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The efficacy of foliar fertilisers on Bollgard II ® cotton

Meredith Errington
Sciences Discipline, Faculty of Agriculture, Food and Natural Resources,
The University of Sydney, Australia, 2006.

Abstract. Newly released, high yielding transgenic cotton cultivars are said to have a higher nutrient demand during the boll-filling period (between flowering and maturity), due to their higher boll retention rate and larger boll load than conventional cultivars. During this period, nutrients are translocated from leaves to bolls, leading to speculation that foliar fertilisation could be used as an effective tool for raising the nutrient status of the leaves at this critical period, and increasing the yield and fibre quality of the cotton crop. The benefits obtained by application of foliar fertilisers on high yielding cotton cultivars is debated, with highly variable responses recorded in both Australia and America. In this study the pattern of nutrient accumulation in the lint, seed and boll wall components of the developing cotton bolls from a Sicot 71BR cotton crop was also measured to assess the critical period of nutrient demand and the pattern of nutrient accumulation in bolls. Nutrient accumulation occurred during the first 500 growing day degrees from flowering, suggesting that the optimum time for foliar fertiliser application to meet this high nutrient demand would be in this period. In this study seven foliar fertilisers (P, K, Zn, Cu, B, Fe and a control) were sprayed onto Sicot 71BR cotton at two sites in north-west New South Wales in the 2005-2006 season. Foliar applications of P, K, Zn, Cu, B and Fe did not increase the plant development, productivity or fibre quality of the Bollgard II ® cotton to which they were applied. While some nutrients (P, Zn, Cu and Fe) were absorbed into the plant leaves there was no consistent pattern in nutrient uptake and little evidence of the nutrients applied being translocated to developing bolls, with no increase in the nutrient content of seeds or lint. There was also no increase in fibre quality or nutrient content. This lack of plant response and ineffective uptake was attributed to the hot, dry environmental conditions and a lack of existing nutrient deficiency at either site.

Key Words: Cotton, *Gossypium hirsutum*, foliar fertilisers, fibre quality, nutrition, boll development, nutrient accumulation, nutrient translocation, seed oil.

List of Tables

List of Figures

Introduction

Salts of many essential plant nutrients are soluble in water and may be applied to plant leaves directly as a foliar fertiliser. This practice has become widespread in the American and Australian cotton industries over the past 20 years as a means of correcting crop nutrient deficiencies and supplying nutrients to plants during peak demand when root uptake may not be adequate (Oosterhuis 2003b). Foliar fertilisation has many advantages over traditional soil fertilisation including:

- low cost of application;
- plant response is fast, and therefore deficiencies may be rectified quickly;
- no soil fixation;
- independent of root uptake, and so may be applied when root functioning is declining or impaired; and
- may be mixed with other agrochemicals.

Foliar applications of micronutrients can overcome short-term deficiencies since the amounts applied are small and the nutrients themselves can be applied directly to the tissues showing signs of deficiencies (Fernandez *et al.* 2005). The risks of foliar fertilisation are phytotoxicity and leaf burn, insolubility of some compounds, high solution pH, difficulty in application of a high volume of nutrient and inefficient plant absorption due to leaf age, crop stage, water stress or climatic conditions (Oosterhuis 2003b).

Correct nutrition of a cotton crop is essential for ensuring high yields and high quality fibre. Newly released Bollgard II ® cotton cultivars are reported to have higher boll numbers and higher boll retention rates than conventional cultivars. It is speculated that these varieties have a higher overall nutrient demand, particularly during the boll development stage, making adequate nutrient supply to these crops a significant factor in achieving high yields (Heitholt 1994c; Pervez *et al.* 2004). During the boll-filling period, translocation of nutrients from leaves to bolls intensifies at the same time as production of assimilates and photosynthates slows and sometimes stops. This halt in photosynthesis is attributed to the translocation of nitrogen to developing bolls (Pettigrew *et al.* 2000). This research proposed that nutrients applied to leaves could prevent their senescence and decline in photosynthesis, allowing photosynthesis and carbon fixation to be extended, thereby increasing seed cotton yields.

Research into the efficacy of foliar fertilisers in cotton has shown a range of plant responses and production benefits in commercial cropping situations, leading to debate as to the usefulness of foliar fertilisation in large scale operations (Ma *et al.* 2004; Nelson *et al.* 2005). Cotton plant responses to foliar fertilisers have been inconsistent and variable. A study conducted at twelve sites across the American cotton belt showed yield increases with foliar applications of potassium at only 40% of the sites (Oosterhuis *et al.* 1994). Similarly yield responses to foliar applications of micronutrients have ranged from an increase of 140kg/ha in lint yield in response to foliar zinc (Sawan *et al,* (1998) to a decrease of 160 kg/ha in lint yield in response to foliar applied boron (Heitholt, (1994b). These inconsistencies have been linked to environmental conditions (Oosterhuis *et al.* 1991; Zhu *et al.* 1992), physiological characteristics of the crops (Oosterhuis 2003a; Wullschleger *et al.* 1989) and chemical properties of the foliar sprays (Howard *et al.* 2000; Howard *et al.* 1998). An understanding of the factors affecting the penetration of foliar sprays and the incorporation of the nutrients into metabolic pathways in cotton plants and other crops may aid in clarifying the effectiveness of foliar applied fertilisers at meeting plant nutritional requirements.

Cotton bolls can be partitioned into the seed, lint and boll walls. These three components mature at different times, reaching full size and volume, and accumulating nutrients and oil at different developmental stages (Benedict *et al*. 1976). The physiological and anatomical development of the boll has not been researched since studies by Leahy (1948) and Tharp (1948) established the pattern of seed and lint development, and Schubert *et al*. (1973) described the lint and seed development dynamics. This pattern of seed and lint development has been accepted in literature about cotton seed for the last 30 years (Reddy *et al*. 1999).

While this physiological pattern of development is well established, the pattern of nutrient accumulation and the source of the nutrients deposited in cotton seeds and lint has not been an area of current research. There is little quantitative data available on the timing of translocation and deposition of nutrients in bolls (Constable *et al*. 1988) and most published data is from conventional varieties, completed before the release of any transgenic cotton cultivars. The developmental pattern is important for establishing the time period in which nutrient demand from the bolls is the highest. Moreover, information about the accumulation of both macronutrients and micronutrients would aid crop management decisions about timing of fertiliser applications to meet the demand from developing bolls.

To determine the efficacy of foliar fertilisation on cotton plants, an understanding of nutrient demands, the pattern of nutrient accumulation within a plant and the partitioning of these nutrients within the plant need to be understood. In cotton crops, particularly in higher yielding varieties, these factors are not completely understood or explained in the literature. Incomplete knowledge about the timing of translocation of nutrients to developing bolls, and if this translocation occurs sequentially across the boll filling period means that fertilisation may potentially create an oversupply of the nutrient if applied before or after the peak demand, or may not provide sufficient amounts to meet plant demands if translocation occurs in a rapid way over a short period of time.

Knowledge about the timing of translocation of nutrients to the developing bolls is one of these areas. Nutrients accumulate in bolls, and the total nutrient content of the mature seed and lint is easy to establish through nutrient analysis at the end of the season. Knowledge about the timing of the accumulation could give insight into peak demand periods from the developing seeds, and the windows of time in which application of these nutrients could enhance the concentration of these nutrients in the seeds or lint.

Among other unanswered questions about foliar fertilisation is the problem of establishing how much of a foliar applied nutrient can be taken up by a crop, particularly since few studies have been carried out on already deficient soil. The hypothesis that higher yielding varieties with an increased boll retention rate also remains an area which could be explored further in the literature. If this were so then the effectiveness of foliar fertilisers at supplying these acute demand phases of higher yielding crops could be investigated further.

Therefore, the aims of this investigation were to establish i) patters of nutrient and oil accumulation in developing cotton bolls from a high yielding cotton cultivar and ii) the effect of foliar applied fertilisers on cotton yield, fibre quality and plant nutrient status when applied to a high yielding cultivar.

Materials and Methods

Experiment 1- Assessing the Efficacy of Foliar Fertilisers on Bollgard II ® Cotton

Site Description and Experimental Design

To test the efficacy of foliar fertilisers on Bollgard II® cotton, a field experiment was carried out at two sites in the cotton-growing region of north-west New South Wales during the 2005-2006 season. Since temperature and water stress have been shown to effect the efficacy of foliar fertilisers (Coker *et al*. 2000; Reickenberg *et al*. 1996) two locations, the Australian Cotton Research Institute (ACRI), Narrabri (149º59'E, 30º12'S) and a commercial cotton property "Longacres", Carroll (150º46'E 30º96'S) were selected, for their variation in climatic growing conditions (Table 1). The locations of these sites are shown in Fig. 1.

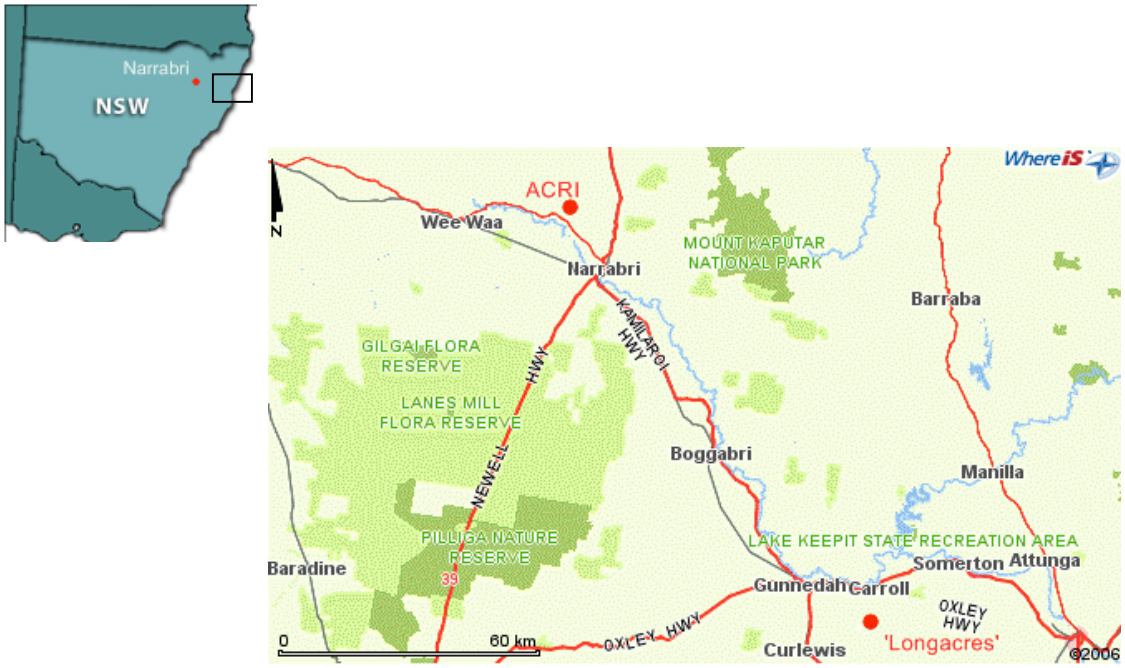

Fig. 1 Location of experimental sites, ACRI, Narrabri and "Longacres", Carroll

ACRI, Narrabri

The soil at this site was a fertile alkaline dark grey-brown cracking medium clay, classified as a fine, thermic, montmorillonitic Typic Haplustert (Soil Survey Staff, 1996), a grey vertosol under the Australian classification system (Isbell 1996). No detailed soil nutrient analysis had been carried out at the site since 2001. Responses by cotton to fertilisers other than N and Zn had not

been recorded at this site, despite irrigated cotton and wheat cropping for 25 years prior to this experiment. Immediately prior to sowing this cotton crop the field had been under a winter fallow as a part of a wheat-cotton-fallow rotation. The average annual rainfall in Narrabri is 643.3mL. The long-term average temperatures and rainfall during the January to March period are shown in Table 1.

'Longacres', Carroll

The soil at the experiment site in Carroll was a fertile alkaline grey cracking medium clay, classified as a Grey vertosol under the Australian Soil Classification System (Isbell 1996). No detailed soil analysis had been carried out for three years prior to this experiment. This field was used in a cotton-sorghum rotation with N and Zn fertilisers applied yearly. The field was sown in 2006 with two cotton cultivars, Sicot 71BR and Sicot 69BR in alternating blocks of 4 rows. The average annual rainfall in Carroll is 636.3 mL. As shown in Table 1 the temperatures and rainfall are lower than at Narrabri. This creates a shorter cotton growing season, with fewer growing day degrees.

Table 1 Long-term average climate data in Narrabri and Carroll

	Narrabri			**Carroll**		
	Jan	**Feb**	**Mar**	**Jan**	**Feb**	**Mar**
Maximum Temp. (ºC)	35.3	33.9	31.3	31.8	31	29.1
Minimum Temp. (ºC)	19.4	18.6	16.3	18.1	18.6	16.6
Rainfall (mL)	81	71.4	55.6	88.7	71.9	42.1

Experimental Design

Seven fertiliser treatments (P, K, Zn, Cu, B, Fe and a control) were applied in a randomised complete block design (RCBD) with four replications at each site. A 16m x 112m area of cotton was split into 4 blocks of 4x112m. Each block consisted of four rows of cotton, the outer two designated as buffer rows to prevent fertiliser drift between blocks. Each 4 x 112m block was divided into seven 4 x 16m blocks. Buffer areas of 1m were cleared of cotton from either end of

each block. Nutrient treatments were randomly allocated to plots within each block. The field layout and field plan is given in Appendix 1.

Cotton Crop

Narrabri

Sicot71 BR cotton (*Gossypium hirsutum*) was sown at a rate of 10 plants/m^2 at the Australian Cotton Research Institute, Narrabri. The cotton was sown with Nitrogen and Zinc starter fertilisers. Nitrogen was applied at a rate of 150kg/ha as urea, zinc applied as zinc sulfate heptahydrate at 1kg / ha.

Carroll

Sicot 71BR and Sicot 69BR cotton (*G. hirsutum*) were sown in alternating 4 rows across the field on 'Longacres', Carroll at a rate of 10 plants/m^2.

Soil Analysis

The top 30cm of soil from the control plot in each block was sampled using a 5cm diameter metal tube. Soil samples were dried at 110ºC and analysed for clay content, Munsell colour (Munsell 1973) and nutrient concentration. Soil pH in 1:5 water was measured. Organic carbon was measured according to the method described by McCleod (1975). Colwell Phosphorus concentration (mg/kg) was measured (Colwell 1963). Nitrate nitrogen was measured using the spectrophotometric method using chromotrophic acid, as described by Sims *et al*. (1971). Soil water extracts were analysed for Ca and Mg by titration with EDTA, for K using a flame photometer and for Na by titration with silver nitrate (Richards 1954). Soil sulphur concentration was measured through extraction of the sulphate-sulphur fraction with 0.15% $CaCl_2.2H_2O$ (Wiliams *et al*. 1959).

Electrical conductivity and CEC were measured using standard laboratory procedures (Richards 1954) and Exchangeable Sodium Percentage (ESP) calculated according to the equation

ESP = [Na]/CEC *100.

Foliar Fertilisers

The seven foliar fertiliser treatments are shown in Table 2.

Table 2 Foliar Fertiliser Sprays

Element	Form	Rate of element applied per spray	pH of foliar fertiliser solution
K	AGRODEX K50*	2 kg/Ha	10.21
P	NaH2PO4	2 kg/Ha	5.31
Fe	AGRODEX Fe*	33 g/Ha	4.28
Zn	ZnSO4.7H2O	75 g/Ha	5.93
Cu	CuSO4.5H2O	16.9 g/Ha	5.64
B	Na2B8.7H2O	0.14 kg/Ha	8.72

*Agrodex K50® and Agrodex Fe®, two commercial foliar fertilisers, were supplied by Agrobest chemicals.

Salts and commercial fertiliser concentrates were mixed with rainwater and a non-ionic surfactant NuFarm ChemWet1000® (1000g/L Alcohol alkoxylate soluble liquid wetting agent) at a rate of 1mL/L. Fertilisers were mixed in the spray tank to a total application rate of 3750L/ha (12L per plot). Fertilisers were sprayed onto plants by hand with a pump action spray pack (Fig. 2).

Fig. 2 Fertiliser application method

Fertilisers were applied three times at Narrabri, at 1280 growing day degrees[1] (GDD) from sowing (10th January), 1523 GDD from sowing (24th January) and 1845 GDD from sowing (11th February). Two fertiliser applications were made in Carroll, at 1166 GDD from sowing (11th January) and 1618 GDD (7th February). The temperature, humidity and radiation conditions of the spray application days are given in Table 3.

Table 3 Climate data from the days on which foliar fertilisers were applied at Narrabri and Carroll

	Maximum Temp. (ºC)	**Minimum Temp. (ºC)**	**Radiation (MJ / m²)**	**Maximum humidity (%)**
Narrabri				
10th January	37.05	22.8	31.29	67.5
24th January	36.95	22.39	30.18	67.47
11th February	32.92	11.13	31.82	79
Carroll				
11th January	36.2	24.63	26.67	68.41
7th February	37.56	22.97	28.35	60.09

[1] Growing Day Degrees were calculated according to the formula given in Appendix 2

Leaf Sampling

The youngest fully expanded leaf (at the 5th node from the top of the cotton plant) was used as the standard leaf sample taken from cotton plants. Initial leaf samples representative of the whole blocks, and then random samples from each plot were taken in Narrabri at 1280, 1503, 1819 and 2031 GDD (10th January, 23rd January, 9th February and 23rd February) and in Carroll at 1166, 1618 and 1823 GDD (11th January, 7th February and 21st February). Sample sizes were approximately 30 leaves (10g dry weight). Fresh leaves were dried at 70°C then ground using a Foss Tecator Cyclotec 1093 sample mill fitted with a 1mm screen and stored in air tight containers to prevent moisture absorption before analysis.

Harvest

Prior to harvesting, 10 plants were randomly selected from each plot, and the number of nodes and bolls per plant counted. Plots in Narrabri were individually mechanically harvested on May 5th, 2006 using a small plot (one row) picker. Random 2 metre sections of plots in Carroll were hand picked on May 11th, 2006 (Fig. 3). Harvested cotton from both sites was ginned individually, and lint percentage, seed cotton turnout, seed and lint weight measured. Small plot measurements were used to evaluate the yield/ha of each plot for analysis.

Fig. 3 Hand picking cotton in Carroll

Fibre Quality

The six important cotton fibre quality parameters according to the Australian cotton classification system were assessed in Narrabri using a High Volume Instrument (HVI). These parameters were:

- Fibre Length (to the nearest 32nd of an inch)
- Fibre Strength (g/tex[2])
- Micronaire (fibre diameter in μm)
- Length Uniformity (a ratio of the average length of the sample fibres to the longer half of the sample fibres)
- Elongation (the percentage that the fibre extends before breaking)
- Short Fibre Index (the proportion of fibres less than one half and inch long, as a percentage)

[2] One tex is equal to the weight in g of 1000m fibre

Oil

The seed oil content was assessed using Nuclear Magnetic Resonance Spectroscopy (NMR). Samples of fuzzy seed of the same volume as 40mL of pure cotton oil were placed in a clear glass tube. The tube was inserted into the NMR machine, and seed oil content measured.

Nutrient Analysis

Leaf samples and random samples of mature seed and lint from each plot were analysed for P, K, Cu, B, Fe and Zn using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS).

Data Analysis

Data was analysed using Genstat ® Version 8.1 (2006) and Microsoft Excel ® (2005). Lint yield, the number of bolls per plant, number of nodes per plant, fibre quality parameters, seed nutrient, lint nutrient and seed oil were analysed using analysis of variance (ANOVA). Data that was not normally distributed (fibre micronaire and fibre elongation) were log and square-root transformed to ensure equal avriances. The two sites were treated as separate experiments for yield, plant growth counts, fibre quality and leaf nutrient accumulation data, since cultivar and climatic differences in Carroll resulted in variation in the data. Cultivar was used as a covariate for Carroll data analysis of yield and plant growth and development, to remove variation due to differences in Sicot 71BR and Sicot 69BR growth and development.

Leaf nutrient data was analysed using ANOVA. The sites were treated as separate experiments due to the unequal time period between leaf samples. Data was analysed for nutrient and date interactions between control plants and plants treated with foliar nutrients.

Experiment 2: Evaluating the timing of nutrient and oil accumulation in cotton bolls.

Site Description and Experimental Design

To examine the timing of nutrient and oil accumulation in cotton bolls, a field experiment was carried out at the Australian Cotton Research Institute (ACRI), Narrabri (Fig. 1), the soil and site as previously described.

The experiment was designed as a Time Series Design, with random sampling from four blocks. Blocks were 1m x 30m areas of Sicot 71BR cotton sown at 10 plants /m. The design included four replicate samples of bolls from 11 time periods.

Boll Tagging and Sample Collection

600 white flowers were tagged on 13th January, 2006 (1341 GDD from sowing). Plastic tape was tied around flower petioles (Fig. 4).

Fig. 4 Tagged Flowers

Random samples of bolls from each block were collected at the sampling dates given in Table 4. Bolls were oven dried at 70°C, then separated by hand into fuzzy seed, lint and boll wall (including internal membranes) components. Seed, lint and wall dry weights were recorded.

Table 4 Sampling Dates in chronological and thermal time

Days From Tagging	Date	Growing Day Degrees
0	13/1/06	1341
7	19/1/06	1439
12	24/1/06	1523
15	27/1/06	1574
20	1/2/06	1660
22	3/2/06	1707
27	8/2/06	1802
32	13/2/06	1876
36	17/2/06	1936
42	23/2/06	2031
50	3/3/06	2132
60	13/3/06	2265

Oil

The seed oil content was assessed using Nuclear Magnetic Resonance Spectroscopy (NMR). Samples of fuzzy seed of the same volume as 40mL of pure cotton oil were placed in a clear glass tube. The tube was inserted into the NMR machine, and seed oil content assessed.

Nutrient Analysis

Seed, lint and wall components were analysed for P, K, S, Ca, Cu, B, Mg, Mn, Fe and Zn concentration using Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS).

Data Analysis

Seed, lint and wall dry weights, oil content and nutrient concentration and content data were analysed using non-linear and linear regression analysis in Genstat® version 8.1 (2006) and Microsoft Excel® (2005).

Results

Soil Analysis

The physical and chemical properties of the top 30cm of soil from both sites is shown in Table 5. Details of the soil from each block are given in Appendix 3. Previous soil surveys classed the soil at ACRI as a fine, thermic, montmorillonitic Typic Haplustert (Soil Survey Staff, 1996). Analysis showed that the soil from ACRI was fairly uniform across all blocks. Block 3 showed a lower clay content, a lower cation exchange capacity (CEC), lower Calcium, Magnesium and Sodium concentrations and a higher Phosphorus concentration than the other three blocks. These findings were consistent with those of previous soil surveys of the farm.

The soil on 'Longacres' was classed as a Grey Vertosol (Isbell, 1996) showed only small variations in chemical properties between the four blocks (Appendix 3). Table 6 shows the properties of this soil compared to the soil nutrient critical values recommended by the Australian cotton CRC for cotton crop growth.

Table 5 Soil description of the top 30cm at Narrabri and Carroll

	Narrabri	**Carroll**
Texture	Medium Clay	Medium Clay
Munsell Colour	Brown	Grey
pH (1:5 Water)	7.9	8.53
CEC (M eq/100g)	38.83	55.48
Electrical Conductivity (Sat. Ext) (dS/m)	1.75	2.38
Electrical Conductivity (dS/m)	0.27	0.38
ESP %	2.18	7.73
Organic Carbon %	0.90	0.70
Nitrate Nitrogen (mg/kg)	17.25	1.70
Sulfate Sulfur (mg/kg)	17.75	21.50
Phosphorus (Colwell) (mg/kg)	28.75	11.95
Potassium (M eq/100g)	1.48	1.95
Calcium (M eq/100g)	26.75	37.00
Magnesium (M eq/100g)	9.75	12.25
Sodium (M eq/100g)	0.85	4.28
Ca:Mg Ratio	2.73	3.03

As described in Table 6 the soil at both Narrabri and Carroll showed adequate nutrient levels for cotton crop growth and development, and could not be classed as deficient or requiring nutrient addition.

Table 6 Critical soil nutrient concentrations for cotton crop growth, and Narrabri and Carroll soil levels

Nutrient	Critical Value	ACRI Soil	'Longacres' Soil
Nitrogen	>20 - 30 ppm	Adequate	Adequate
Phosphorus	10ppm	Adequate	Adequate
Potassium	0.2 - 0.4 meq/100g	Adequate	Adequate
Sulphur	5 -10 ppm	Adequate	Adequate
Calcium	2 - 3.5 meq/100g	Adequate	Adequate
Magnesium	1 – 1.2 meq/100g	Adequate	Adequate

Source: NUTRIPAK, Cotton CRC, 2005.

Experiment 1: Evaluating the Efficacy of Foliar Fertilisers

Yield

There was no difference (P >0.05) in lint yield between plots treated with different foliar fertilisers, at either Carroll or Narrabri (Fig. 5). The mean yield at Narrabri was 1889.4 kg lint/ha (8.32 bales/ha) with an L.S.D. of 208 kg/ha. The mean yield at Carroll was 1420 kg lint/ha (6.25 bales/ha) with an L.S.D of 245.5 kg/ha.

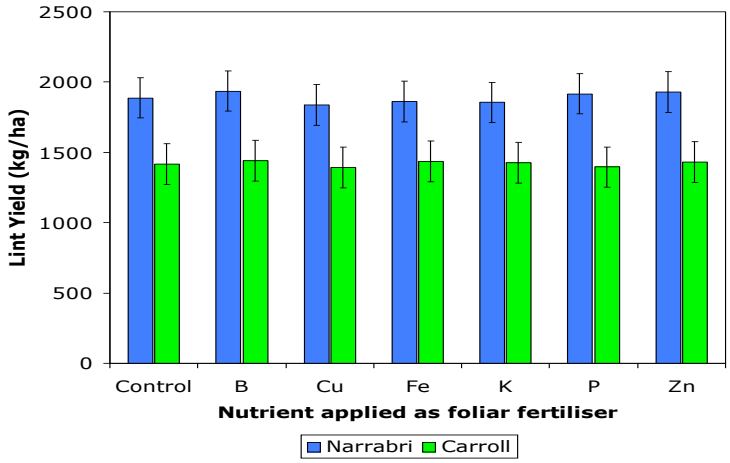

Fig. 5 Lint Yield (kg/ha) from plots sprayed with different foliar fertilisers at Narrabri and Carroll

Plant Growth and Development

As shown in Table 7 was no difference in the average number of bolls per plant at Narrabri ($P > 0.05$) (Table 7). Variety had a significant influence on the number of bolls per plant in Carroll ($P = 0.013$), however there was no difference in the average number of bolls per plant when analysed with variety as a co-variate and the means adjusted ($P > 0.05$) (Table 8).

There was no difference in the average number of nodes per plant at Narrabri ($P > 0.05$) (Table 7). Plants sprayed with copper at Carroll had a higher average number of nodes per plant than plants sprayed with iron and zinc ($P = 0.014$), however there was no difference in the number of nodes per plant between the control plants and the plants sprayed with different foliar fertilisers (Table 8).

Table 7 Average number of nodes and bolls per plant in Narrabri from plots to which foliar fertilisers were applied, *P* values and L.S.D.s

Nutrient	Average number per plant	Standard Deviation	*P* value	L.S.D.
		Nodes per Plant		
Control	27.25	2.61	>0.05	3.25
B	26.95	2.92		
Cu	26.35	3.10		
Fe	25.95	3.02		
K	26.8	2.30		
P	27.45	2.01		
Zn	26.25	2.93		
		Bolls per Plant		
Control	16.85	5.91	>0.05	1.76
B	15.2	3.85		
Cu	15.7	3.26		
Fe	14.6	3.93		
K	14.85	2.52		
P	15.3	4.36		
Zn	17.05	4.24		

Table 8 Average number of nodes and bolls per plant in Carroll from plots to which foliar fertilisers were applied, *P* values and L.S.D.s

Nutrient	Average number per plant	Standard Deviation	*P* value	L.S.D.
		Nodes per Plant		
Control	27.15	3.29	0.014	1.336
B	26.7	2.56		
Cu	27.9	2.34		
Fe	25.65	3.52		
K	27.5	2.44		
P	26.4	2.75		
Zn	25.7	2.01		
		Bolls per Plant		
Control	15.8	3.03	>0.05	2.77
B	15.1	4.50		
Cu	16.45	4.44		
Fe	14.45	3.90		
K	17.05	5.67		
P	17.2	3.15		
Zn	13.5	3.37		

Seed Oil Content

There was no difference in the oil content of mature seeds from plots treated with different foliar fertilisers, at either Narrabri or Carroll ($P > 0.05$) (Table 9).

Table 9 Seed Oil Content (%) in Narrabri and Carroll

Nutrient Applied as Foliar Fertiliser	Seed Oil Content (%)	
	Narrabri	Carroll
Control	21.92	25.14
B	22.30	25.27
Cu	22.09	23.68
Fe	22.03	25.45
K	22.38	25.61
P	21.73	25.14
Zn	22.36	25.37
L.S.D.	0.82	1.94

Fibre Quality

There was no difference in fibre length, fibre micronaire, fibre uniformity, fibre elongation or the short fibre index of lint harvested from plots sprayed with different foliar fertilisers at either Narrabri or Carroll ($P > 0.05$) (Fig. 6). There was a difference in fibre strength between Sicot 71BR fibre and Sicot 69BR fibre at Carroll ($P = 0.022$) but no difference in fibre strength between plots to which nutrients were applied when adjusted for variety ($P > 0.05$). There was no difference in fibre strength between plots to which foliar fertilisers were applied and control plots (Fig. 6).

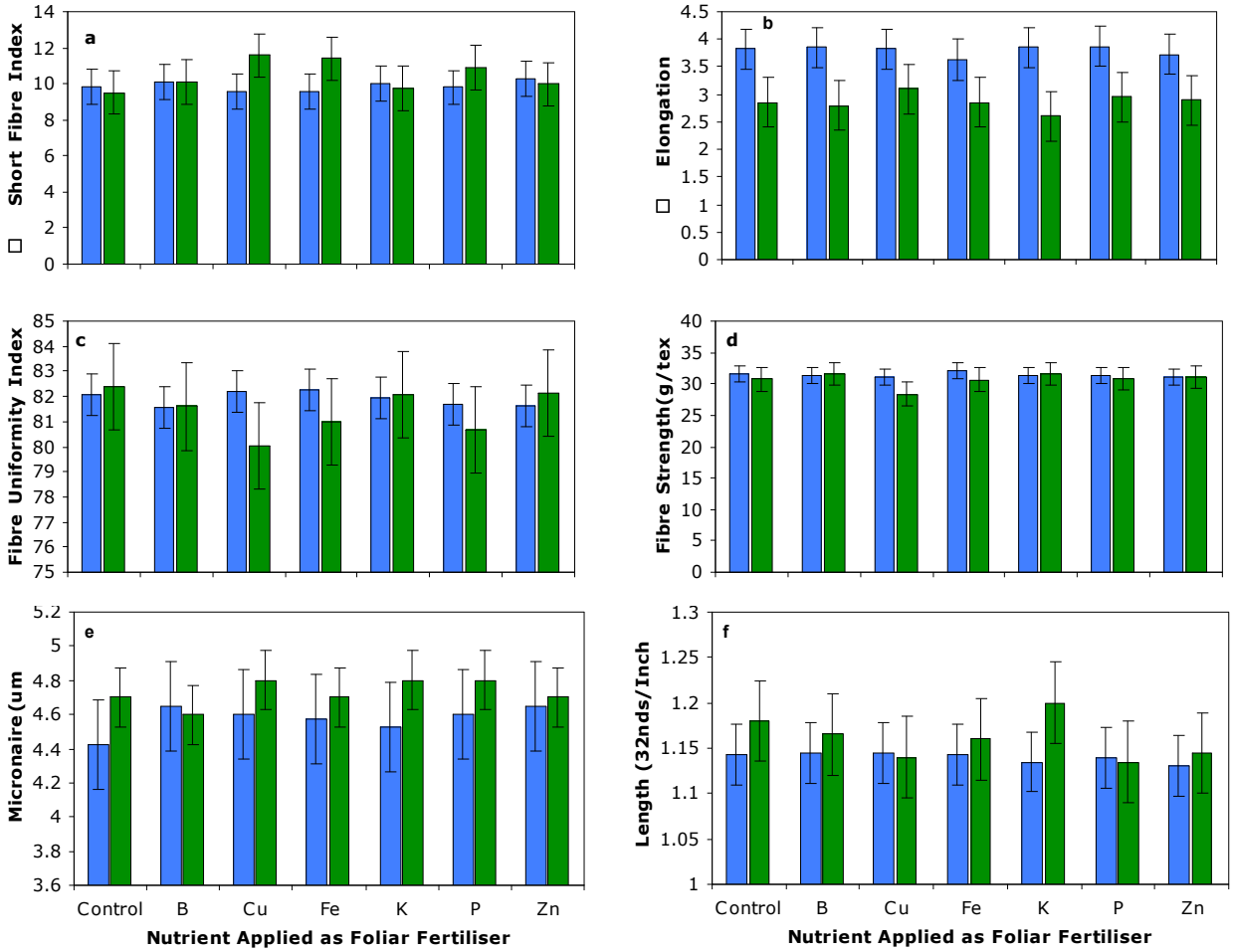

Fig. 6 Comparison of fibre quality parameters of lint harvested from plants sprayed with different foliar fertilisers at Narrabri Carroll.

(a) Fibre elongation (%) (b) Short Fibre Index (%) (c) Fibre Uniformity Index (d) Fibre Strength (g/tex) (e) Fibre Micronaire (um) (f) Fibre Length (32nds of an inch)

Plant Uptake of Fertilisers into Leaf Tissue

Potassium and Boron

There was no difference in the leaf potassium or boron concentration from control plants and plants to which foliar potassium and boron fertilisers were applied at either Narrabri ($P > 0.05$) or Carroll ($P > 0.05$) (Fig. 7).

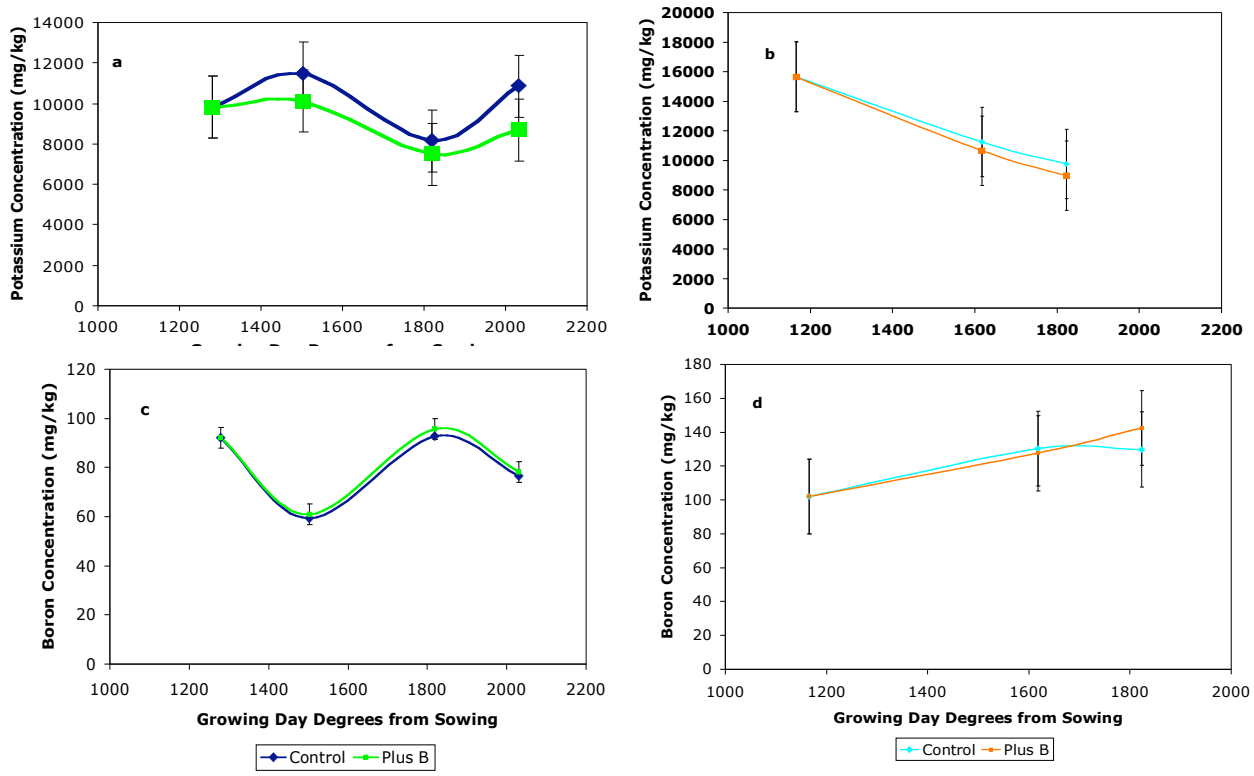

Fig. 7 Potassium concentration in leaf tissue (mg/kg) at (a) Narrabri and (b) Carroll and Boron concentration in leaf tissue (mg/kg) at (c) Narrabri and (d) Carroll prior to any spray application (1280 (Narrabri) and 1166 (Carroll) growing day degrees) and after each application of a foliar fertiliser.

Phosphorus

There was no difference in the leaf phosphorus concentration of plants to which phosphorus fertilisers were applied at Narrabri after spray number 1 and 2, however there was an increase in phosphorus concentration in the leaf tissue after the third fertiliser application at 2031 growing day degrees ($P < 0.001$) (Fig. 8). There was no difference between the leaf phosphorus concentration of fertilised and control plants after either fertiliser application in Carroll ($P>0.05$) (Fig. 8).

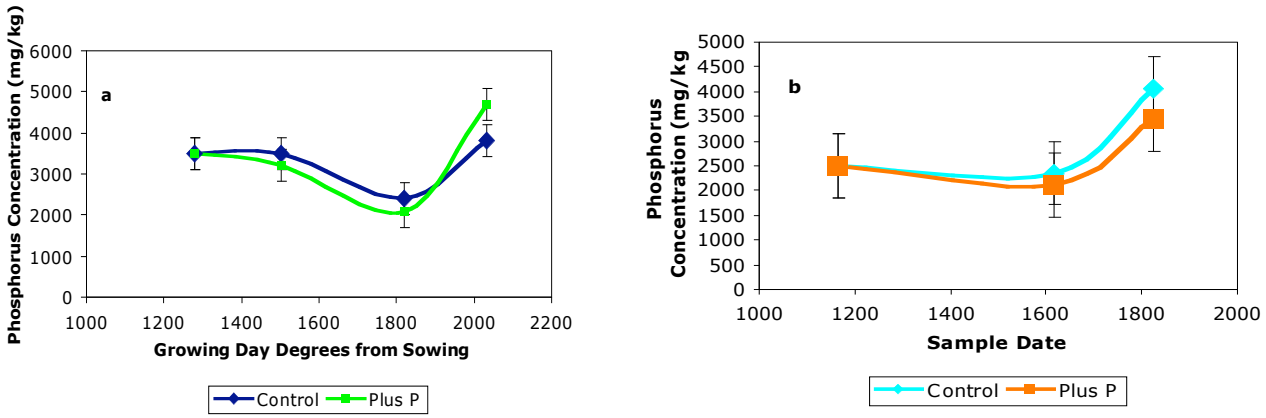

Fig. 8 Phosphorus concentration in leaf tissue (mg/kg) at (a) Narrabri and (b) Carroll prior to any spray application (1280 (Narrabri) and 1166 (Carroll) growing day degrees) and after each application of a foliar fertiliser.

Iron

There was no difference in the leaf iron concentration from control plants and plants to which foliar iron fertilisers were applied at either Narrabri ($P > 0.05$) (Fig. 9). There was no difference in the leaf iron concentration of plants to which iron fertilisers were applied in Carroll after the first application of foliar fertilisers, but fertilised plants showed a higher leaf iron concentration after the second fertiliser application (at 1819 growing day degrees) ($p < 0.001$) (Fig. 9).

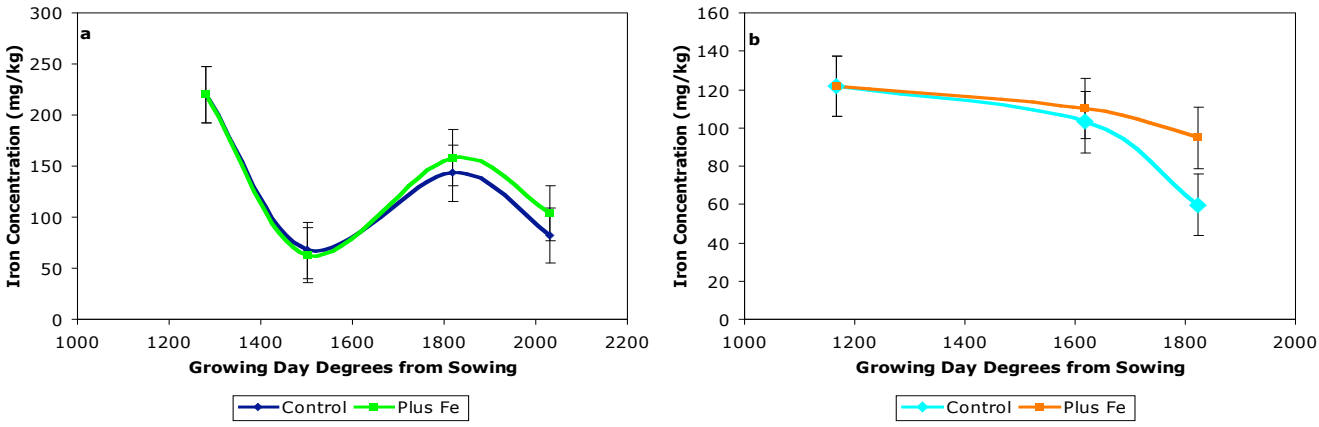

Fig. 9 Iron concentration in leaf tissue (mg/kg) at (a) Narrabri and (b) Carroll prior to any spray application (1280 (Narrabri) and 1166 (Carroll) growing day degrees) and after each application of a foliar fertiliser.

Zinc

There was no difference in the leaf zinc concentration of plants to which zinc fertilisers were applied at Narrabri after the first fertiliser application, however after the second application (at 1819 day degrees from sowing) fertilised plants showed a higher leaf zinc concentration that the control plants ($P < 0.001$). There was no difference after the third application of fertilisers (Fig. 10). There was no difference in the zinc concentration of leaf tissue from control plants and plants to which foliar zinc fertilisers were applied in Carroll ($P > 0.05$) (Fig. 10).

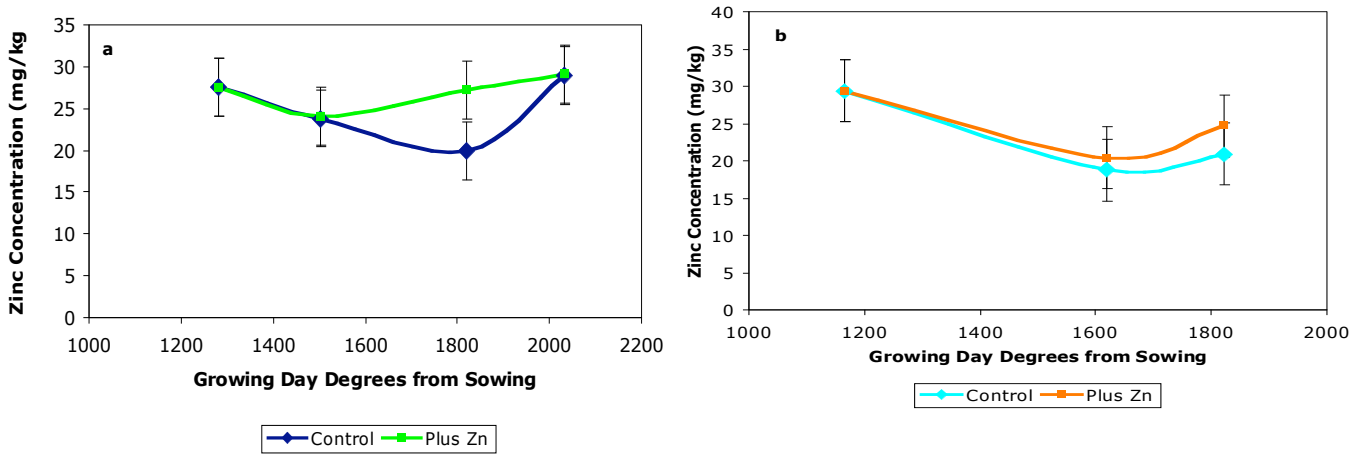

Fig. 10 Zinc concentration in leaf tissue (mg/kg) at (a) Narrabri and (b) Carroll prior to any spray application (1280 (Narrabri) and 1166 (Carroll) growing day degrees) and after each application of a foliar fertiliser.

Copper

There was no difference in the leaf copper concentration of plants to which copper fertilisers were applied in Narrabri after the first and second application of foliar fertilisers, but fertilised plants showed a higher leaf copper concentration after the third fertiliser application at 2031 growing day degrees from sowing ($P < 0.001$) (Fig. 11). There was no difference in the leaf copper concentration of plants to which foliar copper fertilisers were applied and control plants in Carroll (Fig. 11).

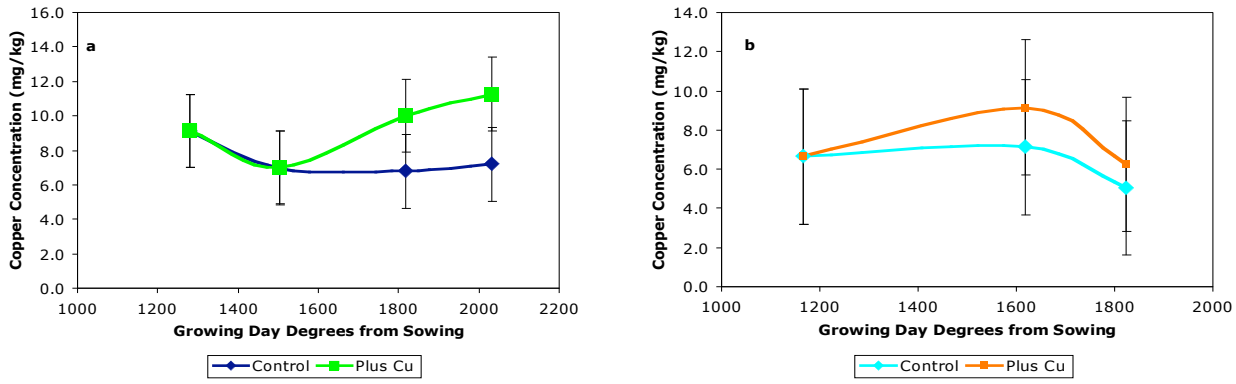

Fig. 11 Copper concentration in leaf tissue (mg/kg) at (a) Narrabri and (b) Carroll prior to any spray application (1280 (Narrabri) and 1166 (Carroll) growing day degrees) and after each application of a foliar fertiliser.

Leaf Nutrient Status

The concentration of nutrient in leaves show that neither crop (at Narrabri or Carroll) was deficient in nutrients measured at 1800 growing day degrees (Table 10). These values do indicate that both crops were slightly deficient in potassium, but had an adequate amount of other measured nutrients in the plant at this developmental stage.

Table 10 Nutrient concentration in plant tissue from control plots, and recommendations for critical concentrations as indicators of deficiencies.

Nutrient	Normal Leaf Range	Leaf Concentration	
		Narrabri	Carroll
Phosphorus (%)	0.28 - 0.5	0.35	0.40
Potassium (%)	1.5 – 3	1	0.90
Sulphur (%)	0.6 - 1.2	0.80	1.30
Calcium (%)	0.4 – 6	0.30	0.40
Magnesium (%)	0.4 - 0.9	0.30	0.40
Zinc (mg/kg)	20 – 60	29	21
Iron (mg/kg)	50 – 350	82	60
Copper (mg/kg)	5 – 25	7.2	5.1
Manganese (mg/kg)	50 – 200	127	119

Boron (mg/kg)	50 - 80	77	130

Plant Uptake of Fertilisers into Mature Seed

Plants to which foliar copper fertilisers were applied in Carroll showed a higher seed copper concentration than control plants (P = 0.031). There was no difference in the seed nutrient concentration of plants to which P, K, Fe, B or Zn fertilisers were applied when compared to control plants. The average nutrient content of mature seeds from plants to which foliar fertilisers were applied are given in Table 11.

Table 11 Summary of nutrient content in mature seed from plants to which different foliar fertilisers were applied, with p values and L.S.D.s

	Narrabri Control	Narrabri Plus Nutrient	P Value	L.S.D.	Carroll Control	Carroll Plus Nutrient	P Value	L.S.D.
P	6150	6150	>0.05	970.7	6350	6700	>0.05	1022.2
K	10300	11650	>0.05	1665.1	11550	11000	>0.05	1041.6
Fe	50.81	46.36	>0.05	9.23	39.77	43.57	>0.05	7.18
B	19.10	16.46	>0.05	3.79	18.05	18.66	>0.05	2.1
Cu	8.69	8.05	>0.05	1.65	7.50	8.95	0.031	1.15
Zn	34.62	34.49	>0.05	9.19	33.38	32.35	>0.05	4.05

Plant Uptake of Fertilisers into Lint

Plants to which foliar copper fertilisers were applied showed a higher lint copper concentration than control plants (P = 0.029). There was no difference in the lint nutrient concentration of plants to which P, K, Fe, B or Zn fertilisers were applied when compared to control plants (Table 12).

Table 12 Summary of nutrient content in lint from plants to which different foliar fertilisers were applied, with p values and L.S.D.s

	Control	Plus Fertiliser	P Value	L.S.D.
P	245	325	>0.05	90.3
K	3850	4600	>0.05	1435.2
Fe	23.40	8.20	>0.05	23.26
B	3.80	7.60	>0.05	4.8
Cu	0.79	1.02	0.029	0.13
Zn	1.85	2.60	>0.05	0.78

Experiment 2: Evaluating the timing of nutrient and oil accumulation in developing cotton bolls

Dry Weight Accumulation in Seed

All three boll components (seed, lint and walls) showed an exponential increase in dry weight from flowering to maturity (Fig.12).

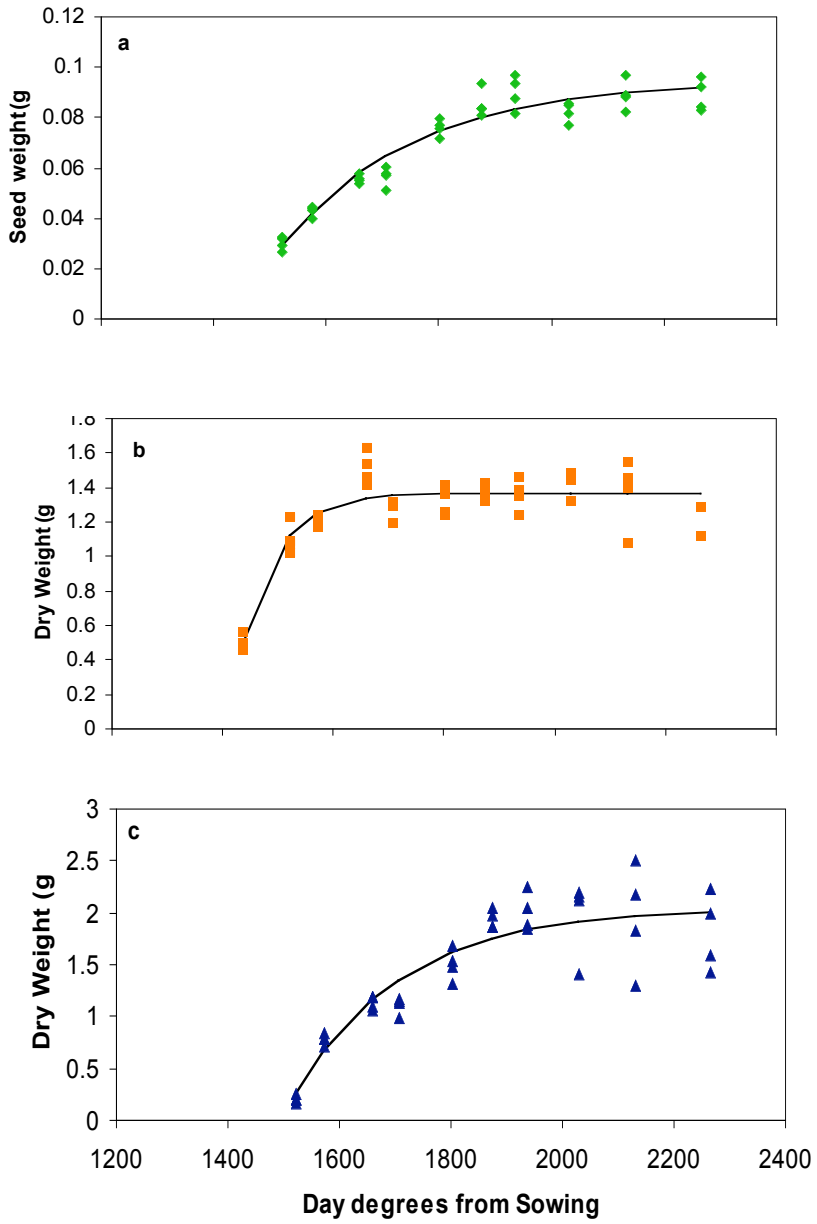

Fig. 12 Dry weight accumulation in (a) seed ◆ (b) boll walls ■ and (c) lint ▲ of Sicot 71BR bolls grown at ACRI showing an exponential increase according to (a) Seed dry weight (g) = 0.09425 + (-41.8 x $0.995769^{\text{Day degrees from sowing}}$) $R^2 = 0.92$ (b) wall dry weight (g) = 1.364 + (-1524847177 x $0.98532^{\text{Day degrees from sowing}}$) $R^2 = 0.83$ (c) lint dry weight (g) = 2.034 + (– 4802 x $0.99483^{\text{Day degrees from sowing}}$) $R^2 = 0.82$

Oil Accumulation in Seed

Oil Accumulated exponentially in seed (Fig. 13). The increase in seed oil concentration slowed after 1936 growing day degrees. This followed the pattern of dry weight accumulation in the seed (Fig. 12).

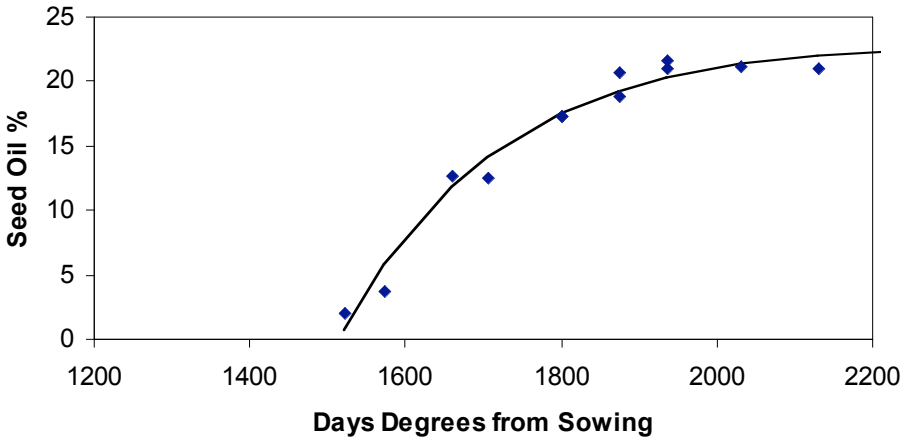

Fig. 13 Oil accumulation (seed oil %) in seeds from Sicot 71BR bolls grown at ACRI, showing an exponential increase according to seed oil % = 22.99 + (-45276 x $0.995014^{\text{Day degrees from Sowing}}$)

Nutrient accumulation in boll components

The pattern of increase in nutrient concentration in boll parts (mg/kg) and in total nutrient content (μg) on a per boll basis varied when plotted on a thermal time or chronological time basis. Since thermal time is the driver of cotton boll development and is linked to the physiological stages of boll development, these results are presented.

Seeds

The concentration of Mn, B, Cu, Zn, Mg, P and S in the cotton seed showed similar patterns of change with thermal time. These nutrients were fitted with quadratic-by-quadratic functions to explain the changes in concentration with time (Fig. 14, Fig. 15 and Fig. 16). The initial decrease in the concentration of nutrients in the seed occurred at the same developmental stage as the rapid increase in seed dry weight (Fig. 12), indicating that this decrease in concentration is explained by growth dilution. The concentration of K in the seed showed an exponential decrease with time

(Fig. 16). Seed Fe concentration could not be adequately explained by fitted regressions. Total seed nutrient content in μg (on a per seed basis) showed an exponential increase in all nutrients measured (Fig. 14, Fig. 15 and Fig. 16). Increase in nutrient content slowed, according to fitted exponential equations, at similar developmental periods to dry weight accumulation (Fig. 12).

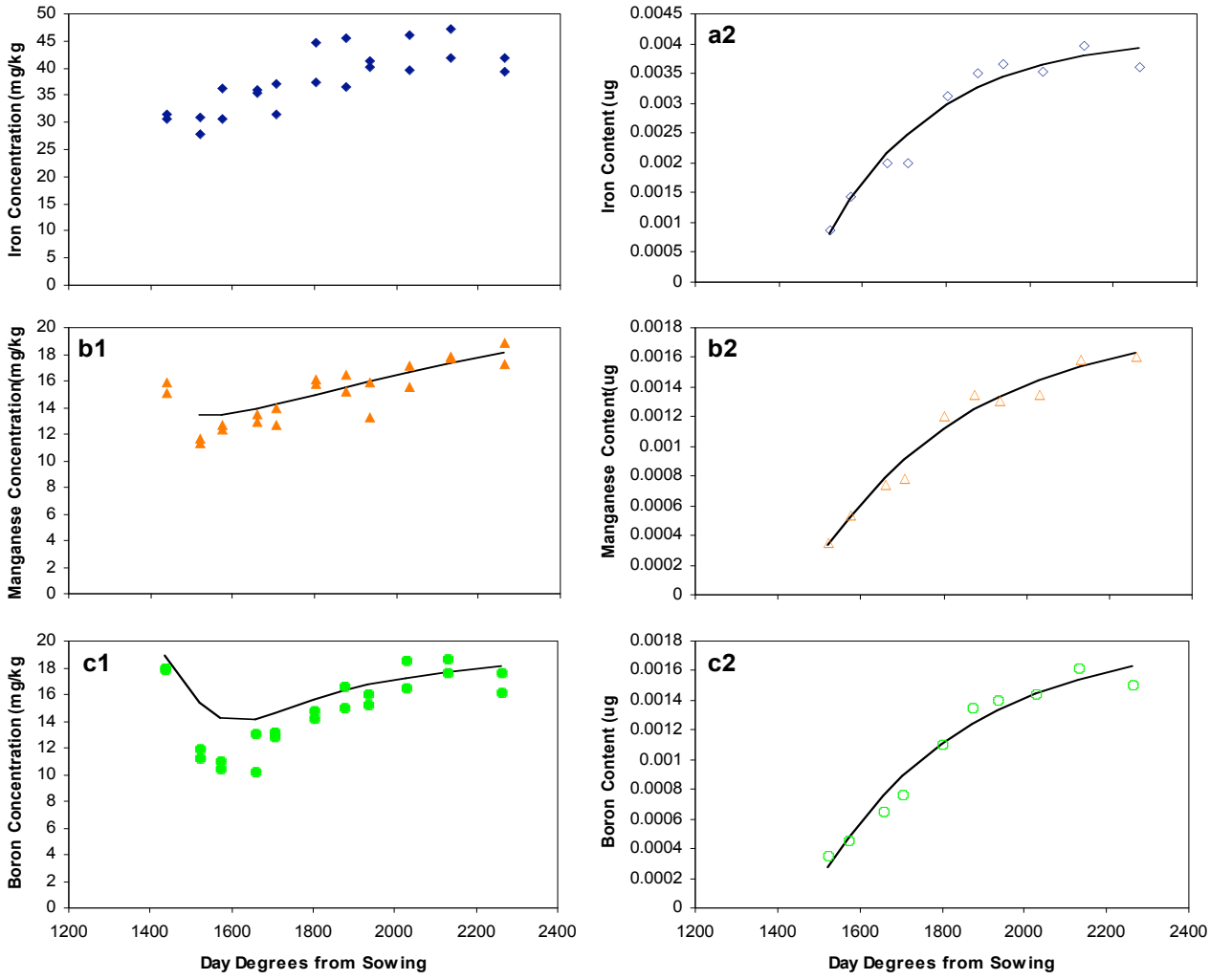

Fig. 14 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) Iron ◆ ◇, (b) manganese ▲ △ and (c) boron ● ○ in seeds from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Iron ◇ Content (μg) = $0.004114 + (-1.08 \times 0.99621^{DDS})$ $R^2 = 0.94$

Manganese ▲ Concentration (mg/kg) = $42.4 + (-61.4 + 0.0423 \times DDS)/(1 - 0.00001 \times DDS - 0.00000047 \times DDS^2)$ $R^2 = 0.78$

△ Content (μg) = $0.001843 + (-0.0819 \times 0.997385^{DDS})$ $R^2 = 0.96$

Boron ● Concentration (mg/kg) = $20.24 + (1.002 - 0.000713 \times DDS)/(1 - 0.0013245 \times DDS + 0.000000443 \times DDS^2)$ $R^2 = 0.85$

○ Content (μg) = 0.001838 + (- 0.096 x 0.997304^{DDS}) $R^2 = 0.95$

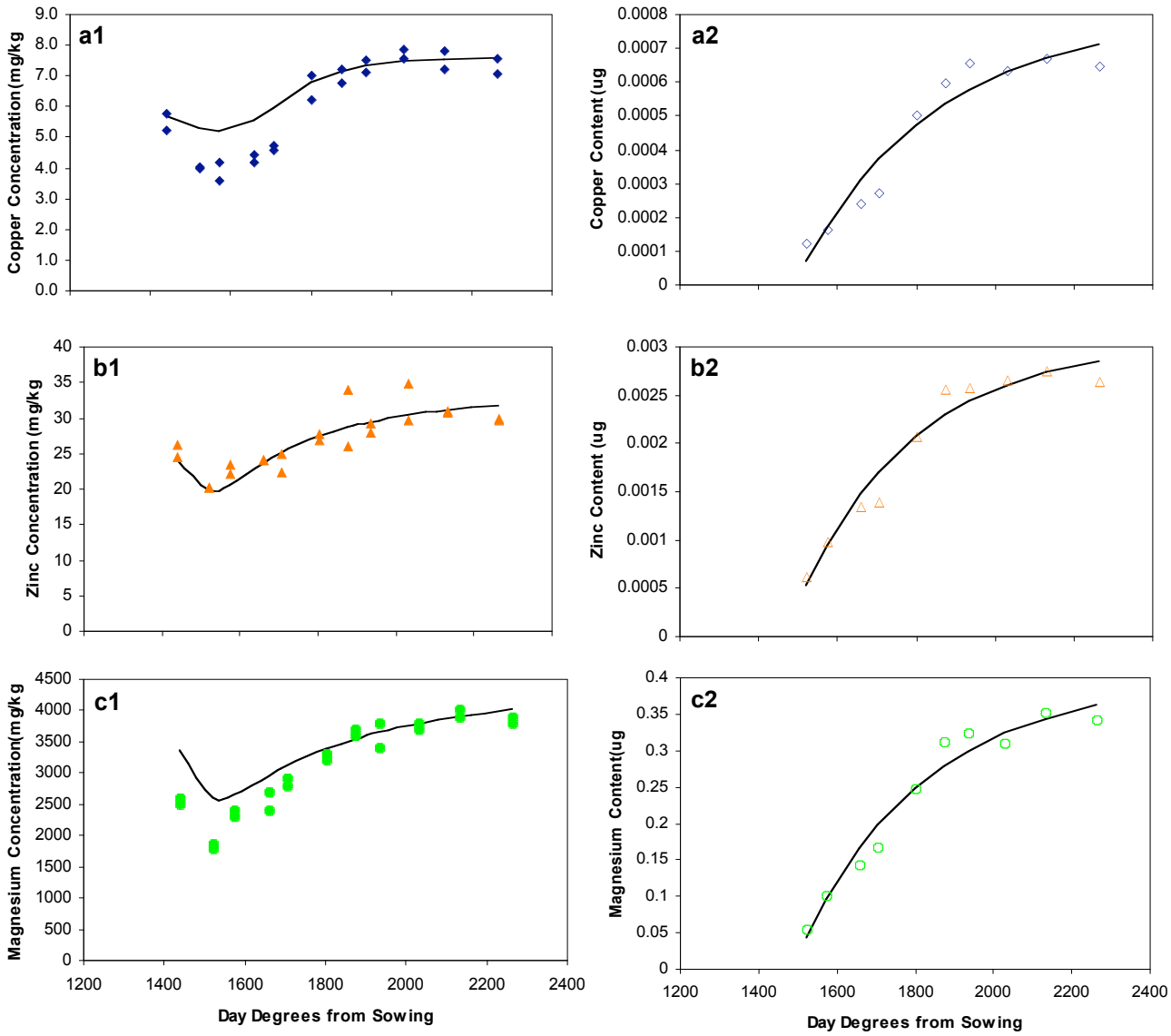

Fig. 15 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) copper ◆ ◇, (b) zinc ▲ △ and (c) magnesium ● ○ in seeds from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Copper ◇ Content (μg) = 0.000789 + (– 0.065 x 0.99705^{DDS}) $R^2 = 0.91$

Zinc ▲ Concentration (mg/kg) = 34.19 + (1.01 - 0.00080 x DDS)/(1 – 0.001340 x DDS - 0.000000455 x DDS^2) $R^2 = 0.73$

△ Content (μg) = 0.003044 + (- 0.448 x 0.996604^{DDS}) $R^2 = 0.94$

Magnesium ● Concentration (mg/kg) = 4666 + (418 – 0.302 x DDS)/(1 – 0.001423 x DDS + 0.00000051 x DDS^2) $R^2 = 0.95$

○ Content (μg) = 0.3971 + (- 38.4 x 0.996928^{DDS}) $R^2 = 0.95$

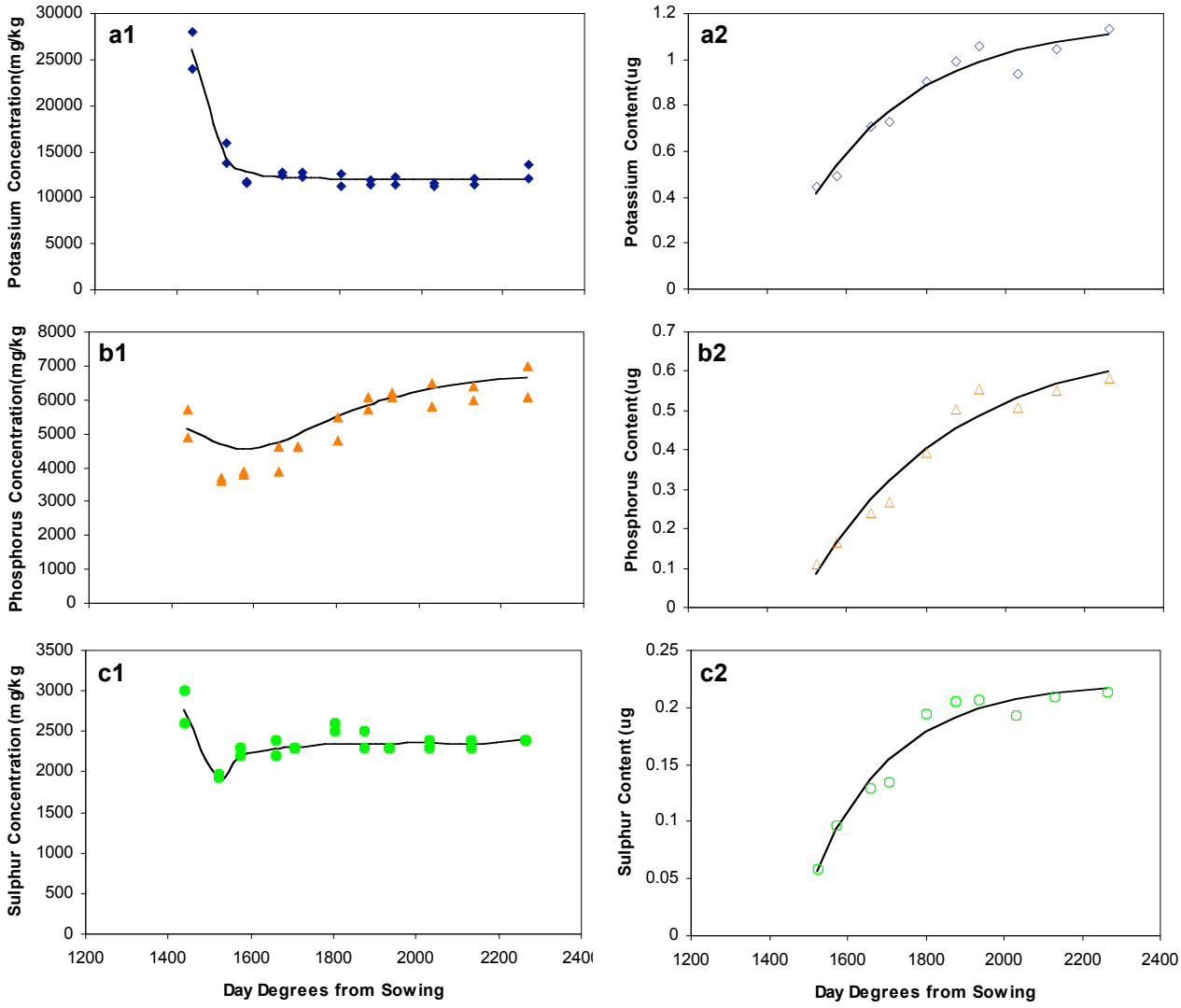

Fig. 16 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) potassium ◆ ◇, (b) phosphorus ▲ △ and (c) sulphur ● ○ in seeds from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Potassium ◆ Concentration (mg/kg) = 11938 + ($2.22E^{17}$ x 0.97910^{DDS}) $R^2 = 0.94$

◇ Content (μg) = 1.1566 + (– 173 x 0.99643^{DDS}) $R^2 = 0.94$

Phosphorus ▲ Concentration (mg/kg) = 7008 + (-47.22 - 0.01362 x DDS)/(1 – 0.001233 x DDS - 0.0000003885 x DDS^2) $R^2 = 0.84$

△ Content (μg) = 0.6745 + (- 39.3 x 0.997247^{DDS}) $R^2 = 0.94$

Sulphur ● Concentration (mg/kg) = 2385.5 + (10.99 – 0.00479 x DDS)/(1 – 0.0011248 x DDS + 0.000000304 x DDS^2) $R^2 = 0.85$

- Content (μg) = 0.2199 + (- 282 x 0.99512^{DDS}) $R^2 = 0.95$

Boll Walls

The concentration (mg/kg) and content (μg) of nutrients in boll walls did not show the same trends of change with thermal time for all the nutrients measured. Fe, Mn and S (Fig. 17 and Fig. 19) showed similar changes in nutrient concentration to the pattern of accumulation in the seed component. These three nutrients were fitted with quadratic-by-quadratic regressions to account for the growth dilution of the nutrient in the early stages of development (before 1600 day degrees from sowing). The content of these three nutrients in the boll walls showed an exponential (asymptotic increase) which followed the same pattern with time as dry matter accumulation in the walls (Fig. 12).

Cu, Zn and P showed an exponential (asymptotic) decrease in concentration with thermal time (Fig. 18 and Fig. 19). Phosphorus followed the exponential increase in wall content, reaching maximum content at 1700 day degrees from sowing, earlier than the maximum phosphorus content in seed (Fig. 16) and at the same time as maximum content in lint was recorded (Fig. 22). The zinc and copper content of the boll walls was not explained by either non-linear or linear regression equations.

B and Mg (Fig. 17 and Fig. 18) content in boll walls were explained by cubic polynomial equations, showing changes in content with time. B reached maximum content at 2031 day degrees from sowing, while Mg showed a continued increase in content with thermal time.

K demonstrated an exponential (asymptotic) increase in both concentration and content in boll walls with thermal time (Fig. 19), not reaching a defined maximum point.

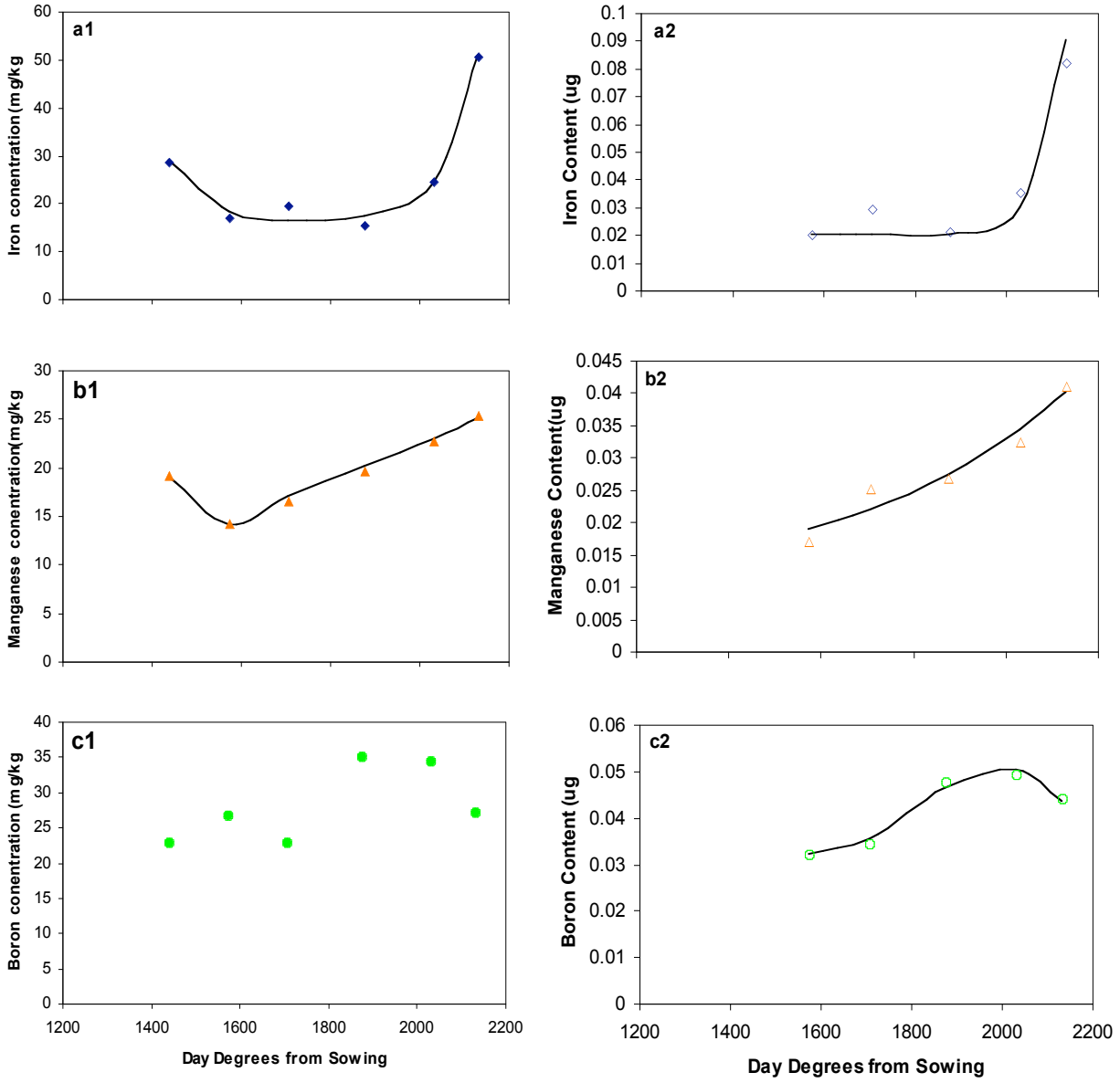

Fig. 17 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) iron ◆ ◇, (b) manganese ▲ △ and (c) boron ● ○ in boll walls of Sicot 71BR Bolls from ACRI

DDS = day degrees from sowing

Iron ◆ Concentration (mg/kg) = 113.4 + 7.85/(1 – 0.0004306 x DDS) -0.742 x DDS $R^2 = 0.93$

◇ Content (μg) = 0.02152 + ($5.54E^{-18}$ x 1.01753^{DDS}) $R^2 = 0.96$

Manganese ▲ Concentration (mg/kg) = -27.7 + (29.1 - 0.01963 x DDS)/(1 – 0.0008907 x DDS - 0.000000144 x DDS^2) $R^2 = 1$

△ Content (μg) = 0.0091 + (- 0.00038 x 1.00206^{DDS}) $R^2 = 0.89$

Boron ○ Content (μg) = Cubic Polynomial Equation, constant = 3.16, day degrees = $-5.58E^{-10}$ $R^2 = 0.93$

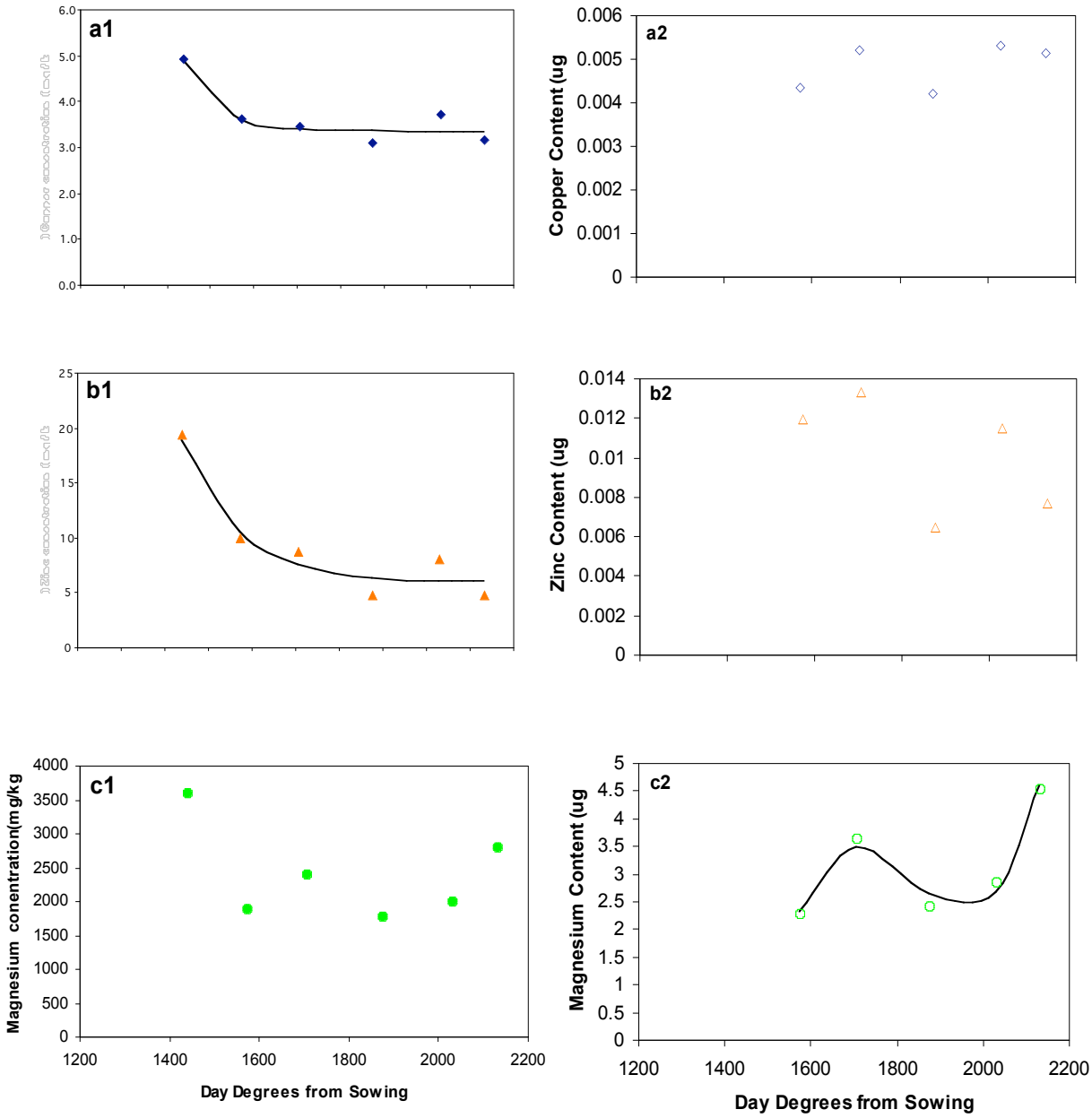

Fig. 18 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) copper ◆ ◇, (b) zinc ▲ △ and (c) magnesium ● ○ in boll walls from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Copper ◆ Concentration (mg/kg) = 3.339 + (360682136 x 0.98671^{DDS}) $R^2 = 0.84$

Zinc ▲ Concentration (mg/kg) = 5.91+ (909447 x 0.99228^{DDS}) $R^2 = 0.88$

Magnesium ○ Content (μg) = Cubic Polynomial Equation, constant = -820, day degrees = $1.36E^{-8}$
$R^2 = 0.87$

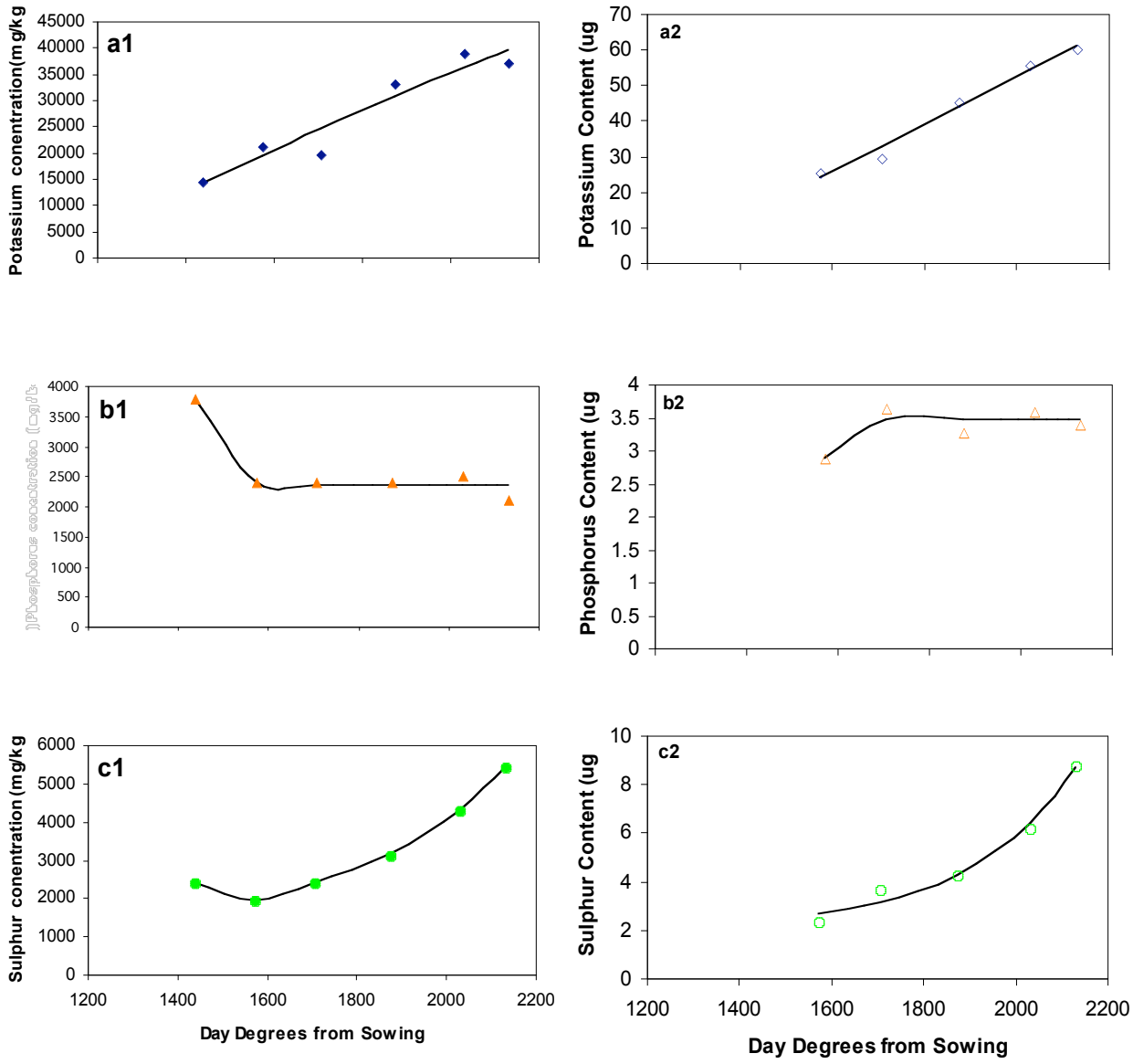

Fig. 19 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) potassium ◆ ◇, (b) phosphorus ▲ △ and (c) sulphur ● ○ in boll walls from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing
Potassium ◆ Concentration (mg/kg) = $11938 + (2.22E^{17} \times 0.97910^{DDS})$ $R^2 = 0.94$
◇ Content (μg) = $-652 + (-581 \times 1.00010^{DDS})$ $R^2 = 0.97$
Phosphorus ▲ Concentration (mg/kg) = $2348.4 + (1.82E^{18} \times 0.9761^{DDS})$ $R^2 = 0.92$

Sulphur

△ Content (μg) = 3.469 + (-4.545E^{73} x 0.89757^{DDS}) R^2 = 0.54

● Concentration (mg/kg) = -836 + (1095 – 0.758 x DDS)/(1 – 0.00108 x DDS + 0.000000268 x DDS^2) R^2 = 0.85

○ Content (μg) = 2.067 + (0.00072 x 1.00429^{DDS}) R^2 = 0.97

Lint

There was an exponential decrease in the Fe, Mn, B, Cu, Zn, Mg, K, P and S concentration in the lint from the Sicot 71BR bolls picked at ACRI (Fig. 20, Fig. 21 and Fig. 22). The nutrient content of the lint (μg) was fitted with quadratic-by-quadratic regressions for all nutrients but boron (which was not explained by either linear or non-linear regression) and potassium, which was explained by a quartic polynomial regression. S, K, P, Mg, Zn and Cu reached maximum lint content at 1660 day degrees from sowing. Fe reached the maximum content earlier, at 1574 day degrees from sowing, while the Mn content of the lint continued to increase with thermal time.

The content of nutrients in the lint declined with time, after maximum content had been reached.

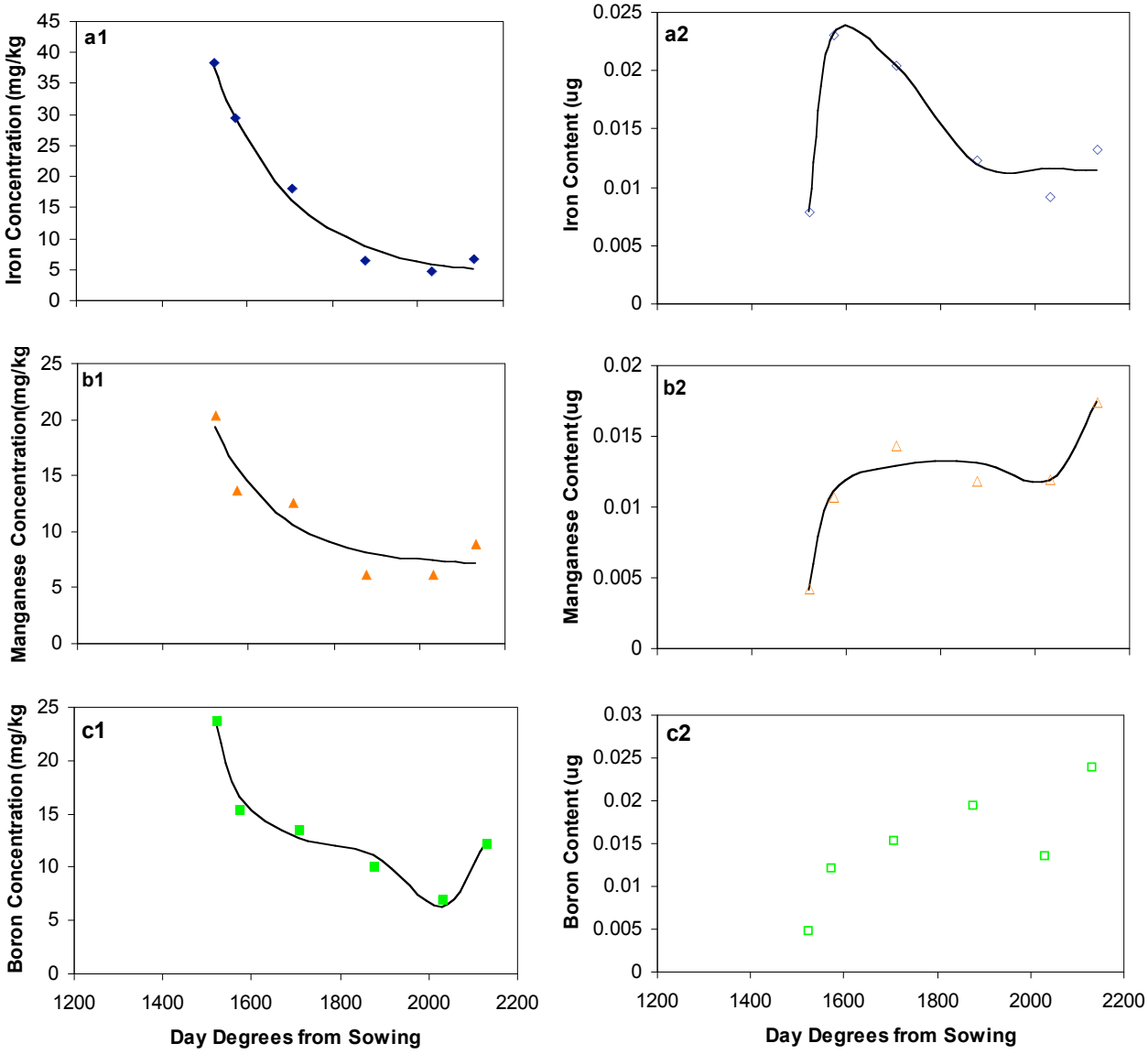

Fig. 20 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) iron ◆ ◇, (b) manganese ▲ △ and (c) boron ● ○ in lint from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Iron ◆ Concentration (mg/kg) = $3.65 + (148336 \times 0.99452^{DDS})$ $R^2 = 0.98$

◇ Content (μg) = $0.01095 + (0.000128 - 0.000000079 \times DDS)/(1 - 0.0012285 \times DDS + 0.000000318 \times DDS^2)$ $R^2 = 0.81$

Manganese ▲ Concentration (mg/kg) = $6.9 + (300638 \times 0.99339^{DDS})$ $R^2 = 0.80$

△ Content (μg) = $0.01418 + (0.000078 - 0.000000026 \times DDS)/(1 - 0.0011429 \times DDS + 0.000000268 \times DDS^2)$ $R^2 = 0.85$

Boron ● Concentration (mg/kg) = Quartic Polynomial Equation, constant = 24894, day degrees = $2.27E^{9}$ $R^2 = 0.82$

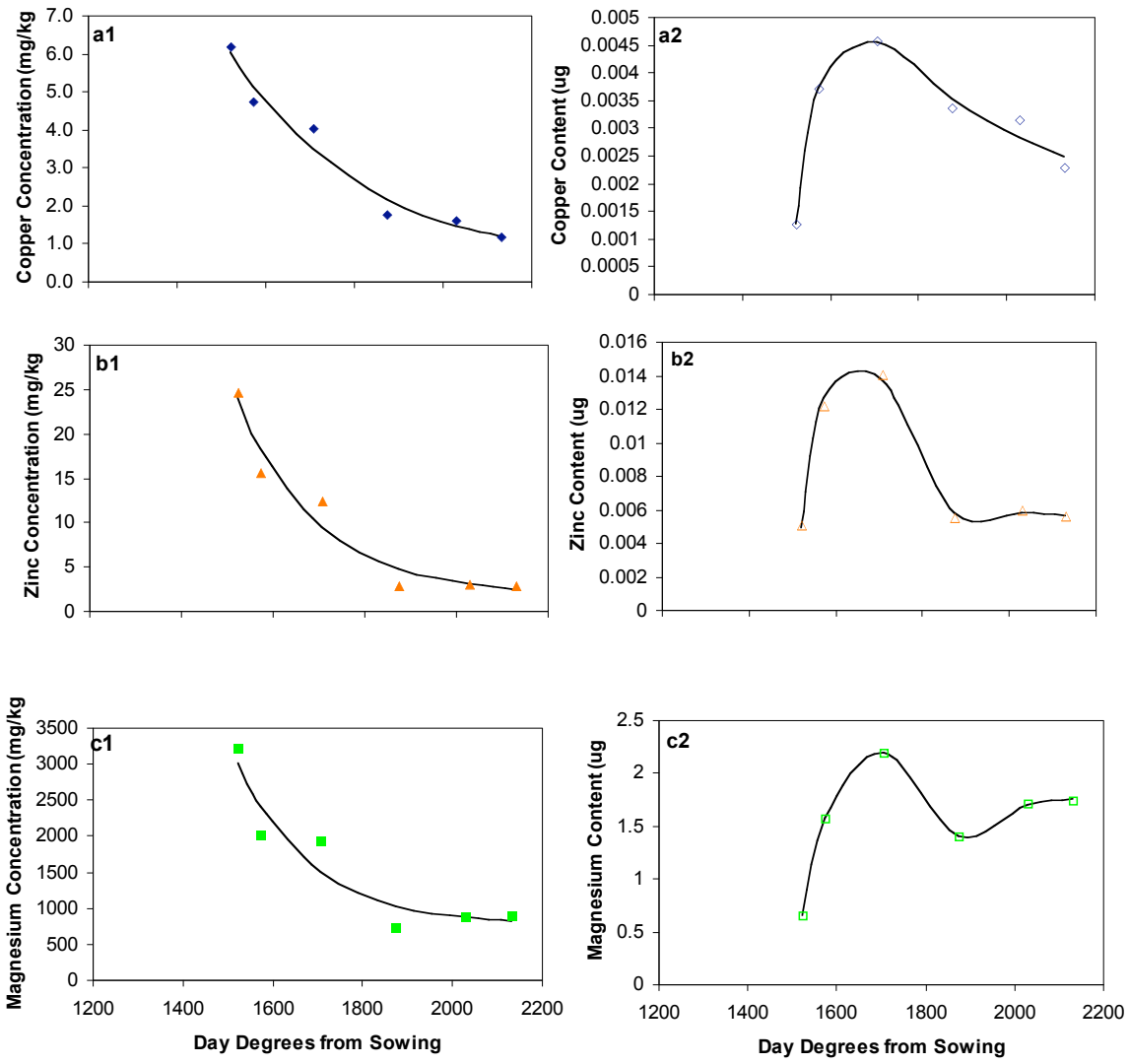

Fig. 21 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) copper ◆ ◇, (b) zinc ▲ △ and (c) magnesium ● ○ in lint from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Copper ◆ Concentration (mg/kg) = 0.42 + (857 x 0.99670^{DDS}) $R^2 = 0.94$

◇ Content (μg) = 0.00012 + (-0.00123 + 0.00000081 x DDS)/(1 - 0.001353 x DDS + 0.000000462 x DDS^2) $R^2 = 0.87$

Zinc ▲ Concentration (mg/kg) = 1.74 + (141327 x 0.99427^{DDS}) $R^2 = 0.93$

△ Content (μg) = Quartic Polynomial Equation, constant = -23.8, day degrees = $-1.92E^{-12}$ $R^2 = 0.97$

Magnesium ● Concentration (mg/kg) = 673 + (25620978 x 0.99389^{DDS}) $R^2 = 0.82$

○ Content (μg) = 1.9291 + (0.05018 – 0.00003039 x DDS)/(1 – 0.00122968 x DDS + 0.000000375 x DDS^2) $R^2 = 0.98$

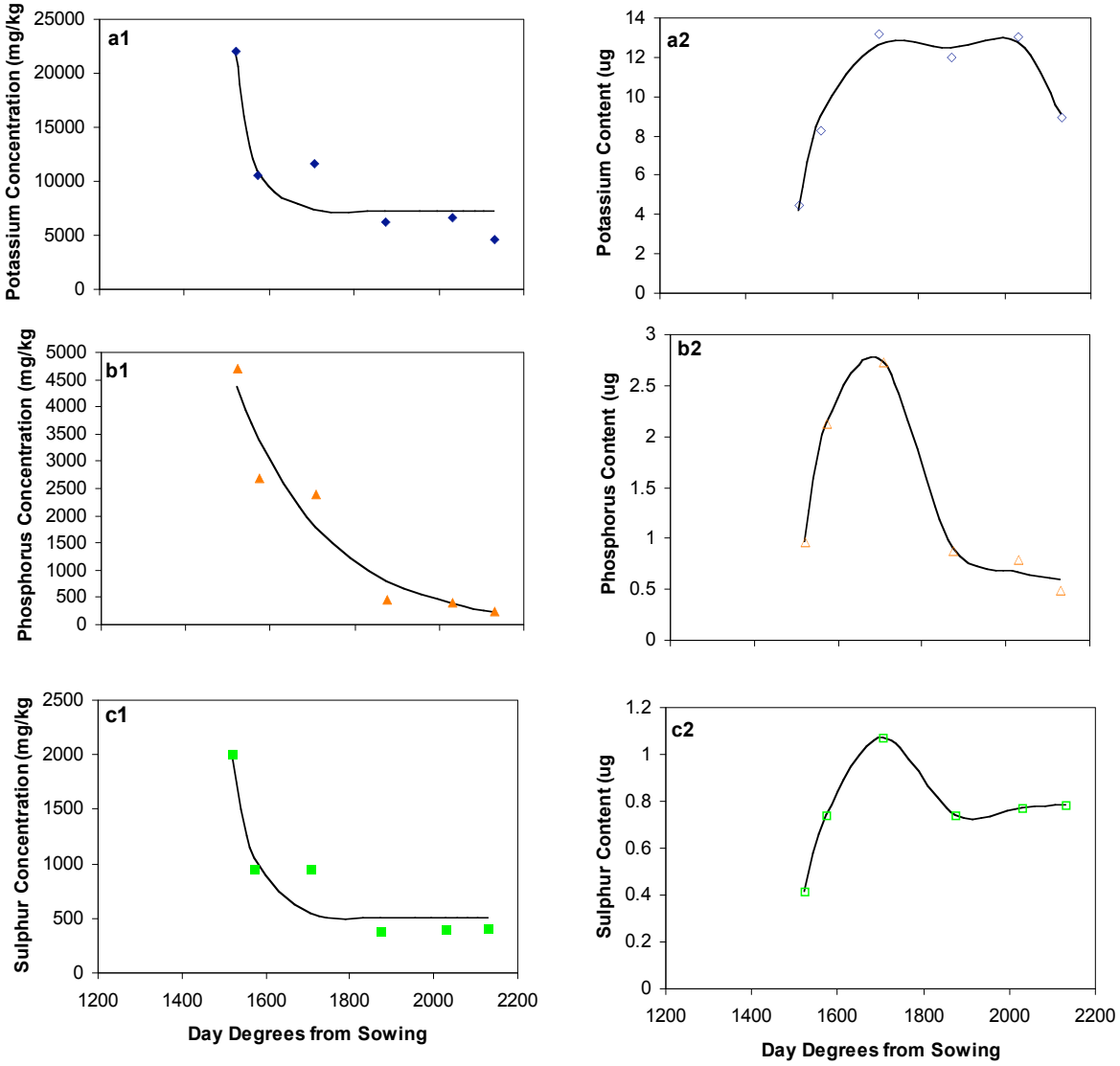

Fig. 22 (1) Concentration (mg/kg) (solid symbols) and (2) Content (μg) (open symbols) of (a) potassium ◆ ◇, (b) phosphorus ▲ △ and (c) sulphur ● ○ in lint from Sicot 71BR bolls from ACRI.

DDS = day degrees from sowing

Potassium ◆ Concentration (mg/kg) = 7127 + ($4.61E^{21}$ x 0.9739^{DDS}) $R^2 = 0.78$

◇ Content (μg) = Quartic Polynomial Equation, constant = -11956, day degrees = $-1.03E^9$ $R^2 = 0.89$

Phosphorus ▲ Concentration (mg/kg) = -11 + (6812191 x 0.99518^{DDS}) $R^2 = 0.88$

	△ Content (μg) = 0.399 + (-0.0332 + 0.0000239 x DDS)/(1 - 0.00122268 x DDS + 0.000000374 x DDS^2) $R^2 = 0.96$
Sulphur	● Concentration (mg/kg) = 496 + ($7.52E^{15}$ x 0.9810^{DDS}) $R^2 = 0.82$
	○ Content (μg) = 0.81220 + (0.010286 – 0.0000006329 x DDS)/(1 – 0.001242329 x DDS + 0.000000384 x DDS^2) $R^2 = 1$

Discussion

Nutrient Accumulation in Bolls

Examination of the sequential accumulation of nutrients in developing Sicot 71BR bolls showed that the accumulation of nutrients in bolls is neither constant throughout boll filling, nor is the pattern the same for all nutrients (Fig. 10-18). While the asynchronous pattern of nutrient translocation to developing seeds in wheat, soybean and maize has been documented (Rashid *et al.* 1992; Ma *et al.* 2004; Haq *et al.* 2005), there has been little previous research into the sequential accumulation of nutrients in cotton seeds. The current findings on nutrient accumulation and asynchronous translocation of nutrients to the developing cotton bolls are significant, both for the development of foliar fertiliser programs as well as a better understanding the links between physiological development of the bolls and demand for specific nutrients.

The bolls examined in these experiments were from a newly released transgenic cotton cultivar, Sicot 71BR. Previous studies carried out on conventional cultivars into the accumulation of nutrients in bolls found that over 60% of the accumulated nutrients were in the walls and seed components of the bolls (Constable *et al.* 1988). The findings of this experiment show that the development of bolls from this modern cultivar was similar to the conventional cultivars. Of the macro and micronutrients examined, between 60 and 80% of the total boll nutrient content was found in the seed and wall components, with the lint accounting for the remainder. The bolls also developed physiologically in similar ways to the patterns established for conventional cultivars by previous research (Schubert *et al.* 1973; Benedict *et al.* 1976).

Overall the bolls showed similar patterns of dry weight accumulation and physiological development when examined as a whole, but this study also examined the development of boll parts when partitioned into seed, lint and walls. Maximum seed dry weight was recorded at 2132 day degrees from sowing (700 day degrees or 50 days after flowering); however, seed dry weight remained relatively constant at about 95% of the maximum from 1936 growing day degrees after sowing (504 day degrees, or 36 days after flowering). This confirms the findings of Benedict *et*

al. (1976) that the fertilised ovule in a cotton boll reaches its full length and volume 18 to 20 days after fertilisation but increases in weight until it detaches from the ovary wall just prior to boll opening.

The high nutrient demand speculated of high yielding transgenic cultivars during boll filling is attributed to rapid seed development (Pettigrew *et al*. 2000). Seed dry weight increased exponentially, showing a relatively constant weight for at least 200 day degrees before boll opening. The nutrient content of seeds increased exponentially, in a similar pattern to the accumulation of dry weight (Fig. 10-12). All nutrients showed a similar pattern of seed nutrient concentration (mg/kg) over time, and accumulation of content (μg) over time. Nutrient concentration showed an initial decline, before increasing, according to a quadratic-by-quadratic regression (Fig. 10-12). This initial decline in concentration represents the growth dilution of the nutrient, as the rapid accumulation of dry weight exceeded the increase in total nutrient content in the seed.

While the pattern of nutrient accumulation was similar for all nutrients examined, the exact timing of nutrient deposition varied between nutrients. P, K, Zn and Mg reached maximum nutrient content at 1936 day degrees from sowing (504 day degrees or 36 days from flowering) (Fig.11 and 12), while S, B, Mn, Fe reached maximum content earlier, at 1876, 2132, 1876 and 1802 day degrees from sowing (370, 444, 700 and 444 day degrees or 22, 32, 50 and 32 days from flowering respectively) (Fig. 10, 11 and 12). This indicates that S, B and Fe are accumulated in seeds earlier than P, K, Zn, Mg and Mn, which accumulated at the same thermal time as dry matter.

The movement of S, B and Fe into the seed before the seed has reached maximum dry weight indicates that the critical time for S, B and Fe fertiliser application would be in this early stage of boll filling, while the other nutrients studied (P, K, Zn, Mg, Mn and Cu) show a critical period for fertiliser application during the period of dry weight accumulation in the bolls, in the first 500 day degrees from flowering.

Oil accumulation in the seed also followed a similar pattern of accumulation to seed dry weight accumulation, reaching maximum oil content at 1936 day degrees from sowing (504 growing day degrees or 36 days from flowering) (Fig.9). This is consistent with previous studies showing that oil deposition in cotton seed from conventional varieties occurred during through a

vascular connection between the ovule and the ovary wall, as carbon assimilates are transported to the developing seeds (Benedict *et al.* 1973; Reddy *et al.* 1999).

Lint and wall structural development also followed a similar pattern to the developmental pattern of conventional cultivars (Schubert *et al.* 1973) reaching maximum dry weight at 1936 day degrees from sowing (497 day degrees or 36 days after flowering). Nutrient accumulation in the lint and boll walls was less consistent than nutrient accumulation in seeds (Figures 13-18). In general the deposition of nutrients occurred before 1936 day degrees from sowing, similarly to the deposition of nutrients in seed.

In all three boll components, K showed a different pattern of change in concentration and the accumulation of content over time, with an exponential decline in the K concentration in seeds and lint, and an exponential increase in boll wall concentration with time (Fig. 12, 15 and 18). The K content of seeds and walls also increased exponentially (Fig. 12). These patterns indicate that the deposition of K in the seeds and walls is not at the same rate of dry weight accumulation, and the traslocation of K to the developing bolls is at a slower rate that the translocation of carbon assimilates and other nutrients. K is a particularly significant nutrient in the cotton industry; for the last 20 years, premature senescence has been reported in Australia and has been linked with K deficiency (Wright 1999). Cotton crops develop this disorder after first flower, during the period of rapid boll filling. Young leaves turn yellow and then red from the margins inwards, and in severe cases the symptoms spread to the whole canopy and can cause defoliation (Wright 1999). The condition shows similar symptoms to severe K deficiencies, although symptoms appear first on young leaves rather than the older leaves as would be expected (Bedrossian *et al.* 2004). The same phenomenon occurs in soybeans, which can develop K deficiencies in leaves subtending developing fruit. Three alternate hypotheses have been suggested as the cause of this problem, that it is; (1) caused by a disease (DeVay *et al.* 1997), (2) a result of poor rooting structure and rooting depth of cotton, contributing to cotton's inability to take up K from surface soil (Cassman *et al.* 1990) and (3) due to an imbalance in K demand from a heavy boll load and the plant's ability to supply K at the required rate (Wright 1999).

In Australia, premature senescence is most likely caused by the increased demand for K from rapidly developing bolls in association with some environmental conditions that predispose the crop to development of the condition. The unusual appearance of symptoms on the youngest leaves first has been attributed to rapid translocation of K to bolls before the nutrient reaches the

top of the canopy (Oosterhuis *et al.* 1994; Wright 1999). Wright (1999) concluded that in Australia premature senescence is primarily a K disorder caused by high boll loads and fruit retention in fast maturing cultivars. This hypothesis was supported by his findings of a 74% decrease in leaf K concentration in severely affected plants. The pattern of accumulation of K in the Sicot 71BR bolls examined in this work support the hypothesis that the translocation of K to the developing bolls does not keep pace with the demand for K from the bolls, since the concentration showed a much sharper decline that other measured macronutrients during the period of dry matter accumulation.

This study examined the accumulation of nutrients and oil in bolls from one cotton cultivar only. To establish a broad picture of nutrient accumulation and the sequence of nutrient movement to bolls in modern cultivars, studies on other cultivars should be carried out. Leaf tissue and petiole sampling of the plants from which bolls were taken could also aid in understanding the nutrient status of the crop from which the bolls were sampled. This was not carried out in this experiment, due to time and logistical limitations. In relation to potassium movement in the plant, an understanding of the leaf nutrient status would give an indication as to where the nutrients were translocated from, and if the limiting factor in boll development is an inability of the plant to translocate nutrients from leaves at a sufficient rate, or if root nutrient uptake is the limiting factor.

This study showed that, since nutrient deposition in the developing bolls occurred in the first 500 growing day degrees from flowering, the optimum time for fertilisers to be applied to increase the nutrient supply to the bolls acting as nutrient sinks would be at this stage. Further investigation into the source of the nutrients deposited in the bolls (if the nutrients are predominantly remobilised from leaves or from root uptake) would aid in establishing if foliar or soil application of fertilisers at this time would be the most effective at supplying the nutrients. Benedict *et al*. (1976) found that the leaf nutrient status influenced the accumulation of oil in the developing cotton seed; however, the link between leaf and boll nutrient status and establishing the proportions of nutrients coming from the leaves and the soil needs to be investigated.

The Efficacy of Foliar Fertilisers

The foliar fertilisers applied in this experiment were ineffective at supplying nutrients to the developing cotton crops during the boll development period, since plant uptake of the nutrients

applied was minimal and inconsistent (Fig. 3-7) and no yield increases or increases in fibre quality were measured (Fig. 1 and 2). This is contrary to the hypothesis that the foliar application of nutrients would increase the productivity of the cotton plants. Similar results have been found in several American studies (Miley *et al.* 1994; Chang *et al.* 1995; Bondada *et al.* 1997; Coker *et al.* 2000) on the efficacy of foliar fertilisers; on the other hand other studies have found yield and quality responses to the application of foliar fertilisers (Sawan *et al.* 1998; Heitholt *et al.* 2001). This practice has not been previously investigated on Bollgard II® cotton in Australia.

Four of the six nutrients applied (P, Zn, Cu and Fe) resulted in an increase of that nutrient concentration in the leaf tissue sampled, but only at one sampling date, and at one of the two sites (Fig.4, 5, 6 and 7). Leaf P, Cu, Zn and Fe were raised by 23%, 56%, 36% and 58% respectively; however, both the control and sprayed leaves contained sufficient nutrients for crop growth and development. These four cases of changes in the leaf nutrient status occurred after only one spray application, not after each application. These results suggest that, for some reason, plant uptake of the foliar fertilisers was reduced or impeded, and thus, the fertilisers applied were not effective at supplying nutrients to the developing crops.

For nutrients to influence either the vegetative or reproductive productivity of a plant, those nutrients must first be effectively incorporated into the plant (through root or foliar uptake). There is little evidence to suggest that the foliar fertilisers applied in this study were absorbed into plant leaves, and thus utilised in plant metabolic pathways. Therefore it follows that no yield or fibre quality response could be expected or explained by the treatments applied.

Ineffective uptake of foliar fertilisers have been linked to environmental conditions (Oosterhuis *et al.* 1991; Zhu *et al.* 1992), physiological characteristics of the crops (Wullschleger *et al.* 1989; Oosterhuis 2003a), chemical properties of the foliar sprays (Howard *et al.* 1998; Howard *et al.* 2000), the plant nutrient status (Constable *et al.* 1988) and to an incorrect timing of application in terms of nutrient accumulation in bolls. In this study, it seems that some of these explanations may give plausible reasons for the lack of uptake of applied nutrients.

Environmental Conditions

The summer of 2005-2006 was an unusually hot summer in North-West NSW. During the period of crop growth at both sites (October 2005 – April 2006) the number of days when the air temperature reached over 36^{o}C was well above the long-term average. In Narrabri air temperature

reached over 36^{o}C sixty times, compared to the long-term average of forty times. In Carroll, these high temperatures occurred fifty-four times, compared to an average of twenty-three. These high temperatures were accompanied by low rainfall and very low humidity. Table 3 shows the air temperature and humidity on the days of spray application.

The high temperatures and low humidity conditions under which the crop was grown may have led to significant periods of water stress being placed on the crops at both sites, despite the application of regular furrow irrigation. Cotton physiological responses to water deficit, such as the dropping of leaves and shedding of bolls and the thickening of leaf cuticles are well documented (Bondada *et al.* 2000). These induced responses to water stress have also been correlated with the plant's ability to both absorb and further translocate foliar applied nutrients (Oosterhuis *et al.* 1991; Wright 1999; Pettigrew *et al.* 2000). Zhu and Oosterhuis (1992) showed that nitrogen absorption decreased as the water stress on the crop increased. This relationship was confirmed by Coker *et al.* (2000) who showed that uptake of foliar applied potassium was reduced with water stress, and by Oosterhuis *et al.* (1991) who showed a 14% reduction in uptake of foliar applied chemicals with the onset of water stress.

Furthermore, the wax content of leaves is affected by environmental factors such as temperature, radiation and humidity. Water stress is a major factor determining the wax content of leaves in sorghum (Jordan *et al.* 1982), wheat (Johnson *et al.* 1983), rice (O'Toole *et al.* 1983) and some pastures (Moseley 1983). Wheet *et. al* (1978) reported that the wax content of cotton leaves increased under water stress, as a physiological response to drought and reduced water uptake. Water stress of cotton plants caused a 33% increase in the thickness of the leaf cuticle when compared to non-stressed plants (Oosterhuis *et al.* 1991). Changes in the types of waxes present in the cuticles of the water-stressed plants also occur, with an increase in longer chain waxes, creating a more hydrophobic cuticle. This thicker, more hydrophobic cuticle, contributed to a measured 34% reduction in the absorption of a defoliant spray (Bondada *et al.* 2000). It could be argued that this would contribute to a reduced uptake of foliar fertilisers in the same way. This theory is supported by Oosterhuis (2003a) who showed a negative relationship between wax content of cotton leaves and absorption of foliar applied ^{15}N, and by Leece (1976) who showed that the absorption of foliar fertilisers in pear and orange tree crops was affected by the leaf cuticle thickness and leaf age.

Since the conditions under which both crops were grown was conducive to placing water-stress on the cotton plants, leaf cuticles may have been thickened and the wax content increased. If these physiological changes had been induced in the crops, the uptake of applied nutrients may have been impeded or reduced.

Nutrient concentration in the spray, pH and the addition of surfactants

Another potential explanation for the lack of plant uptake and incorporation of applied nutrients could be due to the nature of the sprays themselves. A potential problem with the application of nutrients to plants by foliar sprays is the possibility of leaf burn (Oosterhuis 1999). This occurs when sprays are applied at times when temperatures or radiation will cause damage to the leaf due to intensified sunlight through the water on the leaf surface (causing sunburn to the leaves) or through direct damage to the leaves through an excess of salts and chemicals on the surface. Leaf burning disrupts the integrity of the cell membranes and photosynthesis, resulting in a reduction in carbon fixation and dry matter accumulation (Chang *et al.* 1995). This risk is increased if the concentration of the nutrient in solution is too high (Thompson *et al.* 1976; Woodruff *et al.* 1987), or if the solution is at a very low or very high pH (Chang *et al.* 1995).

The fertiliser solutions applied were diluted to concentrations of less than 5% (P and K) and less than 1% (Fe, Zn, Cu and B) concentration (weight:water ratio). At these solutions the risk of leaf burn through direct salt damage to leaves should not have occurred. A more likely problem mav have been created through the fast evaporation rates off the leaves due to the hot conditions and low humidity. This process may have increased the concentration of the solution, reducing its uptake and damaging leaves.

Solution pH may also have influenced the degree of uptake and total absorption of the fertiliser sprays. Chang and Oosterhuis (1995) found that yield benefit obtained through the application of foliar potassium was increased when the pH of the solution was adjusted to 4 and 6. Likewise Howard *et al.* (2000) found that buffering of potassium and boron solutions to a pH of 4 or 6 increased the lint yield by an average of 14%, compared to a non-buffered solution with a pH of 8.5. These findings were consistent with earlier findings by Howard and Gwathmey (1995) and Heitholt (1994a) for the application of potassium and boron. Solutions with a pH of 3 or less cause a reduction in the penetration of the solution through leaf cuticles (Schonherr 2000), yet the results of Chang and Oosterhuis (1995) and Howard *et al.* (2000) indicate an increasing

efficacy with a reduction to a pH of below 7. The K and B sprays in this work, both of which resulted in no difference in leaf tissue K or B concentration in sprayed plants, had pHs of 10.2 and 8.7 respectively (Table 2). These high pHs may have been a factor in reducing the uptake of the fertilisers into leaves. No buffer was added to these sprays due to the difficulty in measuring the spray pH in the field, and the logistical issues with pre-mixing sprays prior to application. The addition of a pH reducing buffer to K and B sprays may have increased their efficacy.

Wetting agents and spreaders have been shown to improve the surface contact and adhesion between the leaf cuticle and the spray, and increase stomatal absorption of the nutrients (Heitholt 1994a; Schonherr 2000). The wetting agent used in this experiment is widely used in the cotton industry and so while it may not have assisted in fertiliser uptake, it also may not have been an impeding factor in the fertiliser efficacy. If the cuticle had been thickened or the wax content of the leaf increased in response to water stress, the wetting agent may not have been as effective.

The form of the nutrient applied

Some forms of Fe, Zn and Mn applied to plants as a foliar spray show less effecive leaf penetration and incorporation than others (Haq *et al.* 2000). Salts (especially sulfates) of some micronutrients show fixation on the surface of the cuticle at the point of application (Rengel *et al.* 1999). This is a result of binding of leaf exudates to cations on the surface of the leaf, and the interaction of epicuticular waxes with metal ions (Schonherr 2000; Ferrendon *et al.* 1988). In cotton, absorption of Zn and Fe chelates is lower than sulfate salts, however the mobility of the chelated form in the phloem sap is higher (Sawan *et al.* 1999; Fernandez *et al.* 2005). Fe was applied in chelated form (as Agrodex Fe50), while Zn and Cu were applied as sulfate salts (Table 2). Some Fe, Zn and Cu were all taken up by leaves, in small amounts and inconsistently. The uptake of Zn may have been increased were it applied in chelated form. Small increases in seed and lint Cu concentrations were recorded (Table 10 and 11) indicating that the absorbed Cu was translocated to reproductive plant structures. Increasing leaf uptake of Cu then, through modifying its form applied could increase the effectiveness of Cu incorporation into the plant.

Soil and Plant Nutrient Status

The efficacy of foliar fertilisers in terms of penetration of nutrient into the cotton leaf cells and incorporation into metabolic pathways within the plant has previously been correlated with the nutrient status of the plant to which the fertiliser is applied (Coker *et al*. 2000). Clarkson and Scattergood (1982) found that foliar absorption and translocation of phosphorus in barley plants was reduced when plants were well supplied with phosphorus at the roots. Tomato, soybeans and corn preferentially take up nutrients from the soil solution through the roots rather than from foliar applied nutrients (Rose *et al.* 1981; Clarkson *et al.* 1982; Harder *et al.* 1982). In cotton, the uptake of foliar applied nutrients was dependent on the availability of these nutrients in the soil (Halevy *et al.* 1988). Constable *et al.* (1988) found no yield response to micronutrient applications when there were no soil nutrient deficiencies. Likewise Bednarz *et al.* (1999) found no yield or fibre quality increase from the application of foliar fertilisers when there was no nutrient deficiency in the soil, and no nutrient limitations to growth. Most yield responses to foliar fertilisers have occurred when the soil applied nutrients or levels of soil nutrient were insufficient to supply the developing boll load of the cotton crop (Oosterhuis *et al.* 2001). A study conducted across the whole of the American cotton belt found no yield responses to foliar applied K on sites where no existing K deficiency existed, but yield increases of up to 23% were recorded at sites where nutrients were limiting in the soil (Oosterhuis *et al.* 1994).

The soil at both sites was not lacking in essential plant nutrients to the stage that they could be limiting to plant growth and development (Table 6). Further investigation of deficiencies can be established through comparing the nutrient concentrations of plant parts from control plots with established critical levels. This is shown in Table 6.

Neither plants at Narrabri nor those at Carroll were deficient in any of the applied nutrients (Table 12). Since both the soil and the plant leaves showed no indicators of nutrient deficiencies, it could be speculated that the lack of plant uptake of fertilisers could be explained through the plant's inability to take up excess nutrients.

These results suggest both a potential for foliar fertilisers in the cotton industry and a limitation to their usage. Since plants will preferentially take up nutrients from the soil through the roots (Hodges 1994) applications of nutrients to the plant foliage in non-deficient conditions may not increase the yield or translocation of nutrients to the developing bolls and fibres where the nutrients are available at the roots. In situations where root growth is restricted or inadequate

for nutrient uptake, foliar application could provide a cheap and environmentally beneficial way to provide these nutrients. Foliar fertilisation may be especially useful in no till cropping systems which reduce root growth and restrict root nutrient uptake (Howard *et al.* 2001). Likewise in higher yielding crops where the soil uptake and supply of nutrients is insufficient for plant demand, foliar application may supply the nutrients needed effectively (Miley *et al.* 1994; Oosterhuis 1999; Pettigrew *et al.* 2000).

Since most cotton in Australia is grown on naturally fertile cracking clays, severe nutrient deficiencies are seldom recorded. The practice of applying nitrogen and zinc fertilisers with sowing reduces the chance of deficiencies in these nutrients developing. If consistently no yield and fibre quality benefits occur from foliar application of nutrients to plants growing under non-deficient conditions, the usefulness of foliar fertilisers in Australia seems questionable. Further research into their effectiveness in deficient conditions, or to overcome short-term acute deficiencies at the end of the season would be most useful in establishing the place of foliar fertilisation in the Australian cotton industry.

Timing of nutrient applications

It is hypothesised that faster maturing cotton and new higher yielding cultivars have a high nutrient demand between flowering and boll opening, as the seed and lint act as nutrient sinks (Patterson *et al.* 1978; Oosterhuis 2003b). Maples *et al.* (1977) found that the optimum time for foliar fertilisation was during boll filling (after flowering and before boll opening) in conventional cultivars. Since the developing boll load has a high demand for both macronutrients and micronutrients which in some cases cannot be supplied through root uptake of nutrients (Benedict *et al.* 1973; Halevy 1976; Constable *et al.* 1988; Unruh *et al.* 1996; Reddy *et al.* 1999), new high yielding cultivars, with increased boll retention rates and higher boll numbers place an increased nutrient pressure on root uptake and translocation of nutrients from leaves (Oosterhuis *et al.* 1994; Bednarz *et al.* 1999; Wright 1999; Oosterhuis 2003b).

While the physiological pattern of boll development is well established, the pattern of nutrient accumulation and the source of the nutrients deposited in cotton seeds and lint is not well documented (Constable *et al.* 1988). The developmental pattern is important for establishing the time period in which nutrient demand from the bolls is the highest, and thus the time at which nutrients are most likely to be limiting in supply. The source of nutrients which end up in the

reproductive plant parts is significant in that raising the nutrient concentration in these plant parts could increase the amount that is translocated to developing bolls, and theoretically increase the yield and quality of these plant parts.

The second experiment in this study was designed to investigate this pattern of nutrient accumulation in boll parts to establish the developmental stage at which nutrients move into seed and lint, and so aid in designing fertiliser regimes around demand from the bolls.

Having concluded that the optimum time for foliar fertilisation occurs in the first 500 degree days after flowering (since this is the time when nutrient demand is maximised), the hypothesis that the lack of plant uptake of fertilisers applied at Narrabri was due to the fertilisers being applied at the wrong developmental stage, outside the period of peak nutrient demand from bolls can be rejected. Flowering, on average, occurs at 707 day degrees from sowing, and so, following this the optimum period of fertilisation would be from 707 to 1207 day degrees from sowing. Since cotton is an indeterminate plant, and flowering continues after this time, the window of optimum fertilisation time could be extended to the 500 day degrees after the last effective flower is produced, which occurred in Narrabri and Carroll at around 1500 day degrees. All three sprays at Narrabri, and both sprays at Carroll occurred between 1200 and 1800 day degrees from sowing, which places them in this window of time at which nutrient movement to developing bolls was maximised.

Since the fertilisers were applied at the correct developmental stage, the most likely explanations for the lack of plant response to the fertilisers applied are that environmental conditions, (the extreme heat, low humidity and water stress on the crops) and the lack of existing plant and soil nutrient deficiencies may have been the most significant factors limiting uptake of the foliar applied nutrients. The efficacy of foliar fertilisers in increasing cotton yields or fibre quality under these conditions is questionable, and their place in the Australian industry, which is generally not characterised by severely nutrient deficient soils should be limited to use in overcoming acute deficiencies at the end of the season and not in supplementing traditional soil fertilisation practices.

Conclusions

Sicot 71BR bolls were found to develop according to the same pattern as that established for conventional cultivars sixty years ago (Reddy *et al*. 1999). The accumulation of seed oil also followed the established pattern (Benedict *et al*. 1973). Nutrients accumulated at different developmental stages in the boll components, reaching maximum nutrient content earliest in the lint (at 1660 GDD), then the boll walls (at 1700 GDD), and lastly the seed component (at 1936 GDD). Different nutrients showed an asynchronous pattern of accumulation, with S, B and Fe accumulating earlier in bolls (by 1800 GDD) than P, K, Cu, Mn, Mg and Zn (by 1936 GDD).

Nutrients accumulate asynchronously within the first 500 growing day degrees after flowering. At this developmental stage nutrients are deposited in the bolls, creating a high nutrient demand supplied through root uptake and translocation from leaves. Foliar fertilisation may be one method of ensuring that this high nutrient demand from bolls is met, through raising the nutrient status of the subtending leaves from which the nutrients are taken. Further research into the source of nutrients in the bolls and the proportional uptake from roots and leaves needs to be completed to assess this potential.

In Australia the effectiveness of foliar fertilisers at supplying nutrients to developing cotton crops during this boll development period is questionable. There was no evidence to suggest that applications of P, K, Fe, B, Cu and Zn in this experiment were absorbed into the plant, and no yield or fibre quality benefits were obtained through their application. Environmental conditions and an abundance of nutrients in the soil are the most likely causes of the ineffective plant uptake of fertilisers. The usefulness of foliar fertilisers in hot, dry condition, such as those typical of the Australian cotton growing region may therefore be limited by climatic factors which create drought responses in the cotton leaves, limiting the penetration and incorporation of foliar applied nutrients. The usefulness of foliar application of nutrients to plants growing in soils with high natural fertility, such as the vertosols typical of the Australian cotton growing region, is also questionable. Plants growing in deficient conditions, where nutrients are limiting to growth and root uptake of nutrients is insufficient for plant demand may show a greater uptake and yield and quality response to foliar applications of nutrients. Further research should be carried out on plants in deficient areas to assess the potential of foliar fertilisers as a means of correcting acute crop nutrient deficiencies.

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Appendices

Appendix 1: Growing Day Degree Formula

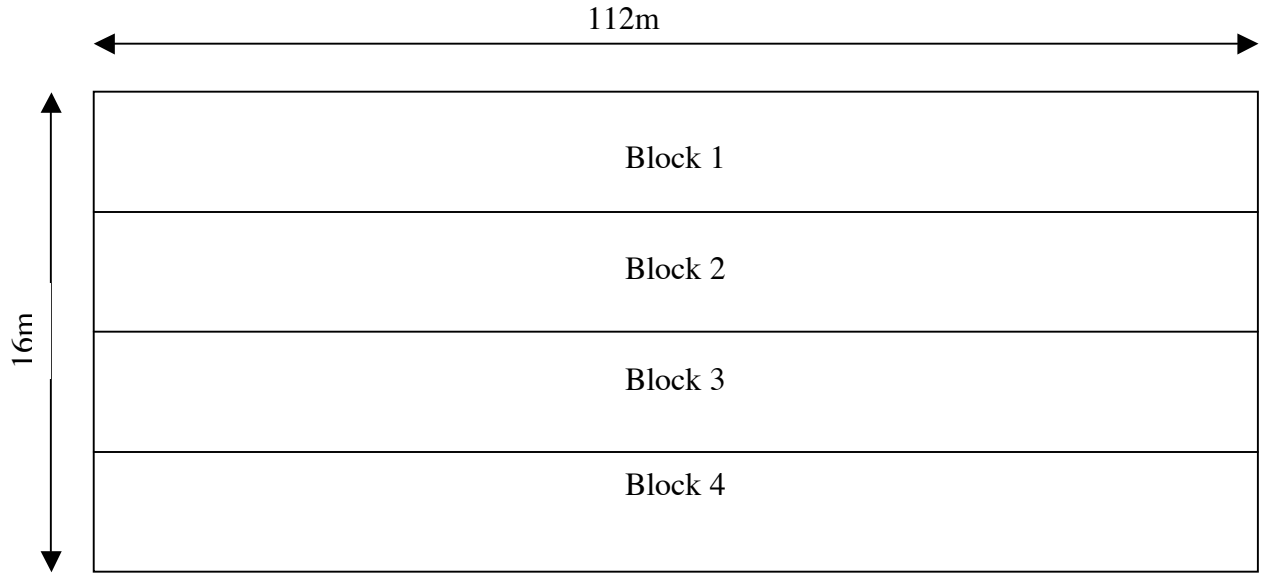
Growing Day Degree Formula

$$GDD = \frac{(T_{max} - 12) + (T_{min} - 12)}{2}$$

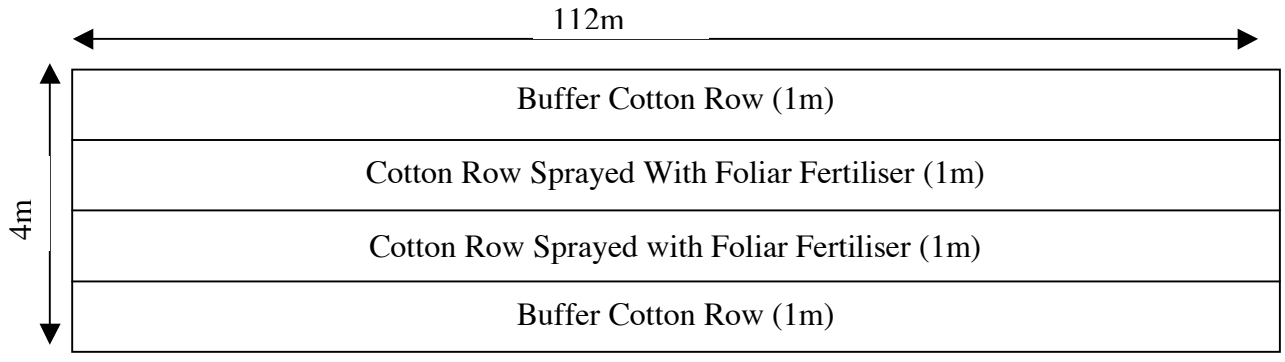
Appendix 2: Field Set up and Field Plan for Narrabri and Carroll

(a) Field Layout showing Block sizes
(b) Field Layout showing rows of cotton in one block
(c) Field Plan, Narrabri
(d) Field Plan, Carroll

(a)



(b)



(c)

	Plot 1	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**Plot 7**
Block 1	P	K	B	Cu	Zn	Fe	Control
Block 2	Zn	Control	Fe	Cu	B	K	P
Block 3	P	K	Zn	Cu	Fe	B	Control
Block 4	Cu	Fe	Control	K	B	Zn	P

(d)

	Plot 1	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**Plot 7**
Block 1	B	P	Cu	Control	Zn	Fe	K
Block 2	Cu	P	Fe	Zn	Control	K	B
Block 3	Cu	Zn	Fe	K	P	Control	B
Block 4	P	Fe	K	B	Zn	Cu	Control

Appendix 3: Soil details from each block, Narrabri and Carroll

Soil from the top 30cm, Narrabri

	Block 1	**Block 2**	**Block 3**	**Block 4**
Texture	Medium Clay	Medium Clay	Light Clay	Medium Clay
Munsell Colour	Brown	Brown	Brown	Brown
pH (1:5 Water)	7.9	8.0	7.8	7.9
CEC (M eq/100g)	41.5	40.1	34.5	39.2
Electrical Conductivity (Sat. Ext) (dS/m)	1.8	1.7	1.8	1.7
Electrical Conductivity (dS/m)	0.29	0.27	0.24	0.28
ESP %	2.1	2.3	2.0	2.3
Organic Carbon %	0.91	0.79	1.0	0.89
Nitrate Nitrogen (mg/kg)	18	12	21	18
Sulfate Sulfur (mg/kg)	18	17	17	19
Phosphorus (Colwell) (mg/kg)	33	15	46	21
Potassium (M eq/100g)	1.6	1.3	1.6	1.4
Calcium (M eq/100g)	28	28	24	27
Magnesium (M eq/100g)	11	9.9	8.2	9.9
Sodium (M eq/100g)	0.87	0.91	0.70	0.91
Ca:Mg Ratio	2.5	2.8	2.9	2.7

Soil From the Top 30cm, Carroll

	Block 1	Block 2	Block 3	Block 4
Texture	Medium Clay	Medium Clay	Medium Clay	Medium Clay
Munsell Colour	Grey	Grey	Grey	Grey
pH (1:5 Water)	8.5	8.5	8.5	8.6
CEC (M eq/100g)	54.3	53.2	55.1	59.3
Electrical Conductivity (Sat. Ext) (dS/m)	2.5	2.0	2.5	2.5
Electrical Conductivity (dS/m)	0.40	0.33	0.40	0.40
ESP %	7.9	8.1	7.6	7.3
Organic Carbon %	0.71	0.69	0.71	0.70
Nitrate Nitrogen (mg/kg)	<1.0	2.2	1.3	1.6
Sulfate Sulfur (mg/kg)	25	20	20	21
Phosphorus (Colwell) (mg/kg)	8.8	13	11	15
Potassium (M eq/100g)	2.0	1.9	1.9	2.0
Calcium (M eq/100g)	36	35	37	40
Magnesium (M eq/100g)	12	12	12	13
Sodium (M eq/100g)	4.3	4.3	4.2	4.3
Ca:Mg Ratio	3.0	2.9	3.1	3.1