of the canopy, with a decline in the number of bolls, boll size and leaf size towards the top and bottom of the plant. The difference in C assimilate export through the canopy was mainly a function of leaf size, rather than a change in leaf functioning in different parts of the canopy. If this finding was extrapolated to the export of N, P and K, it would mean that the proportional redistribution would be the same from different leaves, but the gross amount of redistributed N, P and K would vary with leaf size and nutrient content. This has not been measured in any previous studies.

In this chapter the redistribution of N, P and K in five node segments up the mainstem of a cotton plant will be calculated to establish;

1) The amount of root uptake of N and K, and it's contribution to boll nutrients, particularly late in the fruit development period
2) The variation in the amount of or rate of N, P and K redistribution occurring within and between different sections of the canopy
3) The variation in the source of nutrients in mature bolls through the canopy – if some rely more on root uptake and others on redistribution.

6.2 Materials and methods

To examine the uptake and partitioning of N, P and K during the fruiting period, a field experiment was carried out in the 2010 – 2011 cotton season (Experiment 6). The experimental design and description of the treatments is given in section 3.4.6.

6.2.1 Treatments and sampling method

A solution containing ^{15}N and Rb was applied once, pre-flowering on December 18th 2010 (725 day degrees from sowing) directly to the soil adjacent to the growing cotton crop. Rb

was applied as a 0.02M solution of RbCl applied at a rate of 2.4184 g L^{-1} (equivalent to 0.1795 g Rb per plot). ^{15}N was applied as a solution of 98.47% ^{15}N urea applied at a rate of 0.4432 g per plot (0.1g ^{15}N excess per plot).

The solution was applied into a 30 cm deep trench dug next to the plant line (as shown in Figure 3.5 and Figure 3.6). 105 mL of solution was applied, in 21 aliquots of 5 mL applied at 10 cm intervals along the 2 m subsection of the plot (Figure 3.6 a and c). Control plots received only deionised water and labelled plots received a solution of ^{15}N and RbCl.

Plants were sampled at five dates between application of ^{15}N and RbCl and physiological maturity (see Table 3.8), and partitioned into 5 sub sections based on the mainstem node, from the base to node 6, from node 7 – 11, from node 12 – 16, from node 17 – 21 and 21+, as described in section 3.4.6.2 (shown in Figure 6.1). Sub-samples were partitioned into leaf, stem and fruit, with the exception of section 5, which was not partitioned, but analysed as a whole.

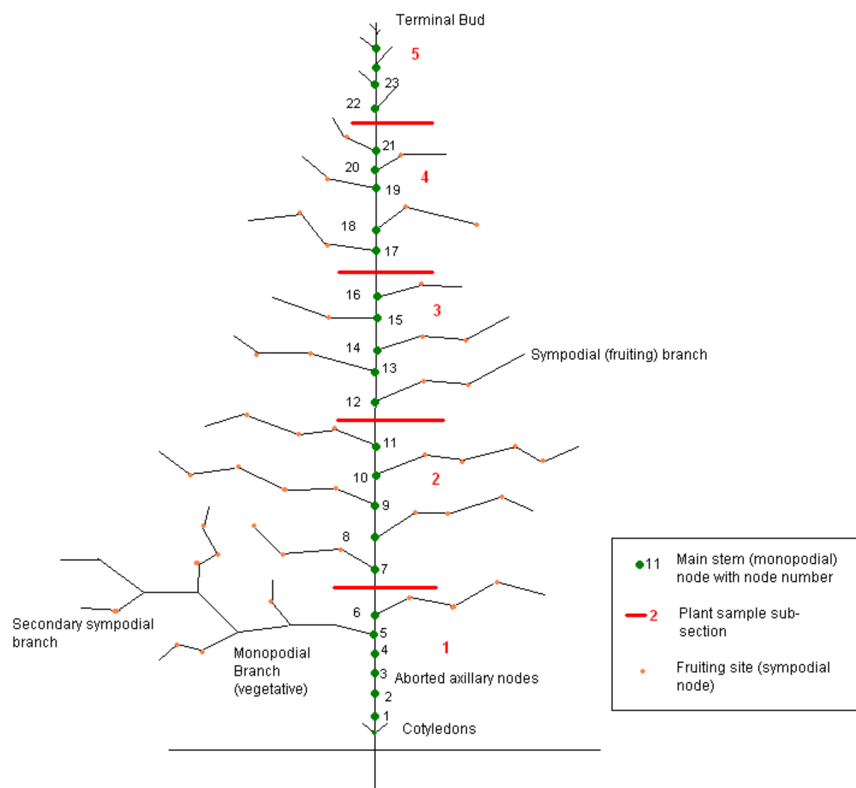

Figure 6.1 Schematic diagram of plant sub-sampling sections for experiment 6

Each sub-sample was dried, ground and analysed for N, P, K and other nutrients as described in section 3.3.1. Isotope analysis and Rb analysis was carried out separately to nutrient analysis as described in section 3.3.2.3 and 3.3.2.4.

6.2.2 Data analysis

Data was analysed using Genstat® 14th edition. The biomass and total accumulation of N, P and K in each section was compared using ANOVA, and the partitioning of N, P and K between leaf, stem and fruit fractions compared between plants to eliminate differences in plant size. Redistribution was calculated by the change in the total vegetative and reproductive concentration of ^{15}N between sampling dates and comparisons between subsections made using ANOVA. The R:V ratio of each section was used as a factor in the analysis to determine if the boll load in the sections influenced the redistribution of N, P or K from the vegetative to the reproductive tissue.

6.3 Results

6.3.1 Plant growth and development

There was no difference in the number of nodes, total dry weight or yield of the labelled plants and the control plants ($P < 0.05$), and so it can be concluded that the application of the solution to the soil adjacent to the plants, and the soil and root disturbance, had minimal impact on the growth and development of the plants. For this reason only the data from the labelled plots is shown.

6.3.1.1 Nodes

The mean number of nodes (Figure 6.2) increased until 158 DAF, after which no new nodes were produced. The growth rate declined from 130 DAS.

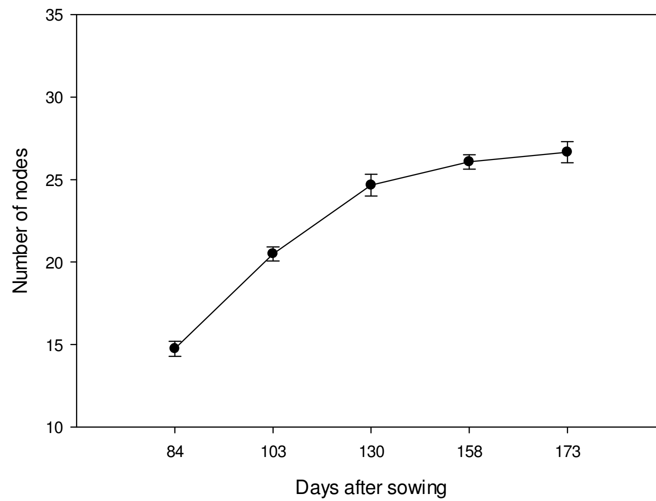

Figure 6.2 The mean number of nodes of the plants in the labelled plots from flowering to maturity. The error bar represents +/- one standard error of the mean.

6.3.2 Biomass accumulation

Biomass accumulation (Figure 6.3) occurred until 130 DAS. There was a decrease in biomass between 158 and 172 DAS. The plants had accumulated 17% of their total biomass by flowering, and 100% of the total biomass at cutout.

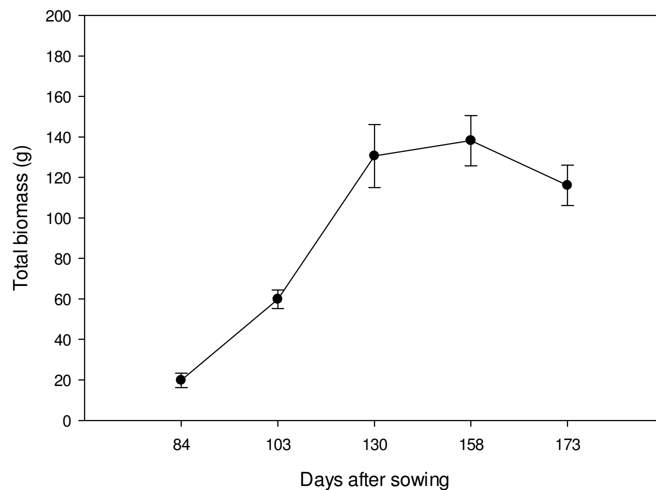

Figure 6.3 Biomass accumulation of treated plants from application of labelled fertiliser at flowering to maturity. Error bars represent +/- one standard error of the mean.

Proportionally, most of the total biomass was in sections 2, 3 and 4 (Figure 6.4). Section 3 contained around a third of the biomass of the plant from 103 DAS till maturity.

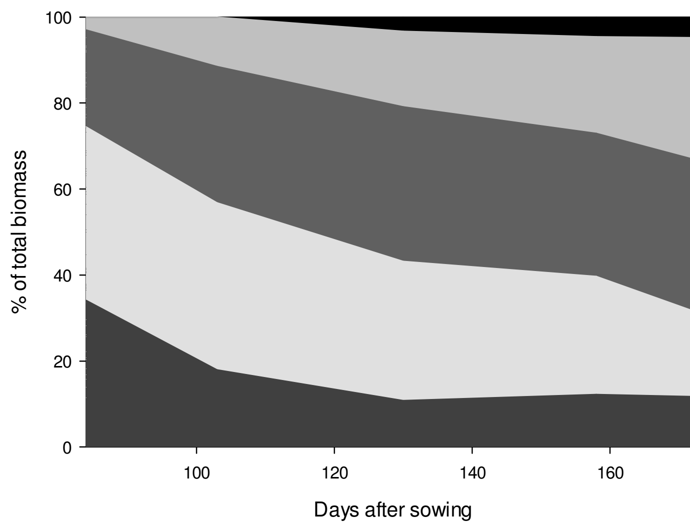

Figure 6.4 Percentage (%) of the total biomass in sections 1 ( ), 2( ), 3 ( ), 4 ( ) and 5 ( ).

The accumulation rate of biomass varied between the sections, and was mostly related to the growth and development of the fruit. Section 1 maintained a slow rate of accumulation until 158 DAS. After 130 DAS the biomass in section 2 decreased, in section 3 it peaked and maintained the same biomass until maturity and in section 4, biomass continued to accumulate. In section 5 the rate of accumulation was constant, but relatively low.

The partitioned dry weight (Figure 6.5) showed that in sections 2, 3 and 4 the fruit accounted for the majority of the dry weight. In section 1 the stems accounted for the highest proportion of the dry weight. Fruit dry weight peaked at 130 DAS in section 2, 158 DAS in section 3 and increased until maturity in section 4. The number of fruit was the highest in section 3 (Figure 6.6), in section 4 there were still a large proportion of boll which were immature (still green) accounting for the continued accumulation of dry weight until maturity.

There was an “apparent” decrease in the biomass of the fruit in section 2 of 50% between 158 and 173 DAS. This decrease is due to a difference in the total boll number of 55% (Figure 6.6) in the time, and can be attributed to variability between the plants sampled. As shown in Chapter 5, a decrease in the boll dry weight of 55% is not typical of boll development, and would not have occurred in these maturing bolls. While there may have been some shedding of fruit, the difference is primarily in older, maturing fruit, rather than small bolls and squares which are likely to be shed. For this reason, since the variability in the dry weight data was

due to plant differences and not a change in the actual dry weight of the fruit, the fruit data from section 2 at 173 DAS was excluded from further analysis of the N, ^{15}N excess, P, K and Rb content.

The number of fruit varied between each section (Figure 6.6), with very few (less than 1) bolls in section 1 and 5 reaching maturity. In section 3 there were no remaining green bolls at maturity, with all fruit in the section mature, open bolls at maturity. In section 4 however, there was an equal number of green and open bolls at maturity, accounting for the continued increase in fruit dry weight in the section. Sections 3 and 4 held the majority of the fruit.

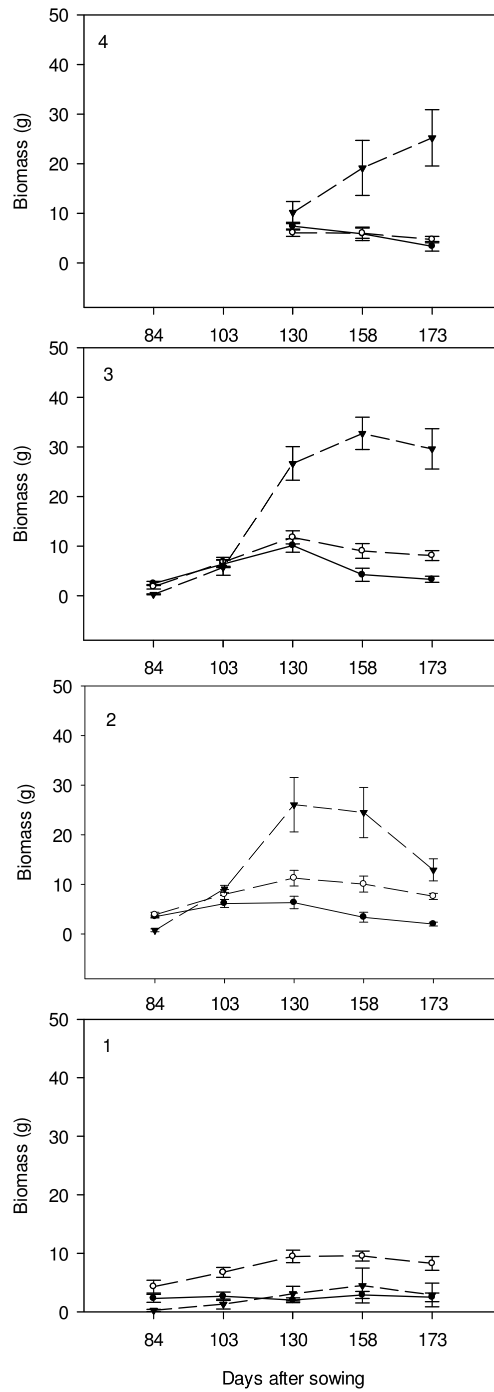

Figure 6.5 The mean leaf ( ), stem ( ) and fruit ( ) dry weight in sections 1-4 of the labelled plants. The error bar represents +/- one standard error of the mean.

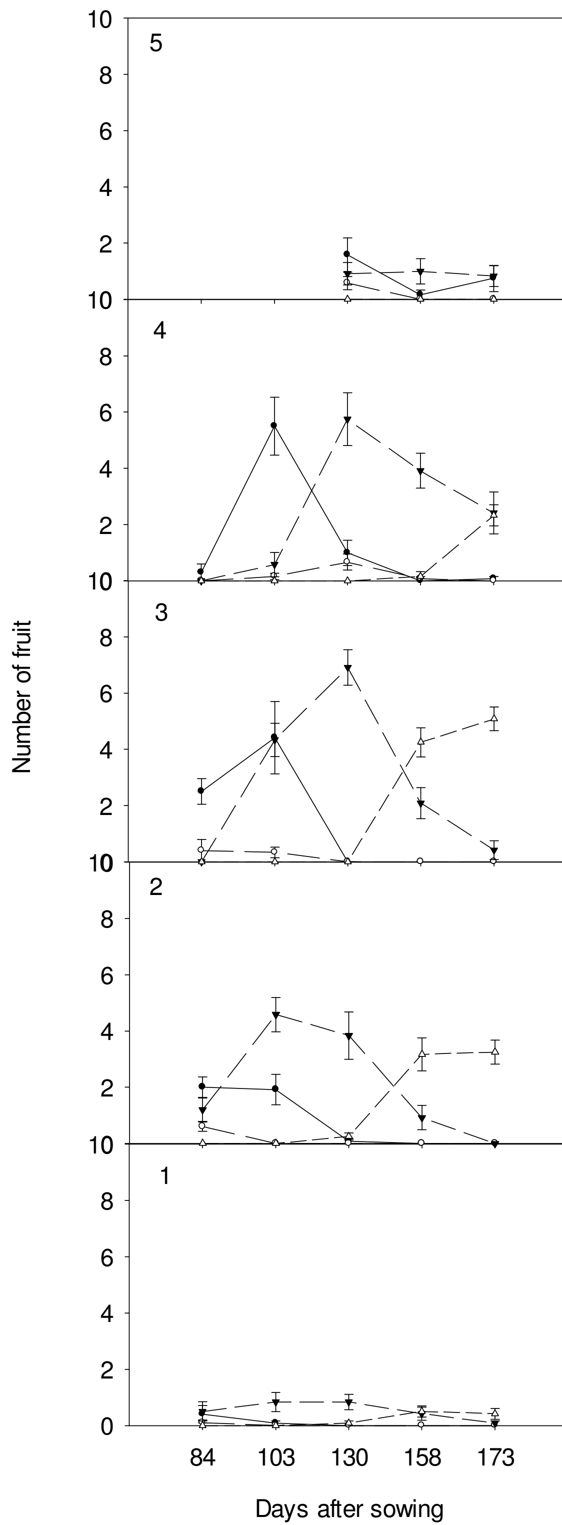

Figure 6.6 The mean number of flowers (– —○— —), squares (——●——), green bolls (– ——▼— –), and open bolls (– —△— — –) in sections 1 – 5. The error bar is +/- one standard error of the mean.

The ratio of reproductive to vegetative biomass increased up the plant, and was highest in section 4 (Figure 6.7). In section 5 all tissues were analysed together so it was not included in the graph. The high stem dry weight (Figure 6.5) and low fruit number (Figure 6.6) in section 1 resulted in the ratio being less than 1:1 from flowering to maturity. Despite the lower fruit dry weight in section 4, the R:V ratio was higher than section 3, indicating that the proportional allocation of biomass to fruit was not the same in different sections of the canopy.

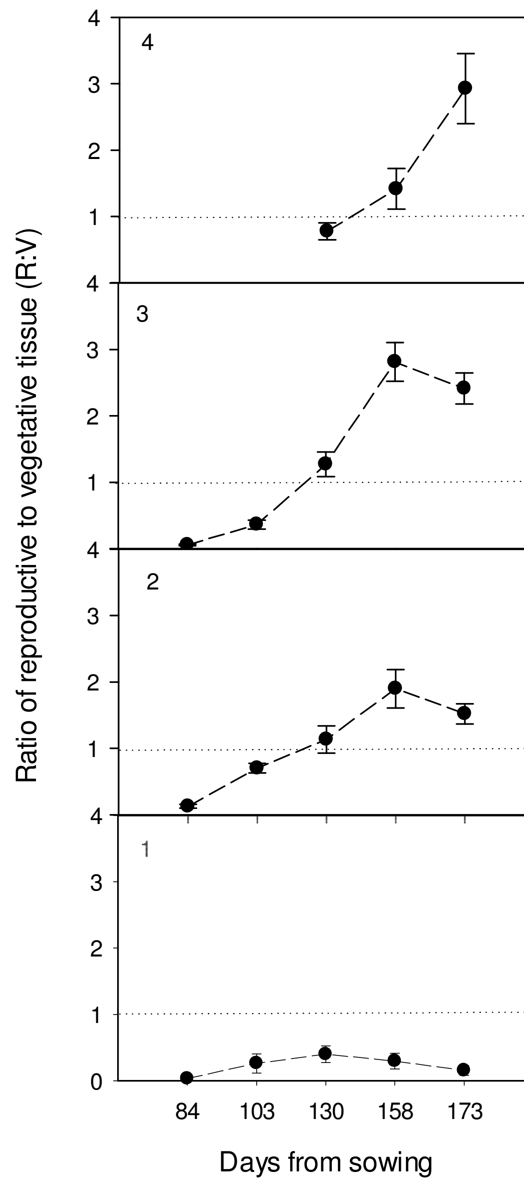

Figure 6.7 The ratio of reproductive to vegetative tissue in sections 1-4. The error bar represents +/- one standard error of the mean and the dotted line represents a 1:1 ratio.

6.3.3 Uptake of N, P, K from soil

The total N content of the plants increased until 130 DAS, after which time there was no change in the N content of the plants ($P > 0.05$). Peak N content occurred at the same time as peak biomass. ^{15}N content peaked at 130 DAS and then declined ($P < 0.001$). The plants took up 17% of the total N at flowering, and 100% of the total N at cutout (130 DAF).

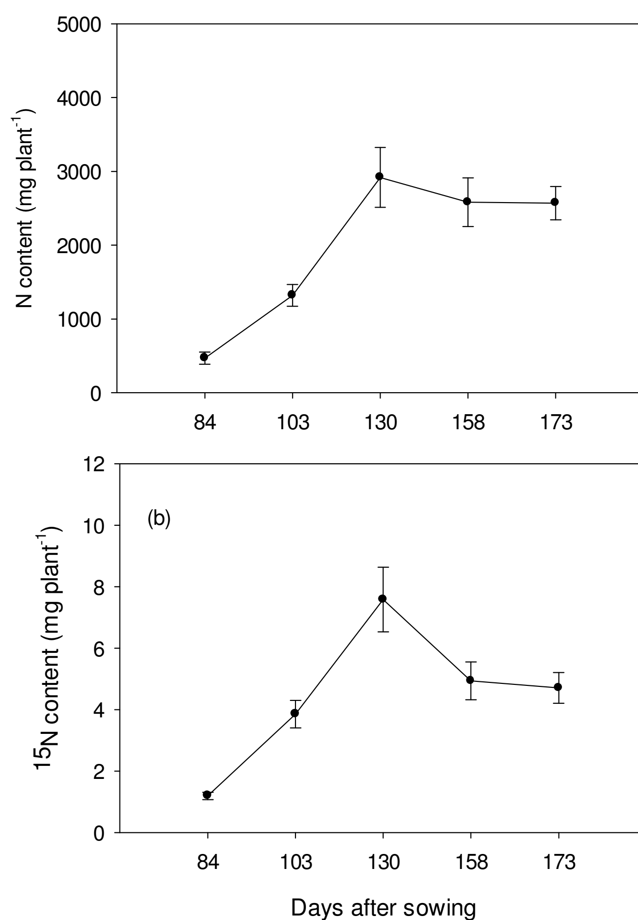

Figure 6.8 The mean (a) N content and (b) ^{15}N content of the labelled plants. The error bar represents +/- one standard error of the mean.

The K and Rb content of the plants (Figure 6.9) increased until 130 DAS and then did not change until maturity ($P < 0.001$). The total Rb applied to each plot was 179.5 mg (8.97 mg per plant), indicating that the total uptake of Rb was only 19% of the total. Uptake of K and Rb was therefore not limited by root access, but stopped or declined from 130 DAS for a different physiological reason. Only about 16% of the total K had been taken up by flowering, and had peaked by 130 DAS.

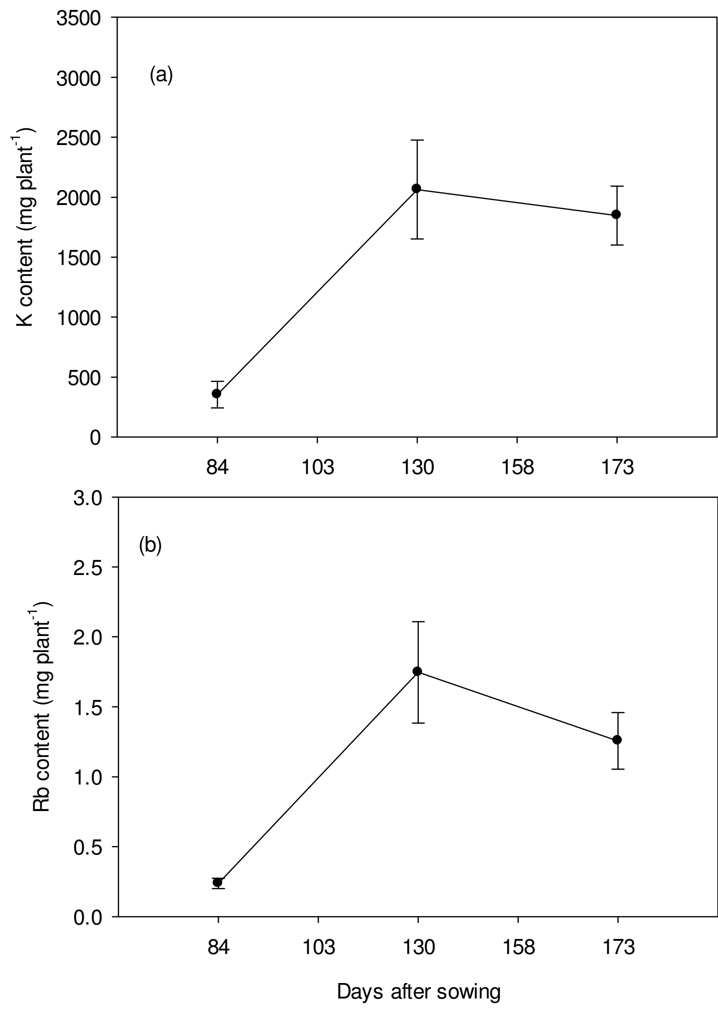

Figure 6.9 The mean (a) K content and (b) Rb content of the labelled plants. The error bar represents +/- one standard error of the mean.

Uptake of P (Figure 6.10) occurred until 130 DAS, after which time there was no change in the total P content ($P < 0.001$). Only about 11% of the total P was taken up by flowering, and had peaked by cutout at 130 DAS.

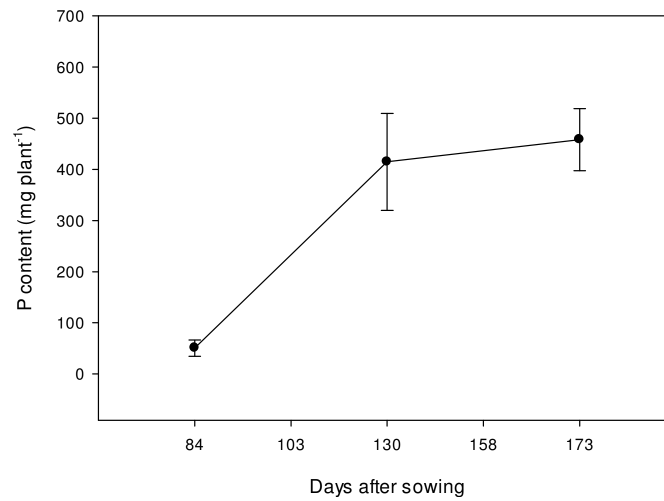

Figure 6.10 The mean P content of the labelled plants. The error bar represents +/- one standard error of the mean.

6.3.4 Distribution of nutrients

There was a similar proportional distribution of N, P and K between the 5 sections of the plants, the majority was found in sections 3 and 4 of the canopy (Figure 6.11), which had the highest number of fruit (Figure 6.6) and largest biomass (Figure 6.5).

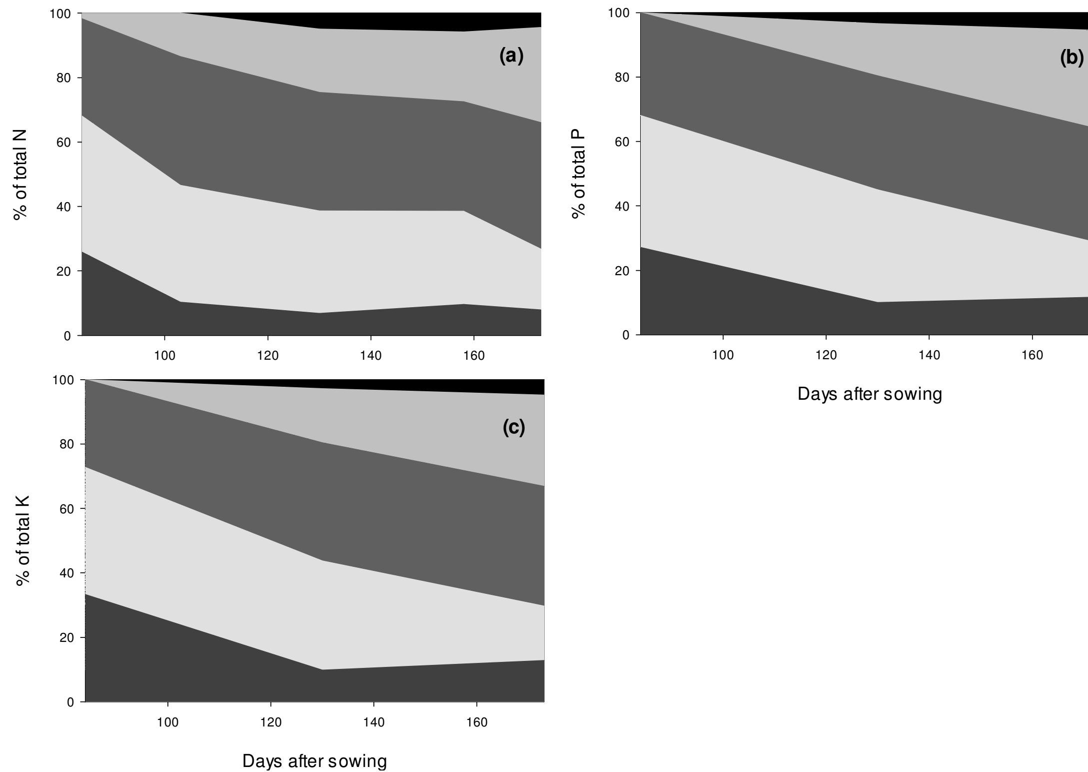

Figure 6.11 The proportion of total (a) N, (b) P and (c) K in section 1 ( ), 2( ), 3( ), 4 ( ) and 5 ( ).

The major sink for N in each section was the fruit (Figure 6.12). While section 5 contained 5% of the total N, it was not partitioned into the leaf, stem and fruit sections and so was not included in Figure 6.12 or in partitioning graphs throughout this chapter.

In sections 2, 3 and 4 the N content of the fruit continued to increase until maturity, while in section 1 the N content of the fruit declined after the peak dry weight was reached (158 DAS).

There was no difference in the concentration of N in any of the tissues between the five sections, except for the fruit in section 1 which had a lower N concentration than the fruit in sections 2 – 4 ($P < 0.05$). In each section there was a consistent decline in leaf and stem N concentration with age.

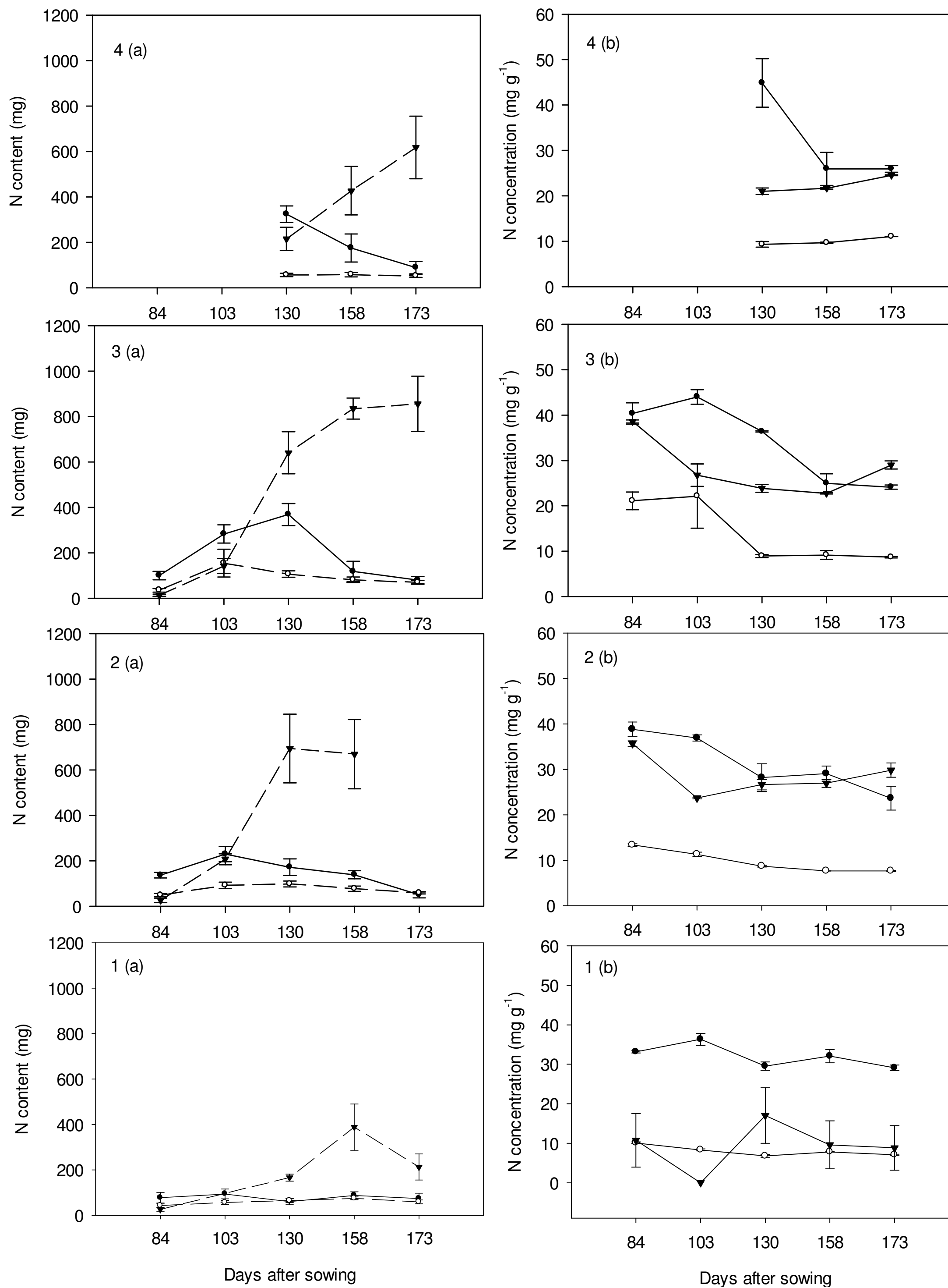

Figure 6.12 The mean N content (mg) (a) and N concentration (mg g^{-1}) (b) of the leaf ( ), stem ( ) and fruit ( ) in sections 1, 2, 3 and 4. The error bar represents +/- one standard error of the mean.

The K content also followed the dry weight of each section, with the total K distributed similarly to the dry weight. The majority of the total plant K was in section 3 at maturity. At 130 DAS 70% of the total plant K was in sections 2 and 3, after which the content of section 2 declined by 57% and the sections 3 and 4 content increased at a greater rate than previously. Section 1 continued to accumulate K until maturity, although it contained a proportionally lower amount than the other sections.

There was no difference in the K concentration of the fruit in each section at maturity ($P < 0.05$). The leaves in section 2 had a lower K concentration than those of the other sections at maturity, and were the only leaves that showed a decrease in the K concentration over time. The K concentration of the leaves of section 3 declined between 84 and 103 DAS, but then increased again, while in sections 1 and 4 the K concentration of the leaves increased until maturity. There was variation in the K concentration of the stems up the plant, with the lower 2 sections (1 and 2) having a lower concentration than the upper two (sections 3 and 4) ($P < 0.05$).

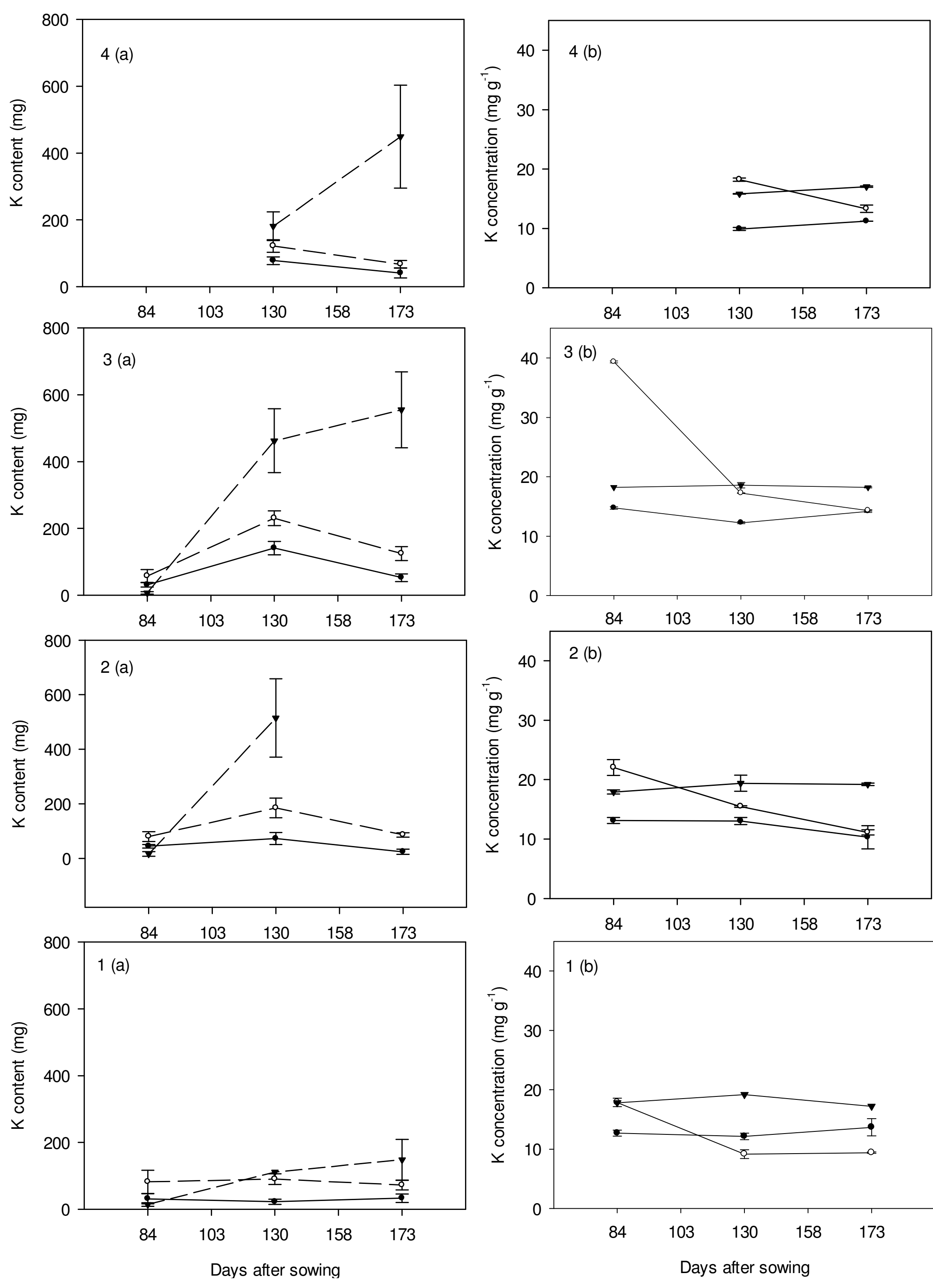

Figure 6.13 The mean K content mg) (a) and K concentration (m g^{-1}) (b) of the leaf (——●——), stem (—◇—) and fruit (– –▼– –) in sections 1, 2, 3 and 4. The error bar represents +/- one standard error of the mean.

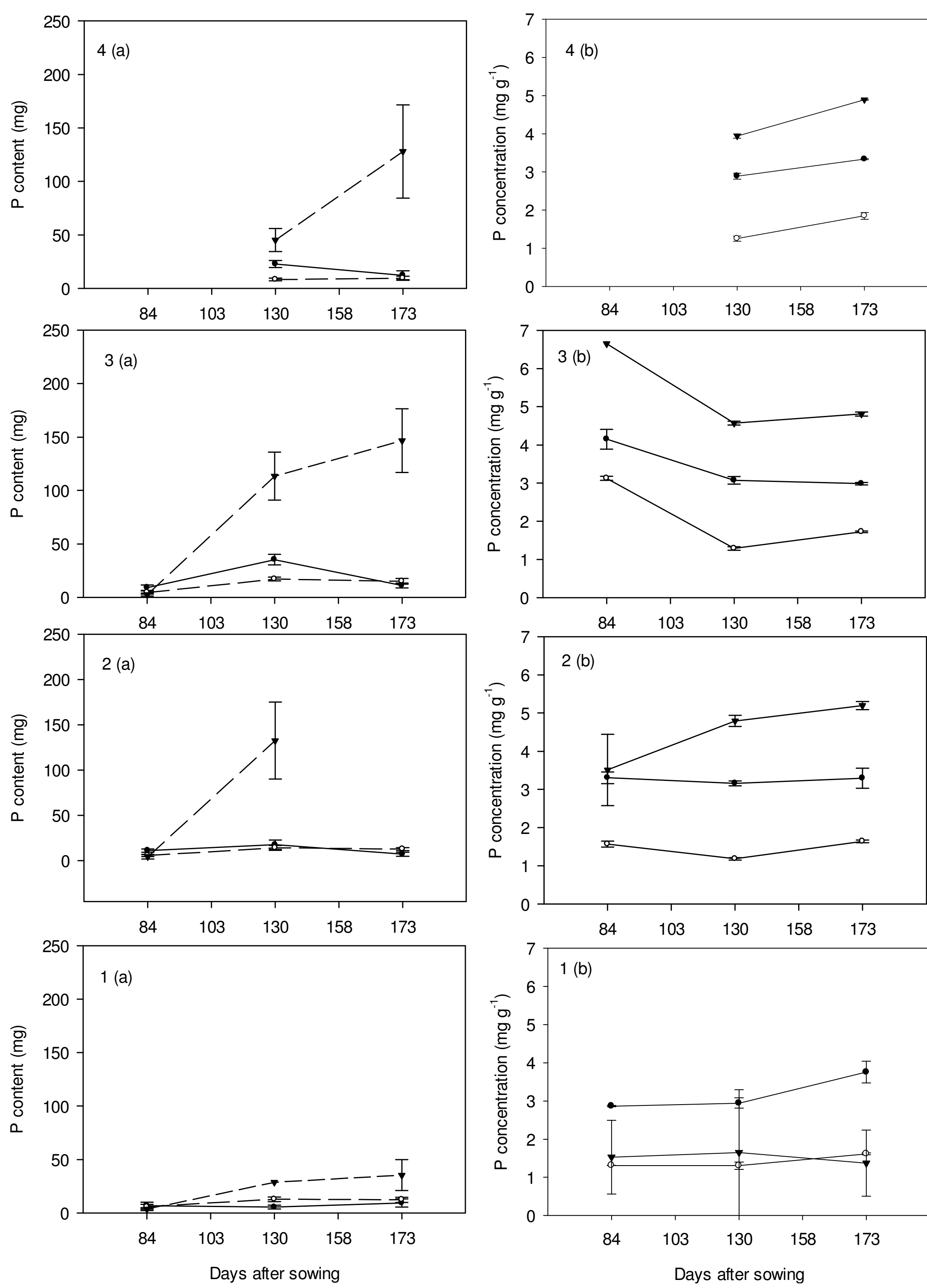

Figure 6.14 The mean P content (mg) (a) and P concentration (mg g^{-1}) (b) of the leaf (—●—), stem (—○—) and fruit (– –▼– –) in section 1 (g and h), 2 (e and f), 3 (c and d) and 4 (a and b). The error bar represents +/- one standard error of the mean.

The P content of the plants followed a similar pattern to the K, with the majority of the plant P in section 2 and 3 at 130 DAS, and a further increase in sections 3 and 4 until maturity (Figure 6.14). The largest sink for P was the fruit in each section. The concentration of P showed very little variation between sections, with no difference in the leaf, stem or fruit concentration between the sections at maturity ($P < 0.05$).

6.3.5 Redistribution of N, P and K

6.3.5.1 Nitrogen and ^{15}N

Unlike the N content, the ^{15}N content of the plants declined between 130 and 158 DAS ($P < 0.001$) (Figure 6.8). The peak ^{15}N content was 7.6 mg per plant, declining to a mean of 4.7 mg. The ^{15}N content mirrored the N content in all sections except the fruit in section 3, where the proportional increase of ^{15}N from 150 to 173 DAS was much higher than the increase in N. In section 4, import of ^{15}N into the fruit commenced at 158 DAS.

A total of 100 mg (0.1 g ^{15}N excess) was applied to the plants, representing a mean of 5 mg plant^{-1} in each plot (at 10 plants m^{-1} for 2 m). Based on this average access of the plants to the fertiliser, plants took up most of the available ^{15}N between the time of labelling and 130 DAS, and the mean ^{15}N content of each plant represented 94% of the total of 5 mg plant^{-1} which had been applied. The high content of the plants at 130 DAS indicates that the removal of plants for sampling at 84 and 103 DAS may have increased the ^{15}N uptake of remaining plants, which had access to ^{15}N placed where the harvested plants had been removed.

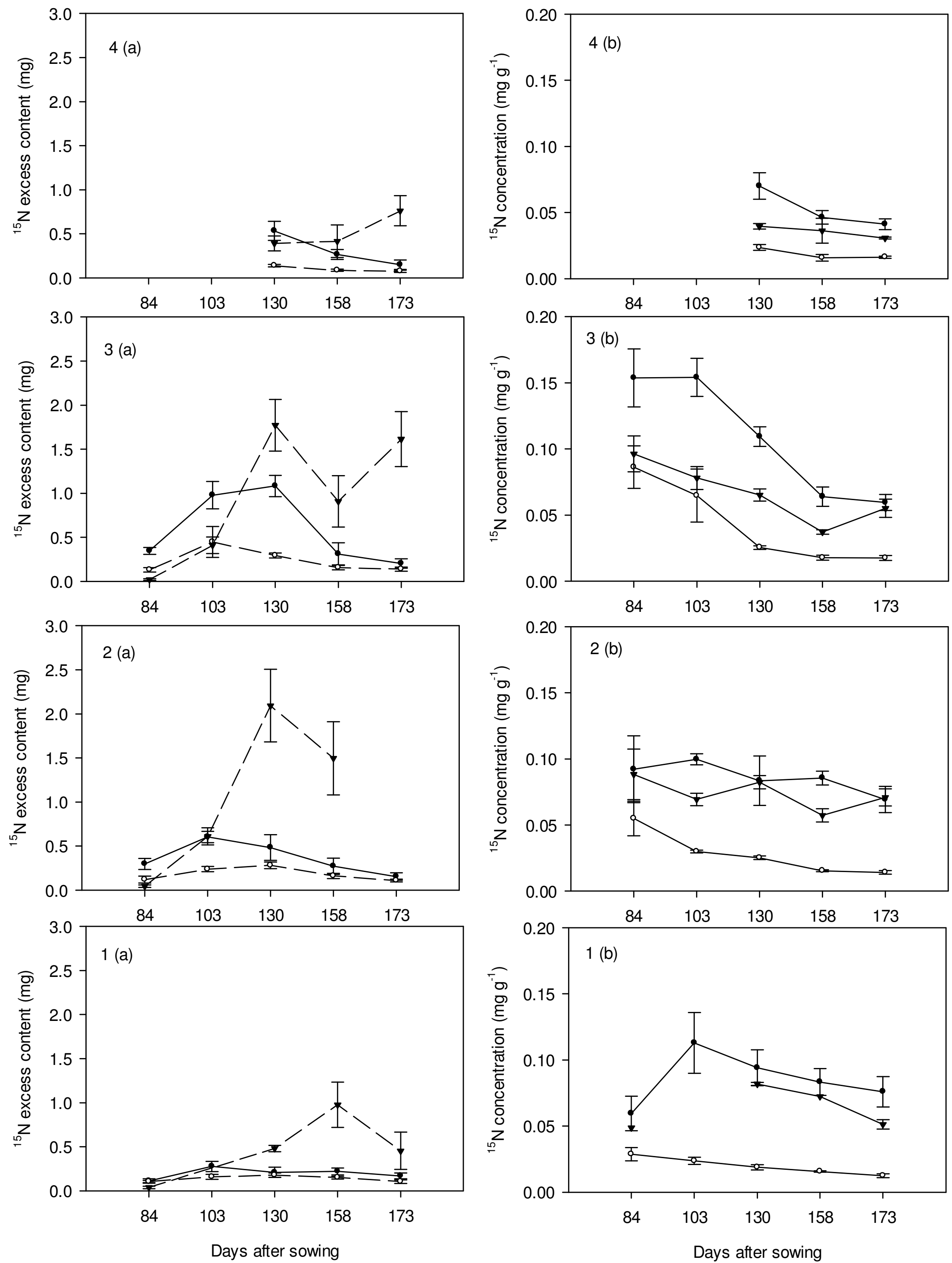

Figure 6.15 The mean ^{15}N excess content (mg) (a) and ^{15}N concentration (mg g^{-1}) (b) of the leaf (—●—), stem (— ○ —) and fruit (– ▼ –) in section 1 (g and h), 2 (e and f), 3 (c and d) and 4 (a and b). The error bar represents +/- one standard error of the mean.

The gross and proportional amount of N remobilised and redistributed varied between sections and tissues (Table 6.1). In sections 2, 3 and 4 the leaves were the largest source of N. In section 1 the fruit remobilised the greatest amount of N, followed by the stems and then the leaves.

There was only a small amount of variation in the redistribution of N in each section up the plant. Proportionally, sections 1, 2, 3 and 5 redistributed the same amount of N (38 - 39%), while section 4 redistributed proportionally much less (7%). This variation is mostly in the redistribution of fruit N, with the fruit in section 4 continued to import N into the fruit until maturity. This is also possibly related to the number of fruit in each section (Figure 6.6), which continued to increase until maturity, particularly the number of green bolls, which would account for the continued import of N.

When the total amount of N exported from each section is calculated as a proportion of the cumulative export of N from each tissue within the section, there was greater variation up the plant. Sections 1, 2 and 3 exported the majority of the remobilised N, in an increasing proportion up the plant, while section 4 redistributed only a small proportion of the total remobilised N from the leaf and stem tissues (16.9%).

Redistribution of N from the fruit occurred only in the lower portions (sections 1 and 2), although the shed fruit was not collected and may have accounted for some of the decrease in total fruit N and ^{15}N after 103 DAS.

Table 6.1 Mean N (mg) redistributed from each tissue in each section, and the combined tissues in each section, extrapolated from the remobilisation of ^{15}N from each tissue from the peak ^{15}N excess content until maturity.

Plant Section	Tissue	Source / Sink for N (total N mg)	Mean ^{15}N redistribution (mg) / %	Mean N redistribution (mg)	Proportion of remobilised N redistributed out of section (%)
5	**Total**	**Source**	**0.089 (38%)**	**68.2**	
4	**Total**	**Sink**	**0.079 (7%)**	**43.9**	**16.9**
	Leaf	Source	0.38 (72%)	233.3	
	Stem	Source	0.063 (45%)	25.35	
	Fruit	Sink	0	0	
3	**Total**	**Source**	**1.191 (38%)**	**422.2**	**103.9**
	Leaf	Source	0.88 (81%)	299.6	
	Stem	Source	0.31 (69%)	106.5	
	Fruit	Sink	0	0	
2	**Total**	**Source**	**1.09 (38%)**	**370.9**	**86.5**
	Leaf	Source	0.449 (75%)	170.6	
	Stem	Source	0.17 (61%)	60	
	Fruit	Source	0.59 (28%)	198.2	
1	**Total**	**Source**	**0.271 (39%)**	**113.7**	**85.9**
	Leaf	Source	0.107 (39%)	36.3	
	Stem	Source	0.074 (42%)	26.7	
	Fruit	Source	0.17 (54%)	69.3	

6.3.5.2 Phosphorus

The redistribution of P from each section can only be calculated as a balance of the total P, and therefore does not account for any simultaneous import and export of P. All sections incorporated the remobilised leaf and stem P into other tissues in close proximity to them, most likely the fruit.

Redistribution of P from the leaves varied up the plant, the leaves in section 3 redistribution the highest gross amount of P and the highest proportion of their P content. Sections 2 and 4

followed, with 59 and 46% of their total P respectively, and the leaves in section 1 did not redistribute any P. A modest amount of P was redistributed from the stems in sections 1, 2 and 3 (4 – 13%).

Table 6.2 Mean mg P redistributed from each tissue in each section, and the combined tissues in each section from the peak P content until maturity.

Plant Section	Tissue	Source / Sink for P (total P mg)	Mean P redistribution (mg) / %	Proportion of remobilised P redistributed out of section (%)
5	**Total**	**Sink**	**0**	
4	**Total**	**Sink**	**0**	**0**
	Leaf	Source	10.6 (46%)	
	Stem	Sink	0	
	Fruit	Sink	0	
3	**Total**	**Sink**	**0**	**0**
	Leaf	Source	24.3 (69%)	
	Stem	Source	2.2 (13%)	
	Fruit	Sink	0	
2	**Total**	**Source**	**0**	**0**
	Leaf	Source	10.4 (59%)	
	Stem	Source	1.3 (9%)	
	Fruit	Source	0	
1	**Total**	**Sink**	**0**	**0**
	Leaf	Sink	0	
	Stem	Source	0.5 (4%)	
	Fruit	Sink	0	

6.3.5.3 Potassium and Rubidium

As with ^{15}N taken up from the soil, there was a decline in the amount of Rb in the plants from 130 DAS (Figure 6.9). The total uptake was only 22% of the total Rb provided in the soil, so the decline in uptake was not related to a reduced Rb concentration in the soil late in the season. There may have been some movement of the Rb through the soil, making further uptake difficult, although Rb is relatively immobile in soil, so this seems unlikely. The decrease in whole plant Rb content may also have been due to the export of Rb to the roots

(since only the above ground material was analysed), or the loss of some Rb from leaf and fruit shedding.

The decrease in whole plant Rb was an additive combination of a decrease in the Rb content of the stems in all sections and the leaves in sections 1 and 2 (Figure 6.16). The uptake of Rb and its distribution through the plant was similar to that of K, and followed the same trends (see Figure 6.13).

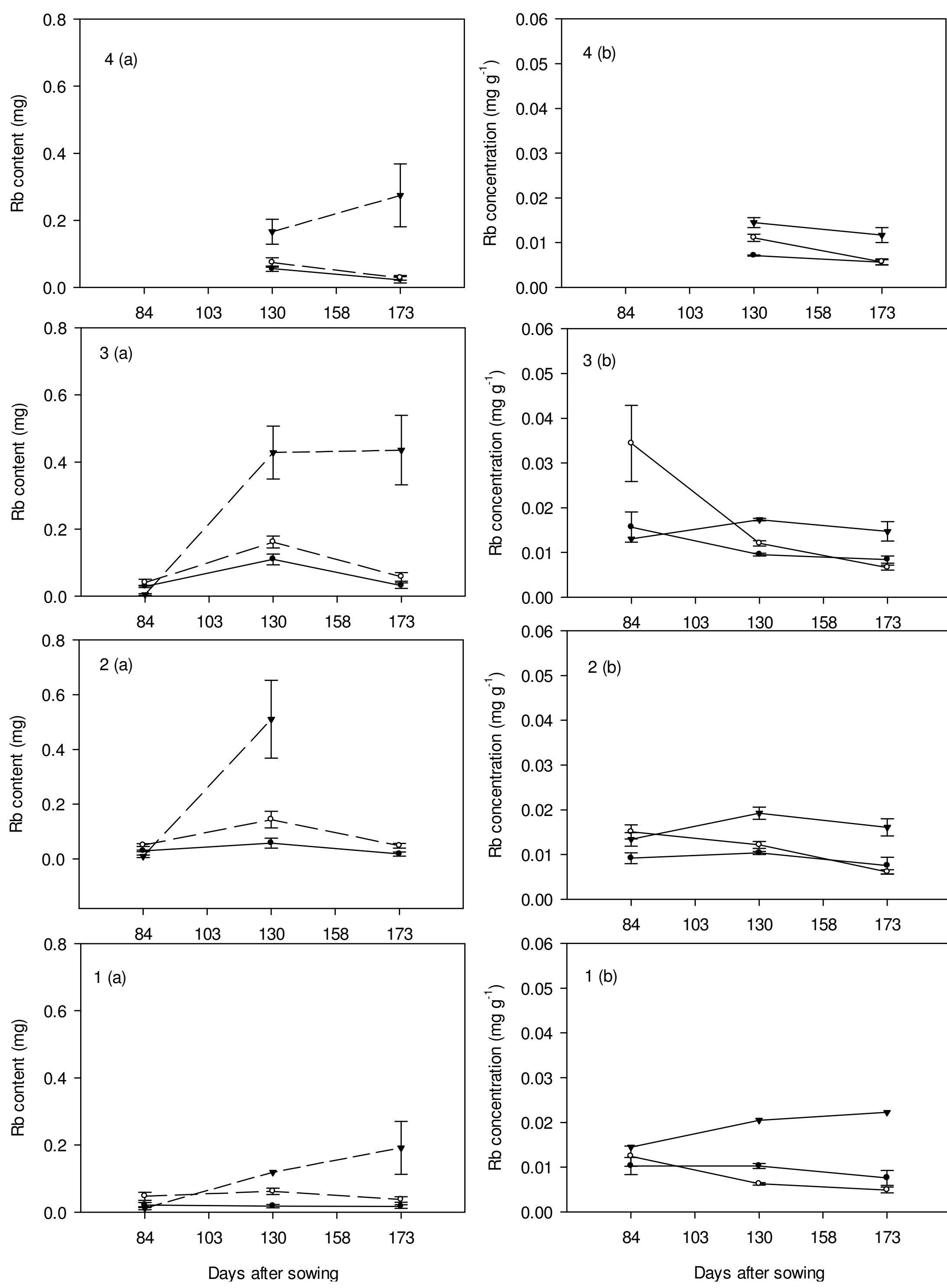

Figure 6.16 The mean Rb content (mg) (a) and concentration (mg g^{-1}) (b) of the leaf ( ), stem ( ) and fruit ( ) in section 1 (g and h), 2 (e and f), 3 (c and d) and 4 (a and b). The error bar represents +/- one standard error of the mean.

As well as variation in the amount of K remobilised from each section, there was a high degree of variation in the distribution of remobilised K from the leaves, stems and fruit within each section (Table 6.3). Proportionally, most of the remobilised K from the leaves, stem and fruit in section 3 was transported out of the section (84%). In contrast, all K remobilised from the leaves and stems in sections 1, 2 and 4, was contained in the section, most likely in the fruit. A similar proportional amount of the leaf and stem K in sections 2, 3 and 4 was redistributed (60 – 70%), much less K was redistributed from section 1 (20 – 40%). The proportion of leaf K redistributed was lower than the potential redistribution calculated in chapter 5 (85%), indicating that there is some variability in the redistribution of leaf K up and down the plant.

Table 6.3 Mean mg K redistributed from each tissue in each section, and the combined tissues in each section, extrapolated from the remobilisation of Rb from each tissue from the peak content until maturity.

Plant Section	**Tissue**	**Source / Sink for K (total K mg)**	**Mean Rb redistribution (mg) / %**	**Mean K redistribution (mg)**	**Proportion of remobilised K redistributed out of section (%)**
5	**Total**	**Source**	**0.002 (4%)**	**2.68**	
4	**Total**	**Sink**	**0**	**0**	**0**
	Leaf	Source	0.033 (60%)	46.5	
	Stem	Source	0.458 (61%)	74.7	
	Fruit	Sink	0	0	
3	**Total**	**Source**	**0.175 (25%)**	**208.1**	**83.7**
	Leaf	Source	0.078 (71%)	100.2	
	Stem	Source	0.104 (64%)	148.2	
	Fruit	Sink	0	0	
2	**Total**	**Sink**	**0**	**0**	**0**
	Leaf	Source	0.0399 (69%)	49.84	
	Stem	Source	0.096 (67%)	122.7	
	Fruit	Sink	0	0	
1	**Total**	**Sink**	**0**	**0**	**0**
	Leaf	Source	0.004 (20%)	6.3	
	Stem	Source	0.024 (40%)	35.7	
	Fruit	Sink	0	0	

6.4 Discussion

The experiment described in this chapter quantified root uptake of a ^{15}N and RbCl solution applied to the soil, and described the distribution of N, P and K over the fruiting period in different sections of the canopy. The aims of the chapter were; 1) to describe the uptake and distribution of nutrients after flowering, 2) to measure the redistribution of nutrients in different sections of the canopy, and 3) to describe the source of nutrients in a mature boll, and if there is any variation in this source based on their node position.

6.4.1 Root uptake through the fruiting period and its contribution to boll development

It is commonly described that root uptake of nutrients declines after "cutout", when the boll demand is greatest, due to the reduction in C, N, K and P to the roots to provide substrates for uptake, transport and assimilation (Krieg and Sung 1986; Guinn and Brummett 1989; Bange *et al.* 2008; Mullins and Burmester 2010). In the previous two chapters it is clear that root uptake through the fruiting period, though it may be slower after cutout, accounts for a significant proportion of the nutrients found in the fruit.

The plants studied in this experiment took up 11% of the total P, 16% of the total K and 17% of the total N and biomass before flowering, a similar amount of biomass to the plants studied in chapter 4, but less N, P and K. More than 80% of the N, P and K in the mature plant were taken up between flowering and cutout, indicating that the bulk of the nutrients in the mature bolls were probably provided by root uptake. To measure if they were directly provided from the roots, or cycled through the leaves would require further research in which the labelled solution is applied to the soil at various stages in the fruiting period, rather than just at flowering.

It can be assumed that the ^{15}N and Rb in the fruit at 103 DAS was imported directly from the roots, since the leaves and stems were still accumulating ^{15}N and Rb at this time in all the sections. This equates to 38% of the N in section 1, 29% of the N in section 2, and 23% of the N in section 3. The amount of N and K in the fruit at sections 4 and 5 was negligible at this point in time. Since there was no analysis for Rb at 103 DAS, a similar estimate of the K provided from the roots by 103 DAS cannot be made, however, at 84 DAS, shortly after flowering, 2% of the total K in section 1 fruit, 5% in the section 2 fruit and 1% in the section

3 fruit was taken up directly from the soil. In section 2 and 3 the leaf and fruit Rb content peaked at 130 DAS, indicating that most, if not all, of the K in the mature bolls was sourced from the soil. This is in line with the conclusions of chapter 5, which found only a very small proportion of boll K at node 11 was provided by redistribution from the leaves immediately surrounding it. Both these estimates show that there is some variation in the supply of N and K from the soil to fruit in different canopy sections, the N supply to bolls from the soil decreasing up the plant and the K supply varying based on the number of bolls developing. Therefore, the bolls lower in the canopy rely more on the roots to supply N and K from the soil than those further up the canopy, and that bolls in different sections of the canopy may derive their nutrients from different sources. This should be further investigated with research into providing labelled fertiliser to the soil at many stages of growth rather than just one, and analysing tissue at more frequent intervals than was possible in this experiment.

In the plants described in chapter 4, where root uptake seemed to continue until maturity in some cases (see section 4.3.3), the redistribution of nutrients from leaves and stems late in the season was inadequate for the supply of the developing bolls. The plants in this experiment did not grow in the same manner as those in Chapter 4, or meet the same conditions in terms of growth and the timing of nutrient uptake, which lead to the conclusion that root uptake occurred late in the season. It is not possible to make conclusions from this experiment about the uptake of nutrients supplementing redistribution late in the season (as in this experiment supplementation was not necessary). By 130 DAS, 85% of fruit N and 92% of plant K had been accumulated, so there was little remaining N and K required for the bolls to develop and mature at the point when leaf and stem tissues exported most nutrients. After this point the export of N, P and K was more than that required for the bolls to develop. The leaf and stem N content of sections 1-4 declined by 718 mg, and the fruit content increased by only 290 mg; the leaf and stem K content declined by 477 mg after 130 DAS, with an increase of K in the fruit in the same time period of only 107 mg. Further experiments on more than one site and in different seasons would be likely to include scenarios such as some described in chapter 4, where root uptake appeared to occur later in the season.

The reason why the plants in this experiment did not take up nutrients late in the season, but those in chapter 4 did, could be explained by the difference in the scale of measurement (that is a single plant vs. a metre of plants), differences in plant spacing (the plants in this

experiment were thinned to 10 m^{-2}), or the impact of plant removal through the season on plants surrounding them. No two neighbouring plants were ever removed, to try to reduce the impact of plant removal, but it is clear that early plant removal increased the access to ^{15}N, at least at 130 DAS (due to their increased ^{15}N uptake). The early thinning of the plants, and subsequent removal of the sampled plants, may therefore have reduced competition between plants and allowed the individual plants to have access to the required nutrients earlier in the season, and therefore not encounter stress or limitation in nutrients to adequately supply their bolls. Rosolem *et al.* (2012) showed that plant density had a significant impact on the rate of uptake of nutrients and the length of the flowering period. In addition, Brodrick *et al.* (2012) found that increased plant density placed more stress on the plants, in terms of biomass accumulation and fruit development at the end of the season. The removal of plants, therefore, may have limited this stress to a point that nutrient uptake before 130 DAS was sufficient to fill most of the bolls on the plants, and removed the late season demands and stresses which other crops studied may have encountered. The bolls in sections 1, 2 and 3 accumulated almost all their biomass, N, P and K before this time, supporting this hypothesis. Experiments evaluating redistribution of nutrients and the efficiency of redistribution under different planting densities, as well as experiments applying labelled fertiliser to plants at set densities (that is, where the removal of the plants does not change the density significantly) could test this hypothesis.

6.4.2 Variability in redistribution in different parts of the canopy

Chapter 5 quantified a potential proportion of redistribution of leaf nutrients, however these measurements were carried out only on one node, which was in the centre of the plant – the site of the highest biomass, fruit and leaf size and nutrient concentration (Thompson *et al.* 1976; Constable and Rawson 1980b; Constable 1991). The second aim of this chapter was to compare the redistribution of nutrients in different sections of the canopy, to see if the amount of redistribution varied between sections, and if the potential defined in chapter 5 applies to the whole plant. There was significant variation between the three nutrients studied in terms of the redistribution of nutrients from the tissues in each section, and the proportional export of the remobilised material to other plant sections.

6.4.2.1 Nitrogen

Export of N occurred from each section, that is, no section redistributed all remobilised N to other tissues within the section. The lower sections (1, 2 and 3) redistributed over 85% of the remobilised nutrients out of the sections, while section 4 only exported a small amount (17%). The low export of N from section 4 may have been linked to the demand for N from the bolls developing, since section 4 had the highest fruit dry weight, boll number and continued to be filling green bolls until maturity. It also had the highest R:V ratio, which may have placed extra pressure or demand on the leaf N resources. From 130 DAS, the fruit in section 4 imported twice the amount as section 3, indicating that the rate of demand for N from section 4 was also the highest. Sections 1 – 3, however, accumulated most of the nutrients in the fruit by 130 DAS, accounting for the export of nutrients from these sections, as more N was remobilised from the leaves and stems than was required by the bolls.

Based on the potential redistribution from leaves calculated in Chapter 5 (81% for mainstem leaves, and 69% for sympodial leaves), the redistribution of N from the leaves in sections 2, 3 and 4 was maximised and reached the potential (based on an average of 75%). The leaves in section 1 redistributed far lower than the potential, indicating some variation in the functioning of leaves through the canopy. Reasons for the low amount of redistribution from section 1 could be related to the number of fruit in the section, the ratio of reproductive to vegetative tissue in the section (which never reached a 1: 1 ratio) the sufficiency of the leaves from other sections, in closer proximity to the developing fruit to supply the N required from bolls further up the canopy, or to the need for the leaves to continue functioning until maturity. A low photosynthetic rate, due to shading, may also have limited their export of N, which is probably linked to the export of carbon assimilates from the leaves (Constable and Rawson 1980b; 1982; Bondada *et al.* 1996; Milroy and Bange 2003). Their lower N content and N concentration also meant that they had fewer reserves to export than the leaves higher up. Apart from the lower leaves, the potential redistribution of 75% of leaf N is accurate for the majority of the leaves in the plant. The average redistribution of all the leaves pooled together was 61%, which though lower than the potential, and much lower than the rate in the middle of the canopy, still gives a good measurement of an average N redistribution from a plant reaching its potential.

The redistribution of N from the stems ranged from 42% in section 1 to 69% in section 3, which could also indicate the importance of the stem in the middle of the plant as a connective tissue transporting N bi-directionally up and down the plant. Redistribution of stem nutrients increased slightly up the plant, which was probably a result of stems low in the plant being constantly used as transport to and from the roots rather than a difference in the redistribution of nutrients from storage organs.

Redistribution of N from the fruit, which in chapter 5 was shown to occur from the walls, lint and bracts occurred only in sections 1 and 2. This earlier developing fruit redistributed N, while there was continuous accumulation in the higher sections. This was probably due to the presence of green bolls in sections 3 and 4 until maturity, and the fact that analysing tissues of a different age reduces the ability of the experiment to detect smaller changes. A similar experiment analysing each node individually would be able to pick up variations in N redistribution from fruit with age, however the cost and time limitations of this experiment made this impossible. Based on the grouped data by 5 node sections it seems as though any variability in fruit redistribution was related to fruit age. Had the plants kept growing past the date of crop "maturity" (when they were defoliated and harvested) and all the green bolls reached maturity, this variation may not have existed, and all fruit may have redistributed some of their N content.

Based on this data it seems that there is some variation in the redistribution of N from the leaves through the canopy, with lower leaves having a lower redistribution rate than those up the canopy. Leaves from nodes 6 to 20 reached their potential in terms of proportional redistribution, showing that in sections contributing to yield (with the most bolls) there was very little variation, and that the measurements made on one node can be extrapolated to the whole middle of the plant. Since the high and low leaves (at nodes 1 – 5 and 21+) did not reach the maximum potential redistribution, any measurement bulking all the leaves from the whole plant may not ever meet the 75% potential figure. Since most leaves in these plants reached their potential, it could be concluded that these plants were an efficient user of N, with an average redistribution of 61% of leaf N across the whole plant. Based on the results in this experiment, a figure of 60% could be used as a measurement of efficiency (although repeated studies should be carried out to confirm this figure). Any redistribution of less than

50% probably indicates inefficient nutrient recycling in the plant, or the allocation of remobilised nutrients to new vegetative growth rather than bolls.

6.4.2.2 Phosphorus

No section transported any of the P remobilised from leaves and stems out of the section, and to any tissue not in close proximity to the site of remobilisation. Since P is a relatively immobile element within the plant (see section 2.2.2), this result makes physiological sense.

There was some variation in the proportion of leaf nutrients remobilised up the plant, with no remobilisation from leaves in section 1 occurring. Section 3 leaves remobilised more leaf P than any other section. Since P is imported into the developing bolls early (see Chapter 5), the sink demand from sections 3 and 4 would have been similar, and the presence of green bolls in section 4 may not have equated to increased demand after 130 DAS. The higher redistribution from the leaves in section 3 was therefore, probably not related to sink demand, but rather so some other physiological mechanism, or the export of C or N from the leaves. The whole plant ratio of reproductive to vegetative structures did not follow the same pattern as the level of redistribution of leaf P – being the highest in section 4 and lowest in section 1, whereas the redistribution was highest in section 3, then 2, then 4.

Based on this data it can be concluded that there was some variation in leaf P redistribution, with the leaves in the middle of the canopy (nodes 11 – 15) redistributing more P than those above and below them, and that there was little variation in stem or fruit P redistribution. A similar experiment, using labelled P fertiliser should be carried out to generate data similar to the N and K data. This would also allow for more accurate estimates of redistribution to be made than the balance method used here.

6.4.2.3 Potassium

As with N and P, there was some variation in the proportion of leaf, stem and fruit K redistributed, and in the proportion of the remobilised nutrients from each tissue redistributed to removed parts of the canopy. Redistribution was highest in the middle of the canopy, as with P and N, with section 3 allocating 84% of the redistributed nutrients from the leaves and stems to other sections. There was no redistribution of fruit K in section 3, indicating that

fruit K was supplied by root uptake, and on balance the leaf and stem K could be transported to other sections. In section 2, although a high proportion of the leaf and stem K was also remobilised the lack of data for the fruit Rb content at 173 DAS means that redistribution out of the section could not be calculated.

There was no export of K outside of the tissues in section 1, 2 or 4. The export of K outside of section 3 concurs with the data presented in chapter 5, that there was significant export of K from the leaves at node 11 to fruit above and below it. This experiment shows that significant variation exists in the allocation of redistributed nutrients from different sections of the plant. The leaves and stems in the middle of the plant seem to be major sources of K for the fruit in other sections, while the leaves and stems in the upper and lower sections of the canopy make little or no contribution to the supply of K to other sections, and remobilised K is allocated to adjacent fruit.

The fruit in sections 1, 2 and 3 would have relied on root uptake as the major source of K for the developing bolls, which confirms the conclusions of chapter 5 that redistribution of K from leaves in close proximity to the developing boll at node 11 contributed only around 1% of the final boll K, and that most of the K in the mature boll must have come from root uptake or other leaves. This experiment shows that other leaves may have contributed only very little of the fruit K in the middle section of the canopy, since all of the section 2 fruit K and 85% of the section 3 fruit K was accumulated by the time root uptake declined.

As well as variation in the allocation of redistributed K from the leaves and stems in each section, there was some variation in the gross and proportional amount of K redistributed from leaves and stems in different sections of the canopy. Section 1, as with N and P, redistributed a smaller proportion of its leaf and stem K than other sections. In sections 2 and 4 there was a similar proportional export of leaf and stem K, the stems redistributing a greater gross amount of K than the leaves. In section 3, however, the leaves exported a greater proportion of their K than the stems.

All the leaves fell short of the redistribution potential of approximately 85% calculated in chapter 5. This may have been a result of pooling leaves from 5 nodes together, or of more variation between the nodes than for the other nutrients. It also may have been a reflection of

the fact that most of the fruit K had already been taken up by the time root uptake declined, when there was limited demand for K from the bolls to be supplied by redistribution. An experiment in a limited K environment or under a range of K conditions should be carried out to evaluate the redistribution of K from leaves and stems when K content from the soil could not meet, or almost meet, boll requirements as in this experiment. An analysis of a variety of plants with a range of ratios of reproductive to vegetative tissue could examine the impact that sink size has on redistribution, although there was no correlation between the whole plant R:V ratio and K redistribution in chapter 4.

6.4.3 Conclusions

There is some variability in redistribution of N, P and K from leaves, stems and fruit in different parts of the canopy. For all three nutrients, the middle section of the canopy redistributed a greater proportion of the nutrients in the leaves and stems, which declined towards the top and the bottom of the plant. Because of this variability between nodes, the maximum potential redistribution calculated in chapter 5 for N and K may not be accurate when analysing whole plant tissues. As suggested, a figure of 60% of leaf N may represent efficient recycling of leaf N, and less that 50% inefficient recycling. Similar benchmarks for K are difficult to hypothesise, since the leaves in this experiment did not reach the potential measured in chapter 5, and there seems to be more variation between individual nodes and plant sections for K than for N. Over 50% may also be an arbitrary cut-off for K, based on the variability in this experiment (between 20 and 71% of leaf K remobilised, and 40 – 67% of stem K).

While further data is needed, there was some evidence to suggest that the bolls lower in the canopy (below node 15) were supplied to a greater extend with N, P and K from root uptake through the flowering period than those higher up the canopy. More experiments examining the uptake of labelled fertiliser applied at different stages, and with different numbers of bolls lower in the canopy could further explain this hypothesis, and help to predict the retention of bolls in different sections of the canopy based on nutrient supply and redistribution efficiencies. Understanding the source of nutrients in the mature bolls throughout the canopy could assist in designing fertiliser strategies and management systems to optimise nutrient use efficiency through the fruiting period.

Some questions remain and still require further research:

1) What is the impact of nutrient stress on the redistribution of nutrients from one plant part to another?
2) What is the impact of water stress on the redistribution of nutrients from one plant part to another?
3) Does the removal of, or presence of fruit lower in the canopy change the nutrient redistribution patterns in different sections of the plant?

The first two of these questions will be addressed in the following two chapters, evaluating the redistribution of nutrients at a whole plant scale under different nutrient and water supply treatments.

CHAPTER 7

Nutrient redistribution in high-yielding cotton grown with varying levels of N, P and K supply

7.1 Introduction

At a single plant level, the redistribution of nutrients from leaves to other plant parts represents the major internal mechanism by which a plant can conserve nutrients (Chapin *et al.* 1990), increase their nutrient use efficiency (Hocking and Mason 1993; Ma *et al.* 2004; Semenov *et al.* 2007; Covelo *et al.* 2008) and potentially increase their competitiveness in nutrient poor environments (Aerts and Chapin 2000). As shown in chapters 4 – 6, the remobilisation and redistribution of N, P and K from leaves in cotton plants varies considerably between crops, even of a similar yield, plant size and total nutrient content. This variability means that conclusions as to the effects of variations in nutrient supply, water supply, temperature, light and pest pressure cannot be confidently made without intra-seasonal comparisons of plants exposed to different levels of stress. The effect of these factors needs to be better understood in order to explain differences in the redistribution from similar crops. This chapter will examine the effect on redistribution of changing the supply of N, P or K to the roots of a developing cotton plant.

The few published reports quantifying the redistribution of nutrients from leaves with varying nutrient contents have mainly focussed on the cycling of nutrients in ecosystems. Killingbeck (1996) and Aerts (1996) both published wide-ranging reviews on redistribution in woodland ecosystems and came to a similar conclusion that it was difficult to establish a clear relationship between nutrient redistribution and nutrient supply. This is because variation between species in a similar ecosystem may limit conclusions with respect to nutrient supply. Kobe *et al.* (2005) used a different approach through allometric scaling of global data sets to quantify the redistribution of nutrients in ecosystems. They showed that taxa with a higher green leaf N concentration generally redistribute a smaller fraction of their tissue N than those with a lower green leaf N concentration, indicating that in environments with a low N supply, or species with a low tissue N status, redistribution is increased. Similarly Norris and

Reich (2009), again examining a woodland ecosystem, showed that species have a modest enhancement of N redistribution in response to limited soil N supply.

Studies linking the N, P or K supply with the remobilisation and redistribution of leaf nutrients in an agricultural system are rare, and have mainly focussed on determinate crops. Hocking and Steer (1995) examined the effect of N supply, and the timing of N application on the remobilisation of leaf N in sunflower. They reported that the amount of N redistributed from leaves to fruit did not vary with increasing N supply, but was primarily influenced by the timing of N application. Guitman *et al.* (1991), however, reported that remobilisation of leaf N in wheat is accelerated and occurs in greater proportion under N deficiency, and decreases with increasing N supply. The findings of Guitman *et al.* (1991) seem to be in line with assertions made in many other studies, indicating the N remobilisation is inversely related to N supply in wheat and other grain crops (Semenov *et al.* 2007; Subasinghe 2007; Gotz *et al.* 2008). In their study examining N dynamics in profiles of leaves up the canopy in cotton, Milroy *et al.* (2001) found that lower leaves in the canopy, with a lower initial N concentration, exported a higher proportion of N than those further up the canopy with a higher peak N concentration. This finding, however, is contradicted by the previous chapter, where the export of N was proportionally similar in the middle sections of the crop, and lower in the lowest leaves. The effect of fertilisation or modification of the N supply was not examined in chapter 6 or the study by Milroy *et al.*, and so differences may have been due to N supply, water supply or another environmental factor not measured. Studies examining the effect of P and K supply on redistribution of these nutrients are limited to woodland ecosystems and large trees, probably because of the more significant P and K deficiencies found in old ecosystems.

While there are a limited number of studies measuring the effect of nutrient supply on their redistribution from vegetative to reproductive plant parts, there have been many studies examining the effect of varying nutrient supply on nutrient accumulation and partitioning in a developing plant or crop. Nutrient rate studies of cotton plants have not widely reported redistribution amounts or rates, but show several common themes from which assumptions about redistribution in can be made. Many studies have shown that increasing the nutrient supply to the plant results in a higher concentration of N, P or K in the plant, particularly in the leaves, without always causing an increase in yield or changing the reproductive:

vegetative ratio of the plant (Halevy *et al.* 1987; Pettigrew *et al.* 1996; Read *et al.* 2006). An increase in plant size, yield, seed size and number of fruiting sites is however, broadly attributed to the application of fertilisers. Excess nutrient supply, particularly of N, promotes increased vegetative growth and may reduce gin turnout (Boquet *et al.* 1994; Boquet and Breitenbeck 2000; Fritschi *et al.* 2004b; McConnell and Mozaffari 2004; Girma *et al.* 2007). If the assumption that leaves with a higher peak nutrient content redistribute a smaller amount of nutrients, it would follow that with increasing fertilisation, redistribution will decrease.

The effect of more or less available N, P and K on the dynamics of N, P and K distribution and redistribution in high-yielding Australian cotton is unclear. Based on the results of previous studies several assumptions about the effect of changing the supply of N, P and K to the developing crops can be made; firstly that remobilisation and leaf senescence is enhanced and accelerated under nutrient deficiency, secondly that a change in the ratio of sinks to sources resulting from varying the nutrient supply would alter the demand for remobilised nutrients to supplement root uptake, and thirdly, that the efficiency of nutrient use will be a function of both the uptake and remobilisation of assimilated nutrients by a plant. This chapter will examine the nutrient distribution and redistribution in high-yielding cotton crops given varying amount of N, P and K fertiliser, addressing the question raised in previous chapters about the effect of a change in the nutrient supply at the roots on the redistribution of that nutrient within the plant. The main aims of this chapter are;

1) To quantify the effect of variation in N, P and K supply on N, P, K and biomass distribution between plant parts.
2) To investigate the effect of variation in N, P and K supply on N, P and K redistribution between vegetative and reproductive tissue in high-yielding cotton.

7.2 Materials and Methods

To compare the N, P and K uptake, distribution and redistribution in plants exposed to nutrient stress and exposed to no nutrient stress, three experiments were carried out in the 2007-08, 2008-09 and 2009-10 cotton seasons. These experiments are described in detail in Chapter 3; experiment 2 (described in section 3.4.2), experiment 3 (described in section 3.4.3) and experiment 5 (described in section 3.4.5).

7.2.1 N rates

Two N rate experiments were carried out at ACRI, Narrabri (see section 3.1.1 for site description). In experiment 2, three N rates were applied to the soil as pre-planting N fertiliser, 0, 125 and 200 kg N ha^{-1}. N was applied by spreading urea (46% N) across the plot at the rate of 0 kg ha^{-1} (nil plots), 271 kg ha^{-1} (125 kg N plots) and 434.78 kg ha^{-1} (200 kg plots). Sicot71BRF cotton plants were sown at a rate of 12 plants m^{-2} on the 13th October, 2008. The experimental design and plot description were as described previously (section 3.4.2.1).

In experiment 5, two N rates were applied, "low" and "high", as pre-planting and in crop side-dressed fertiliser. A 0 kg ha^{-1} plot was not used. N was applied at a rate of 50 kg N ha^{-1} and 200 kg N ha^{-1}, as anhydrous ammonium (82% N) at a rate of 61 kg ha^{-1} (50 kg N) to all plots and an additional 326 kg ha^{-1} urea (150 kg N) applied to 200 kg plots as a side-dressing. Sicot71BRF seeds were sown at a rate of 12 plants m^{-2} on the 15th October, 2009. The experimental design and plot description were as described previously (section 3.4.5.1).

7.2.2 P and K rates

A P and K rate experiment (experiment 3, described in section 3.4.3) was carried out at 'Cardale', Narrabri (see section 3.1.2 for site description). Two P and K rates were applied, "nil" and "plus PK", as pre-planting fertiliser. P and K were applied at 0 kg ha^{-1}, or at 60 kg P ha^{-1}, and 160 kg K ha^{-1}. Sicot71BRF plants were sown at a rate of 12 plants m^{-2} on the 1st October, 2007. The experimental design and plot description were as described previously (section 3.4.3.1).

7.2.3 Plant sampling and analysis

The above ground plants from a 1 m^2 area were harvested from each plot at regular intervals between flowering and defoliation of the crop. Sampling dates are given in Table 3.3, Table 3.4 and Table 3.7.

Whole plants were partitioned into leaves, stems (including petioles) and fruit (squares, flowers and bolls including seed, lint, boll walls and bracts). Samples were dried, ground and

analysed for N, P and K as described in sections 3.3.1 and 3.3.2. After defoliation yield was determined by handpicking as described in section 3.3.3.

7.2.4 Data analysis

Data was analysed using Genstat® 14th edition. Total biomass, N, P and K were compared using ANOVAs. Bernacchi *et al.* (2007) and Gedroc *et al.* (1996) found that accounting for developmental stages, more differences in dry matter accumulation and partitioning between plants as they develop can be demonstrated. Analysis of plants at a specific growth stage accounts for some of the differences in growth rate and seasonal environmental effects. To account for these differences between the crops, three separate ANOVAs were carried out to compare the dry matter accumulation and nutrient uptake and partitioning at flowering, 4 NAWF and maturity, rather than using plant age or thermal time as a factor. Correlation coefficients were calculated using Genstat® 14th edition. Redistribution was calculated using the method described in Ch.4, using Sigma Plot® and calculated redistribution was compared using ANOVA.

7.3 Results

7.3.1 Crop growth and development

The dates of the key growth and development stages of the cotton crops are given in Table 7.1. Crops were representative of normal irrigated cotton growth and development in Australia. There was no difference in the fruiting time period between treatments at the same site, so one set of data is presented.

Table 7.1 Dates of key developmental stages reached in the three experiments.

Growth Stage	Cardale	F6	A3
Sowing	1st Oct, 2007	13th Oct, 2008	15th Oct, 2009
Emergence	10th Oct, 2007	21st Oct, 2008	23rd Oct, 2009
Squaring	22nd Nov, 2007	29th Nov, 2008	22nd Nov, 2009
First Flower	9th Dec, 2007	21st Dec, 2008	10th Dec, 2009
Open Boll	12th Feb, 2008	7th Feb, 2009	29th Jan, 2010
4 NAWF	3rd Mar, 2008	3rd Feb, 2009	27th Jan, 2010
Maturity	4th Apr, 2008	17th Mar, 2009	8th April, 2010

7.3.2 N rates experiments (2 and 5)

7.3.2.1 Yield, boll number and size

The addition of N fertiliser increased the yield and number of bolls at each site (Table 7.2). Increasing N fertilisation decreased gin turnout and average boll size at both sites. There was significant seasonal variation in the yield response to N fertiliser ($P < 0.05$).

Table 7.2 Mean yield (kg lint ha^{-1}), number of bolls m^{-2}, boll weight (g) and gin turnout from each N rate. Significance calculated at a 0.05 level.

N Rate	Experiment	Yield (kg lint ha^{-1})	Bolls m^{-2} at maturity	Average Boll weight at maturity (g)	Gin turnout (% lint of seed cotton)
Nil	F6	1942.2^{c}	112^{c}	6.3^{a}	43.6^{b}
50 kg N ha^{-1}	A3	2422.7^{b}	128^{c}	4.6^{b}	44.5^{a}
125 kg N ha^{-1}	F6	2489.9^{b}	164^{b}	5.1^{ab}	42.2^{c}
200 kg N ha^{-1}	F6	2394.4^{b}	158^{b}	5.3^{ab}	41.8^{c}
200 kg N ha^{-1}	A3	2983.3^{a}	191^{a}	5.1^{b}	41.6^{c}
L.S.D.		351.1	24.8	1.158	0.771
P value		< 0.001	< 0.001	0.042	< 0.001

7.3.2.2 Total N and biomass

Biomass and N accumulation throughout the season followed a logistic curve at each site (Figure 7.1). The peak period of biomass accumulation and N accumulation occurred between flowering and 4 NAWF, with N accumulation (Figure 7.1b) preceding biomass accumulation (Figure 7.1a).

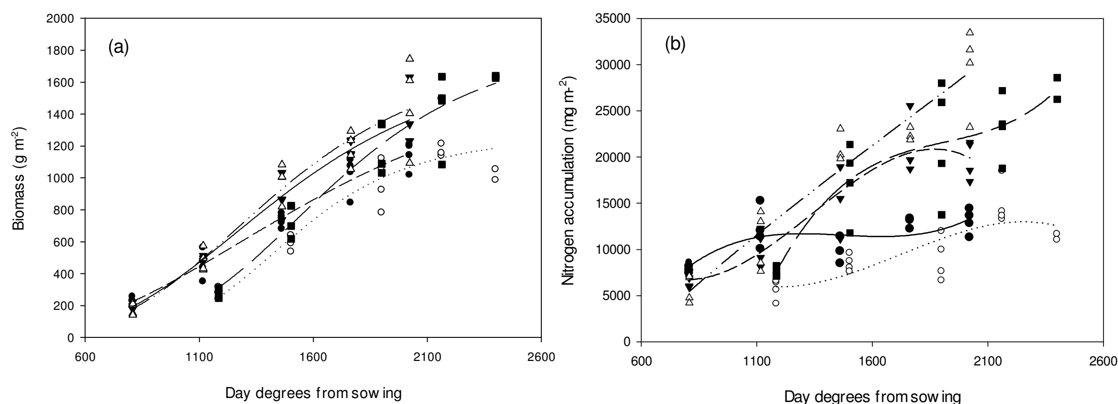

Figure 7.1 (a) total biomass accumulation (g m^{-2}) and (b) total N accumulation (mg m^{-2}) at —●—F6, 0 kg N ha^{-1}, ···○··· A3 50 kg N ha^{-1}, —▼—F6 125 kg N ha^{-1}, —△—F6 200 kg N ha^{-1} and —■—A3 200 kg N ha^{-1} with fitted logistic curves .

Biomass and N uptake were analysed according to standardised growth stages (flowering, 4 NAWF and maturity) to account for seasonal variation and the changes in plant size or growth rate between the N rates (Figure 7.2). The addition of fertiliser increased the total biomass ($P < 0.001$) and N uptake ($P < 0.001$) at each site (Figure 7.2). At maturity, total N uptake was related to biomass uptake, with more biomass equating to a higher N uptake (Figure 7.2), although there was no difference in biomass accumulation or N uptake between the nil and 50 kg N ha^{-1} plots. There was no significant seasonal effect on N uptake or plant size ($P > 0.05$).

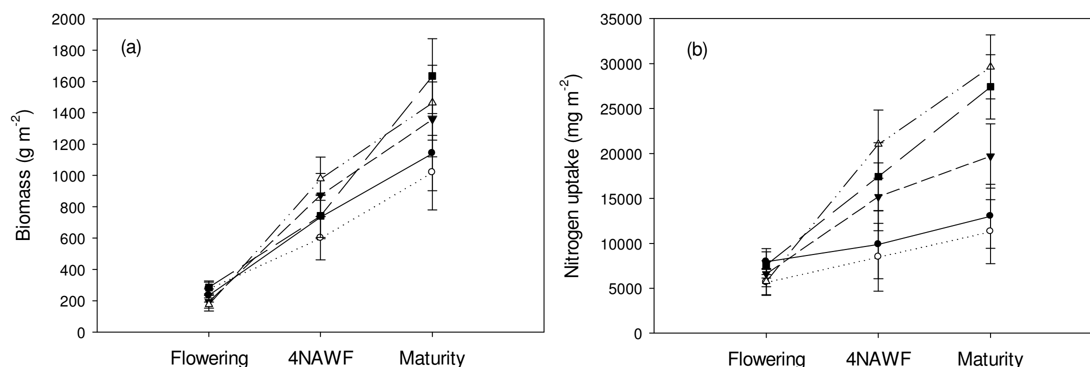

Figure 7.2 (a) biomass (g m^{-2}) and (b) N accumulation (mg m^{-2}) at flowering, 4 NAWF and maturity at —●—F6, 0 kg N ha^{-1}, ···○···A3 50 kg N ha^{-1}, —▼—F6 125 kg N ha^{-1}, —△—F6 200 kg N ha^{-1} and —■—A3 200 kg N ha^{-1}, vertical bars represent the LSD at 0.05.

At flowering N concentration seemed to be related to the site more than the N fertilisation rate (Figure 7.3). There was no difference in N concentration between plots within each site; the F6 plots had a higher N concentration than the A3 plots. Peak N concentration occurred at flowering in all plots. The decline in N concentration between flowering and 4 NAWF at each site was inversely related to N supply. In the nil plots the mean N concentration declined from 34 to 13 mg N g^{-1} m^{-2}, in the 125 kg N plots from 34 to 17 mg N g^{-1} m^{-2} and at the F6 200 kg plots, from 32 to 20 mg N g^{-1} m^{-2}. At A3, the 200 kg plots declined during the same period by only 3 mg N g^{-1} m^{-2}, while the 50 kg N plots declined from 20 to 14 mg N g^{-1} m^{-2}. At 4 NAWF and maturity N concentration was related to N supply, with plants supplied with more N maintaining a higher N concentration. There was no difference in N concentration at maturity between the plants fertilized with 50 kg N ha^{-1} and no fertiliser (Figure 7.3).

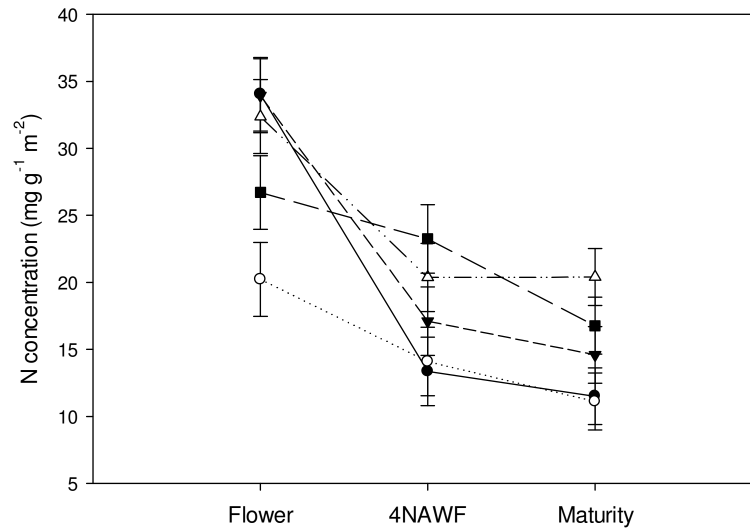

Figure 7.3 N concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity at —●—F6, 0 kg N ha^{-1}, ○ A3 50 kg N ha^{-1}, —▼—F6 125 kg N ha^{-1}, —△—F6 200 kg N ha^{-1} and —■—A3 200 kg N ha^{-1}, vertical bars represent the LSD at 0.05.

Table 7.3 shows the proportion (%) of the total N and biomass accumulated between sowing and flowering and between flowering and 4 NAWF. Plants supplied with more fertiliser took up a higher proportion of the total N and biomass after flowering than those supplied with no or a low amount of N ($P < 0.001$). In general, the largest plants continued biomass accumulation after flowering, and the largest plants in the 200 kg N ha^{-1} plots at A3 took up more biomass after 4 NAWF than all other plots. There was no difference in the proportion of

total N taken up by 4 NAWF. N accumulation occurred earlier than biomass accumulation at all sites.

Table 7.3 The proportional uptake (%) of the total N and total biomass at flowering, 4 NAWF and maturity.

N Rate	Expt.	N			Biomass		
		Flowering	4 NAWF	Maturity	Flowering	4 NAWF	Maturity
Nil	F6	61.2	79.9	100	20.5	64.3	100
50 kg N ha^{-1}	A3	49.9	74.8	100	27.3	58.9	100
125 kg N ha^{-1}	F6	33.5	77.0	100	14.3	64.4	100
200 kg N ha^{-1}	F6	19.4	71.0	100	12.0	66.9	100
200 kg N ha^{-1}	A3	27.7	67.2	100	17.5	45.4	100
L.S.D.		9.81			4.58	16.57	
P value		<0.001	n.s.	n.s.	<0.001	0.05	n.s.

7.3.2.3 *N and biomass partitioning*

The proportional (%) distribution of biomass and N between the leaf, stem and fruit fractions of the plants at flowering, 4 NAWF and maturity is given in Table 7.4. There was a consistent difference in the biomass partitioning between the two sites ($P < 0.05$) indicating that seasonal differences may have altered the growth pattern more than just the N supplied. Plants at A3 partitioned less biomass and N to fruit between flowering and 4 NAWF than the F6 treatments. Between the F6 treatments plants supplied with more N partitioned less biomass and N to fruit at 4 NAWF, and accumulated more N and biomass in fruit after 4 NAWF than those supplied with limited N. N supply had no consistent affect on the partitioning of biomass and N between the seasons. At F6 plants supplied with more N had a higher allocation of biomass and N to leaves and stems at maturity. There was no difference in partitioning between N treatments at A3.

Table 7.4 The proportional distribution (%) between the leaves, stems (including petioles) and fruit (including boll walls, bracts, seeds and lint) of N and biomass at flowering, 4 NAWF and maturity.

N Rate	Expt.	Tissue type	Biomass			N		
			Fl.	4 NAWF	Mat.	Fl.	4 NAWF	Mat.
Nil	F6	Leaf	45.5	20.4	14.4	61.4	37.0	20.0
		Stem	50.4	26.8	23.3	33.6	8.8	9.3
		Fruit	4.2	52.9	62.4	5.0	54.1	70.7
50 kg N ha^{-1}	A3	Leaf	39.5	27.2	13.0	63.5	49.5	18.7
		Stem	52.2	41.8	29.8	24.8	14.6	12.3
		Fruit	8.3	31.1	57.2	11.7	35.9	69.0
125 kg N ha^{-1}	F6	Leaf	50.9	21.8	15.5	68.1	41.1	21.8
		Stem	44.6	26.7	23.0	26.5	10.2	9.7
		Fruit	4.5	51.5	61.5	5.3	48.8	68.5
200 kg N ha^{-1}	F6	Leaf	45.9	25.0	18.9	62.9	45.1	28.7
		Stem	48.4	29.1	23.6	30.2	12.7	11.3
		Fruit	5.7	45.9	57.6	6.9	42.2	60.0
200 kg N ha^{-1}	A3	Leaf	41.9	31.9	12.1	62.4	53.9	17.6
		Stem	51.3	44.3	27.8	28.7	22.0	9.1
		Fruit	6.8	23.8	60.1	8.9	24.1	73.3
P* values and LSD** *** = *P* < 0.05** *= *P* < 0.001**		**Leaf**	* **6.05**	** **1.85**	** **2.02**	**n.s.**	** **4.81**	** **5.43**
		Stem	**n.s.**	** **5.18**	** **3.37**	**n.s.**	** **4.82**	**n.s.**
		Fruit	**n.s.**	** **6.55**	**n.s.**	* **4.15**	** **7.57**	* **8.02**

While there was no difference in the total amount of N in the leaves at flowering, there was a difference in the N concentration ($P < 0.001$) (Figure 7.4). Plants at F6 had a higher N concentration at flowering than those at A3. At cutout, plants from both sites supplied with less N had a lower leaf, stem and fruit concentration ($P < 0.05$). The decline in the leaf

concentration between flowering and maturity of the plants supplied with less N was also greater than those given 200 kg N ha^{-1} (Figure 7.4). Between 4 NAWF and maturity there was variation between the amount and the rate of N concentration decline in the leaves, stems and fruit. The change in leaf concentration was least in plants supplied with 200 kg N ha^{-1}, and greatest in the nil plots. In the A3 200 kg N ha^{-1} plots, leaf N concentration increased until 4 NAWF, indicating N uptake was faster than leaf growth, although the decline after 4 NAWF was at a greater rate than any other treatment. These plants also showed a decrease in the N concentration of the stems between 4 NAWF and maturity during which time the N concentration was constant at all other treatments.

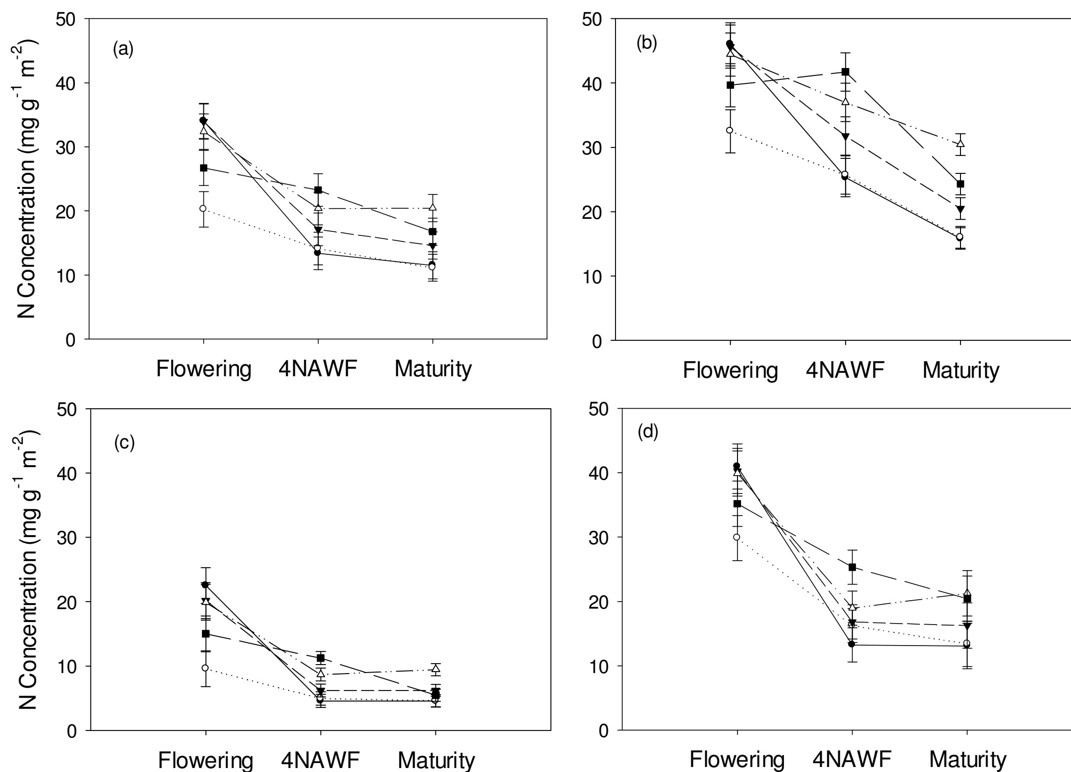

Figure 7.4 N concentration (mg g^{-1} m^{-2}) at flowering, 4 NAWF and maturity in the (a) Whole plants, (b) Leaves, (c) Stems and (d) Fruit at —●—F6, 0 kg N ha^{-1}, ···○··· A3 50 kg N ha-1, —▼— F6 125 kg N ha^{-1}, —△—F6 200 kg N ha^{-1} and —■—A3 200 kg N ha^{-1}, vertical bars represent the LSD at 0.05.

7.3.2.4 N redistribution

Logistic curves were fitted to the total N and fruit N accumulation data for each plot. The derivative of these curves was calculated to give the daily uptake rate of N by the whole plant, and by the fruit fraction. The area between the fruit accumulation curve and the total

accumulation curve after the point at which fruit accumulation was equal to total plant accumulation was calculated until maturity to give the amount of N in the fruit supplied by redistribution of vegetative nutrients. The mean uptake rates and redistribution values are given in Figure 7.5 and Table 7.5. All curves were a statistically significant fit ($P < 0.05$), although the N accumulation curve in the nil plots at F6 showed a limited variation in N uptake rate through the season, with the mean daily uptake rate declining throughout the season, indicating a deficiency in N supply limiting growth (Figure 7.5a).

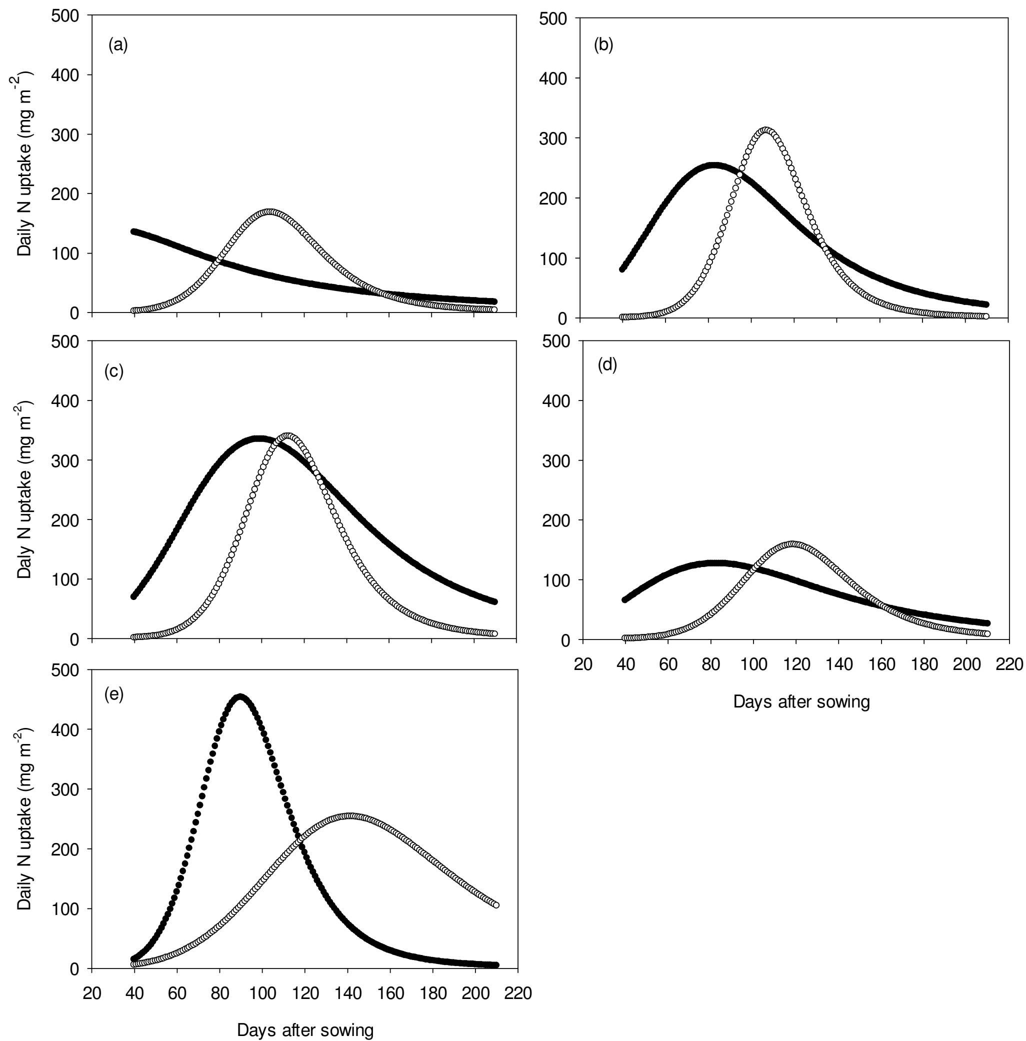

Figure 7.5 Derived N accumulation curves for the daily total plant accumulation of N (◆) and the daily fruit accumulation of N (◇) in plants from (a) nil plots, plots fertilised with (b) 50 kg N ha^{-1}, (c) 125 kg N ha^{-1}, (d) 200 kg N ha^{-1} (F6) and (e) 200 kg N ha^{-1} (A3).

Redistribution was greatest in the plots supplied with 200kg N ha^{-1} at A3, where 3807 mg N m^{-2} more N was redistributed than any other plot (Table 7.5). There was no difference in the

amount of N redistributed in any of the other treatments. Analysing each site separately showed that at F6 redistribution declined with increasing N supply ($P < 0.001$), although the opposite result was observed at A3 ($P < 0.05$). Similarly at F6 the proportion of fruit N supplied from redistribution was higher in plants given less N fertiliser, and at A3 the opposite trend occurred ($P < 0.05$) (Table 7.5).

Table 7.5 The total amount of N redistributed from vegetative to fruit tissue (mg m^{-2}) and the proportion (%) of the fruit N at maturity supplied by redistribution

N rate	Total N (mg m^{-2})	% of Fruit N at maturity from redistribution
F6 0	5484^{b}	59.2^{a}
A3 50	2188^{b}	23.9^{b}
F6 125	2630^{b}	20.1^{b}
F6 200	1639^{b}	9.4^{b}
A3 200	9291^{a}	54.3^{a}
LSD	3529	27.81
P value	0.002	0.005

7.3.3 P and K fertiliser

7.3.3.1 Lint Yield

The addition of P and K fertiliser had no effect on the lint yield or the % lint of the seed cotton (Table 7.6).

Table 7.6 Mean lint yield (kg lint ha^{-1}) and % lint of seed cotton from plants given no P and K fertiliser or 60 kg P and 160 kg K ha^{-1} fertiliser. Significance measured at $P = 0.05$.

P / K rate	Yield (kg lint ha^{-1})	% lint of seed cotton
Nil	2097	41.9
60 kg P ha^{-1} / 160 kg K ha^{-1}	2188	42.8
P value	n.s.	n.s.

7.3.3.2 Total P, K and biomass

There was no difference in the biomass, P or K accumulation between the plants from the two treatments (Figure 7.6). The biomass, P and K accumulation followed a logistic curve, with plants supplied with P and K fertiliser showing a higher accumulation of P and K early in the season.

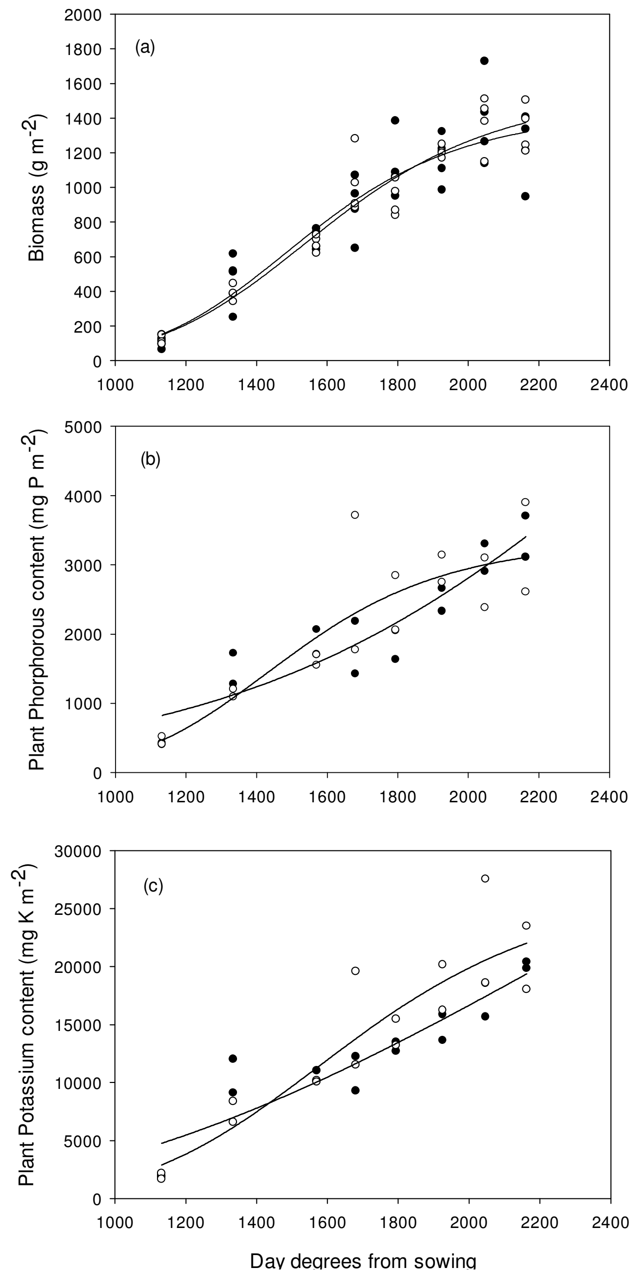

Figure 7.6 (a) total biomass accumulation (g m^{-2}) in ● plus PK plots and ○ nil plots with fitted logistic curves; (b) total P accumulation (mg P m^{-2}) in ● PK plots and ○ nil plots with fitted logistic curves; and (c) total K accumulation (mg K m^{-2}) int ● PK plots and ○ nil plots with fitted logistic curves.

As with the N rate experiment, P, K and biomass were compared at flowering, 4 NAWF and maturity. There was no difference between the plant size or P and K content between the two treatments at any growth stage (Figure 7.7).

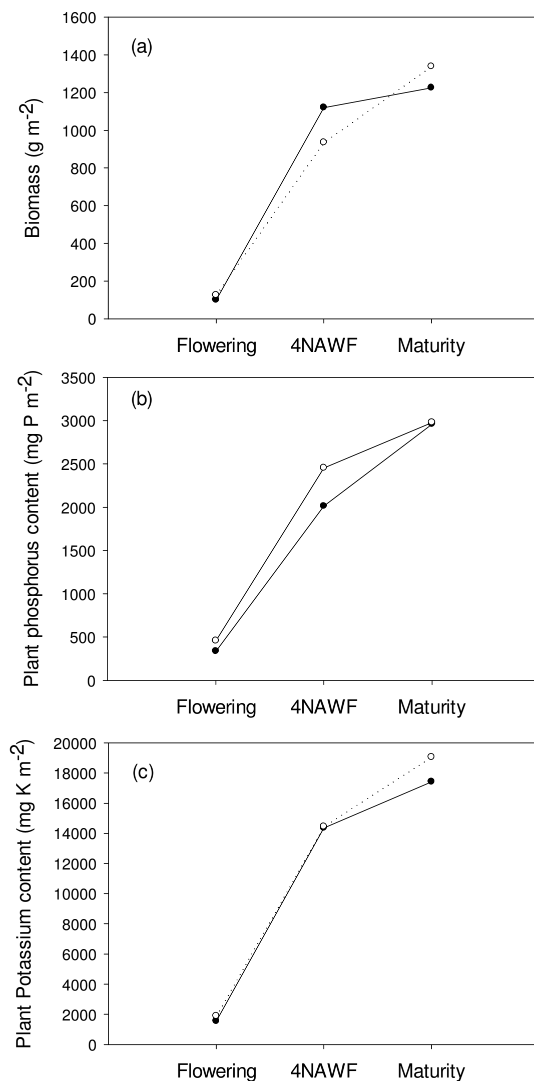

Figure 7.7 (a) biomass; (b) P and (c) K accumulation at flowering, 4 NAWF and maturity at —●— nil plots, ··○·· PK plots.

While there was no difference in the size or the total uptake of P and K between the plots, there were some differences in the proportion of the total accumulation of biomass, P and K taken up by flowering and 4 NAWF (Table 7.7). Plants supplied with P and K fertiliser took

up proportionally more P and K before 4 NAWF than the nil plots, despite accumulating the same proportion of the total biomass. This was the opposite trend to the N rate experiment, where higher rates of supply equated to continued uptake throughout the fruiting period and after 4 NAWF.

Table 7.7 The proportional uptake (%) of the total P, total K and total biomass at flowering, 4 NAWF and maturity.

P / K Rate	% of total P uptake			% of total K uptake			% of total biomass uptake		
	Fl.	4 NAWF	Mat.	Fl.	4 NAWF	Mat.	Fl.	4 NAWF	Mat.
Nil	12.3	56.4	100	9.4	65.0	100	8.3	73.4	100
60 kg P ha^{-1}/ 160 kg K ha^{-1}	14.4	75.9	100	9.2	69.5	100	9.4	70.4	100
L.S.D.	1.38	11.72			3.86				
P value	0.01	0.01		0.25	0.03		0.48	0.64	

Since plants given more P and K took up the same total amount of P and K, but at an earlier stage, the concentration of P and K in the plant must have been higher. When compared at flowering, 4 NAWF and maturity, the plants supplied with P and K fertiliser had a higher concentration of both P and K than those given no fertiliser (Figure 7.8).

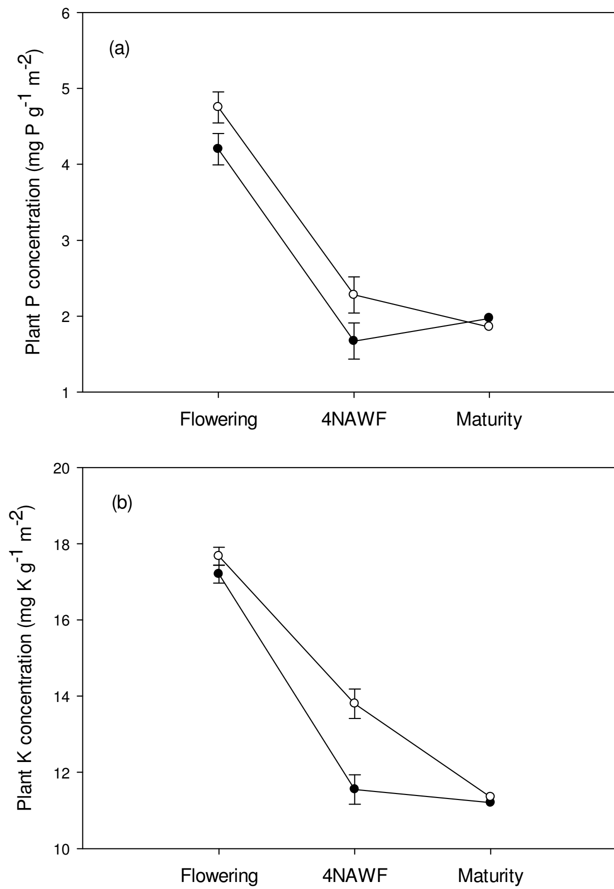

Figure 7.8 The concentration of (a) P an (b) K in the whole plants at flowering, 4 NAWF and maturity in —●— nil plots, ··○·· PK plots. Error bars represent the LSD at 0.05, no bars indicate n.s.d.

7.3.3.3 P, K and biomass partitioning

As with comparisons of the whole plants, there was little variation in the accumulation of biomass in the leaf, stem and fruit fractions of the plants. Both the nil plots and the P/K plots partitioned an equal biomass in the same way at flowering and maturity. There was no difference in the leaf and stem dry weight at 4 NAWF, however the plants in the nil plots had a higher mean fruit dry weight at 4 NAWF than those given P/K fertiliser (Figure 7.9).

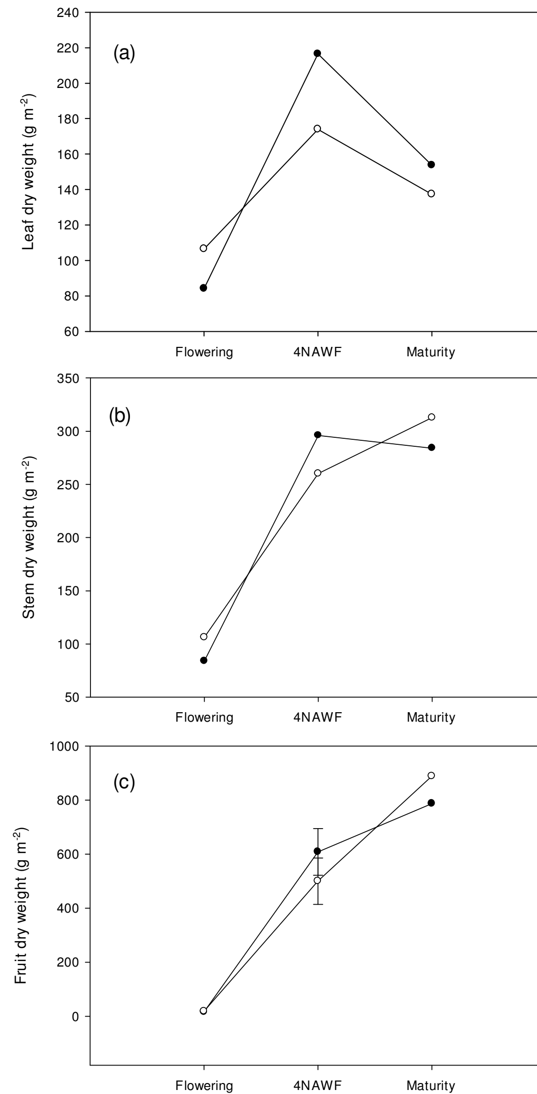

Figure 7.9 (a) leaf, (b) stem and (c) fruit dry weight at flowering, 4 NAWF and maturity in —●— nil plots and ···○··· PK plots.

Comparison of the P content and concentration (Figure 7.10), and the K content and concentration (Figure 7.11) of the plants showed that while no additional P and K was taken up by 4 NAWF in the PK plots (Figure 7.7), the increased concentration in the whole plant (Figure 7.8) was observed in the leaf, stem and fruit tissues (Figure 7.10 b, d and f and Figure 7.11b, d and f). These differences must, therefore, have been the result of minor differences in plant dry weight and P and K contents, that were not significant individually but when combined into a concentration figure (mg P or K per g dry weight m^{-2}) highlighted differences in the uptake and partitioning of these nutrients. Fertilised plants had a higher P

concentration in the leaves and fruit at flowering (Figure 7.10b and f), and a higher K concentration in the fruit at flowering (Figure 7.11f). There was no difference in the concentration of P or K in any tissue at maturity, indicating a similar partitioning of nutrients despite differences in concentration at 4 NAWF. There were similarly no differences in the P content of any tissue except for the P content of the fruit at 4 NAWF, which was higher in the plants supplied with P and K fertiliser (Figure 7.10e). The higher content and higher concentration of P in these fruit indicate a preferential partitioning of P to the developing fruit. There was no difference in the K content of any tissue at any of the growth stages measured (Figure 7.11a, c and e).

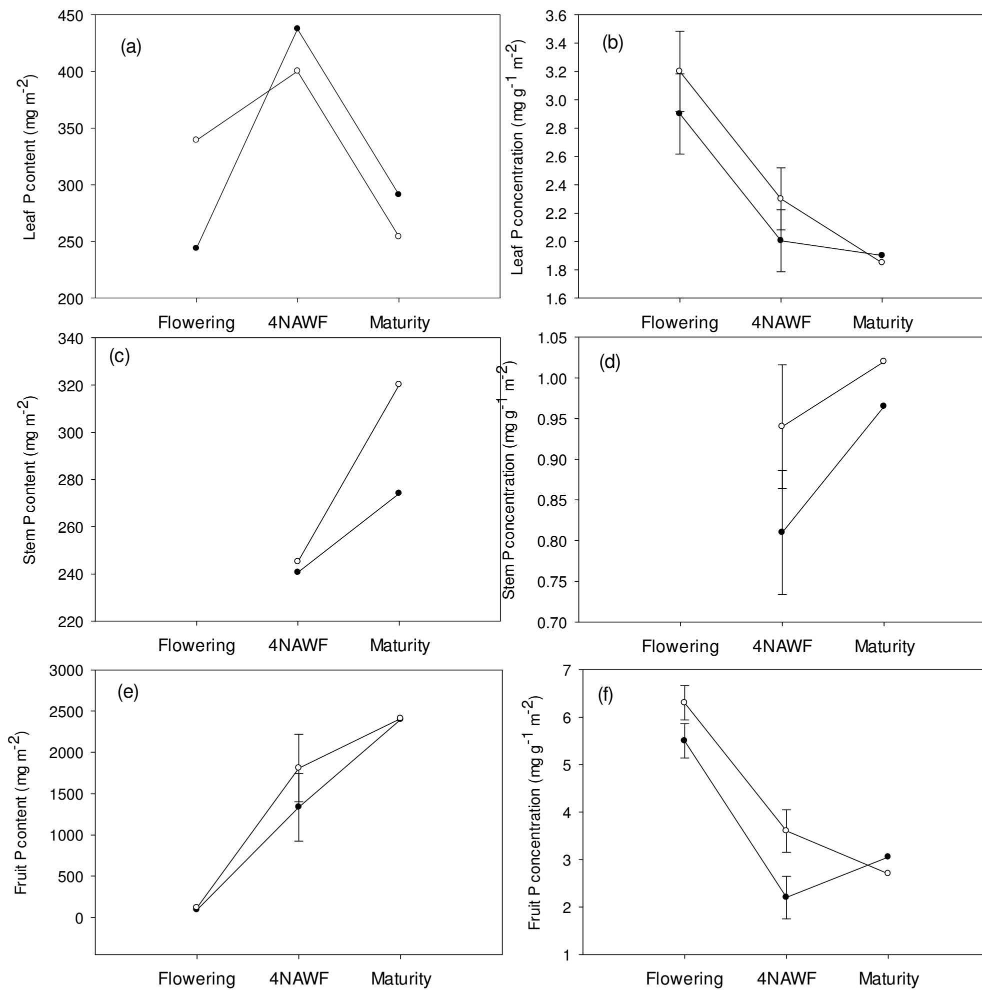

Figure 7.10 The leaf (a and b), stem (c and d) and fruit (e and f) P content (mg P m^{-2}) (a, c and e) and concentration (mg P g^{-1} m^{-2}) (b, d and f) in —●— nil plots and ···○··· PK plots. Vertical bars show the LSD at 0.05, lack of vertical bars denotes no significant difference.

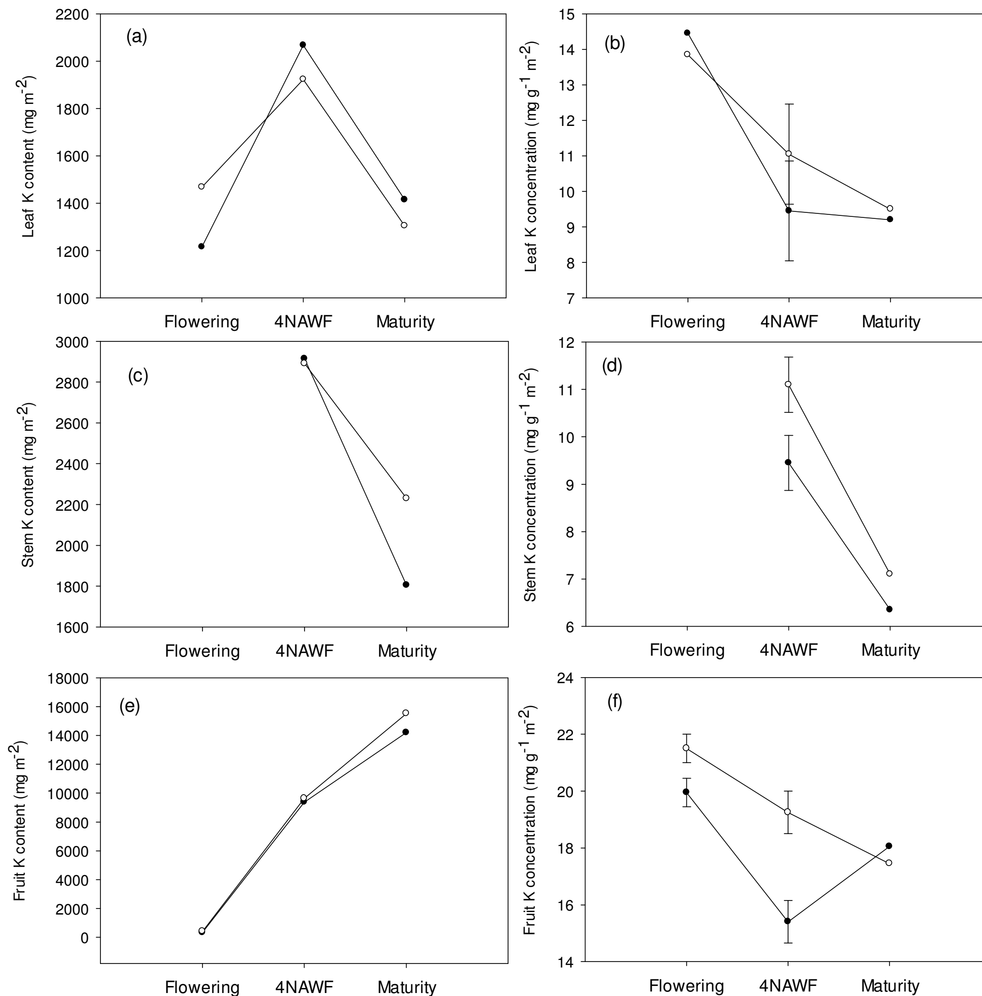

Figure 7.11 The leaf (a and b), stem (c and d) and fruit (e and f) K content (mg K m^{-2}) (a, c and e) and concentration (mg K g^{-1} m^{-2}) (b, d and f) in —●— nil plots and ···○··· PK plots. Vertical bars show the LSD at 0.05, lack of vertical bars denotes no significant difference.

There were no differences in the biomass accumulation, P or K uptake at flowering, 4 NAWF or maturity ($P > 0.05$) and limited differences in the P or K content of the leaf, stem ad fruit tissue. There were some differences in the partitioning of the same amount of biomass, P and K between the leaf, stem and fruit tissue which were not captured in a comparison of the dry weight and nutrient content data.

Table 7.8 shows the partitioning of the biomass, P and K as a percentage between the leaf, stem and fruit at flowering, 4 NAWF and maturity. Plants supplied with P and K fertiliser

consistently partitioned more of the total P and K to vegetative structures than the nil plots. The nil plots had a higher mean percentage of both P and K in the fruit from flowering to maturity, a trend consistent with the changes in partitioning observed in the N rate experiment.

Table 7.8 The proportional uptake (%) of the total P, total K and total biomass distributed between the leaf, stem and fruit at (A) flowering, (B) 4 NAWF and (C) maturity.

P / K Rate	Tissue type	P			K			Biomass		
		A	B	C	A	B	C	A	B	C
Nil	Leaf	70	20.6	7.4	76.2	14.0	6.1	84.5	19.1	12.8
	Stem		10.5	8.4		18.3	9.28		26	23.3
	Fruit	30.4	67.5	85.3	23.83	67.8	84.7	15.5	55	63.9
60 kg / 160 kg	Leaf	76.7	16.5	8.3	80.6	13.5	6.6	85.3	18.6	10.3
	Stem		9.6	10.3		19.3	11.2		27.8	23.4
	Fruit	23.3	73.8	81.3	19.4	67.3	82.3	14.7	53.6	66.3
P value and L.S.D. * < 0.05 ** <0.001	Leaf	* 5.85	* 4.07	* 0.718	* 0.036	n.s.	* 0.4	n.s.	n.s.	n.s.
	Stem		n.s.	** 0.785		n.s.	n.s.		n.s.	n.s.
	Fruit	* 5.83	* 5.92	* 2.398	* 0.04	n.s.	* 2.362	n.s.	n.s.	n.s.

7.3.3.4 P and K redistribution

Logistic curves were fitted to the total N and fruit N accumulation data for each plot (shown in Figure 7.6). Redistribution was calculated as described in section (Chapter 4 section 4.3.5). The mean uptake rates and redistribution values are given in Figures 12 and 13 and in Table 7.9. All curves were a statistically significant fit ($P < 0.05$), although the P accumulation in the fruit of the nil plots reached no apparent peak, indicating that the curve was not an ideal fit to the data, or that the fruit continued to accumulate P until defoliation (Figure 7.12a). Peak K accumulation occurred earlier than peak P accumulation in both the nil and the fertilised plots.

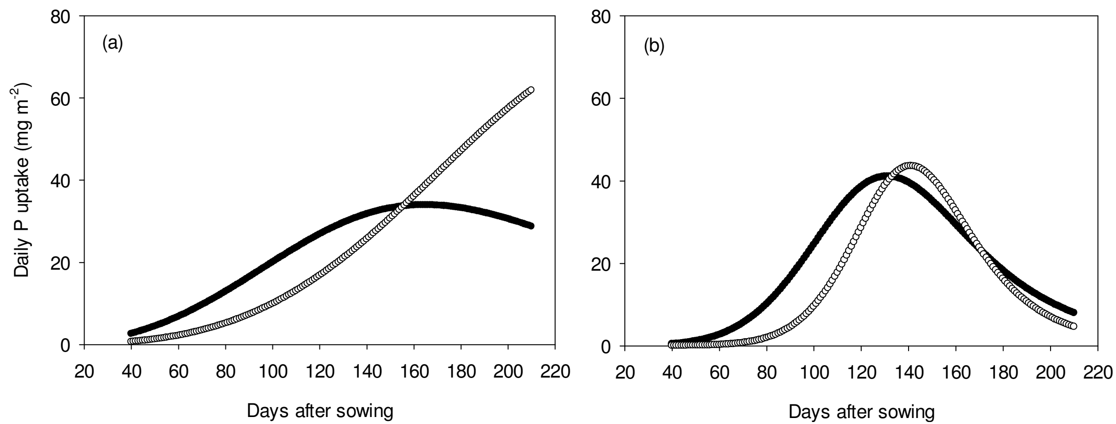

Figure 7.12 Derived P accumulation curves for the daily total plant accumulation of P (◆) and the daily fruit accumulation of P (◇) in plants from (a) nil plots and (b) plots fertilised with 60 kg P and 160 kg K ha^{-1}.

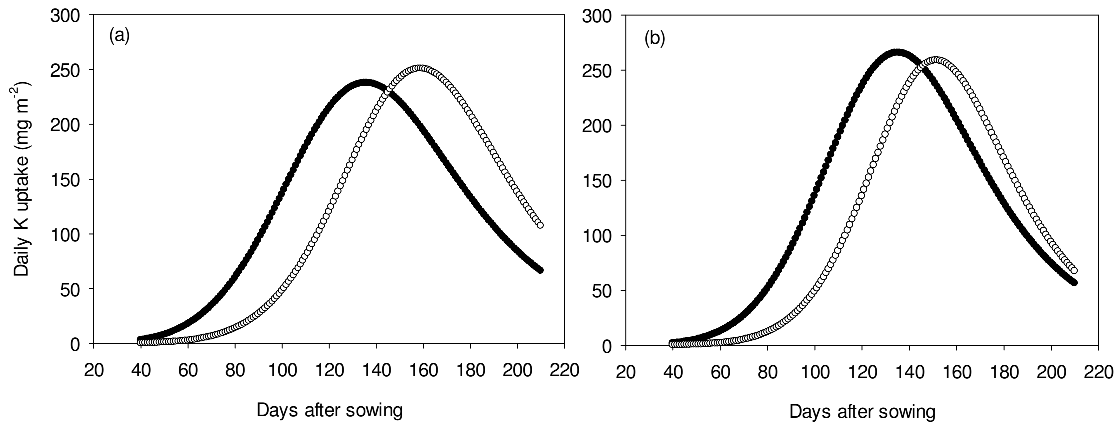

Figure 7.13 Derived K accumulation curves for the daily total plant accumulation of K (◆) and the daily fruit accumulation of K (◇) in plants from (a) nil plots and (b) plots fertilised with 60 kg P and 160 kg K ha^{-1}.

The redistribution of P from vegetative to reproductive tissue (from leaves and stems to bolls) was higher in the nil plots. There was no statistical difference in the proportion of redistributed P from redistribution between the plots, nor was there a difference in redistributed K or the contribution of redistribution to fruit K (Table 7.9).

Table 7.9 The total amount of P and K redistributed from vegetative to fruit tissue (mg m^{-2}) and the proportion (%) of the fruit P and K at maturity supplied by redistribution

P / K rate	Redistributed P (mg)	% fruit P at maturity from redistribution	Redistributed K (mg)	% fruit K at maturity from redistribution
Nil	339.8[a]	11.8	2607.1	15.3
60 kg P ha^{-1}/ 160 kg K ha^{-1}	129.5[b]	4.9	1639.6	9.6
L.S.D.	155.3			
P value	0.02	0.08	0.18	0.25

7.4 Discussion

The effects of changes in the nutrient supply on plant morphology, nutrient uptake, distribution and redistribution in the high-yielding cotton crops studied varied considerably between years, and between the different nutrients. The hypothesis that N, P and K supply changes the uptake and concentration of these nutrients in plant tissue, as well as changing the partitioning of biomass and nutrients within the plants was tested. The effect of these changes, as well as the nutrient supply, on the remobilisation of nutrients from vegetative to reproductive plant parts was also examined.

7.4.1 N, P and K supply and the accumulation and partitioning of N, P, K and biomass

The first aim of this chapter was to establish the effect of changes in the N, P and K supply on nutrient uptake, tissue concentration, biomass and nutrient partitioning. These were examined in terms of both the timing of growth and uptake and the relative changes within plants from any treatment.

7.4.1.1 N fertilisation

N application ranging from 0 to 200 kg N ha^{-1} had significant effects on the growth, N uptake, N concentration, biomass partitioning and N partitioning of high-yielding cotton plants. Confirming much previous research, the addition of N fertiliser to the crops resulted in increased plant dry weight, yield, boll number m^{-2}, a higher total N uptake by the plants, and a higher concentration of N in the leaf, stem and fruit tissue (Jones *et al.* 1974; Thompson *et al.* 1976; Oosterhuis *et al.* 1983; Leffler and Hunter 1985; Halevy *et al.* 1987; Mullins and

Burmester 1990; Guitman *et al.* 1991; Boquet *et al.* 1994; Boquet and Breitenbeck 2000; Chua *et al.* 2003; Fritschi *et al.* 2004b; Mussaddak 2004; Dreccer 2006). This increase in yield, boll number and plant size is beyond that measured in many of these studies, and demonstrates that in modern, high-yielding, transgenic cultivars a similar response to N fertilisation is observed as in the older cultivars described in these studies. These effects of increasing N supply were consistent across the two years, although to a different degree, for example the yield increase per additional kg of N was much higher in 2008-09 than in the previous year.

The changes in biomass partitioning in the plants was more varied, and did not follow previously reported trends, or the assumptions that were made about how extra N would influence growth. Excess N application (that is, more than is required to support the yield of a particular crop) has been linked to continued vegetative growth through the boll filling period, delayed cutout and a lower ratio of reproductive tissue to vegetative tissue (R:V ratio) (Boquet *et al.* 1994; Boquet and Breitenbeck 2000; Fritschi *et al.* 2004b; McConnell and Mozaffari 2004; Girma *et al.* 2007). N uptake by a crop has also been directly correlated with flower bud production, leaf production and expansion, and fruit retention (Marcus-Wyner and Rains 1982; Zhu and Oosterhuis 1992). It follows that the reduction in the R:V ratio observed in crops given excessive amounts of N would be due to the preferential allocation of extra N to vegetative structures over reproductive ones, or an inability of the plant to produce more fruit without an initial increase in vegetative biomass. In this experiment, there were differences in the R:V ratio at 4 NAWF ($P < 0.001$), but by maturity all plants had reached the same ratio of 1.5 ($P = 0.067$).

Plants supplied with more N did partition more (as a % of total uptake) N and biomass to vegetative parts than to the fruit at flowering and at 4 NAWF. This indicates that at least at earlier growth stages the theory holds true, however the changes in the plants during boll filling indicates that the extra dry weight allocated to vegetative matter was used to support additional reproductive structures. If a season was continued past the anthropogenic end it usually reaches in Australian irrigated systems (that is through the application of a defoliant) it could be assumed that even the plants given “excess” N, resulting in rank vegetative growth, could potentially produce higher yields, if an average ratio of 1.5 were assumed.

Without many more years of repeated experiments, it remains unclear if this equal relative allocation of biomass to reproductive structures under high and low N supply is a function of the environment, that is that the plant growth is retarded at a certain point in the season by declining temperatures and shorter day length, or a function of the agronomic management of the crop. N rate experiments would need to be combined with other agronomic stresses across a variety of environments to confirm if there is a limit to the ratio of reproductive to vegetative tissue to which cotton plants will naturally reach. If this ratio could be increased, particularly in large plants, significant yield benefits and efficiencies of nutrient use could occur.

While there were no differences in the partitioning of biomass between vegetative and reproductive structures, the partitioning of N showed more variation between the treatments, ranging from 0.3 to 1.1 at 4 NAWF and 1.6 to 2.8 at maturity. This shows that there is considerable variability in the distribution of N even within plants of a similar yield and boll number. The ratio of reproductive N to vegetative N was not related to N supply. The largest, highest yielding (in the 200 kg ha^{-1} plots at A3) plants having a ratio of 2.8 at maturity, and the plots given the same amount of N at F6 with a ratio at maturity had a ratio of 1.6. It can be concluded that N partitioning does not follow biomass partitioning, particularly at maturity and also that N partitioning does not reflect the N stress or supply to the growing plant. Considerable seasonal variation also exists in the partitioning of N.

7.4.1.2 P and K fertilisation

In this experiment, P and K were added together as a combined fertiliser. More accurate conclusions as to the effect of P or of K on the growth, development and partitioning of biomass and nutrients in high-yielding cotton could be made if the two nutrients had been added separately. This is a limitation of the experiment and may influence the conclusions that can be made, however the treatments were combined for several reasons. Firstly, identifying a site with P and particularly K deficiencies in the cotton growing regions of NSW and QLD is difficult, due to the inherently fertile soils on which cotton is grown. Secondly, financial constraints as to the number of sites and samples limited the scope of the experiment. While the combining of P and K makes identifying the effects of individual nutrient supply on the growth and development difficult, several observations can be made and conclusions drawn.

There was no difference in lint yield, or lint % of the seed cotton between the plants given P and K fertiliser and those without. Similarly there was no difference in the size of the plants at flowering, 4 NAWF or maturity, or in the partitioning of biomass between the leaf, stem and fruit tissues. The main affect of increasing the P and K supply was increasing the concentration of P and K in the plants, particularly at 4 NAWF, presumably while root functioning and nutrient uptake was still occurring.

Similarly to the N experiment, the addition of P and K did not alter the R:V ratio of the plant at maturity but showed some influence on the partitioning of biomass and P and K during the earlier growth stages. This increased R:V ratio early in the season could be a sign of stress or that growth was being limited by access to P and K in the nil plots.

7.4.2 N, P and K supply and redistribution

The second aim of this chapter was to quantify the effect of variation in N, P and K supply on the redistribution of nutrients from vegetative to reproductive structures. Any changes were hypothesised to be a product of morphological changes or changes in the uptake and concentration of N, P or K in the plant resulting from a change in the nutrient supply. Previous research has shown variable results. In summary, changes in redistribution have been shown to be 1) a function of nutrient supply, 2) a function of the timing of nutrient application or stress, 3) a result of changes in the source-sink ratio (the R:V ratio), or 4) a function of the nutrient concentration of the exporting tissue. As discussed above, an increase in the supply of N, P and K did result in an increase of the concentration of N, P or K throughout the early parts of the fruiting period till 4 NAWF, after which time cotton plants are said to rely on leaf nutrients to supplement boll nutrient demand (Pettigrew *et al.* 2000).

7.4.2.1 Nitrogen

The variation in N redistribution between the N supply in these treatments was limited, with two different amounts (mg N redistributed m^{-2}) and proportions of fruit N supplied through redistribution recorded (Table 7.5). There was no difference between the proportion of fruit N redistributed in the 0 kg ha^{-1} plots at F6, and the 200 kg N ha^{-1} at A3, or between the other three rates of N supply (125 kg ha^{-1}, 200 kg N ha^{-1} (F6) and 50 kg N ha^{-1}). There was no consistent relationship between the amount of N supplied to the plant, the amount of N taken up by the plant, especially when plants were supplied with a large amount of N (200 kg N ha^{-}

^{1}). Likewise there was no consistency between the N concentration of the leaves and stems and the redistribution of N from vegetative to reproductive tissue. The lack of correlation between these parameters across two seasons indicates that environmental and agronomic factors may have as much, or perhaps more, effect on the movement of N around the plant than the supply of N.

Based on these results the hypothesis that increasing N supply decreases redistribution can be rejected. In experiment 2 (in 2007-08), N redistribution decreased with N supply, but in experiment 5 in the following season (2008-09), the opposite occurred. This indicates that the relationship between N supply and N redistribution is at least in part environmentally controlled or modified and the effect of N supply on redistribution interacts with other factors affecting plant growth and the internal recycling of nutrients. The relationship between N supply and redistribution as suggested by other authors (Guitman *et al.* 1991; Killingbeck 1996; Sawan *et al.* 1998; Semenov *et al.* 2007; Subasinghe 2007), was based primarily on glasshouse or controlled environment studies. The field data in this experiment did not measure temperature, water, humidity or other stresses which may have altered the functioning of the plants, and highlights the difficulties in applying theoretical models of unstressed plant physiology to variable field conditions.

These results also contradict the reported trends that N remobilisation and redistribution is increased in plants with a low vegetative N concentration, and that increasing the supply of nutrients to a plant will decrease the remobilisation of that nutrient from vegetative to reproductive tissue (Guitman *et al.* 1991; Killingbeck 1996; Sawan *et al.* 1998; Semenov *et al.* 2007; Subasinghe 2007). There was no correlation at either site between redistribution and N concentration at flowering, 4 NAWF or maturity. There was, however, a strong correlation between N supply and N concentration, which presents the question, if redistribution is not consistently related to the N supply (or subsequently N concentration), what characteristic of the crop at A3 stimulated the significant increase in N remobilised when compared to the plants fertilised with only 50 kg N ha^{-1}? And what characteristic of the crop given 200 kg ha^{-1} at A3 was similar to the 0kg N ha^{-1} treatment at F6?

Factors contributing to the increased remobilisation and redistribution of N from leaves to bolls could be;

1) water supply
2) environmental factors such as radiation, temperature and relative humidity,
3) plant morphology (the ratio of reproductive to vegetative tissue, the distribution of fruit up the plant or root growth, development and functioning),
4) plant growth rate
5) the rate of nutrient uptake, OR,
6) the concentration of nutrients in vegetative plant parts.

Both crops were watered as required throughout the season. Any variability in water supply resulted from rainfall. There was a significant difference in the amount of rainfall that fell on the crops throughout the growing season. In the 2007-08 season the total in crop rainfall was 354 mm, predominantly falling in February. In the 2008-09 the total in crop rainfall was 513 mm, with a prolonged period of rainfall before and during the flowering period. This extra water would have significantly reduced the stress on the plant and may have resulted in extra growth and increased N transport throughout the boll filling period. The effect of water supply on redistribution will be examined in Chapter 8.

Since the ratio of reproductive to vegetative tissue was the same in the crops showing both low and high redistribution rates, it can be concluded that in this experiment the proportion of biomass allocated to reproduction was not the driving factor behind the increased redistribution. The distribution and retention of fruit on the plant was not recorded. The relative redistribution of N from different sections of the canopy was discussed in Chapter 6, concluding that the middle sections of the plant are more likely to export a higher amount of N from the leaves than the extremities of the plant, the very bottom 5 nodes and those about node 21. In Chapter 6 this was not related to the R:V of individual sections. Therefore, a more likely factor affecting redistribution and explaining the similar redistribution from the two crops could be the functioning, or decline in functioning of the root system (Cakmak *et al.* 1994; de Groot *et al.* 2001; Andersson *et al.* 2005; Andersson and Johansson 2006; Dong *et al.* 2008). As shown in Chapters 4 and 5, and discussed by several authors (Hall and Brady 1977; Hendry 1988; Guitman *et al.* 1991; Wright 1999; Pettigrew *et al.* 2000; Andersson and Johansson 2006; Yasumura 2009), redistribution is a *supplementary* process supplying

nutrients to developing reproductive structures when root uptake is inadequate. A crop of a similar yield may, therefore, redistribute a high or a low amount of a particular nutrient depending on both its supply to the roots and the ability of the roots to take the nutrient up. The decline in root functioning of cotton crops at cutout is attributed to competition for carbohydrates as leaf functioning is declining, limiting their supply (Schwab *et al.* 2000; Mullins and Burmester 2010). Since N uptake is a highly energy dependent process, if this carbohydrate competition were occurring in any crop, N uptake would be reduced.

Unlike in the crop fertilised with 200 kg N ha^{-1} at F6, the N uptake in the 200 kg N ha^{-1} crop at A3 declined and stopped after approximately 1800 day degrees, with the peak daily rate of uptake occurring earlier and declining much more rapidly than in the F6 200 kg N ha^{-1} crop, which maintained a higher rate of N uptake through the boll filling period (Figure 5 c and e). This indicates that the root functioning of the 200 kg N ha^{-1} crop at A3 may have declined, resulting in a decrease in the uptake of N, and consequently a reliance on supply from the leaves instead. This may also have been related to the large amount of rainfall early in the season at this site, which can result in shallow root systems forming and a reduced root functioning later in the season. This process does not seem to have occurred in the nil plots, which showed a low, and consistently declining, rate of N uptake throughout the season. Proportionally more of the total plant N was taken up before cutout in the nil plots, indicating that there was limited access to N in the soil and that the roots may not have been able to take it up. There is no indication that there is a relationship between the N concentration of the leaves, stems or fruit at any growth stage and the redistribution of nutrients, as the 200 kg N ha^{-1} plots consistently had the highest N concentration in each tissue and the nil plots the lowest.

Based on a brief examination of the proposed factors which contribute to an increased redistribution rate it seems likely that the reason for the similar redistribution in the high N plots at A3 and the nil plots at F6 may have been very different. The crop at A3 probably redistributed a large amount of N due to either environmental conditions or a reduction in root functioning after 4 NAWF. The extra redistribution when compared to the 50 kg N ha^{-1} plots may be attributed to the higher boll numbers and boll weight, as they seem to have followed very similar growth patterns in other respects. The crop at F6 seems to have relied

on redistribution to supply developing bolls due to a lack of available N in the soil, other environmental stresses or a reduction in root uptake and assimilation of new N.

While none of the assumptions about the effect of N supply on N redistribution seem to have held true across the two seasons, it seems clear that in some cases the reported factors that stimulate redistribution could have been a significant factor in driving the movement of N between tissues. It is clear that this is not a consistent relationship, and that the complex interaction of climatic and agronomic factors with nutrient supply and plant morphology results in changes in redistribution. As a result the simplification of one process only driving redistribution, in isolation from other factors, will not consistently explain plant N use.

7.4.2.2 Phosphorus and Potassium

As well as increasing the P concentration and uptake, the addition of P fertiliser decreased the total amount of P redistributed from leaves and stems into the fruit, and reduced the proportion of fruit P supplied by redistribution (at a significance level of 0.1). Since the experiment was at a small scale, and was only carried out over one season, the degrees of freedom for the ANOVA to identify differences in redistributed P and proportional fruit supply were low. This reduced the power of the experiment to detect small changes in the redistribution, an inherently variable process (see Chapters 4 and 6), since the standard error of the mean was relatively high. To further investigate this process, and confirm the trend that redistribution is decreased in plants given P fertiliser more field experiments should be carried out in P deficient and non-deficient conditions.

Similarly the power of the analysis to identify differences in the redistribution of K was limited by the variability of the data. While the means showed a similar trend to that observed with P, that the fertilised plants redistributed less K, the result was not significant. More and larger scale experiments with a larger number of replicated samples should be carried out to confirm this trend.

The redistribution of P and K has been cited as the cause of premature senescence symptoms observed in cotton around the world, in which very low P and K concentrations are recorded in the senescing leaves. It has been speculated that premature senescence is exacerbated by low P and K levels in the soil (Bedrossian and Singh 2004), a conclusion which would be

(tentatively) supported by these results. Premature senescence has also been attributed to a high R:V ratio in the plant at cutout and during boll filling (Wright 1999; Pettigrew *et al.* 2000). Since there was no difference in the R:V ratio at flowering, cutout or maturity this conclusion cannot be confirmed from these results, although further experiments would add to understanding this conclusion and if the redistribution of P and K is indeed a sink driven process.

7.4.3 Conclusions

The effects of changes in the nutrient supply on plant morphology, nutrient uptake, distribution and redistribution in the high-yielding cotton crops studied varied considerably between years, and between the different nutrients. Comparisons of these three crops, and the various ways that the same cultivar responded to fertilisation at different sites and in different seasons highlights the contribution of environmental and seasonal factors to the efficiency of nutrient use. This study also supports the proposition that plant responses to nutrient supply are not uniform, or necessarily predictable in a field context, but rather are highly variable between seasons (Read *et al.* 2006). To reduce the impact of climatic and soil variability, the best method of evaluating plant responses to nutrient supply would be to have many seasons of data over a long time period. In this thesis more than 3 years of field experiments to measure plant responses were outside the scope of the project.

It is clear from this data that the models and assumptions about nutrient supply and the resulting changes in redistribution in field conditions cannot be accepted as an adequate explanation of the variability in nutrient redistribution observed between different crops. While in a non-stressed, climatically and water controlled environment, the addition or removal of a nutrient from the system can be isolated, in field conditions the effects of plant competition, climatic variability and management stresses cannot be controlled. In the field environment the observed variability in nutrient redistribution is much higher. This indicates that, in a variable climate, plants are able to modify their use of nutrients to optimise their fitness and reproductive output through initiating or inhibiting certain processes such as the redistribution of assimilated nutrients. While hypotheses about the contribution of one or more factors to the process of redistribution can be made, further research into the causes of this process, particularly genetic controls, the optimisation of root-functioning and the internal mechanisms regulating the R:V ratio need to be carried out.

CHAPTER 8

Nutrient redistribution in high-yielding cotton grown under varying soil water deficit irrigation

8.1 Introduction

In previous chapters it was hypothesised that differences in water supply may have contributed to the variability in N, P and K redistribution between otherwise similar crops. In this chapter water supply will be examined in isolation from other factors to investigate the effect of water supply on N, P and K redistribution from leaves to bolls. The effect of variability in water supply on the production of biomass and the partitioning of this biomass to harvestable seed cotton has been identified in many studies that relate soil water supply with boll size, square production and retention, lint yield and plant size (Hearn 1975a; 1979; Krieg and Sung 1986; Ball *et al.* 1994; Van Iersel and Oosterhuis 1996; Singh *et al.* 2006a). Less research has linked the effect of water supply on other physiological processes within the plant, especially in the remobilisation and transport of nutrients from one tissue to another.

Water stress can have a range of impacts on a developing cotton plant depending on the duration, severity and timing of the stress. In general the major effect of a reduction in water supply is a reduction in plant size (Hearn 1975a; 1979; Constable and Rawson 1982; Krieg and Sung 1986; Ball *et al.* 1994; Poorter and Nagel 2000; Silber *et al.* 2003; Bange *et al.* 2004; Neumann 2005; Singh *et al.* 2006a). While plant size is reduced by water supply, no difference in the partitioning of dry matter between plant parts has been observed, both in terms of their ratio and the relative change in each tissue type in either waterlogged (Bange *et al.* 2004) or drought-stressed conditions (Krieg and Sung 1986). Water stress, therefore, promotes the growth of smaller plants with the same ratio of reproductive to vegetative tissue as larger, non-stressed plants. The reduction in total biomass is the result of a reduction in leaf area, attributed to a decline in leaf expansion and lower leaf numbers, resulting in a subsequent reduction in carbon assimilate production limiting new vegetative or reproductive growth.

Reduced lint yields from water stress have been attributed to a reduction in boll numbers, particularly on each sympodial branch (Krieg and Sung 1986). Water-stressed plants generally do not support more than one boll position along a fruiting branch, with sympodial leaves and 2nd or 3rd position fruit being abscised early to reduce competition for resources along a branch. Krieg and Sung (1986) showed that under severe water stress mainstem leaf numbers were reduced by only 10%, but sympodial leaf numbers reduced by almost 50%. Van Iersel and Oosterhuis (1996) observed increased ethylene production in young fruits grown in water-stressed conditions, which contributed to the abscission of young fruits and a reduction in boll numbers. Biomass and yield changes therefore, are driven by the reduction in carbon supply from reduced leaf area, and through a limitation to boll numbers by hormonal changes and a reduction in respiration and translocation of carbon assimilates. There are no reports of research linking the reduction in biomass production or development of new tissue with a change in the transport rate, efficiency or concentration of nutrients around the cotton plant.

The effect of water stress on the uptake, assimilation, translocation and re-translocation of nutrients around a cotton plant has not been widely studied. Mostly changes in nutrient uptake observed under water-stressed conditions are correlated with reductions in biomass accrual, with several studies noting little variation in nutrient concentration or partitioning patterns under water-stressed conditions, but a reduction in total uptake associated with a smaller plant size (McConnaughay and Coleman 1999; Coker *et al.* 2000; Poorter and Nagel 2000; Bernacchi *et al.* 2007; Hou *et al.* 2007). This 'functional equilibrium' theory, that is that biomass and nutrient partitioning remain relatively consistent under varying levels of resource availability (Poorter and Nagel 2000), has been supported by biomass partitioning experiments under varying levels of water supply in cotton plants (Ball *et al.* 1994; Bange *et al.* 2004). This partitioning of nutrients under water stress and water logging has been less widely studied in cotton plants, although it can be assumed that the relative changes in biomass and nutrient concentrations observed would indicate that water effects nutrient partitioning in a similar manner to biomass.

Changes in nutrient uptake and transport in developing plants have been observed under water stress. Uptake may be limited by a reduction in root functioning, or a limitation of supply in soil solution to the root-soil interface; though root growth is often promoted under

water stress (Krieg and Sung 1986), water-dependent uptake and apoplasmic transport to the cortex and vascular system may be reduced (Neumann 2005; Vandeleur *et al.* 2005). The effect of water stress on nutrient transport through the plant is a result of, (but not necessarily limited to);

1) Changes in the water potential (ψ) of the vegetative plant parts (Hearn 1979; Van Iersel and Oosterhuis 1995);
2) Altered xylem and phloem composition, both as a signalling mechanism and as a response to limited supply (Nobel *et al.* 1994; Bahrun *et al.* 2002);
3) Changes in the hormone production from specific plant parts (Van Iersel *et al.* 1995; Van Iersel and Oosterhuis 1996); and,
4) The early triggering of tissue senescence (Hearn 1979; Ball *et al.* 1994; Hortensteiner and Feller 2002; Dumka *et al.* 2003; Bange *et al.* 2004; Dong *et al.* 2008).

A rapid reduction in xylem-borne nitrate, phosphate and K of up to 50% has been observed in maize plants grown in glasshouse pot experiments to which water-stress was applied through withholding irrigation (Bahrun *et al.* 2002). The reduction in the uptake of these minerals may act as a signal to leaves and other above ground plant parts to reduce photosynthesis and respiration and to conserve nutrients and energy under water stress. A change in the water potential of the leaves, stems and bracts of cotton plants is a response to water stress, which in turn changes the rate and amount of nutrients unloaded from the xylem and phloem vessels and transported through the apoplasm or reduced to other organic molecules (Van Iersel and Oosterhuis 1995; Van Iersel and Oosterhuis 1996). Both waterlogging (Belford, 1981) and drought stress (Mothes 1928) have been shown to trigger leaf senescence in several plant species, although through different mechanisms. Prolonged drought may increase the rate of protein degradation, probably as a response to the limited nutrient uptake of nutrients from the soil solution (see section 1.2). Waterlogging may cause root anoxia and prevent nutrient uptake, stimulating the same response as drought stress through the mobilisation of leaf nutrients through cell and tissue senescence and the mobilisation of these nutrients to supply young leaves and fruit.

In summary, drought-induced changes to nutrient transport fall into two categories. Firstly changes in the uptake of nutrients from the soil and the subsequent signalling of reduced supply, and secondly in terms of stimulating leaf senescence for the supply of nutrients to

preferential sites of growth, that is the roots and the surviving fruits. The cessation of vegetative growth under drought stress can cause premature senescence of leaves and the rapid remobilisation of previously assimilated nutrients to supply roots and fruits. Since the size and nutrient content of drought-stressed plants is smaller, it would be assumed that the gross amount of N, P and K redistributed would be equally smaller. If, however, leaf senescence were triggered by drought, the proportion of fruit nutrient supplied by redistribution could be higher. The limitation imposed on transport by the rapid changes in water potential, and the reduction in photosynthesis, may lead to a reduction in the transport of nutrients from leaves in water stressed conditions. In this chapter the effect of a variation in the soil water deficit on cotton growth and development, on the distribution and redistribution of nutrients in the plant will be researched. The two main aims of the chapter are;

1) to quantify the effect of variation in water supply on N, P, K and biomass distribution between plant parts; and,
2) to investigate the effect of variation in water supply on N, P and K redistribution between vegetative and reproductive tissue in high-yielding cotton.

8.2 Materials and Methods

To compare the total nutrient uptake and distribution of high-yielding cotton grown in conditions with varying degrees of water stress, and to quantify the impact of water supply on the redistribution of nutrients from vegetative to reproductive plant parts a field experiment was carried out at ACRI in the 2008-09 cotton season. Sicot71BRF cotton (*Gossypium hirsutum* L.) was sown on the 15th October, 2008. The experimental design and the irrigation treatments applied are given in section 3.4.4, and summarised here.

8.2.1 Irrigation treatments

Two irrigation treatments were applied, with irrigation occurring when a pre-determined soil water deficit was reached. The treatments were “wet”, irrigated at a 40 mm deficit, and “dry, irrigated at a 120 mm deficit. Soil water deficits were measured weekly to a 120 cm depth in 15 cm intervals, using a CPN Corpotation Hydroprobe®, model 503DR, neutron attenuation meter (NAM). The NAM was calibrated using the methodology of Tennakoon and Hulugalle

(2006). Since soil water was monitored frequently, rainfall was accounted for in determining deficits and irrigation scheduled accordingly. Total rainfall throughout the growing season totalled 327 mm. Irrigation details are given in section 3.4.4 and Table 3.5.

8.2.2 Plant sampling and analysis

The above ground plants from a 1 m^2 area were harvested from each plot at regular intervals between flowering and defoliation of the crop. Sampling dates are given in sections 3.4.4.

Whole plants were partitioned into leaves, stems (including petioles) and fruit (squares, flowers and bolls including seed, lint, boll walls and bracts). Samples were dried, ground and analysed for N, P and K as described in section 3.3.1. After defoliation yield was determined by handpicking as described in section 3.3.3.

8.2.3 Data Analysis

Data was analysed using Genstat® 14th edition. Total biomass, N, P and K uptake and concentrations were compared using ANOVAs. Bernacchi *et al.* (2007) and Gedroc *et al.* (1996) found that accounting for growth stages, more differences in dry matter accumulation and partitioning between plants as they develop can be demonstrated. Analysis of plants at a specific growth stage accounts for some of the differences in growth rate and plant size. To account for these differences, three separate ANOVAs were carried out to compare the dry matter accumulation and nutrient uptake and partitioning at flowering, 4 NAWF and maturity, rather than using plant age or thermal time as a factor. Correlation coefficients were calculated using Genstat® 14th edition. Nutrient redistribution was calculated using the method described in Chapter 4, using Sigma Plot® to fit logistic curves and calculated redistribution was compared using ANOVA.

8.3 Results

8.3.1 Irrigation treatments

Accounting for rainfall and the time taken to schedule and apply irrigation water on a large research property, the actual deficits at which the plants were irrigated were different from

the intended deficits. The mean actual deficit at which the plants were irrigated is given in Table 8.1.

Table 8.1 Measured soil water deficits to which the cotton was irrigated in the 2008-09 cotton season

Treatment	Intended Deficit	Actual Deficit	Number of irrigations
Wet	40 mm	35 mm	11
Dry	120 mm	105 mm	2

8.3.2 Yield, boll number and size

The number of bolls, average boll weight and lint percentage were highly variable as indicated by the high standard error of the mean (Table 7.2). For this reason, though the total yield was higher in the wet treatment ($P < 0.001$), there was no significant difference between the boll number, boll weight or boll lint percentage.

Table 8.2 Mean lint yield (kg lint ha^{-1}), number of bolls m^{-2}, boll weight (g) and lint % from each irrigation treatment. Significance calculated at P < 0.05.

Irrigation treatment	Lint yield (kg lint ha^{-1})	Bolls m^{-2} at maturity	Average boll weight at maturity (g)	% lint of seed cotton
Wet	2745.4^{a}	139	6.2	42.0
Dry	2023.5^{b}	126	5.4	42.6
s.e.d.	79.5	16.31	0.552	0.269
L.S.D.	194.4			
P value	<0.001	n.s.	n.s.	n.s.

8.3.3 Total biomass and nutrient uptake

Crop biomass, N, P and K uptake followed logistic curves (for each curve $P < 0.05$) (Figure 8.1).

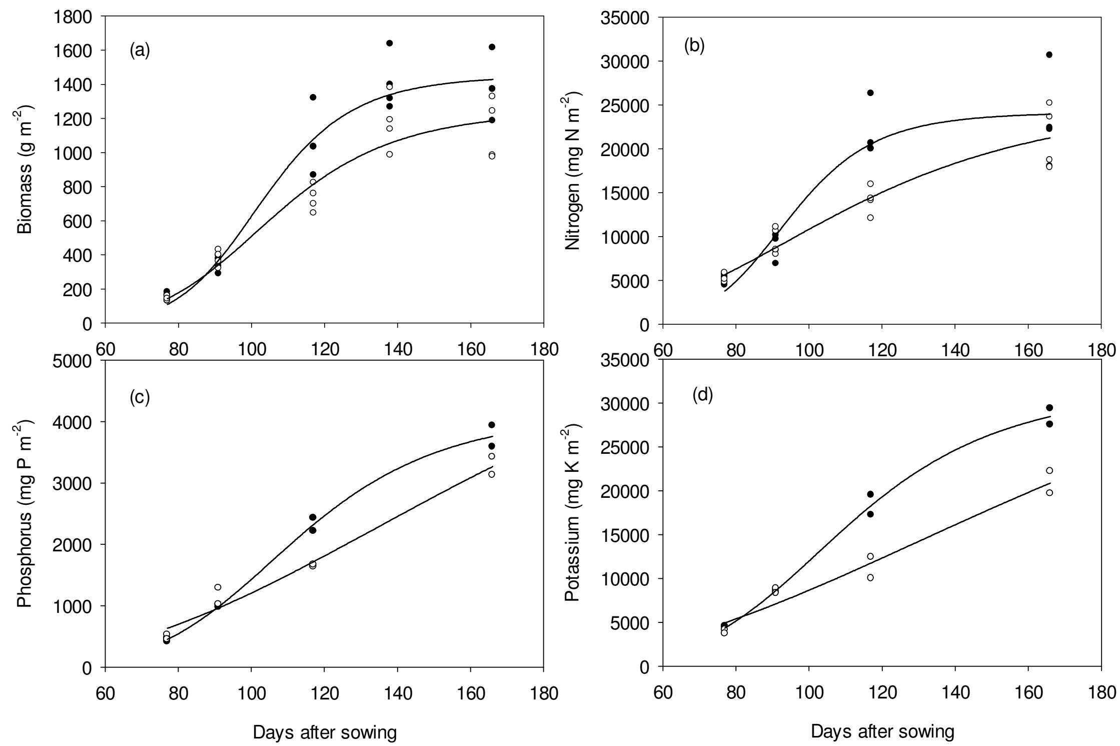

Figure 8.1 Mean (a) biomass, (b) N, (c) P and (d) K uptake in • wet and ○ dry plots. Lines show fitted logistic curves.

Comparison of the biomass, N, P and K accumulation at flowering, 4 NAWF and maturity highlighted differences between the plants grown in the two irrigation treatments (Figure 8.2).

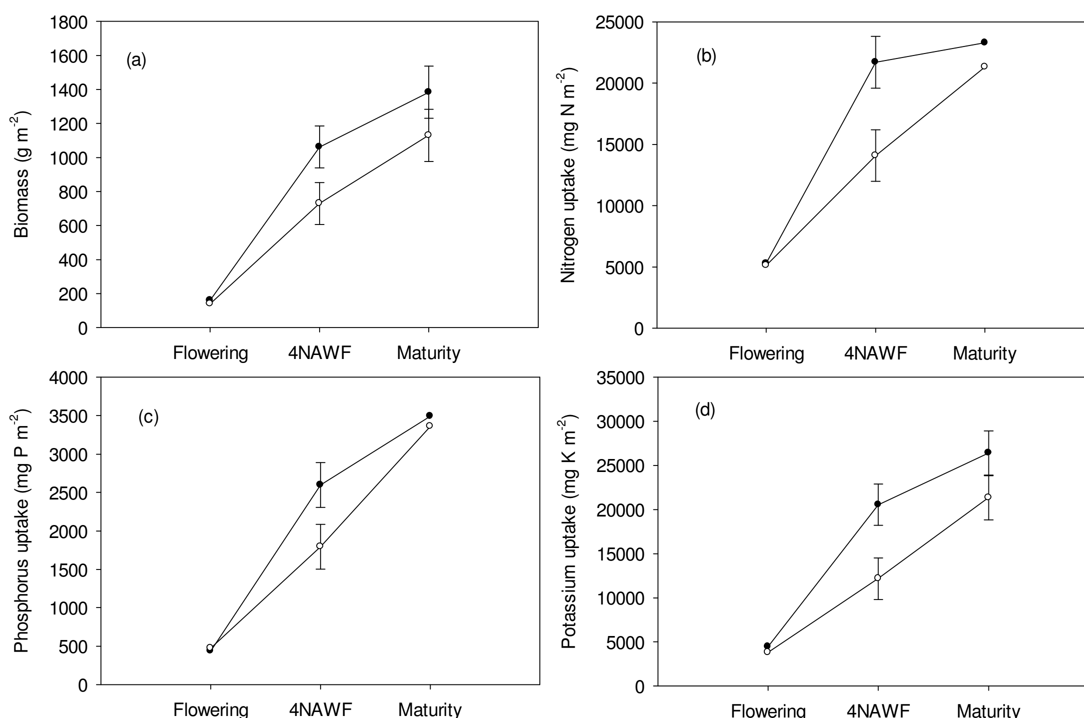

Figure 8.2 Mean (a) biomass (g m^{-2}), (b) N, (c) P and (d) K uptake (mg m^{-2}) in • wet and ○ dry plots at flowering, 4 NAWF and maturity. Error bars represent the L.S.D. at 0.05, and are not presented where there is no significant difference.

There was a consistent pattern of difference in the uptake and accumulation of biomass, N, P and K between the wet and dry treatments. At flowering plants were of a similar size and contained the same amount of N, P and K in the two treatments. By 4 NAWF the wet treatment had a higher biomass ($P = 0.017$), N uptake ($P = 0.004$), P uptake ($P = 0.015$) and K uptake ($P = 0.005$). Between 4 NAWF and maturity the dry treatment took up more N and P than the wet treatment, with no difference in the total N and P uptake at maturity (P = 0.560 and 0.678 respectively). The wet treatment maintained a higher biomass and K uptake at maturity (P = 0.009 and 0.048 respectively).

While there were differences in the nutrient content and biomass at 4 NAWF, there was no difference in the proportional accumulation of biomass and uptake of N, P or K by flowering or 4 NAWF (Table 8.3), indicating that the plants in the wet and dry treatments took up proportionally the same amount of nutrient and biomass after 4 NAWF. While there was a much higher uptake of N and P after 4 NAWF in the dry plots (Figure 8.2 b and c) the

variation in the data lead to high standard errors of differences in the means, contributing to the lack of difference found in the ANOVA analysis

Table 8.3 The proportional uptake (%) of the total N, P and K and total biomass at flowering, 4 NAWF and maturity.

Treatment	Proportion of total at maturity accumulated at growth stage (%)		
	Flowering	**4 NAWF**	**Maturity**
		Biomass	
Wet	11.6	76.7	100
Dry	12.4	64.6	100
***P* value**	n.s.	n.s.	
		N	
Wet	22.7	93.2	100
Dry	24.0	66.1	100
***P* value**	n.s.	n.s.	
		P	
Wet	11.8	61.7	100
Dry	14.9	50.4	100
***P* value**	n.s.	n.s.	
		K	
Wet	15.8	64.6	100
Dry	18.7	53.5	100
***P* value**	n.s.	n.s.	

8.3.4 Nutrient and biomass partitioning

There were differences in the partitioning of biomass, N, P and K at flowering, 4 NAWF and maturity, even though there was no difference in the total N and P uptake at flowering and maturity. Table 8.4 shows the proportional distribution of biomass and nutrients between the leaf, stem and fruit tissue (%).

Table 8.4 The mean proportional distribution (%) between the leaves, stems (including petioles) and fruit (including boll walls, bracts, seeds and lint) of N and biomass at flowering, 4 NAWF and maturity.

	Flowering			4 NAWF			Maturity		
	Leaf	**Stem**	**Fruit**	**Leaf**	**Stem**	**Fruit**	**Leaf**	**Stem**	**Fruit**
	Biomass								
Wet	46.2	48.8	5.06	26.2	32.9	40.9	14.4	24	61.6
Dry	48.7	44.9	6.39	24.2	24.1	51.7	13.6	26.9	59.5
***P* value** **L.S.D.**	0.022 2.05	0.023 3.14	n.s.	n.s.	0.003 4.71	0.029 9.89	n.s.	0.004 1.59	n.s.
	N								
Wet	59.7	34	6.22	49.6	12	38.5	24.3	12.4	63.3
Dry	61.7	30.7	7.59	42.1	8.76	49.1	21.6	16.2	62.2
***P* value** **L.S.D.**	0.06 2.06	0.05 3.34	n.s.	0.03 6.73	n.s.	0.04 10.6	n.s.	0.002 1.83	n.s.
	P								
Wet	50.5	39.9	9.63	31.80	15.25	55	12.20	10.7	77.1
Dry	52.5	33.5	14	20.21	7.17	72.6	15.3	17.5	67.2
***P* value** **L.S.D.**	n.s.	0.007 3.23	n.s.	0.015 6.24	0.001 2.95	n.s.	n.s.	n.s.	n.s.
	K								
Wet	28.1	67.8	4.1	21.8	40.6	37.7	10.2	15	74.8
Dry	29.5	64.5	6.1	18.4	23.7	57.9	12.3	17.3	70.4
***P* value** **L.S.D.**	n.s.	n.s.	n.s.	n.s.	0.003 6.73	0.022 10.8	n.s.	0.008 1.56	0.044 4.95

In addition to differences in the partitioning of biomass, N, P and K between the leaf, stem and fruit tissue, comparison of the ratio of reproductive to vegetative biomass and nutrients showed how water supply altered the allocation of resources between plant structures. The dry treatment allocated more biomass, N, P and K to reproductive tissue at 4 NAWF and at maturity supported the same ratio of reproductive to vegetative biomass and N as the wet treatment. The wet treatment had a much higher R:V P and K ratio than the dry, despite the lack of difference in the proportional P allocation (Table 8.5) at maturity.

Table 8.5 The ratio of reproductive to vegetative (R:V) biomass, N, P and K at flowering, 4 NAWF and maturity

	R:V Ratio		
	Flowering	**4 NAWF**	**Maturity**
	Biomass		
Wet	0.05	0.69	1.61
Dry	0.07	1.09	1.47
L.S.D.		0.31	
***P* value**	n.s.	0.02	n.s.
	N content		
Wet	0.07	0.63	1.75
Dry	0.08	0.99	1.65
L.S.D.		0.33	
***P* value**	n.s.	0.04	n.s.
	P content		
Wet	0.11	1.39	5.0
Dry	0.15	2.48	2.06
L.S.D.		0.71	2.28
***P* value**	n.s.	0.01	0.02
	K content		
Wet	0.04	0.73	3.07
Dry	0.06	1.28	2.39
L.S.D.		0.41	0.53
***P* value**	n.s.	0.016	0.02

At flowering the dry treatment had a consistently higher N concentration (mg N g^{-1} m^{-2}) in each tissue, which subsequently declined to be the same as the wet treatment (Figure 8.3). The N concentration in the fruit and stem tissue increased between 4 NAWF and maturity in the dry treatment, resulting in a higher N concentration of the whole plant, the stems and the fruit at maturity. The wet treatment maintained the N concentration of the leaves, resulting in a higher concentration at 4 NAWF, before a rapid decline (Figure 8.3b).

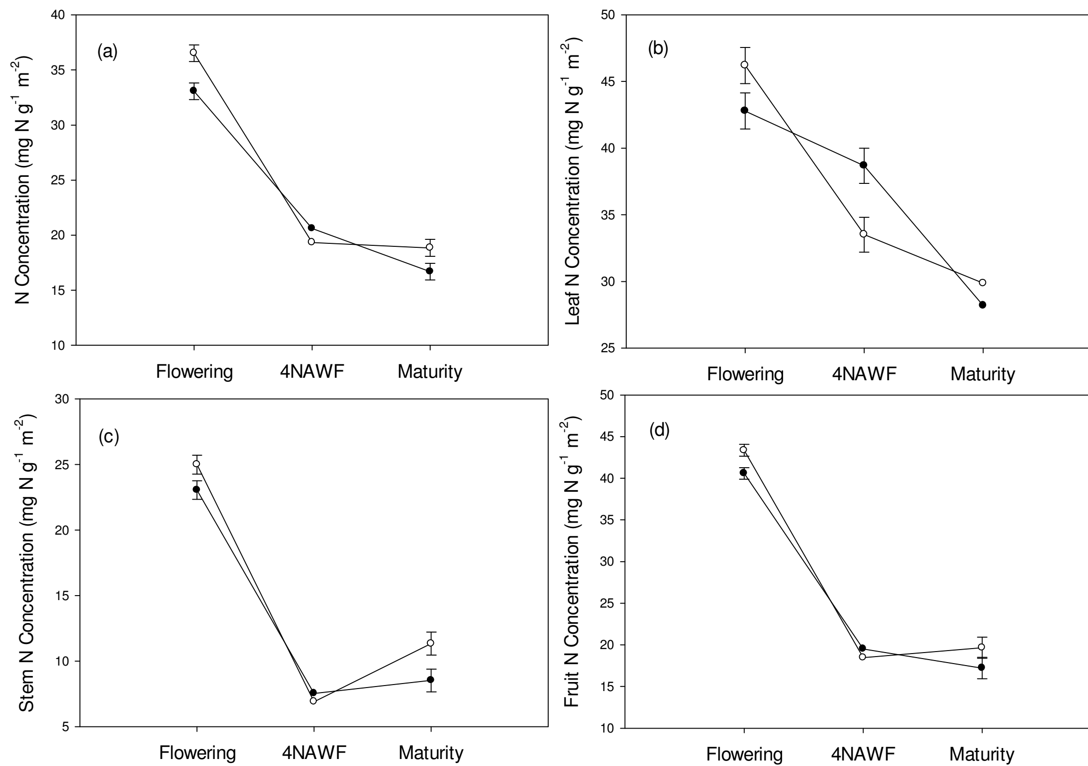

Figure 8.3 Mean N concentration (mg g^{-1} m^{-2}) in (a) whole plants, (b) leaf, (c) stem and (d) fruit at flowering, 4 NAWF and maturity in ● wet and ○ dry treatments. Error bar represents the LSD at 0.05.

The concentration of P in the leaf, stem and fruit tissues showed distinctly different patterns between the two treatments (Figure 8.4). As with N, the concentration of P in each tissue type was higher in the dry treatment at flowering. The wet treatment maintained a higher P concentration in the leaves, stem and fruit at 4 NAWF, after which point there was a slight decline in their concentration. In the dry treatment however, after 4 NAWF there was a rapid increase in the leaf and stem P concentration (Figure 8.4b and c), resulting in a much higher concentration at maturity than the in wet treatment. There was no difference in the P concentration in the fruit at maturity (Figure 8.4d).

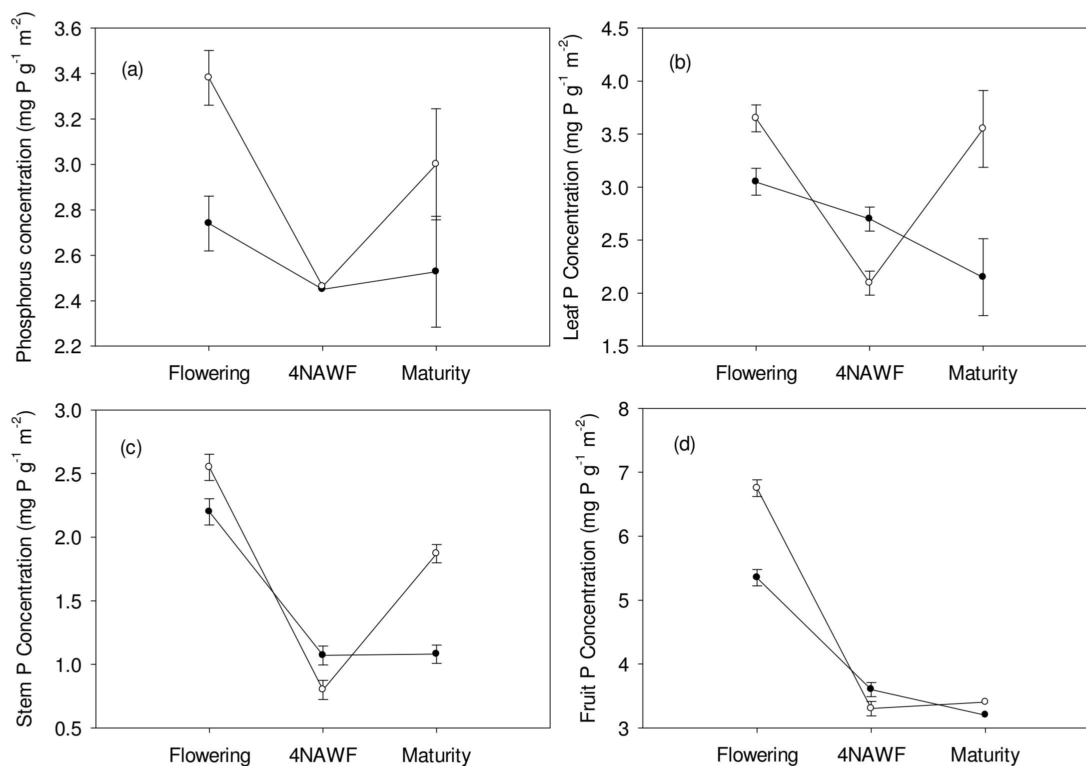

Figure 8.4 Mean P concentration (mg g^{-1} m^{-2}) in (a) whole plants, (b) leaf, (c) stem and (d) fruit at flowering, 4 NAWF and maturity in ● wet and ○ dry plots. Error bar represents the LSD at 0.05.

The pattern of changes in the concentration of K in each tissue was different again. At flowering there was no difference in the leaf or stem concentration, while the dry treatment had a marginally higher fruit K concentration (Figure 8.5d). There was no difference in the stem K concentration throughout the season (Figure 8.5c), but the wet treatment maintained a higher K concentration in the leaves and fruit at 4 NAWF (Figure 8.5 b and d). As with P, there was an increase in the concentration of P in the dry treatment leaves after 4 NAWF, resulting in the dry treatment having a higher leaf K concentration at maturity than the wet treatment (Figure 8.5b). Unlike for P there was no accumulation in the stems. In both treatments the concentration of K increased in the fruit after 4 NAWF, at a faster rate in the dry treatment, resulting in no difference in the fruit K concentration at maturity (Figure 8.5d).

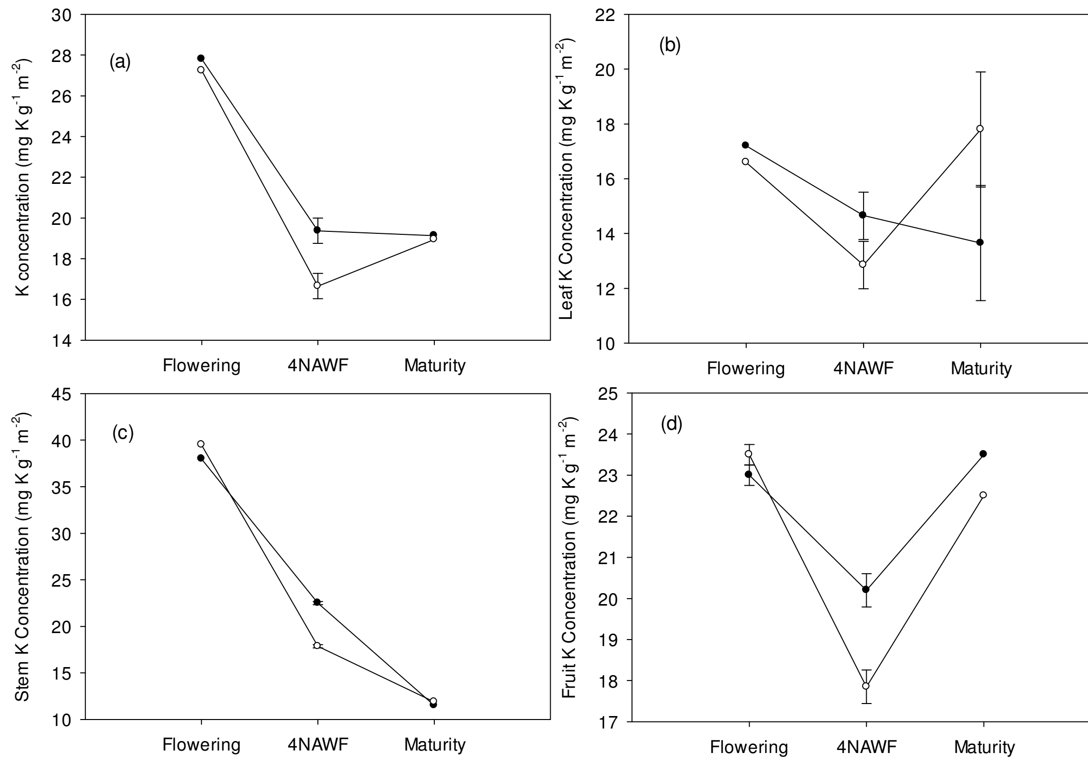

Figure 8.5 Mean K concentration (mg g^{-1} m^{-2}) in (a) whole plants, (b) leaf, (c) stem and (d) fruit at flowering, 4 NAWF and maturity in ● wet and ○ dry plots. Error bar represents the LSD at a 0.05 significance.

8.3.5 N, P and K redistribution

Redistribution was calculated by the method described in Chapter 4, using the logistic curves fitted to the uptake data given in Figure 8.1.

8.3.5.1 Nitrogen

Figure 8.6 shows the derived daily uptake of N in the whole crop and the fruit from sowing until maturity.

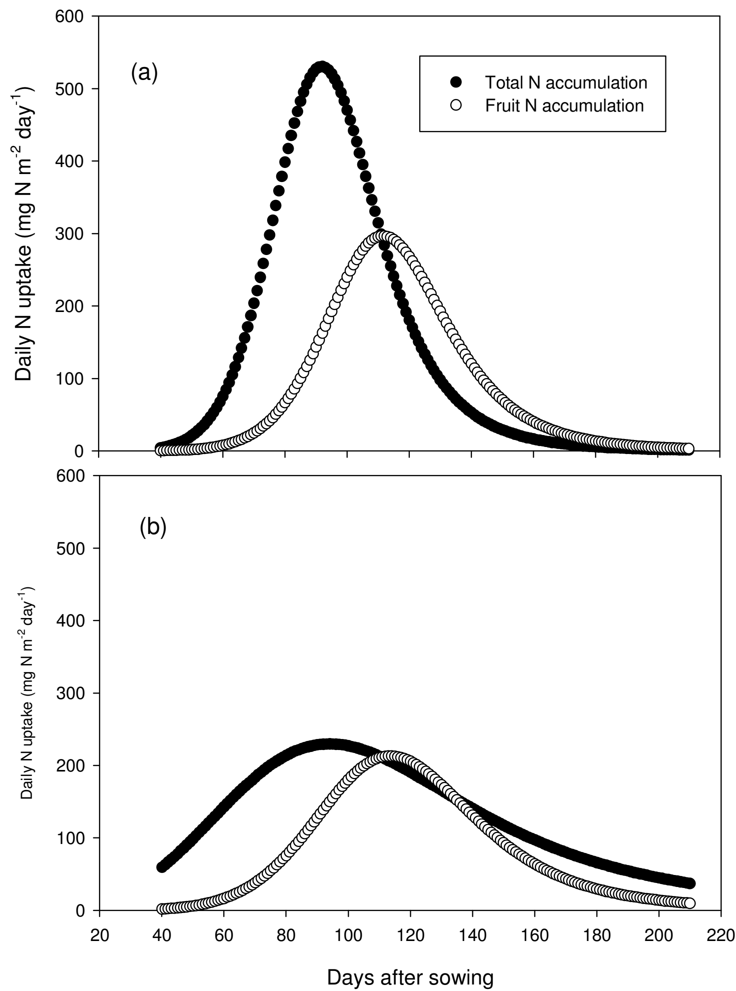

Figure 8.6 Mean daily N uptake in • the whole plant and ○ the fruit (mg N day^{-1} m^{-2}), calculated as the derivative of the logistic cure fitted to the accumulation data in Figure 8.1.

There was a significantly higher amount of N redistributed from the vegetative plant parts of the wet treatment, which redistributed approximately 3000 mg m^{-2} more N than the dry treatment (Table 8.6). The peak daily N uptake rate was much higher in the wet treatment in both the whole plants and the fruit. Proportionally, redistribution accounted for more of the fruit N in the wet treatment than in the dry.

Table 8.6 Total N redistribution and the proportion of fruit N supplied through redistribution

	Total N redistribution (mg N m^{-2})	Proportion of fruit N supplied by redistribution (%)
Wet	3228	21.9
Dry	235	1.8
L.S.D.	1580	
P value	0.022	

8.3.5.2 Phosphorus

The derived daily uptake curves used to calculate P redistribution are shown in Figure 8.7.

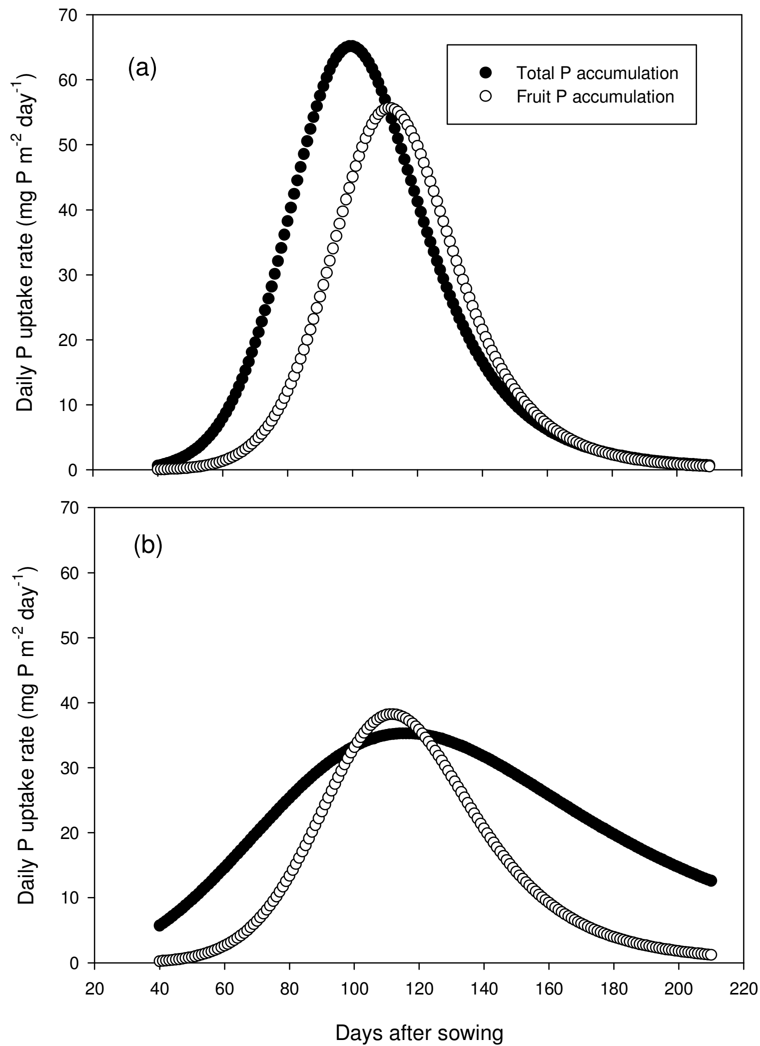

Figure 8.7 Mean daily P uptake in • the whole plant and ○ the fruit (mg P day^{-1} m^{-2}), calculated as the derivative of the logistic cure fitted to the accumulation data in Figure 8.1.

As with N, the wet treatment redistributed more P than the dry, although in both treatments the proportion of fruit P supplied by redistribution was very low (Table 8.7). The daily P uptake rate peaked at a much higher rate in both the whole plants and the fruit in the wet treatment, although uptake continued in the dry treatment at a higher rate than in the wet treatment, accounting for the increase in vegetative P concentration (Figure 8.4) and low proportional uptake of P by 4 NAWF (Table 8.3).

Table 8.7 Total P redistribution and the proportion of fruit P supplied through redistribution

	Total P redistribution (mg P m^{-2})	**Proportion of fruit P supplied by redistribution (%)**
Wet	257	4.06
Dry	43	1.9
L.S.D.	241	
P value	0.05	n.s.

8.3.5.3 Potassium

The derived daily uptake curves used to calculate K redistribution are shown in Figure 8.8.

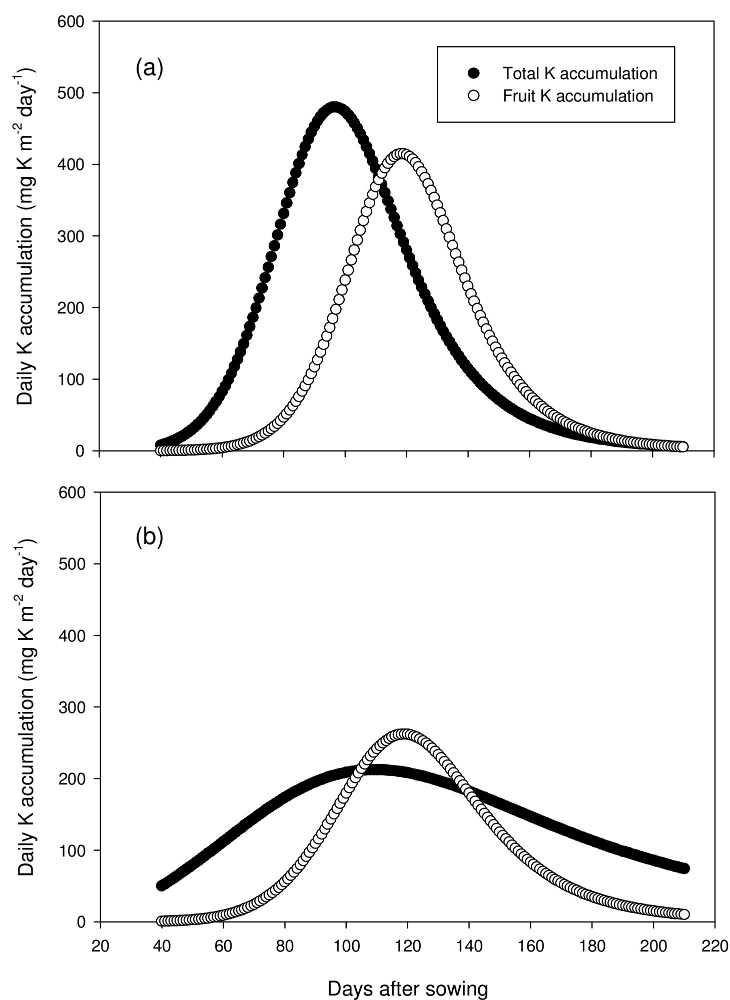

Figure 8.8 Mean daily K uptake in ● the whole plant and ○ the fruit (mg K day^{-1} m^{-2}), calculated as the derivative of the logistic cure fitted to the accumulation data in Figure 8.1.

As with N and P, the wet treatment redistributed more K than the dry and redistribution accounted for more of the wet treatment fruit K. Similarly the peak uptake rate was higher for the wet treatment for both the whole plant and the fruit. As with the P, the uptake of K in the whole plant continued through until maturity at a higher rate, accounting for the rapid increase in the leaf K concentration and the increase in the fruit K concentration (Figure 8.5b and d).

Table 8.8 Total K redistribution and the proportion of fruit K supplied through redistribution

	Total K redistribution (mg K m^{-2})	**Proportion of fruit K supplied by redistribution (%)**
Wet	5159	25.8
Dry	1189	7.9
L.S.D.	2237	
P value	0.003	

8.4 Discussion

8.4.1 Biomass and nutrient uptake and distribution

The first aim of this experiment was to evaluate the effect of varying water supply on the biomass, N, P and K distribution between plant parts. Based on previous research, the withholding of water to produce water stress should produce smaller plants with a correspondingly lower N, P and K uptake, a similar N, P and K concentration, no difference in the R :V ratio and similar biomass and nutrient partitioning (McConnaughay and Coleman 1999; Poorter and Nagel 2000; Enquist and Niklas 2002; Bernacchi *et al.* 2007). A lower yield, lower boll number and lower average boll weight have also been reported as the result of water stress in cotton plants (Hearn 1975a; 1979; Constable and Rawson 1982; Hou *et al.* 2007; Hake and Grimes 2010).

8.4.1.1 Yield and boll number

A comparison of the biomass, N, P and K uptake and partitioning followed some of these reported trends and not others. As previously observed, the wet treatment had a higher yield, though the difference in boll size and boll number was not significant. Since yield is a product of boll number, boll size and lint %, it can be assumed that the lack of statistically

significant differences in these variables between the wet and dry treatments were an artefact of the variability in the data, since the difference in yield was considerable (the wet treatment yielding 136% of the dry treatment).

8.4.1.2 Biomass and nutrient accumulation

In line with previous studies, the wet treatment produced bigger plants, with a higher K content but unlike in previous studies, there was no difference in plant N or P uptake between the wet and dry treatments. Until 4 NAWF, plants followed reported trends, with biomass, N, P and K uptake being lower in the dry treatment. The rate of uptake of N and P after 4 NAWF was much higher in the dry treatment than the wet, with 34.9% of the total N being accumulated after this point, and 49.6% of plant P. A similarly large proportion of K (46.5%) and biomass (35.4%) was accumulated in the dry treatment plants after 4 NAWF. The wet treatment, in contrast, took up only 6.8% of total N, 23.3% of biomass, 38.3% of P and 35.4% of K after 4 NAWF – indicating that some environmental or agronomic conditions stimulated a different response late in the season in the dry treatment, and not the wet. The plants in the dry treatment did produce some late-season re-growth, that is, they began a new phase of growth after 4 NAWF and bolls were opening, producing new nodes up the main stem and developing additional fruiting branches. This was probably in response to rainfall during this period, which may have stimulated new growth. Total in crop rainfall was 354 mm, of which 88 mm fell after 4 NAWF, and 188 mm fell between flowering and 4 NAWF. Saturated soil during the flowering period and then rainfall late in the season would have reduced the stress on the dry treatment plants, and it is hypothesised that water supply late in the season promoted the extra growth.

Several studies have shown a consequence of water stress is the expansion of a root system, enabling the rapid response to a change in conditions (Gedroc *et al.* 1996; Jackson 1997; McConnaughay and Coleman 1999; Raats 2007). This may explain the rapid growth and nutrient uptake late in the season in the dry treatment, and not the wet, which had not experienced water stress prior to the late season rainfall. Since this was a field based experiment, variable climatic conditions are inevitable. A similar experiment, repeated over several seasons to produce a data set large enough to account for seasonal conditions would explain this factor. While very difficult to assess in a field context, experiments accounting for root growth, in terms of biomass and nutrient accumulation would also shed light on the

different growth dynamics of water stressed and well watered cotton. In glasshouse experiments root growth, and the allocation of carbon to root tissue has been recorded (Constable and Rawson 1982). Plants grow differently in a controlled environment and so it could not necessarily be assumed that they would respond in a similar way in the field, and allocation of carbon and nutrients to roots should be researched.

8.4.1.3 Biomass and nutrient partitioning

Many studies have shown that water stressed and well watered plants partition biomass in a similar way (McConnaughay and Coleman 1999; Poorter and Nagel 2000; Pline *et al.* 2003; Janat 2004). The only difference in the ratio of reproductive to vegetative (R:V) biomass in this experiment occurred at 4 NAWF, when the wet treatment had a lower R:V biomass. This may be explained by an early limitation of leaf expansion and growth in the dry treatment, and an investment in the vegetative growth to support a high boll load in the wet treatment, resulting in the lower R:V figure. Excess, or luxurious supply, of water can also delay the onset of fruiting (Hearn 1975a) and increase allocation to vegetative structures. At maturity, however, the water treatments had no effect on biomass partitioning between vegetative and reproductive tissue, despite the difference in plant size, in line with previous studies.

Since nutrient movement often follows biomass accumulation, it is assumed that nutrient partitioning between tissues would be the same under variations in water supply, in line with biomass partitioning results (Poorter and Nagel 2000). Theoretically, the smaller plants produced in water stressed conditions, would take up a correspondingly smaller amount of nutrients, but partition them in the same way, and produce the same ratio of reproductive to vegetative structures. In this experiment, however, there were some differences in the partitioning of N, P and K where no differences in biomass partitioning occurred. The R:V of N partitioning was similar to biomass, which is a consequence of N export and accumulation being closely linked with carbon allocation and production. There was no difference in total N uptake, meaning that the same amount of N was partitioned to vegetative and reproductive structures at maturity. The partitioning of P and K however showed a different trend, in that the wet treatment plants accumulated a much high proportion of both nutrients in the fruit than in the vegetative tissue at maturity. The fruit of the wet treatment contained 75% of plant K and 77% of P, compared to 70% of plant K and 67% of plant P in the dry treatment. The wet treatment plants contained more K overall, while there was no difference in the P uptake

(see Figure 8.2). Therefore, regardless of the timing of uptake, the dry treatment contained far less P and K in the fruit than the wet treatment.

The decreased allocation of P and K, but not N or biomass to fruit in water-stressed plants has not been reported in other studies. Coker *et al.* (2000) reported K uptake and partitioning in well watered and dryland cotton, although combined the water treatment with a foliar application of K, confounding the effect of the water stress on K partitioning. Singh *et al.* (2006a) examined the growth of plants, and P distribution and uptake under drying conditions, although they did not report total plant P partitioning in this or a related study (Singh *et al.* 2006b). Assumptions about the reasons for this increased allocation of P and K to the fruit can be made, taking into account the physiological functions of each nutrient, their role in fruit development, and the growth and development of the crops after 4 NAWF.

The dry treatments took up a large proportion of the total P and K content (around 50%) after 4 NAWF. During this time the wet treatment took up some P and K, but far less than the dry treatment (only 38.3% of total P and 35.4% of K). The leaf and stem concentration of P (see Figure 8.4b and c) and the leaf concentration of K (see Figure 8.5b) increased in the dry treatment during this time, indicating that the P and K taken up from the soil were allocated to the existing and new vegetative tissue. Interestingly, the N concentration of the leaves and stems in the dry treatment did not increase during this period, indicating that N uptake was either consistent with growth or the concentration declined due to growth dilution. However, the P and K uptake rate, clearly exceeded the growth rate. The extra P and K uptake and their allocation to vegetative tissue may be primarily due to their increased availability to the roots late in the season. These nutrients are taken up in solution, and may be less available to roots during water scarcity. Soil water content is particularly important for P uptake, since P is relatively immobile in the soil. Similarly, cotton is generally regarded as inefficient at taking K up from the soil (Bednarz and Oosterhuis 1999), and so the lack of soil water, and poor access of roots to the nutrients in soil solution would hinder uptake.

The allocation of P and K to vegetative tissue, over reproductive tissue may be related to the timing of uptake. Since after cutout (4 NAWF) relatively few new bolls are produced, the extra uptake could not have been allocated to new reproductive tissue, but to existing structures. In the wet treatment, the R:V of P and K increased during this time, probably due

to a combination of some uptake allocation and then redistribution. In the dry treatment the R:V of P decreased, indicating that almost all uptake accumulated in vegetative structures, and the K ratio increased, though by a smaller margin than the wet – indicating a difference in the partitioning preference of the plants. Chapter 5 showed that the uptake of P and K, particularly of K, occurs early in the boll development. The late increase in supply, therefore, may have been outside of the optimum window for allocation of P and K to the bolls. The pattern of fruit K and fruit P concentration changes was similar for both treatments, with the P concentration of the fruit remaining relatively steady after 4 NAWF and the K concentration increasing. This similarity supports the hypothesis that the dry treatment plants were either unable to allocate the extra P and K to fruit, or the fruit did not require the extra P and K when they became available. More studies on the timing of nutrient accumulation in bolls, particularly paired with fertiliser timing treatments could further examine this hypothesis.

8.4.2 Nutrient redistribution

The second aim of this experiment was to investigate the effect of variable water supply on the redistribution of nutrients from vegetative to reproductive structures. In this experiment, the redistribution of N, P and K (when calculated using the method described in Chapter 4) was significantly reduced in the dry treatment plants, exposed to prolonged drying and grown at a large soil water deficit.

8.4.2.1 Physiological responses to water stress and their relationship to nutrient redistribution

Transport of nutrients to developing bolls occurs primarily in the phloem (see lit review section 3.1.2.1). The flow of nutrients and water to developing bolls, particularly before the xylem connections between the boll and the rest of the plant become functional at around 20 days after flowering, has been shown to be relatively unresponsive to water stress and changes in the water potential of the surrounding tissue (Van Iersel and Oosterhuis 1995; Van Iersel and Oosterhuis 1996). The flow rate of water and nutrients therefore, may not be the limiting factor driving changes in nutrient redistribution from vegetative to reproductive structures under varying water conditions, either before or after the vascular connections between the boll and the rest of the branch become fully functioning. More likely changes

would be induced by both a limitation to the amount of carbon exported from leaves, due to a reduction in photosynthesis, and a change in the partitioning of exported carbon and nutrients from the leaves, to support root growth at the expense of new vegetative or reproductive tissue. The smaller plants observed in this experiment in the dry treatment indicate that carbon substrates for growth were limited by the physiological consequences of water stress.

Based on previous research it could be assumed that the total amount of nutrients redistributed from leaves would be less, under water stressed conditions, than under non-stressed conditions. Water stress has been shown to reduce photosynthesis, reduce the transport of carbon from leaves to bolls, and to reduce the nutrient content of the xylem and phloem sap (Constable and Rawson 1982; Schaefer *et al.* 1987; Iersel *et al.* 1994; Bahrun *et al.* 2002). Water stress can enhance leaf senescence and promote the internal recycling of nutrients to supply roots and developing fruits (Hearn 1979). These two findings seem to contradict one another, although if it is assumed that leaves are smaller, and their total nutrient content is lower, then enhanced senescence may still result in a smaller net movement of nutrient from vegetative to reproductive structures.

In this experiment, there was a significant reduction in the amount of N, P and K redistributed from vegetative tissue with the application of water stress (Figures 6, 7 and 8). This reduction in the amount of N, P and K redistributed from vegetative to reproductive tissue in the dry treatment plants could be due to;

1) A change in the priorities of the plant, resulting in the allocation of redistributed nutrients to new vegetative growth, which would not be accounted for in the balance method applied to the calculation of redistribution used.
2) A real reduction in the amount of nutrients redistributed from leaves, similar to the previously reported reduction in carbon export.
3) A late season increase in water supply to the plants from rainfall which increased root uptake, negating the need for the plant to supply the previously limited number of bolls with nutrients from the leaves, and allowing redistributed nutrients to supply new vegetative growth.

When examined in the context of the changes in biomass, nutrient uptake, tissue nutrient concentration and nutrient and biomass partitioning, it seems the most likely explanation that

a combination of these factors resulted in the decreased redistribution. The extra growth and nutrient uptake from increased water supply late in the season indicate that the roots of the plants in the dry treatment remained functional for longer than those in the wet treatment, or else functioned at a comparatively higher level in terms of N, P and K uptake. In other experiments where root functioning appeared to continue through the season, redistribution was reduced (see chapters 4 and 7).

The allocation of the nutrients taken up late in the season in the dry treatment, particularly for P and K, was mostly to vegetative growth. This allocation was probably added to by redistribution of nutrients from older vegetative tissue – although the method quantifying changes in the total vegetative and reproductive nutrient content used to calculate redistribution would not capture this movement. At the end of the season, plants responded rapidly to a change in conditions and allocated acquired nutrients to vegetative structures. If the growing season were longer, the plants could have produced more fruit. Extra fruiting structures on existing branches were not produced, but rather the nutrients used for the production of structures to support potential new fruit at the top of the plant. Allocation to the roots of the plants may also have declined, when the stress placed on the plant by water stress was removed, enabling more nutrients to be allocated for vegetative growth. Plants in the dry treatment did not, however reach the same size as the wet treatment plants, but increased their R:V biomass ratio to be the same as the wet treatment in the mature plants.

To further examine the redistribution of nutrients in water-stressed plants, an experiment similar to that in Chapter 6, should be carried out under different water conditions. This experiment did not capture redistribution from vegetative to vegetative structures, which may have occurred late in the season. Labelling experiments would give a better quantitative measurement of redistribution, and if applied to the soil could also highlight late season uptake of nutrients.

In this experiment it is likely that reduced water supply lead to reduced nutrient redistribution by several mechanisms; firstly, through limiting uptake of nutrients early in the season, secondly, through reducing the functioning of leaves, especially their export of carbon and other nutrients, and thirdly, through promoting root development. This increased root development lead to an increase in late season vegetative growth, when the dry treatment

plants were supplied with adequate water again through rainfall. Further field experiments, examining both non-stressed and water-stressed plants which remain stressed until the end of the season would be helpful in quantifying the effect of water stress on nutrient redistribution. Though there were differences in the redistribution measured between the treatments, the differences were confounded by the rapid growth and nutrient accumulation of the dry treatment plants after 4 NAWF.

8.4.3 Conclusions

Water stress reduced plant size, yield and N uptake in this experiment, and significantly reduced the amount of N, P and K redistributed from vegetative to reproductive structures. The late season increase in biomass, N, P and K uptake in the dry treatment confounded results, as late season rainfall stimulated uptake of nutrients and resulted in new vegetative growth. The reduction in N, P and K redistribution from vegetative to reproductive structures was significant, and the hypotheses of previous chapters that variability in water supply, due to water management at different sites, and changes in rainfall between seasons could have significantly impacted the redistribution of nutrients in the plants is confirmed.

The dry treatment plants, while yielding far less than the wet treatment, contained a similar amount of nutrients, leading to the conclusion that water stress or supply has an impact on both the distribution and redistribution of nutrients within a high-yielding cotton plant. Further experiments, using a larger range of soil water deficits, or amounts of water supplied could show the relative impact of water stress on nutrient movement in the plants, and could quantify its effect.

CHAPTER 9

General Discussion

The uptake of essential nutrients by roots, their transport and allocation to various above ground organs plays a pivotal role in optimising plant growth, yield and quality at many growth stages (Boquet *et al.* 1994; Unruh and Silvertooth 1996; Velemis *et al.* 1999; Zhao and Oosterhuis 1999; Drake *et al.* 2002; Wahid *et al.* 2004). In modern, high-yielding Australian cotton cropping systems, the supply of developing crops with nutrients and the optimisation of nutrient use- efficiency is essential for long term sustainability and profitability.

Since the 1930' s there have been many studies describing the uptake and distribution of nutrients in cotton plants (Crowther 1938b; 1941b; a; Olson and Bledsoe 1942; Richards 1944; Crowther 1947; Boynton 1954; Bassett *et al.* 1970; Jones *et al.* 1974; Hearn 1975a; b; Halevy 1976; Leffler and Tubertini 1976; Leffler and Hunter 1985; Cassman *et al.* 1989a; Unruh and Silvertooth 1996; Pervez *et al.* 2004; Geng *et al.* 2005). Research has focussed on describing the response of cotton plants to fertiliser inputs, differences in the uptake and distribution of nutrients between cultivars, and the effect of crop nutrition on yield. Less research has focussed on linking nutrient inputs with plant physiological responses or on linking nutrient inputs with other management operations such as irrigation. There have been few studies quantifying the proportion of nutrients redistributed from one plant organ to another, or including redistribution in the definition or discussion of nutrient use efficiency (NUE). NUE is generally described in terms of lint yield per unit of nutrient uptake (kg ha^{-1}), rather than examining the efficiency of the plant's use of the nutrients in terms of it's recycling of accumulated nutrients from one tissue to another, or its efficiency of redistribution to supplement a high boll load.

This study adds to the previous knowledge about cotton plant nutrient demands in terms of total uptake, partitioning and yield. Redistribution of N, P and K was quantified, and based

on the data presented should be incorporated into the concept of NUE, as a primary mechanism by which a plant can improve its efficiency of nutrient use.

9.1 Quantifying redistribution

The redistribution of N, P and K from vegetative tissues was examined at three different levels; 1) from a single leaf (Chapter 5), 2) from leaves in five node segments up the mainstem of a cotton plant (Chapter 6), and 3) from the pooled tissues or various ages from a whole plant (Chapter 4). There was a significant variation in the gross and proportional amount of redistributed N, P and K between leaves and stems from different nodes and between crops of a similar yield, size and nutrient content. There was also some variation in the redistribution of each nutrient, as N, P and K showed distinct patterns, which could be related to their function, relative mobility, accumulation in various sinks and the timing of demand for each from different organs.

The method used in chapter 4 to quantify redistribution was effective as a means of comparing the nutrient redistribution between different crops. The large range in redistribution calculated using this method shows that the process is highly variable. Further experiments showed that redistribution is also variable within a single plant, and that the potential remobilisation of N, P and K within a whole plant is influenced by many interacting factors. Measuring redistribution at a whole plant scale using the method developed in Chapter 4 is useful for comparing different crops, as in Chapters 7 and 8. The variability in redistribution between leaves, and between leaves and stems in different sections of the canopy shows that any measure of whole plants will not be as accurate as measurement of tissues of the same age. A complete budget for N, P and K in a developing plant should be developed, based on the boll accumulation, and potential redistribution from tissues within the bolls, as well as from individual leaves and stems. This model could be developed to help predict nutrient demands of plants, to optimise boll development, and to account for redistribution in any model of nutrient uptake and efficiency.

9.1.1 Nitrogen redistribution

Two methods were used to calculate N redistribution; firstly, analysing the N content and concentration of each tissue and calculating redistribution as the balance between the peak content and the content at maturity and, secondly, by using ^{15}N-labelled urea to trace the movement of N between tissues. Redistribution of N occurred in all plants examined in these experiments. N is highly mobile nutrient, with between 6 and 80% of the leaf N content being recycled and redistributed to other tissues. While other tissues (the bracts, boll walls, lint and petioles) were exporters of N the leaves were the tissue exporting the largest amount of N both as a gross figure and as a proportional amount. The bracts and lint exported a small amount of N, which was not consistent between bolls at different positions. Likewise the stem N content exported was highly variable along branches and at different nodes up the mainstem. Therefore, on the basis of variability and low gross N export from other tissues, potential N redistribution was calculated as potential leaf redistribution. The range in leaf N export measured in these experiments was similar to that reported in previous studies (20 – 70%), but some data were higher than previous research suggests for cotton leaves and whole plants (Oosterhuis *et al.* 1983; Zhu and Oosterhuis 1992; Boquet *et al.* 1994; Fritschi *et al.* 2004b).

The first aim of this study was to quantify redistribution and to propose an average baseline figure against which to compare plants as being efficient or inefficient. At a single leaf level, the mainstem leaf exported a mean of 81% of the peak N content and the 1st position sympodial leaf exported 69%. In the three sections of the plant contributing the most to the lint yield (Constable 1991), from node 6 to node 20, N redistribution from the leaves was between 72 and 81% - reaching the average proportional redistribution suggested by the single leaf study. As described in chapter 6, the potential leaf N redistribution in the whole plant is more like 60% of leaf N contents. This value accounts for the lower redistribution from the leaves on nodes 1 – 5 and the assumed lower N redistribution at the top of the plant (since the leaves are younger, and did not reach maturity). A value of 60% N redistribution from the leaf tissue allows for accumulation to continue at the higher nodes (21 and above), and reflects the variation in redistribution between plant parts, despite being lower than the potential proportional redistribution from a single leaf.

This hypothesis, that 60% leaf N redistribution represents efficient redistribution, is based on the N export from leaves described in chapters 5 and 6. Using these results to calculate a potential for redistribution efficiency makes several assumptions, the most significant of which is that the results of experiments 6, 7 and 8 are representative of a "standard" cotton plant, which can be used as a benchmark for other plants to be compared with. It is clear from the experiments described in chapters 4, 7 and 8 that seasonal differences in climatic and environmental conditions as well as nutrient and water supply have a significant effect on the redistribution of N in the plant. This conclusion confirms previous research reporting the partitioning of nutrients under different water and nutrient treatments (Hearn 1976b; Oosterhuis *et al.* 1983; Guitman *et al.* 1991; Hocking and Steer 1995; Bange *et al.* 2004). To account for variability between seasons, climatic and environmental conditions the results of experiment 9 should be verified over a range of seasons, in different cultivars and under different irrigation and nutrient treatments.

The leaves and bolls in experiments 7 and 8 used to calculate the potential redistribution were similar to those in the few previous studies reporting similar data. The N concentrations of the fruit in experiments 7 and 8 were similar to those reported by both Zhu and Oosterhuis (1992) and Thompson *et al.* (1976), ranging from 45 mg g^{-1} to 15 – 20 mg g^{-1} at maturity. The peak N concentration of the vegetative tissue was slightly lower than that reported by Zhu and Oosterhuis (1992), but declined to the same concentration (around 20 mg g^{-1}). This difference in peak N may have changed the proportion of N recorded as export, however they found that up to 42 days after the peak N was reached the mainstem leaf exported 60% of its N, and the sympodial leaf "appeared to export only a small amount of N". As they did not account for the simultaneous import and export of N observed in experiment 8, they may have underestimated the total export from the leaf, and some export from the mainstem leaves may not have been accounted for.

Other assumptions made to calculate a 60% leaf N redistribution potential, and to use it as a benchmark to compare to other plants and studies are that plants with a different number of nodes, leaf number, leaf area or leaf size will redistribute the same proportion of N and that measurements made following ^{15}N applications from a single point in time (peak N content)

are reflective of continued leaf functioning. Other sympodial leaves were not included in experiment 9, which would redistribute less N than the first position and mainstem leaf, based on their lower N concentration, N content and the reduction in redistribution between the first position and mainstem leaves. This may decrease the accuracy of 75% of single leaf N being the potential average redistribution and further studies should define the redistribution potential of the 2nd, 3rd and 4th sympodial leaves, as well as of the cotyledons and leaves from monopodial branches. Replicate experiments applying ^{15}N-labelled urea at various times through the leaf growth and development both before and after flowering are needed to define the period of peak export and calculate the rate of export during leaf growth and development. In experiment 8 only two time points were compared to calculate export from the labelled leaf, so the rate of export and variability in the timing of export between leaves could not be calculated. Repeated measurements are needed to rectify this limitation.

Based on the data from experiments 6, 7 and 8, a redistribution of less than 60% of leaf N represents inefficient N redistribution. Since the experiments described in chapters 4, 7 and 8 include stem redistribution, this benchmark cannot be applied to the redistribution calculations made there. It is, however, helpful in terms of identifying where inefficiencies may come from, and in terms of how NUE may be increased in cotton production systems. A redistribution of less than 60% indicates that the plant could have supported more bolls, since there was N available to developing bolls which was not used. These data are useful for modelling plant nutrient use and for the linking of nutrient inputs with the plant's physiological potential NUE.

9.1.2 Phosphorus redistribution

Since no experiment using labelled P was carried out, it is not possible to quantify P redistribution with the same accuracy as for N and K. As P is a relatively immobile nutrient in the plant, its redistribution was predictably much lower than for N and K. The single branch experiment showed that the P content of the leaves declined during the peak period of P import into the bolls, and then increased. Likewise most tissues along the single branch acted as sources of P and then as sinks. Calculation of the redistribution of P during the

"source phase" showed relatively little export. At a whole plant scale the redistribution of P was between 0 and 20%, accounting for up to 36% of fruit P.

The data shows that, even for an immobile element like P there is significant movement between tissues. Chapter 6 shows that there is also some variation in the amount of P exported from leaves in different parts of the canopy, the middle section redistributing a higher proportion of the leaf and stem P than the lower or upper sections. The stems were relatively neutral throughout the plant in terms of their behaviour as a source or a sink. Since there is a low concentration of P in the xylem and phloem sap, and P is mainly stored as ATP or in phosphate compounds in high energy use areas of the plant, this reflects the areas of demand for P. There was some evidence of P remobilisation from the lint after the first few weeks of boll filling, and a small amount of export of P from the boll walls and bracts. This data suggests that, as for N and K, there is movement of P from one tissue within the boll to another, depending on the timing of and role of the nutrient in development. Further experiments using a stable P isotope (^{33}P) should be carried out to clarify the amount of P remobilised from leaf tissue and boll tissue and to define a proportion of leaf P which is remobilised and redistributed. The results of the whole plant study are too variable to calculate a potential redistribution.

Comparison of the leaf export in different parts of the plant showed that proportional leaf export ranged from 0 – 69%, with far more export occurring in the middle of the plant than at the top or the bottom. Single leaf studies should be carried out to calculate potential P redistribution from a leaf. As P is less mobile than N or K, potentially more of the exported P would be allocated to the subtending boll, or those in close proximity to the leaf. Labelling studies would confirm this hypothesis.

9.1.3 Potassium redistribution

Potassium, being a highly mobile nutrient stored mainly as an ion in solution, was remobilised and redistributed in large quantities between tissues. Unlike N and P, the stems were a significant source of K which was redistributed to other tissues, exporting up to 71%

of their K content. The potential remobilisation of K from both the mainstem and first position leaves was found to be 85%, with little difference in the proportional redistribution of the peak K content between the leaves. There was more variation in K redistribution in different parts of the plant than between the leaves, with the middle of the plant exporting 71% of leaf K and the bottom only 20%.

As suggested in chapter 6, an arbitrary measure of 50% of leaf K could be used as a benchmark for redistribution efficiency. Since the plants in different experiments did not reach the same proportional redistribution of K the assumptions made in calculating the potential N redistribution cannot be met. The variability in the plants shows that the plants described in experiment 8 were not necessarily equivalent to those in experiment 6, and therefore differences from the 85% potential reached in experiment 8 could be due to other factors changing the redistribution of K in other plant parts.

Further experiments to validate the 85% of leaf K which was exported in experiment 9 and to compare the redistribution of K from leaves at various nodes should be carried out. Since there are few studies estimating the redistribution of K from single leaves, or pooled leaves within the canopy, similar experiments to experiments 6 and 8 in a controlled environment should be carried out. This would eliminate much of the variation in growing conditions in the field experiments, through controlling the water, temperature and light conditions. This would help explain the variability in K redistribution between experiments and help to estimate a proportion of leaf K which represents efficient or inefficient redistribution.

9.2 The accumulation and source of N, P and K in bolls

A detailed description of the accumulation pattern of N, P and K in the developing bolls was given in chapter 5, describing the timing of uptake and the changes in nutrient content and concentration as the different boll components developed. This work focussed on the bolls at position 1 and 2, defining a clear hierarchy of the sinks along a single branch, and showing that the accumulation of N, P and K in the boll at position 2 occurred mostly after the boll at position 1 had reached peak nutrient content. The concentration and content of the boll

components was similar to those previously reported for N (Thompson *et al.* 1976; Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999; Boquet and Breitenbeck 2000; Chua *et al.* 2003), P and K (Leffler and Tubertini 1976; Thompson *et al.* 1976; Zhao and Oosterhuis 1999; Wahid *et al.* 2004).

The contribution of the mainstem and first position leaf to the developing boll has not been previously quantified, beyond studies estimating redistribution as the balance between peak nutrient content and the content at maturity. These studies all made the assumption that all or most of the redistributed nutrients from subtending leaves were allocated to the subtending boll, which the data of experiment 9 contradict. Of the ^{15}N recovered in the plant, over 90% had been exported to tissues above or below the labelled node, from both the mainstem and first position leaves, rather than the subtending boll. The mainstem leaf supplied the first position boll with 4.9% of seed N and 1.8% of the boll wall N (the lint and bracts accounting for very little N, as shown in experiment 8). The first position leaf supplied a further 6.7% of seed N and 5.5% of boll wall N. In terms of K supply, none of the K in the mature boll came from the mainstem leaf, but 7.8% of the seed K and 13.1% of the boll wall K came from the first position leaf. This data shows that the assumption made by many authors about the role of the subtending leaf in the supply of bolls with nutrients is overestimated, and that for bolls on node 11, around 90% of the total N and K content is from other sources.

This finding has several implications for cotton nutrient use and growth models. Firstly, the hypothesis that the cessation of growth and the production of new fruiting sites (at ‘cutout’) are related to the nutrient demand from the bolls (Hearn 1975a; Hearn 1976a; 1981; Rosolem and Mikkelsen 1989; Wright 1999; Oosterhuis and Bondada 2001; Baker and Baker 2010) is not supported by this data. Secondly, circumstantial evidence suggesting that the N export for boll development is the cause of photosynthetic decline and leaf senescence (Constable and Rawson 1980b) is also unsupported by this data. The experiments in chapters 5 and 6 showed that, across the whole plant, the nutrients supplied to bolls by the redistribution of leaves are supplementary to root uptake, providing only 10% of the N and K in the bolls in the middle of the plant, and a small amount of P. Export from the mainstem and first position leaf at node 11 to fruit at other nodes (experiment 9) shows that a similarly small proportion of the

nutrients in the mature boll are provided by leaves on other nodes. Redistribution of leaf nutrients is therefore not the limiting factor for boll development, nor can the decline in leaf functioning or the slowing or stopping of growth be attributed to export of N or other nutrients from the leaf. The labelling of leaves at different stages, based on the timing of demand from the developing bolls described would provide more information about the export of nutrients from the leaves. The labelling of the leaves with Rb prior to flowering, and the measurement of the Rb content in the first two weeks after flowering would show the contribution of the leaf to these tissues, before they exported some of their K. Equally, the redistribution of K between tissues in the boll should be measured, since circumstantially the boll wall and lint may provide a significant proportion of the seed K.

Since root uptake of nutrients has been shown to be the source of the majority of nutrients in the mature boll, it is likely that the size and functioning of the root system is the limiting factor for yield and the driver for cutout. The reasons for the decline in photosynthesis and stomatal conductance of the leaves late in the season may be linked to hormonal and biochemical changes, rather than N export, which may be involved in a negative feedback mechanism with the roots to limit new growth. Further research to identify the cause of cutout in cotton plants should be carried out, and growth models should be modified to decrease the emphasis on boll demand for nutrients as a limitation to growth late in the season.

9.3 How does agronomic management effect N, P and K redistribution?

The management of a cotton crop has a significant impact on the realised and potential lint yield, size, phenotype and its growth rate and development. The experiments described in chapter 7 and 8 show that two key management factors in cotton crop production also have an impact on the plant's mechanisms for the recycling and partitioning of N, P and K.

Water stress, applied as a reduction in soil water to a 120 mm deficit, significantly reduced N, P and K redistribution from vegetative to reproductive tissues. The addition of P and K fertiliser decreased the total P and K redistribution from vegetative to reproductive tissue.

There was no clear relationship between the two seasons in which N rate experiments were carried out to conclude that N stress increased redistribution. In experiment 2, N redistribution decreased with increasing N supply, but in experiment 5, carried out in the proceeding season, N redistribution increased with increasing N supply. The high-yielding crop supplied with 200 kg N ha^{-1} in experiment 5 is an interesting anomaly. Continued root uptake of N until late in the season was accompanied by a high proportion of vegetative N being redistributed to the reproductive tissue. The reason for this high redistribution and high root uptake of N was hypothesised to be due to interacting management and environmental factors, and the continuation of root uptake until the end of the season. Why some non-stressed crops redistributed a high proportion of their leaf N could help to increase the N use efficiency of cotton production systems, and the circumstances leading to this plant behaviour should be determined. Experiments combining several factors at once, including water, temperature, light interception and nutrient supply in a controlled environment could identify a set of conditions likely to promote root uptake and redistribution of N through the boll filling period.

The nutrient rate and deficit irrigation experiments both highlight the role of root uptake, growth and functioning as a significant variable in the nutrient uptake and redistribution within the cotton crop. Further experiments are needed to identify the conditions promoting deep early season root growth, such as that hypothesised to have occurred in the dry treatment in experiment 4, and also to promote the functioning of roots late into the season such as was hypothesised to occur in the high N plots in experiment 5 and in several of the crops described in Chapter 4. This emphasis on root growth and linking it to the above-ground use of nutrients could help to maximise the crop productivity and the efficiency of nutrient and water use.

9.4 The source-sink ratio in cotton plants and its effect on N, P and K redistribution

Having defined the redistribution of N, P and K in cotton crops, identified the source of nutrients in a mature boll and described the effect of nutrient and water management on nutrient redistribution in high-yielding cotton crops, the question remains; what are the

drivers for redistribution? If redistribution is not a limiting factor to the yield and boll development of high-yielding cotton crops, it is logically a secondary buffer to maintain nutrient supply to bolls if the primary source of nutrients from the roots becomes limited. Therefore, is root functioning, particularly late in the season, the limiting factor for boll development and yield?

The answer to the second question is more straightforward. The experiments in this study indicate that redistribution is clearly a supplementary process for the supply of developing bolls with the nutrients required, and that the bolls do not primarily rely on redistributed nutrients. Root uptake, throughout all the experiments, has been cited as a significant source of nutrients, and root functioning related to the total uptake of nutrients and redistribution. In no instance was yield or boll size correlated with redistribution, indicating that redistribution is not limiting to yield or boll development under the circumstances described in these experiments.

The first question, relating to the drivers of redistribution is more complex. If the redistribution of N, P and K were source-driven (that is determined by the functioning of the leaf and stem or by other processes occurring in them) then the expected pattern of redistribution would be;

1) Out-of sync with the demand from the sinks.
2) Similar in all parts of the plant, between leaves of similar ages and growth stages.
3) Regulated by processes such as photosynthetic decline or hormonal changes associated with aging.
4) Related to the nutrient concentration or content of the source itself.
5) Have no correlation with the sink size either locally or in the whole plant, in terms of the redistribution rate or the gross or proportional amount of nutrients redistributed.

If it were sink-driven (that is, determined by the demand from the bolls and driven by their requirement for nutrients) then the pattern of redistribution would be;

1) In-sync with the demand from the sinks.

2) Variable in different parts of the plant and related to the distribution of sinks.
3) Regulated by feedback mechanisms from the sinks in terms of allocation and demand.
4) Related to the nutrient concentration or content of the sink.
5) Be correlated with the sink size, either locally or in the whole plant.

Previous research suggests that the export of carbon from leaves is not driven by sink demand, nor does the demand for carbon from the developing boll drive the decline in photosynthesis in the mainstem or sympodial leaves (Constable and Rawson 1980b). The question of sink demand for nutrients playing a role in the decline in photosynthesis in leaves (particularly since a large fraction of leaf N is associated with photosynthetic enzymes), and driving the export of nutrients from leaves has been raised by many authors (Constable and Rawson 1980b; Wright 1999; Wahid *et al.* 2004; Zhang *et al.* 2007; Li *et al.* 2009), although in cotton crops there are few studies presenting more than circumstantial evidence.

This study adds both circumstantial and quantitative evidence to the question of sink- or source-driven export of nutrients from cotton leaves and stems. In chapter 5 a detailed description of the timing of accumulation of nutrients in each tissue along a sympodial branch was given, confirming previous research that the export of N is out of sync with demand from bolls (Zhu and Oosterhuis 1992; Zhao and Oosterhuis 1999). The same is true for both P and K, where peak demand occurs shortly after flowering. This would suggest that the demand for N, P and K is not the main driving force for the export of these nutrients from the subtending leaves – especially for K and P which are accumulated early in the bolls development. Despite being out of sync with the subtending leaves, data from chapter 5 also showed that the contribution of the mainstem and first position leaves to the first position boll was around 10% of the seed N and 6% of the boll wall N, and 8% of seed K and 13% of the boll wall K. As discussed previously, this means that a significant amount, about 90%, of all the N and K in the major sinks of each branch, was sourced from other places. Clearly, the demand from the bolls did not drive the export from the subtending leaf.

Data from chapters 5 and 6 also show that a significant amount of N and K exported from leaves and stems are translocated to tissues, and plant sections, removed from the leaf or stem

exporting the nutrient. In chapter 5, both N and K from the mainstem and first position leaves moved both up and down the plant, and to leaves and fruit. There were differences in the allocation of N and K from each tissue. This is particularly evident for K which was allocated only to fruit removed from node 11 from the mainstem leaf, and to tissues below node 11 from the first position leaf. This long-distance movement was also out-of sync with the development of bolls at each node, since N and K were allocated to both older and younger tissue from both leaves.

The export of N, P and K did appear to show some variation in different parts of the plant, as shown in Chapter 6. This variation was not correlated with the number of developing bolls in each section, or with the R:V ratio of biomass in each section; indeed, the section in which most of the bolls were located exported the most N and K, followed by the section immediately below it. The proportional export from the leaves and stems in the middle of the plant was higher than the lower; this could possibly have been driven by the number of fruit in each section, except that the middle section of the plant exported most of the N and K from the leaves and stems into other sections, not to the bolls. This evidence confirms the finding of Chapter 5 that N and K are highly mobile in the plant and move long distances, but does not suggest that the export from the leaves and stems is driven by the sink demand.

The third criteria for the sink- or source-driven export from the leaves and stems or cotton plants was not addressed in this study and is outside the scope of the project. Several other authors have speculated on the relationship of nutrient export with the decline of other processes in leaves (Constable and Rawson 1980b; Landivar *et al.* 1983; Wullschleger and Oosterhuis 1990a; Bondada *et al.* 1996; Pettigrew *et al.* 2000) and have linked the production of specific hormones, free radicals and oxidative chemicals with nutrient export and senescence (Pettigrew *et al.* 1993; Pettigrew *et al.* 2000; Djanaguiraman *et al.* 2009). These have mainly described the export of N in relation to the senescence of the leaf, and did not include the cycling of nutrients through the leaf, and its role as a temporary storage organ or site of reduction and synthesis for nutrients then redistributed to other tissues, which was identified in Chapter 5. Never-the-less, this research tends to argue that the sink demand for

nutrients and the interaction of shading, chemical accumulation and cell damage all play a role in leaf senescence.

The fourth criteria listed above, linking nutrient redistribution to the nutrient concentration in either the source or the sink can be addressed circumstantially by the experiments in this study. To be sink driven, export from sources would increase during the period of highest demand from the sink, and the rate would decrease when the sink demand declined or was met. The concentration of the nutrient in the sink would feedback to the source to speed or slow redistribution. This pattern was not observed on a single branch scale, since the export of N, P and K did not follow the timing of import into the first position boll, nor was the export or import of N, P or K into the different sections of the canopy correlated with the development of the bolls, or the export of N, P or K from the leaves and stems in sync with the import into the bolls. At a whole plant scale, there seems some evidence to suggest that redistribution, at least of N, was linked to the demand for N from the developing bolls. In chapter 4 the redistribution of N was highest in those crops which had a high rate of increase in R:V biomass, that is, in those crops which rapidly increased the sink size after 4 NAWF. While there was no evidence to suggest that N redistribution was limiting to the source development, there was a clear trend across the six crops linking those that had a low R:V at 4 NAWF and a subsequent rapid development of bolls with high N redistribution. Similar trends were not evident for P or K redistribution showing that the redistribution of the three nutrients operate independently of one another to some extent.

Previous research has suggested that the export of N is increased from tissues with a lower peak N concentration (Guitman *et al.* 1991; Milroy *et al.* 2001; Semenov *et al.* 2007). The findings of Chapter 7 support this theory, although Chapter 6 showed that, within a single plant the export of N from leaves lower in the canopy, with a lower initial N concentration was lower than those higher up with a higher N concentration. Similarly, the P and K data did not support this claim. Further experiments examining the relationship between concentration in the leaf and the export of N, P and K should be carried out, especially examining leaves in different parts of the canopy and under different rates of nutrient supply (to increase the N concentration). This would help explain the apparent contradiction in the data from Chapters

6 and 7, and define the effect of N concentration on the potential N export from leaves. An analysis similar to that of Killingbeck (1996) could define a minimum N, P or K concentration in the mature leaf post-export which indicates complete redistribution, which could explain differences in redistribution based on the difference in the amount of "excess" nutrients in the leaf.

The last criteria addressing the question of source- or sink-driven export from leaf and stem tissue is the relationship between sink size and source size. If the redistribution of N, P or K were sink-driven in cotton plants, whole plants, or sections of plants, with a higher R:V would have a correspondingly higher proportional redistribution of nutrients. This relationship has been suggested as the driver for redistribution by many authors, but is seldom reported (Krieg and Sung 1986; Rosolem and Mikkelsen 1989; Wright 1999; Mullins and Burmester 2010). In a study manipulating the source – sink ratio in wheat by removing source organs (the flag leaf of the wheat plants) Guitman *et al.* (1991) showed that increasing the R:V did not increase the redistribution from the remaining vegetative structures, but rather decreased it. In their experiments, senescence was delayed, as was the remobilisation of N under two N rate treatments. Sink removal however, has been shown to accelerate senescence in both wheat and maize (Christensen *et al.* 1981). Both these findings, while describing determinate plants with a very different structure and growth pattern to cotton, indicate that a change in the R:V stimulates a change in the functioning of the tissues. Increasing the efficiency of the source, in terms of delaying senescence and prolonging photosynthesis or stimulating the premature senescence of the leaves to promote new growth seem to be the illogical consequences of source and sink removal respectively, instead of the increase in R:V placing "extra demands" on leaf resources and increasing nutrient export.

The R:V biomass ratio for each experiment was reported. In Chapter 4 the ratio of R:V at maturity was not correlated with the redistribution of N, P or K, although the crop with the lowest R:V at 4 NAWF did redistribute the most N, indicating that a low ratio may have stimulated export and leaf senescence in cotton as shown for other crops (Christensen *et al.* 1981). Under the different N, P, K or water treatments the R:V at 4 NAWF or maturity was not correlated with N, P or K redistribution. A high R:V at 4 NAWF and during the boll

filling period has been cited as the cause of increased redistribution of P and K from leaves and the premature senescence of cotton plants (Wright 1999; Pettigrew *et al.* 2000). This hypothesis is not supported by the findings of this study, with no indication that a high R:V stimulates redistribution or a plant with a high R:V at 4 NAWF would be likely to redistribute more P or K to developing bolls.

The relationship between the R:V and redistribution should be further investigated, experiments involving the removal of both leaves and bolls and then the subsequent recording of redistribution and nutrient export should be conducted. A study similar to experiment 6, with the removal of bolls from different sections of the plant, or the removal of half the fruit from the entire plant at flowering would be useful to compare the rate and proportional amount of redistribution from different sections of the plant and in a whole plant under different R:V ratios. The hypothesis that senescence is delayed by source removal should be tested, and the conclusions from the experiments on determinate plants examined in indeterminate cotton plants.

In summary, based on the criteria proposed above, there seems little evidence to suggest that the remobilisation of leaf N, P or K is a primarily sink driven process. However, without detailed examination of leaf and whole plant export under different R:V ratios and without measuring other indicators of source functioning, carbon assimilation and gas exchange, it cannot be concluded that it is a source-driven process. It seems likely that the relationship between sinks and sources and the transfer of nutrients between them is a complex one, regulated by the environmental conditions, nutrient and water supply and on biochemical, hormonal and chemical relationships between the tissues. It is clear that the redistribution of N, P and K from leaves and stems is complex, variable and is not influenced by any single factor alone, but by a complex of factors, interacting with one another.

9.5 Suggested future work

This study, while producing data to address the hypotheses and questions which were raised, presents the opportunity for further work and investigation of nutrient redistribution and

nutrient use efficiency. Many of these suggestions have been made in the context of the discussion of the individual results, but some more limitations of these experiments and areas outside the scope of this project should be addressed.

1) What is the genetic basis for nutrient remobilisation? It is clear from many studies that there is an irreversible genetic component to the nutrient remobilisation from leaves due to senescence. This study has shown that there are significant amounts of nutrients in leaves which are not remobilised before the bolls subtending them mature, and which remain in the leaves until plant maturity. If there was a genetic component to nutrient remobilisation, which could make the process more efficient, it should be identified and the factors affecting its expression and function identified as a potential for increasing the nutrient use efficiency of cotton crops.

2) Does nutrient remobilisation vary between cultivars? The experiments described in this study were all carried out using one cultivar, representative of much of the Australian cotton industry. There may be cultivar differences in the potential redistribution of N, P or K, or in the efficiency of transport of those nutrients.

3) How is root growth promoted, and how could cotton plants be grown in such a way as to continue root uptake of nutrients until late into the boll-filling period, or until maturity?

4) To what extent are nutrients remobilised either to or from roots, and how do nutrient and water supply, soil structure and type and environmental factors affect this?

9.6 Conclusions

This study has 1) quantified the redistribution of N, P and K from vegetative to reproductive tissue in high-yielding transgenic cotton plants, 2) suggested a benchmark against which to class cotton crops as efficient or inefficient users of N and K in terms of redistribution, 3) quantified the contribution of single leaves to the subtending bolls, 4) described the nutrient

accumulation pattern of bolls and 5) provided significant evidence to suggest that N, P and K redistribution is not a primarily sink-driven process.

The contribution of N to a developing boll by the subtending leaf, and mainstem leaf on the corresponding node has been shown to be around 10%. The potential export of leaf N has been quantified at 75% peak content, and a cut-off figure for N remobilisation of 60% of peak leaf N has been proposed as a measure of N use-efficiency. The contribution of K to a developing boll from the subtending leaf has been shown to be around 10%, and no contribution was recorded from the mainstem leaf. The potential export of leaf K has been quantified at 85% for a leaf in the middle of the plant, and a figure of 50% redistribution of peak leaf K proposed as a measure of K use-efficiency. Root uptake has been identified as the source of the majority of N, P and K in a mature boll, and factors affecting root uptake proposed as the drivers for redistribution.

Two widely held assumptions in cotton growth and nutrient use models have been called into question. Firstly that there is a link between the R:V biomass ratio and the redistribution of nutrients from vegetative to reproductive tissue, which has been shown to have no correlation with N, P or K redistribution in any of the experiments described. Secondly, the assumption that the subtending leaf is a major source organ for the supply of nutrients to developing bolls, and that export from leaves are partitioned to the closest sink, are not supported by these findings. Cotton growth and nutrient use models should incorporate these findings, with a new assumption that the whole plant, including the roots, is far more interconnected than previously thought, and that nutrient remobilisation and redistribution is a response to a complex of factors throughout the season, not just to the partitioning of above ground biomass.

Further understanding the physiological basis for the variation in nutrient use-efficiency of cotton, and the reasons why some plants redistribute far less than the proportions proposed here helps in two ways. Firstly to identify why cotton crops are sensitive to variations in nutrient supply under certain conditions, and secondly it contributes to understanding how to

increase the nutrient use efficiency and contribute to high yields and high-quality cotton. The experiments presented in this study, and the conclusions drawn from them increase the understanding of how modern, very high-yielding crops use the nutrients supplied to them and help to describe the physiological mechanisms of nutrient use and nutrient use efficiency in high-yielding cotton.

Nutrient distribution and redistribution was shown to be influenced by a range of factors including agronomic management, environmental and seasonal conditions and in the interactions between them. The study has not measured or described the interaction of biochemical, hormonal or genetic factors, but it is clear that nutrient redistribution is a result of all these factors interacting to produce a specific set of conditions to which the cotton plant responds. The adaptability of the cotton plant to produce high yields, and partition biomass and nutrients to support those yields has been highlighted by these experiments.

The management of cotton crops to promote both nutrient remobilisation, and the continuation of root uptake until late into the season, as occurred in some of the crops studied here, presents the opportunity for cotton growers to produce high-yielding and nutrient use efficient plants. As resource use becomes more costly, and the imperative for producing cotton in the most environmentally friendly way increases, the pressure on cotton growers to increase the nutrient use efficiency of their production systems will grow. Understanding the factors which affect the internal nutrient dynamics of the cotton plant will assist future researchers and growers to link their inputs with the plant's demands and to manipulate the plant's physiological processes to meet desired outcomes.

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