EARLY WATER STRESS ON GROWTH, DEVELOPMENT AND YIELD OF HIGH RETENTION COTTON

By

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Paytas, M., Yeates, S. Fukai, S. and L. Huang (2007) **Early production of biomass in high retention cotton.** In; Proceeding for The **World Cotton Research Conference-4** September 2007 Lubbock, Texas, USA http://wcrc.confex.com/wcrc/2007.

Paytas, M; Yeates, S; Fukai, S; Huang, L. (2008) **Effect of early moisture deficit on growth, development and yield in high retention BT cotton.** In: **14th Australian Agronomy Society Conference** in Adelaide (South Australia) September 2008. http://www.agronomy.org.au/events/2008

Paytas, M; Yeates, S; Fukai, S; Huang, L. (2008) **Modulation of Pre-flowering vegetative biomass by water and nitrogen supply to enhance assimilates supply for high fruit retention of BT cotton.** In**: CRC Science Cotton Forum** in Narrabri (Australian Cotton Institute), New South Wales, Australia. October 2008. http://www.cottoncrc.org.au

Paytas, M; Yeates, S; Fukai, S (2009) **Responses of high retention cotton to enhanced pre-flowering vegetative biomass.** In**: CRC Science Cotton Forum** in Narrabri (Australian Cotton Institute), New South Wales, Australia. August 2009. http://www.cottoncrc.org.au

Paytas, M, Fukai, S and Yeates, S (2009) **Effect of early water stress and flower bud removal on growth and development of high retention cotton.** In**: InterDrought-III Conference** in Shanghai, China. October 2009. http://www.interdrought.org

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Abstract

The Bollgard II cotton varieties, which contain two genes from Bacillus thuringiensis var kurstaki (Bt) that express proteins toxic to Helicoverpa spp. were recently released in Australia, and they have increased insect protection compared with conventional (non-Bt) varieties with similar genetic backgrounds. Irrigation programs in Australia have been tailored to the lower retention conventional varieties and incorporated a long period of water stress until squaring, followed by full irrigation during the reproductive stage. This management, while proven for low retention conventional varieties may not produce sufficient early biomass to support the higher boll load due to high retention in Bt varieties and may limit their yield potential due to a high competition for assimilates between organs under water stress.

This thesis aimed to understand the differences in growth, development and yield of different levels of water availability at pre-flowering in high retention cotton. To achieve this general objective, eight field experiments, seven at Gatton in southeast Queensland and one at Narrabri, New South Wales, were conducted in three seasons (2006/07, 2007/08 and 2008/09). Four of them (Exp. 1, 2, 3 and 4) compared the effects of pre-flowering soil water deficits on fruit retention, boll distribution and yield, and quantify differences on biomass growth, partitioning and phenological development. In four experiments (4, 5, 6 and 7), the effect of early water availability was examined for high and low fruit retention cases (the latter achieved by flower buds removal), and responses on the dynamics of fruit sink development and assimilate supply were studied. A single Experiment (8) at Narrabri, NSW was conducted to study the responses of pre-flowering irrigation management under under furrow irrigation. The effects of water

treatments were examined using rainout shelters or plastic cover of inter-row space for designated time period in all the experiments.

Even modest early soil water deficits affected lint and components of yield in high retention cotton. Increased pre-flowering water availability impacted significantly on the crop, increasing retention of boll load, with changes in boll distribution on lateral and vertical fruits positions, and increased in final yield. The number of reproductive organs was negatively related to duration and severity of the stress period. Early water stress hastened plant development and reduced boll number, as a result of reduced fruiting sites. Irrigation at pre flowering extended the time to cut out and maturity as the result of higher biomass at pre-flowering that could support a greater number of reproductive organs.

The number of fruiting sites increased under irrigated conditions (high availability of resources), mainly in first position on fruiting branches and concentrated in the middle and upper part of the canopy. The absolute number of flower buds and bolls, and the percentage of fruit retention were higher in irrigated compared with stress treatments in high retention cotton. Without flower removal (Bt), the effect of early water stress reduced seed cotton yield by about 20%, however with flower removal (conventional) the reduction of yield was 5-8%. This suggests that early irrigation increased the supply of assimilates (before flowering) which was important for the high retention cotton, whereas plants can be stressed during early stages in conventional cotton varieties (low retention) where source-supply is relatively large and can tolerate early water stress compared with stressed Bt cotton.

The artificial canopy opening to exposure to higher light showed that the period of exposure of 42 days after flowering and until the end of the crop, increased vegetative dry matter production, boll dry matter and TDM, and fruit retention in second position on fruiting branches by 13-15% and total fruit retention by 10%, with a much larger number of fruits retained in the lower part of the plant thus increased significantly final seed cotton yield compared with control (no canopy exposure). This result indicates that high retention cotton has a capacity to respond to increased source supply even after flowering.

These observations show the advantages of early water availability in high retention cotton in order to improve final lint yield, and support the general hypothesis that insufficient early growth, produced under soil water deficits at pre-flowering, reduces the assimilates supply to a higher boll demand in high retention cotton.

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Chapter 1 Introduction

Cotton is grown as an annual crop, but has a xerophytic, woody perennial nature (Hearn, 1980). More than 90% of the world's cultivated cotton consist of two species: *Gossypium hirsutum* (upland cotton) and *Gossypium barbadense* (Pima or extra-long staple cotton) (Heitholt, 1999b).

Cotton production forms one of the world's most important agricultural cash production systems. The lint is universally used as a textile raw material, while cottonseed is the second most important source of vegetable oil; further, cottonseed cake is a rich source of quality protein for incorporation in animal feeds (Eisa, 1994).

Cotton production, like most major agricultural crops, is negatively impacted by moisture deficit stress. About 53 % of world cotton production is from irrigated conditions, while the remainder is produced under rainfed conditions (Hearn, 1994). Almost all production under 'Mediterranean' or 'desert climates' is from fully irrigated cropping environments, and includes almost all production in Spain, Greece, Morocco, Israel, Egypt, Turkey, Syria, China, India, Pakistan and the Central Asian Republics, together with extensive areas in the west of North and South America (Hearn, 1994). In tropical and subtropical summer rainfall zones, including much of Sub-Sahara, Africa, Central and South America, cotton is more commonly grown under rainfed conditions (Hearn, 1994).

Australia is responsible for about 12% of the world's cotton production, and is the third largest exporter of cotton fibre. Seventy per cent of Australian's cotton is grown in the state of New South Wales, with the remainder being produced in the state of Queensland (Fig. 1.1) (CRDC, 2005), in an area that extends from Emerald in Queensland to Hay in New South Wales (Fitt, 1994). Less than 20% of the Australian cotton crop is grown under rainfed conditions (CRDC, 2005). Over the last 30 years, the Australian cotton industry has grown dramatically, from 45,000 tonne in the 1970's to 600,000 tonne in the 2000's. Over the same period, average yield increased by 1.8% per year (Constable, 2004), reflecting the adoption of new higher yielding varieties and more intensive cotton cropping practices. Cotton yields currently being achieved in Australia of up to 1,700 kg/ha of lint, are the highest from intensive production systems in the world (Constable, 2004).

Figure 1.1 Cotton growing regions of Australia (Cotton Research and Development Corporation, Australian Government, 2005)

Cotton is attacked by a range of insect pests, the most significant of which is the larvae of *Helicoverpa* spp (Fitt and Wilson, 2000). These larvae feed on the developing fruit (flower buds or squares and bolls), causing them to be shed. The reduced fruit retention, especially early in the growing season, delays cut-out, the point at which the boll load (sink) is sufficiently high for the demand of assimilates for fruit development to equal assimilate supply from photosysnthesis (source), and at which point the plant ceases to set additional bolls (Hearn, 1972; Hearn, 1994).

Cotton is one of many crops that have been genetically modified to increase their performance with respect to weed, insect pest and disease control, the modifications being aimed on improved tolerance of pests and diseases, together with better weed control, and thereby reduce the need for application of synthetic pesticides and herbicides (Constable, 1998). The Bollgard II cotton varieties containing two genes from *Bacillus thuringiensis* var *kurstaki* (Bt) that have proteins toxic to *Helicoverpa* spp., were released in Australia in 2005. These Bollgard II varieties have increased insect protection when compared with conventional (non-Bt) varieties with similar genetic backgrounds, resulting in higher early fruit retention and boll load, together with faster accumulation of boll weight, while having a lower leaf area than their conventional equivalents (Yeates et al., 2006).

The higher sink demand of the smaller Bollgard II cotton plants has lead to early cut-out and lower yields of these high retention varieties, when compared with the equivalent conventional varieties (lower retention), when grown using traditional irrigation management practices. The irrigation programs in Australia have been tailored to the lower retention conventional varieties, and incorporate a long period of water stress until the time of squaring, followed by full irrigation during the reproductive phase. This form of irrigation management, while proven suitable for the low retention conventional varieties, may not produce sufficient early biomass to support the higher boll load resulting from high fruit retention of the Bollgard II varieties, and may limit yield potential due to high competition for assimilates among organs. Therefore, to achieve the higher yield potential of the genetically modified varieties, changes in some aspects of crop management, such as pre-flowering water regimes, need to be investigated to ensure the sustainability and high productivity of cotton production systems.

The primary objective of the study reported in this thesis, was to investigate options for pre-flowering irrigation as a production practice in Australian systems, that is aimed to assist with the development of a larger canopy during the early stages of growth, in support of a higher rate of fruit retention in Bt cotton.

The specific objectives of the study were to:

i) Compare the effects of pre-flowering soil water deficits on fruit retention, boll distribution and yield in high retention cotton (Chapter 3).

ii) Achieve an understanding of the potential effects of pre-flowering soil water deficits on high retention cotton in relation to phenological development, biomass production and partitioning, all of which may influence final yield (Chapter 4).

iii) Compare the effects of early water availability on sink development and source availability, in high and low fruit retention (low retention being simulated by flower removal) cotton, with specific reference to:

(a) Dynamics of fruit development, distribution and retention, and yield (Chapter 5).

(b) Phenological stages and biomass accumulation and partitioning (Chapter 6).

iv) Investigate the responses of early-irrigated high retention cotton to canopy exposure to light on growth, development and yield (Chapters 5 and 6).

v) Test the responses of pre-flowering irrigation management under furrow irrigation at different growing environment (Chapter 7).

Chapter 2 Literature Review

2.1 Introduction

The use of biotechnology for the development of transgenic crops like cotton, has greatly improved the productivity and sustainability of Australian agricultural systems. The introduction of high yielding cotton to Australian farming systems is one of the technological advances that have improved tolerance to pests and diseases, and allowed better control of weeds. Despite these improvements, issues relating to water management during the early stages of the crop growth, aimed at achieving larger plants and potentially larger source of assimilate to meet the higher demand associated with higher fruit retention in potentially high yielding transgenic cotton varieties, have yet to be investigated. There is strong interest in the Australian cotton industry in the improvement of water use efficiency, to ensure the sustainability and profitability of production under conditions of increased limitations to inputs such as water.

This review firstly considers the growth, development and physiological processes that affect cotton yield and its components. The review then considers how water supply influences growth and development of conventional cotton varieties. Finally, the review summarises what is currently known about Bt cotton, and its response to early biomass production.

2.2 Growth and development

Cotton is grown as an annual crop (Hearn, 1980), with *Gossipium hirsutum* (upland cotton) and *G. barbadense* (Pima cotton) accounting for more than 90% of the world's cultivated cotton crops (Heitholt, 1999a).

Modern cotton varieties are indeterminate, with vegetative and reproductive development following an orderly and regular pattern. Vegetative growth is characterized by the successive development of the main stem (primary axis) nodes. A new node is produced every 2 to 4 days, depending on temperature during growth (Hearn and Constable, 1984). Axillary branches differentiate from the main stem. At the lower nodes, monopodial branches (similar to the primary axis) can develope, but from approximately the fifth main stem node and upward, only sympodial branches develop (Heitholt, 1999b). Fruiting sites are produced at regular intervals, about every 5 to 6 days, along the fruiting branch (Hearn, 1994).

Cotton's phenological development is controlled primarily by temperature, as modern varieties are photoperiod insensitive (Lee, 1984). Both the rate of branch development (monopodial and sympodial branches) and fruit development are controlled by temperature (Hearn, 1992). Temperature summations (degree-days) are commonly used to predict the development of the crop during the growing season. Constable (1976), working with cotton over three seasons in Australia (1972-1975), quantified the heat unit summations or Growing Degree Days (GDD), and demonstrated their ability to predict the length of the development phases. Constable and Shaw (1988) found that about 505 degree-days are necessary to reach first square, 777 degree-days first flower, and 1527 degree-days first open boll, in the Australian cotton growing environments. GDD was considerably less variable than days, in predicting phenological development in Australia, particularly for the growth phase, planting to squaring (Constable and Shaw, 1988). However greater variability was found for predictions of the reproductive stage. The base temperature used in this methodology was 12^{o}C.

The reproductive stage starts with the formation of squares, followed by flowers. About 830 degree days produce peak vegetative growth, just before the first flower. Peak dry weight production is reached at about 1000 degree days, shortly after first flower (Kerby, 1986). When boll growth requirements equal the carbohydrate production of leaves, cut-out will occur. In unstressed plants, cut-out occurs when NAWF (number of nodes above the highest 1st position white flower) is 4.5. NAWF is defined as the number of nodes from a first position flower (counted as zero) when moving towards the terminal of the plant (Kerby, 1986).

During vegetative growth, production of carbohydrates through photosynthesis increases. As the plant continues growing, the demand for carbohydrates by different organs in the plant also increases. In this way a balance is achieved between carbohydrate supply and demand. The time of maturity is determined by the capacity of the cotton plant to continue the production of new vegetative organs relative to the demands of the reproductive organs (Hearn, 1994). The assimilate supply by the leaves is the primary determinant of yield and essential for the support of vegetative and reproductive growth. Radiation interception by the canopy is therefore a major determinant of crop growth and yield (Monteith, 1977).

2.3 Yield and components of yield

The primary harvest product for cotton is lint rather than seed, but because of the close association between lint and seed biomass, with the seed epidermis supporting fiber growth, some researchers support the concept of seed cotton biomass to refer to cotton yield, while others refer only to cotton lint yield. Most definitions of yield are related to the number of cotton bolls (Pettigrew, 1994) and the amount of lint per boll (Hearn and Constable, 1984). Yield can be also defined as the product of total aerial biomass and the percentage of that biomass that is lint (this is called the harvest index), with increasing yield being associated with increased partitioning of the biomass to the fruit (Meredith and Wells, 1989).

2.3.1 Nutritional and hormonal hypothesis

The number of bolls is directly affected by the balance between assimilates supply and demand during the growing season of the crop (Bange and Milroy, 2000), as well as by other factors such as temperature. The balance of assimilates available for boll production basically determines lint yield (Hearn, 1972; Hearn, 1994). This approach is explained by the nutritional hypothesis in conjunction with the hormonal balance within the cotton plant.

The nutritional hypothesis in combination with hormonal influences plays an important role on the changes in growth patterns during the cotton ontogeny, with a negative correlation between vegetative and reproductive growth (Guinn, 1986). Vegetative production on the main stem and reproductive branches, can continue indefinitely under favourable conditions because the cotton plant is indeterminate with no morphological limit to its size and development (Hearn and Constable, 1984). However, due to the demand on the resource supply by reproductive organs, the plant eventually stops producing new leaves and fruiting branches at a time which is called 'cut-out' (Hearn, 1994).

The hormonal hypothesis refers to the balance between auxins produced by the plant, and auxins inhibitors produced by the developing bolls which regulate the retention of fruit in cotton when fruit shedding is not determined by assimilates supply - demand relationship (Eaton and Rigler, 1945). Guinn (1998) also found that a balance between hormones can affect growth during the reproductive stages, with fruit retention being affected by growth promoting substances such as auxins and gibberellins.

Guinn (1998) concluded that the nutritional and hormonal theories are not contradictory or mutually exclusive. Consistent with this, much recent work has integrated these hypotheses, supporting the concept that assimilate supply is the primary regulator, with hormones playing an important role in the whole system, determining the time of cut-out and fruit shedding (Guinn, 1998; Mauney, 1986).

2.3.2 Flower bud production and shedding

Once the reproductive phase of growth has been initiated with the development of flower buds or squares, a number of factors can potentially affect the processes that determine flower bud number and boll retention which, in turn, can have a significant impact on lint yield (Guinn et al., 1981; Heitholt et al., 1992). About 400 degree days, using a 12 degree base, are necessary for the square to reach anthesis (Constable, 1991).

During the squaring stage, it is more likely that small flower buds will be shed, rather than larger and fully expanded squares, especially during the ten days before anthesis. Shedding during the early stages of squaring can be explained by two possible and conflicting hypotheses (Heitholt, 1999a). The first assumes that shedding of small squares is strictly due to biotic stresses such as that caused by insect damage, rather than being due to physiological causes. This hypothesis is supported by the assumption that small squares require a small assimilates supply, which is not a resource limitation during this early stage of development. The second hypothesis assumes that physiological, abiotic (Ungar et al., 1989) or biotic stresses (Sadras, 1996) can cause the shedding of small flower buds.

Constable (1981) concluded that older squares and flowers are less likely to be shed, as up to 50% of their assimilate requirement can be produced from the bracts of the flower buds. A similar conclusion was drawn for bolls older than 10 days.

2.3.3 Boll retention, distribution and yield

Boll retention and distribution within a plant play an important role in determining final yield, and is linked to the allocation of assimilates produced during vegetative growth by the plant. If the availability of assimilates is adequate to support the developing bolls, then the bolls will be retained (Constable, 1991; Jenkins et al., 1990a; Jenkins et al., 1990b). However, if the demand from growing bolls exceeds the assimilates supply, the retention of bolls will decline as a result of an increase in the number of boll abortions or shedding (Guinn, 1998; Mason, 1922). Nevertheless, the retention or otherwise of fruits is ultimately dependant on a number of physiological factors of greater complexity than the simple relationship between assimilates supply and demand (Constable, 1991; Jenkins et al., 1990a; Jenkins et al., 1990b).

At the stage of boll development, hormonal concentrations and factors involving assimilate supply are important, and can affect fruit retention (Hearn and Constable, 1984). Depending of the cultivar and growing conditions, the boll carrying capacity of the crop can be calculated as a function of photosynthetic capacity of the crop and the potential growth rate of the fruits (Hearn, 1994). Studies by Hearn (1984) estimated the carrying capacity of conventional cotton varieties to be about 100 fruit per meter of plant row.

The boll distribution within a plant is an important determinant of final yield. Under favourable environmental conditions, the first fruiting position of a fruiting branch produces the largest fruit in terms of size and number (Heitholt and Schmidt, 1994), while also having a significantly greater impact on final yield than other fruiting positions within the plant (Jenkins et al., 1990a). Kerby (1981) also found that bolls retained in the first position (position 1) reduced the fruit retention in position 2, resulting in a higher number of bolls being retained and more fruits in position 1 than for position 2. Jenkins (1990b) found that 70% of the total yield was produced from the first position on the fruiting branches of the plant. Boll size is generally largest in the first position on the fruiting branches in the middle part of the canopy of the plant (Jenkins et al., 1990a; Jenkins et al., 1990b). Consistent with this, the largest component of leaf area of the plant develops in about the same part of the canopy, increasing the proportion of assimilates supplied to fruit, while on the other hand leaves on lower part of the canopy export a greater part of their assimilates to the development of the root system.

The first fruiting position has a competitive advantage for assimilates over other fruiting positions (Constable and Rawson, 1980b; Wullschleger and Oosterhuis, 1990a; Wullschleger and Oosterhuis, 1990b). Bolls on the first position of a fruiting branch are higher sinks of assimilates close to the main stem with older leaves, compared with those further out on the branch (Kerby and Buxton, 1981). The solar radiation that can be intercepted is also different throughout the canopy, so the production of assimilates and competition for these assimilates also differs. The older leaves on the main stem and the subtending leaf have an advantage of being less shaded from leaves higher in the canopy (Constable and Rawson, 1980b) than leaves on second and third positions which develop later with less competitive advantage for accessing assimilates (Kerby and Buxton, 1981; Wullschleger and Oosterhuis, 1990b; Wullschleger and Oosterhuis, 1990c).

Compared with non-Bt cultivars, Bt cultivar has a shorter vegetative cycle and higher early fruit retention rate for the first and second positions on fruiting branches when availability of resources is favourable (Ahuja, 2006; Hofs et al., 2006).

Crops grown under higher solar radiation have a higher photosynthetic capability and assimilate more carbon, than those growing at lower solar radiation (Patterson et al., 1977). Environments with lower radiation levels (e.g. cloudy days) can directly affect the production of assimilate, with resulting reductions in both yield and fibre quality (Pettigrew, 1994). Guinn (1974) concluded from studies under controlled environmental conditions that, under low solar radiation, young bolls were more likely to be shed immediately due to reduced photosynthetic activity and hormonal action. Constable (1981) also concluded that the shedding of young bolls happens when the radiation levels decreased; even though the plant had enough assimilates to support growing bolls.

Many studies have been undertaken of the impact of modifications of canopy configuration to test whether the amount of radiation intercepted improves crop performance. Brown (1971) found increases in the shedding of squares and young bolls in experiments using narrower row spacing and higher plant population, due to a lower light flux density in the lower part of the canopy. A similar situation develops within the canopy as the crop grows, with newer leaves higher in the canopy shading older leaves. The net effect is that the older leaves may intercept lower levels of radiation, thereby reducing assimilate production and supply for growing bolls (Constable and Rawson, 1980a; Constable and Rawson, 1980b).

2.3.4 Fruit shedding and compensation

Cotton yield can be affected by the shedding of fruit, the magnitude and effect of which depends on when it happens, resulting in from moderate to severe yield loss (Sadras, 1995).

Four types of compensation responses were defined by Sadras (1995) based on the earlier research findings (Brook et al., 1992b; Hearn and Room, 1979; Kletter and Wallach, 1982a). One response is passive and instantaneous, in which the reproductive structures are damaged and shed physiologically. A second response is passive and time dependant, where the reproductive organs are supposed to be aborted but, instead, are retained to replace those previously damaged, causing a net delay in fruit setting. A third response is active and instantaneous, in which resources are partitioned to damaged organs instead of undamaged ones, increasing fruit weight but no increase in the number of fruiting sites. A fourth is active and time dependent in which the loss of reproductive organs prolongs flower bud production, increasing the rate of late flowering and number of fruiting sites. These four responses are neither mutually exclusive or easy to separate, but may be important from an agronomic perspective (e.g. for the determination of time to maturity) (Sadras, 1995).

Early fruit retention and growth may not be so critical in non-Bt cotton cultivars, due to cyclical compensatory growth of vegetative biomass and fruit in response to early loss of fruit caused by biotic or abiotic factors such as water deficit or insect attack (Sadras, 1996). Leaf area is one major variable affected by fruiting loss, extending the duration of canopy expansion and growth (Brook et al., 1992b).

2.4 Water relations of cotton plants

2.4.1 Influence of water supply on morphogenesis and phenological development

Water availability is potentially one of the most limiting factors to profitable cotton (*Gossipium hirsutum* L.) production. Cotton appears to be well adapted to the production of lint under a range of water regimes (Hearn, 1979), and is therefore able to be grown in areas throughout the world with variable rainfall and limited water for irrigation. However, adequate soil moisture through the correct timing of irrigation or precipitation events is essential for successful commercial production of cotton.

When the water supply is plentiful, phenological development continues for longer, resulting in larger plants and higher yields; when the supply is limiting the opposite occurs. The key adaptation of the cotton plant is that when water supply starts to becoming limiting, the plant responds and stops further morphological development and focuses on the maturation of fruit already set (Hearn, 1994).

Cotton plants respond to soil water deficits by reducing leaf area expansion (Constable, 1981; Gerik et al., 1996; Hearn, 1979; Turner et al., 1986). However, this response depends on the timing, duration and severity of the soil water deficit. For example, in a four year study reported by Constable (1981), it was found that leaf expansion was affected only after 60% of available soil moisture was depleted.

Hearn (1979) reported that in cotton the processes dependent on cell expansion, such us expansion of leaf area and increases in plant height, are more sensitive to water deficits than those associated with stomata closure, such us photosynthesis and transpiration. The effect of water stress on leaf area is to reduce the interception of photosynthetically active radiation (PAR), hence canopy photosynthesis (Ball et al., 1994; Ennahli and Earl, 2005; Turner et al., 1986). Radiation interception is directly affecting the production of photo-assimilates by leaves and a major determinant of crop growth and yield (Monteith, 1976). Light penetration and interception are important in cotton due to the earliest fruit production taking place on the lower branches of the plant in the bottom half of the canopy (Constable, 1986). The balance of production of biomass directly affects the source-sink relationship and partitioning of assimilates, thereby contributing to the timing of phenological stages of the crop growth, such as timing to cut-out. Generally, any reduction in biomass production in cotton decreases final yield.

2.4.2 Leaf area index, leaf shape and radiation interception

Constable (1977) investigated how both leaf area and crop growth are influenced by season, cultivar, row space and node position within the crop canopy, with special reference to the growth of the boll fraction. The studies were undertaken in the cotton growing area in the Namoi Valley in the state of New South Wales in Australia. He found that, during wetter than average growing seasons, the LAI was the highest and increased rapidly. A positive association was established between rapid vegetative growth and high rainfall. During the vegetative phase, a consistent positive association was found between LAI and crop growth rate for LAI values of less than 2. On the other hand, during the reproductive phase, the relationship between LAI and CGR changed; although still positive, there were lower CGR values for a given LAI. The decreased CGR during the reproductive phase was attributed to leaf ageing, causing a decline in photosynthesis, and a greater respiratory load.

Studies using okra leaf cotton cultivars have showed greater production of flowers (Wells and Meredith, 1986) and lower yield potential (Meredith, 1985) than achieved with normal leaf cultivars. It may be that the okra leaf cultivars have insufficient LAI to support boll growth during adverse growing conditions caused by water deficits. However, recent Australian studies have found the opposite with okra leaf cultivars. Stiller *et al.* (2004) evaluated the relationship between morphological and phenotypic characteristics, such as leaf type and maturity, on performance under dryland conditions, to develop breeding strategies for water stress situations. The okra leaf cultivars were higher yielding than normal leaf cultivars under most dryland experimental conditions. Full season okra leaf cultivars had the highest water use efficiency. Also, the strong positive association between crop maturity and lint yield suggests that the phenological plasticity of later maturing cultivars is an advantage under dryland conditions in Australia.

Pettigrew (2004c), working with eight cotton genotypes, including an okra leaf type near-isoline pair and transgenic lines paired with their recurrent parents in humid southeastern USA, found that drought stress reduced overall LAI by 35%, resulting in a 8% reduction in solar radiation interception. Similar results were reported by Gerik *et al.* (1996) from studies with two short-season cultivars in a rain shelter-lysimeter facility.

Increased early season light capture and growth in cotton before peak flowering and the boll filling stage, produces a larger canopy that can provide more assimilates to reproductive organs and can, in turn, result in higher yields (Heitholt et al., 1992).

2.4.3 Boll growth and retention

It is well documented that boll retention declines when the boll load is high and bolls compete for assimilates. However, after a period of limited assimilates supply, if the carbon supply is increased through enhanced photosynthesis, the crop will increase boll retention (Hearn and Daroza, 1985). Constable (1977) studied the relationship between LAI and boll growth rate (BGR) in different seasons and showed that a high LAI was required for a high early BGR.

Under stress conditions, with limited carbon available in the plant, preference is given to larger bolls, causing the smaller bolls to be shed. When water availability is favourable in the early stages of plant growth, and a large number of bolls are produced, the BGR have been found to significantly exceed CGR after 120 DAS, although some bolls are shed due to the limitation in assimilates supply (Hearn, 1972, Constable, 1977). For example, Constable (1977) found a 8% loss of bolls between 130 days and maturity in dry years.

Pettigrew (2004a) found differences in flower production with differing water relations. Flowering was primarily affected late (after 90 DAS) in the growing season, with plants in irrigated plots being found to consistently produce significantly more flowers per unit ground area than plants in the dryland plots. However, the plants in dryland plots had higher flowering rates early in the growing season relative to plants grown under irrigated conditions.

During the early stages of fruits growth, vegetative growth also occurs in other parts of the plant, resulting in competition for assimilates between lower bolls and upper leaves (Constable, 1977). Moisture deficit stress affects both the final number of bolls and their distribution. Pettigrew

(2004a) found that, under irrigated conditions, additional bolls produced are primarily located at higher plant nodes and in more distal positions on sympodial branches, resulting in higher yields than from stressed plants.

2.4.4 Root growth

Cotton root systems are capable of penetrating to a depth of 3m. Up to 80% of the root system of cotton plants may be developed by flowering time, imposing the greatest demand for excess carbohydrates during early plant growth. During boll development the rate of root expansion declines on account of competition with the bolls for assimilates (Constable, 1995).

Ball *et al.* (1994) studied the root growth dynamics in growth chambers under both well watered and stressed conditions. Under stressed conditions the number of growing roots was reduced to 50% of all roots, averaged across the upper and lower zones. Cotton rooting density decreased in a drying soil when the water content declined below a soil water potential of -0.1MPa.

As moisture levels decrease to the wilting point, the roots have difficulty in extracting water from the soil and plant demand cannot be satisfied. While in light soils the available moisture for roots is less than 30mm, for heavier clays it is greater than 100mm. Other factors such us soil compaction and soil structure affect the water availability at different depths. During periods of soil water deficit, the capability of crop roots to extract soil water is primarily dependent on the distribution and depth of the root systems.

2.5 Physiological responses to water stress

Water is essential for plant metabolism, at both cellular and whole plant levels, directly affecting plant growth and processes, ranging from photosynthesis to solute transportation and accumulation. However, plants have evolved physiological responses, of stress avoidance or stress tolerance, as well as ecological strategies, to cope with water deficits (Pugnaire, 1994).

2.5.1 Leaf water status

Experiments undertaken in Arkansas have shown that some indicators of water stress in cotton are leaf water potential, leaf expansion and canopy temperature (Wullschleger and Oosterhuis, 1990b; Wullschleger and Oosterhuis, 1990c). Leaf water potential is reduced under drought conditions, although the cotton plant has the ability to osmotically adjust and maintain a high leaf turgor potential (Nepomuceno et al., 1998).

Under water stress conditions, leaf water status declines as the result of water loss through transpiration exceeding water uptake from the soil (Boyer, 1985). Pettigrew (2004b), working in field experiments in the southeastern USA, found that water relations in leaves during late flowering were altered by soil moisture treatments. The afternoon water potentials were 36% more negative in leaves of plants grown under dryland conditions, than the leaves of plants grown in an irrigated environment. Turner (1986) studied the influence of soil water deficits on flowering, boll set and yield. In studies of the impact of water deficit stress during flowering, and from first flower to first pick and to final pick, the predawn leaf water potential was found to decrease to -2MPa, reducing photosynthesis and leaf expansion, affecting directly the carrying capacity of the crop and causing a carbohydrate shortage for boll growth. Further, in experiments conducted on a Wasco sandy loam and a Panoche clay loam in the San Joaquin Valley, California, USA, Grimes (1994) found that water stress reduced midday leaf water potential below -1.2 MPa. It was also found that that young boll abscission was initiated at -2.0 MPa, while reduced boll growth was observed when the leaf water potential declined below -2.3 MPa.

Water stress has been found to reduce leaf relative water content, leaf area and nitrate reductase activity, while increasing stomatal resistance, leaf temperature and leaf proline content, in an outdoor pot experiments, with cotton cv. SRT 1, when grown under a rain shelter in India (Singh and Sahay, 1992). Plants grown under water stress conditions have been found to exhibit a capacity to adjust to the depletion of available soil moisture through significant reductions in both stomatal conductance and transpiration rates (Zakaria, 1993; Singh, 1992; Faver, 1996).

2.5.2 Photosynthesis

Under severe stress levels, the rate of photosynthesis decreases during the middle of the day. These changes in photosynthesis with water deficits are associated with changes in leaf conductance (Hearn, 1994; Turner et al., 1986).

In field studies conducted under dryland and irrigated conditions, Pettigrew (2004b) showed that dryland cotton leaves had 6% greater CO_2 exchange rates (CER) and 9% higher photosystem II (PSII) quantum efficiency, than the leaves of irrigated plants during the morning. However, the water potential of dryland plants declined in the afternoon, with resulting lower CER than for the leaves of irrigated plants.

Water stress during flowering and fruit setting significantly reduce photosynthesis and increase photorespiration (Singh and Sahay, 1992 ; Turner et al., 1986). In addition, water stress reduces canopy expansion, resulting in a canopy of older leaves with lower photosynthetic ability (Peuch Suanzes, 1988; Rosenthal et al., 1987).

2.6 Nitrogen and its interaction with water stress

2.6.1 Nitrogen

The importance of mineral nutrition is briefly reviewed in relation to increasing final cotton yield in sustainable systems. Joham (1986) reviewed the importance of mineral nutrition in cotton and developed a mainly nutrient element balance hypothesis. According to this hypothesis, plant growth reflects mineral nutrition in two ways. The first is 'intensity', which is the concentration of nutrient elements in the plant tissues at different stages of crop growth. The second is 'balance', which refers to the relationship between essential nutrient concentrations. An optimum balance will produce a higher yield, while a 'less than optimum balance' can potentially affect yield.

Nitrogen is an essential element for plant growth and development. It is a component of many biomolecules including proteins, nucleic acids, amino acids, coenzymes, vitamins, and pigments, as well as being essential for photosynthesis and leaf development. The requirement for optimal cotton yields under different environment conditions can vary, reflecting the indeterminate growth habit of cotton and the complexity of N cycling in the soil (Gerik et al., 1998). In N fertilizer studies by Ockerby *et al.* (1993) at Emerald in Central Queensland on cracking clay soils (vertisols), LAI was found to increase linearly with crop N content, and then yield was linearly related to LAI. N deficiency had a direct impact on yield through decreasing leaf area expansion and CO_2 assimilation capacity (Reddy et al., 2004), the net result of which was the production of fiber of low quality (Read et al., 2006).

Many researchers have concluded that, in addition to the adverse effects of low levels of N (Gerik et al., 1994), higher than optimum levels of N (Pettigrew *et al.,* 2006) can also affect the performance of a cotton crop. Lower than optimum available N levels may affect the biomass production by reducing leaf area and photosynthesis, decreasing the amount of assimilates available for growing bolls. A reduction in N can also lead to earlier maturity, thereby decreasing yield. On the other hand, higher than optimum N levels can delay maturity due to excessive vegetative growth (Weir, 1996). It is therefore important to optimize the N application rate to reflect the soil characteristics and crop N needs.

Low N and K supply can significantly shorten the duration of boll growth by limiting leaf photosynthesis and hence, photosynthate supply for boll growth (Pettigrew, 1999, 2000 & 2003). Nutrients such as P and K can significantly affect assimilates and fruiting production (Joham, 1986). For all nutrients, it is important to quantify the uptake and export from the cotton systems, especially in high yielding crops in which the uptake may limit the productivity of future crops unless the nutrients removed are replaced (Rochester, 2007). The status of N is very important in transgenic cotton (Bt, glyphosate resistant; or both genes) because of the role of N in protein synthesis and metabolism. An alternation in the N status can potentially affect the expression of the transgenic trait. Rochester *et al.* (2006) conducted N related studies in New South Wales, using the new transgenic variety Bollgard ll. In response to the application of 150 kg N ha^{-1} as anhydrous ammonia it was found that the Bollgard II cultivar used nutrients more efficiently (N applied-N uptake ratio) than conventional cultivars, while the application of high levels of nutrients did not necessarily produce greater lint yields.

2.6.2 N-water relations

Nutrients are less mobile in a drying soil due to the pores between soil particles being replaced by air, and the pathway from the soil to the root surface is less direct than under saturated conditions. A low water potential in the soil as well as inside the plant, inhibits plant growth, reduces developmental activities of cells and tissues, decreases the uptake of essential nutrient elements, and causes a variety of morphological and biochemical modifications (Pessarakli, 2002). Nitrogen is one of the most widely limiting elements for crop production and, when plants are subjected to water stress, N uptake and utilization are likely to be more severely affected than for other mineral nutrient (Dubey, 2002). Plants growing in water stressed environments show reduced N uptake. The roots affected are unable to absorb $NO_{3,}$ due to decreased transpiration as a result of stomata closure (Shaner and Boyer, 1976). Under water stress conditions, roots reduce their uptake of nutrients from the soil due to reduced root activity and slower rates of ion diffusion and water movement. Plant recovery in response to irrigation is generally much faster under conditions of high fertility than under unfertilized conditions (Garg et al., 1990; Ockerby et al., 1993).

Fernandez (1996) evaluated cotton plant responses in terms of leaf area production and water relations when exposed to water and N deficits during pre-flowering stage. Leaf water status and leaf production were sensitive to soil water deficits, and showed an interaction with N deficits. Leaf turgidity declined faster in N starved plants than in N supplied plants, when plants were exposed to water deficits. Water and nitrogen deficits decreased the daily production of mainstem leaves, branch leaves and the final area of individual mainstem and branch leaves (Fernandez et al., 1996). When plants were exposed to water deficits, the leaf water potential declined, although the N status had no effect on the time course of this decline. However, Radin (1979) found that leaf water potential of low N plants under well watered conditions was 0.1 to 0.2 MPa lower than in high N plants; it was suggested that this reflected a greater resistance to water flow in the low N plants, possibly at the root level.

Both water stress and N deficits affect total biomass accumulation and partitioning in cotton (Hutmacher et al., 1995). Water stress alone and N deficit alone, have been found to inhibit the growth of leaves, petioles, and branches, but not growth of the stem. When both water and N were limiting, McConnell *et al.* (2004) found that both yield and plant growth was influenced more by irrigation than N fertilization. In years when drought conditions caused water stress and limited plant growth, dry-land cotton gave only a limited response to the N fertilization treatments.

2.7 Cotton yield responses to water stress

2.7.1 Water stress during early and late stages of crop growth

Depending of the timing of water stress, the growth of cotton can be potentially affected in different ways. While there is substantial evidence that soil water deficits during critical growing stages, such as reproductive stage, can significantly affect growth and yield (Kaur and Singh, 1992; Kock et al., 1990; Marur, 1991; Rosenthal et al., 1987; Turner et al., 1986), less is known about the impact of water availability during early growth, particularly for high retention cotton. Potentially it might be anticipated that there might be a greater impact on Bt cotton cultivars if there is a reduction in the availability of assimilates for the support of early growth and early fruiting demands. Early stress reduces sink capacity, and so, even if the plants have good water supply later, the reduced sink may become a limitation to achieving high yield.

Grimes *et al*. (1978) compared the effects of the first irrigation after sowing in experiments conducted on a Wasco sandy loam and a Panoche clay loam in the San Joaquin Valley, California, USA, and found that an early first irrigation extended the period of vegetative growth, delaying maturity. Water stress before flowering (40 days after sowing - DAS) was found to accelerate development and maturity, with the effect being greatest when the plants were also stressed at flowering/fruiting (60 DAS).

Pettigrew (2004a) found that early irrigation delayed cut-out, which occurs as a result of the slowing of vegetative growth due to strong reproductive demand for assimilate. This delay in maturity enabled those plants to sustain flowering later in the growing season relative to plants grown under non-irrigated conditions. This difference in plant development was demonstrated by more nodes above white flower (NAWF) in the irrigated than the dryland plots. Turner *et al.* (1986) also found that plants in water stressed treatments flowered 4.3 days earlier when compared with continuously irrigated conditions.

The consequences of water stress during the reproductive stages of cotton growth have been investigated over the past 30 years and are well documented. Some researchers found that water deficits are critical during the reproductive stages. Using a rain shelter to produce severe water stress, (Turner et al., 1986) studied the influence of soil water deficits on flowering, boll set and yield. In the water stress treatments during flowering, and from first flower to first pick, and to final pick, LAI, number of fruiting sites, number of bolls and lint yield, were all reduced when compared with the unstressed treatments. Compared with unstressed conditions, water stress around flowering also reduced the proportion of flowers that set bolls, with the number of bolls picked declining from 90 to 68 m^{-2} and resulting lint yield dropping from 205 g to 140 g m^{-2}.

The results of studies reported by Cook and Elizik (1993) also showed that water deficits after flowering reduced the total number of bolls and increased the shedding of fruiting buds and bolls.

Stressed plants shed double the number of fruiting buds compared with unstressed plants Comparing boll retentions in two regimes of water treatments (stress after flowering and full irrigation), boll retention of different cultivars was reduced by 20-21% in stress treatments.

Field studies in Orissa, India, with hybrid cotton varieties showed that yield and its attributes decreased more significantly in all varieties evaluated, in response to water stress imposed at flowering , when compared with other stages, indicating this stage is the most sensitive to drought conditions (Kar et al., 2001). Luz (1998) reported similar results from an evaluation of a number of varieties grown under Brazilian conditions. Using rain shelters, Rabey (1982) found that stress during flowering significantly reduced final yields, the level of the reduction being greater than when the plants were stressed at either the vegetative or boll-filling stages. Plant water stress during square formation and early flowering resulted in fewer bolls reaching maturity (Singh and Sahay, 1992), although the bolls were bigger and had greater lint growth (Kock et al., 1990).

On the other hand some people found larger impact of water stress during the vegetative period for conventional cotton varieties. Anac *et al.* (1999), working in Turkey with six levels of irrigations and three levels of nitrogen, found that the vegetative period of cotton should be given preference for irrigation over the other growth stages. Boll formation was the least affected stage of development, and it was concluded that omitting irrigation during this stage could potentially result in water savings of between 4.3 to 9.1%. In experiments conducted on a Wasco sandy loam and a Panoche clay loam in the San Joaquin Valley, USA, Grimes (1994) found that the expansive vegetative growth phase was more sensitive to water stress when compared with other stages of development, for Pima upland cultivars.

2.7.2 Water supply on early biomass production in high retention cotton

Under current Australian commercial cotton growing conditions, it is a common management practice to limit irrigation until flowering, thereby applying a subtle level of moisture stress. This irrigation management regime was designed for conventional varieties, which have lower boll weights at early flowering than do Bt transgenic varieties (Yeates et al., 2006). The main limitation of long periods of water stress on high retention cotton cultivars is the potential impact on the production of sufficient assimilates for the higher number of fruits retained, relative to conventional varieties.

Early fruit retention and growth may not be so critical in non-Bt cotton cultivars due to the cyclic compensatory growth of vegetative shoot and fruits, in response to the early loss of fruit caused by biotic or abiotic factors, such as low water and/or nutrient availability (Sadras, 1996). However, this compensatory mechanism seems to be weak in Bollgard cotton cultivar. Compared to non-Bt cultivars, Bt cultivars have a shorter vegetative cycle and higher early fruit retention rates at the first and second positions on the fruiting branches when the availability of irrigation and nutrients is high (Ahuja, 2006; Hofs et al., 2006). Cotton yield is dependent not only the total number of fruiting sites and fruit retention rates, but also the growth capacity of individual fruit. Inadequate resource availability (assimilates and nutrients) during early development of reproductive organs greatly limits the growth capacity of individual fruit (Stewart, 1986).

The high fruit retention of Bt cotton cultivars creates a high demand for the supply of assimilates and nutrients from the relatively small vegetative shoot biomass under conditions of moisture stress or low levels of available nutrients, particularly early in the season. The high fruit/vegetative shoot biomass ratio in Bt cotton can lead to imbalances between sink demand and source supply from the vegetative shoots. There is a linear relationship between the maximum vegetative shoot biomass and fruit growth rates in cotton plants with high levels of available water and nutrients (Sadras, 1996). As a result, studies are warranted of the mechanisms for up-regulating the pre-flowering vegetative shoot biomass for increasing plant's capacity to supply the assimilates and nutrients required for the early and high boll retention rates in Bt cotton, in order to realize its high yielding potential under irrigated conditions. Potential advantages associated with a relatively large vegetative shoot by the flowering stage may include a canopy ready for the intensive demand for assimilates for rapid and intensive fruit growth, including the high requirements of floral buds prior to anthesis (Zhao and Oosterhuis, 2000).

The higher sink demands of water stressed smaller plants until squaring or flowering, may risk early cut-out and reduce yields in Bollgard II crops. However, it may be possible to manipulate water supplies in the period before flowering, to increase the vegetative biomass for increasing the provision of assimilates for the development and maturation of the early bolls, and thereby increase the yield potential of Bt crops.

Chapter 3 The effect of pre-flowering soil water deficits on fruit retention, boll distribution and yield of high retention cotton

3.1 Abstract

While the current practice of irrigation water management appears suitable for traditional low retention cotton varieties in Australia, it is unclear whether manipulation of water prior to flowering to increase the vegetative biomass for enhanced provision of assimilates for the development and maturation of bolls can maximize the yield potential of high retention Bt varieties. Two years of experiments with the Bollgard ll variety Sicot 71BR (Bt cotton producing two insecticidal Cry proteins) were conducted at Gatton in Southeast Queensland, Australia, to determine whether differences in early soil water deficits impacted on fruit production, fruit retention, boll distribution, seed cotton and lint yield, in high retention cotton. The water treatments included: (i) irrigation (I) over the whole crop growth; water stress periods with (ii) no irrigation until squaring (NIS) followed by irrigation; and (iii) no irrigation until flowering (NIF) followed by irrigation until the end of crop growth.

Even modest early soil water deficits affected lint yield and yield components in high retention cotton. Greater pre-flowering water availability had a significant increase in production and retention of boll load and the bolls were set at higher node positions. Decreased number of reproductive organs was associated with the duration and severity of the stress period. NIF with a longer stress period than NIS, produced a smaller number of reproductive organs in all four experiments. The level of fruit retention was 85 - 92% for all treatments at early flowering stage and decreased to 65 - 68% by the irrigated treatment and 53 - 59% in the stressed treatments at the time of crop maturity. Early sowing date in the first season (Exp.1) was associated with better

recovery after the period of stress, relative to the late sowing stressed treatments (Exp.3). The NIS and NIF treatments, when associated with early sowing, had a smaller yield reduction (7 - 20%), compared to late sowing (41 – 44% reduction).

These results show the advantages of increased early water availability for high levels of fruit retention and yield in high retention Bt cotton.

3.2 Introduction

Cotton (*Gossipium hirsutum* L.) is attacked by a range of insect pests, the most significant of which is the larvae of *Helicoverpa* spp (Fitt and Wilson, 2000). These larvae feed on the developing fruit (flower buds or squares and bolls), causing them to shed. This reduces fruit retention, especially early in the season, and delays cut-out, the point at which the boll load (sink) is high enough that their demand for fruit equals assimilates supply from the photosynthates and vegetative biomass of plants (source) and the plant essentially ceases to set more bolls (Hearn 1972, Hearn 1994). Bt transgenic cotton (Bollgard II™®) contains two genes from *Bacillus thuringiensis* var *kurstaki* (Bt), producing the Monsanto Cry1Ac and Cry2AB proteins that are toxic to some key lepidopteron pests. Bollgard II also provides additional mechanisms to set earlier fruiting structures, increasing fruit retention and earlier cut-out (Mills et al., 2008). Some comparisons of Bt and non-Bt lines have shown yield advantages in favor to transgenic varieties, most of them on Bollgard with only one gene from Bt expressing the endotoxin (Hofs et al., 2006; Mills et al., 2008).

Water availability is one of the most limiting factors to profitable cotton production. From wild cotton lines to modern cotton varieties, adequate soil moisture through correct timing of irrigation or precipitation events is essential for a successful production of cotton (Hearn, 1992). Cotton yield is dependent on boll number and their size. Inadequate resource availability, such us soil water deficit, during early development of reproductive organs greatly limits the growth capacity of individual bolls (Stewart, 1986). Low early fruit retention may not be so critical in non-Bt cotton cultivars, due to the cyclic compensatory growth of vegetative shoot and fruit in response to the early loss of fruit caused by biotic or abiotic factors (e.g low water availability) (Sadras, 1996). However, this compensatory mechanism seems to be weak in Bollgard cotton varieties. Compared to non-Bt varieties, Bt varieties have a shorter vegetative cycle and higher early fruit retention rates at the first and second positions on the fruiting branches with the full availability of irrigation water and nutrients (Ahuja, 2006; Hofs et al., 2006).

The enhanced efficacy of the Bollgard II varieties to caterpillar pests has led to very high early fruit retention in Australian cotton crops (Yeates et al., 2006). Such high levels of retention and the subsequent early development of the fruit load may restrict plant canopy development and subsequently yield potential. In particular, there is concern that the high retention rates may limit maximum potential yield through an early cut-out, or result in higher levels of susceptibility to premature senescence. Depending of the timing of water stress, cotton growth can be affected in different ways. While there is substantial evidence that soil water deficit during critical growth stages, such as the reproductive stage, can significantly affect crop growth and final yields (Kaur and Singh, 1992; Kock et al., 1990; Marur, 1991; Rosenthal et al., 1987; Turner et al., 1986), less is known about the potential impact of variation in water availability early in the growth of the crop, particularly for high retention cotton. Early stress with resultant smaller leaf canopy might potentially reduce plants capacity to supply assimilates to developing reproductive organs, and even if the plants have a good water supply later, the reduced assimilates source may limit yield in high retention cotton. A potential approach to reduce this impact would be to increase potential boll number and increase canopy size through increased early water availability and to meet the higher demand for assimilates from the higher early fruit retention during the latter part of growth. Therefore increased early water availability would result in an increase in fruit retention and final cotton yield.

The main objective of the work reported here was to determine whether differences in pre-flowering soil water deficits impacted on site and fruit production, fruit retention, boll distribution, seed cotton and lint yields, in high retention cotton.

3.3 Materials and Methods

3.3.1 Experimental sites and growing conditions

Four experiments were conducted over two years (Exp.1 from October 2006 to March 2007, Exp.2 and Exp.3 from November 2006 to April 2007, and Exp.4 from October 2007 to March 2008) at the Gatton campus of the University of Queensland (91m, 27^{o}33'S, 152^{o}20'E), in the Lockyer Valley in Southeast Queensland, Australia. The soil in the area where the experiments were undertaken is a Lawes clay loam (Powel, 1982), with heavy dark cracking clays, black vertosol. Average annual rainfall is 760 mm with a summer dominance; evaporation rates are high, almost double the average rainfall.

3.3.2 Cultural practices

The Bt transgenic Bollgard ll®™ variety Sicot 71BR (producing the Monsanto Cry1Ac and Cry2AB proteins) was sown in all the experiments. Experiments 1, 3 and 4 were sown using a Nodet Gougis vacuum planter, while Experiment 2 was sown by hand. High seeding rates were used, with seedling number then being reduced to obtain the target population of plant density of 140,000 plants ha^{-1} (12-15 plants m^{-1} with row spacing of 1 m). The land was prepared a month before sowing using conventional tillage practices. The plots were fertilized with 100 kg ha^{-1} of N spread on surface at sowing. Herbicides were used for weed control, pendimethilin being applied pre-planting and glyphosate post-emergence. Insects were controlled through regular monitoring of the crop and strategic insecticide applications based on thresholds derived for cotton in temperate Australia (Farrell 2006).

3.3.3 Experimental design and water deficit treatments

Irrigation water was applied using overhead sprinklers, based on the following schedules for the different treatments:

I (Irrigation throughout the growth): Irrigation was applied to meet the water requirements for a cotton crop, calculated as the product of daily class "A" pan evaporation by a crop coefficient depending on the phenological stage of the crop (CRDC, 2003).

NIS (No irrigation until squaring): No water applied from establishment to squaring (water stress period), followed by irrigation through to maturity.

NIF (No irrigation until flowering): No water from establishment to flowering (water stress period) and then irrigation through to maturity.

3.3.3.1 Experiment 1

The experiment was sown on 6th October 2006. Plots were 100 m^2 (10 rows, 10m in length) with a 1m row spacing with sufficient buffer areas to ensure that there was no lateral water movement between plots. The total area of the experiment was 1600 m^2. The experiment employed a randomized complete block design with four replications.

Water stress was achieved in the non-watered treatments by intercepting rainfall with the use of plastic covers which were placed on the ground between the rows within 1cm of the plant stems, with the covers then being secured using wire pegs. The water stress treatments were covered from the two first true leaves up to beginning of squaring (**NIS**) and beginning of flowering (**NIF**). The covers were removed when the treatments periods were finished and irrigation commenced.

To ensure uniform plant establishment, every plot received 25 mm of irrigation water immediately after sowing. The number of irrigations from sowing to maturity was 10, 6 and 4 for I, NIS and NIF, respectively, with an average of 30 mm being applied at each irrigation.

3.3.3.2 Experiment 2

The experiment was sown on 16th November 2006. An automatic rainout shelter was used to ensure the exclusion of rainfall. The area of each rainout shelter was 140 m^2. Every plot received the same volume of irrigation water immediately after sowing (50mm) for plant establishment. An overhead sprinkler system was used for irrigation. The number of irrigations was 6 and 4 for NIS and NIF, respectively.

3.3.3.3 Experiment 3

The experiment was sown on 21st November 2006. The experimental design and methodology were the same as described for Exp.1. The number of irrigations was 5 and 3 for NIS and NIF, respectively

3.3.3.4 Experiment 4

The experiment was sown on 16th October 2007. The experimental design and methodology were the same as described for Exp.1. The area used for the experiment was 2400 m^2. For Exp.4, due to the higher rainfall in the second season, the number of irrigations after the stress period was reduced to 3 and 1 for NIS and NIF, respectively.

3.3.4 Measurements

3.3.4.1 Meteorological conditions and soil water

Daily temperature, relative humidity, precipitation, pan evaporation and solar radiation were measured in a weather station adjacent to the experimental field. Volumetric soil water content was measured periodically using a neutron probe calibrated in the experimental fields. A 2 m long x 50 mm diameter access tube was placed within a row at the center of each plot. Measurements were made at soil depths of 30, 50, 70, 90, 110, 130, 150 and 170 cm.

3.3.4.2 Mapping of fruit retention

The dynamics of reproductive organ development in cotton plants was studied in the experiments. One of the most important components is fruiting site production (which is the total number of fruiting sites produced per plant including sites with fruits and abortions) and retention of fruits. Mapping of fruit retention was undertaken for the different phenological stages of crop development (flowering, cut-out and maturity) on a 1 m row (Kerby and Hake, 1996). Vegetative branches were not included in the study.

The retention rates in three different fruiting positions on branches were studied - FS1, the first position closest to the main stem; FS2, the position adjacent to FS1; and FS3+, FS3 and beyond, a position further out on the branch. The distribution of retention rates for fruiting sites on the vertical positions (nodes) of the plant was collected only during the second season (2007/2008) in Exp.4.

3.3.4.3 Lint yield

To measure lint yield, all open bolls from 5 m^2 (Exps.1, 2 and 3) and 4 m^2 in Exp.4, were hand-picked in each plot. For Exps.1 to 3, this sampling commenced when about 60% of bolls had opened (bolls were defined as having opened when two sutures on the boll had dehisced) and continued weekly until last boll had opened. In Exp.4 there was only one hand picking about the time most bolls had opened.

3.3.4.4 Fibre quality

The seed cotton samples were ginned using facilities of the Department of Primary Industries (DPI) in Toowoomba, Southeast Queensland. Fiber quality was tested using High Volume Instrumentation (HVI) based on 300 g sub-samples of lint from each plot.

3.4 Results

3.4.1 Meteorological conditions

Daily maximum and minimum temperatures, solar radiation and rainfall are illustrated in Figures 3.1a, 3.1b and 3.1c, respectively. Mean maximum air temperature was 31.1^{o}C and mean minimum was 16.8^{o}C during 2006/07, while in 2007/08 they were much lower at 28.7^{o}C and 15.5^{o}C, respectively. Cumulative solar radiation, cumulative degree days and total rainfall during the period of each experiment are summarized in Table 3.1.

During 2007/08, more rain was recorded in terms of absolute amount and frequency, than in 2006/07. During the period of water stress, the rainout shelters and plastic covers were effective in preventing water infiltration into the soil from the rainfall.

Table 3.1 Cumulative degree days, mean maximum and minimum temperatures, total rainfall and cumulative solar radiation during the period of the four experiments at Gatton, SE QLD. Base temperature of 12^{0}C is used (Constable and Shaw, 1988)

Variable	Exp1	Exp2	Exp3	Exp4
Cumulative degree days	2099	1854	1816	2163
Mean maximum temperature (^{0}C)	31.1	31.6	31.8	28.7
Mean minimum temperature (^{0}C)	16.2	16.7	16.8	15.5
Total rainfall (mm)	278	237	236	606
Cumulative solar radiation (MJ m^{-2})	4130	3464	3304	3544

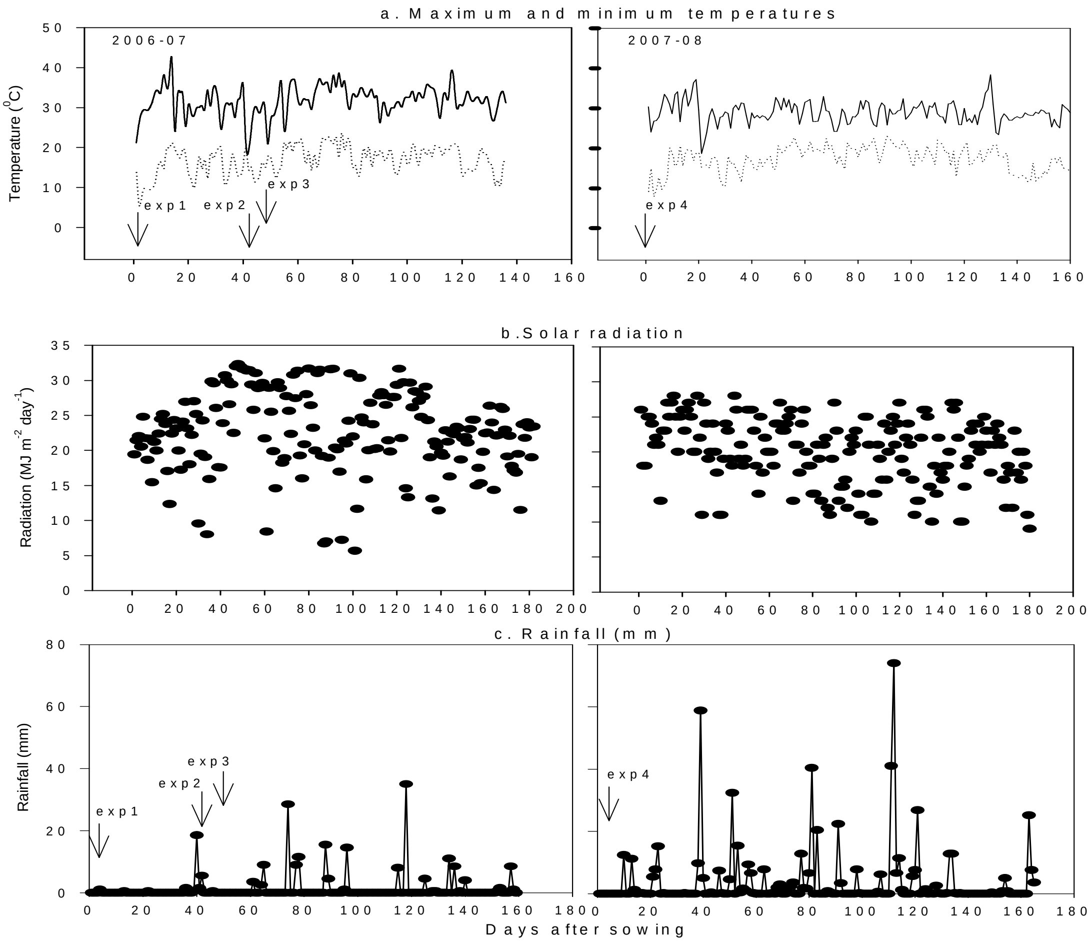

Figure 3.1 Daily minimum and maximum temperatures (^{o}C), (b) daily incident solar radiation (MJ m^{-2} day^{-1}) and (c) rainfall (mm) at Gatton, during 2006-07 (left) and 2007-08 (right). Arrows indicate the sowing date for each experiment

3.4.2 Water received and soil water extracted at termination of stress

Fig. 3.2 shows that the stress period (no inputs of water) for the NIS and NIF treatment in Exp.1 was from 14 to 65 and 91 days-after-sowing (DAS), respectively. Similarly, the duration of the water stress period for Exp.2 and 3, was respectively from 11 to 51 and 83 DAS and from 9 to 58 and 78 DAS in NIS and NIF. However, the water stress period was slightly shorter in Exp.4, NIS being 35 days and NIF being 58 days.

Considering the difference in degree of severity between the stress treatments, the difference in water input between NIS and NIF in Exp.2 in 2006/2007 was the largest (169 mm). The difference between the NIS and NIF treatments in Exp.3 was less pronounced, being only 89 mm. The total amount of water received by the crop in Exp.1 (mainly from irrigation) for the I treatment was 627 mm, while for Exp.2 and Exp.3, the total amount of water received from irrigation and rainfall in the same treatment was 610 and 620 mm respectively.

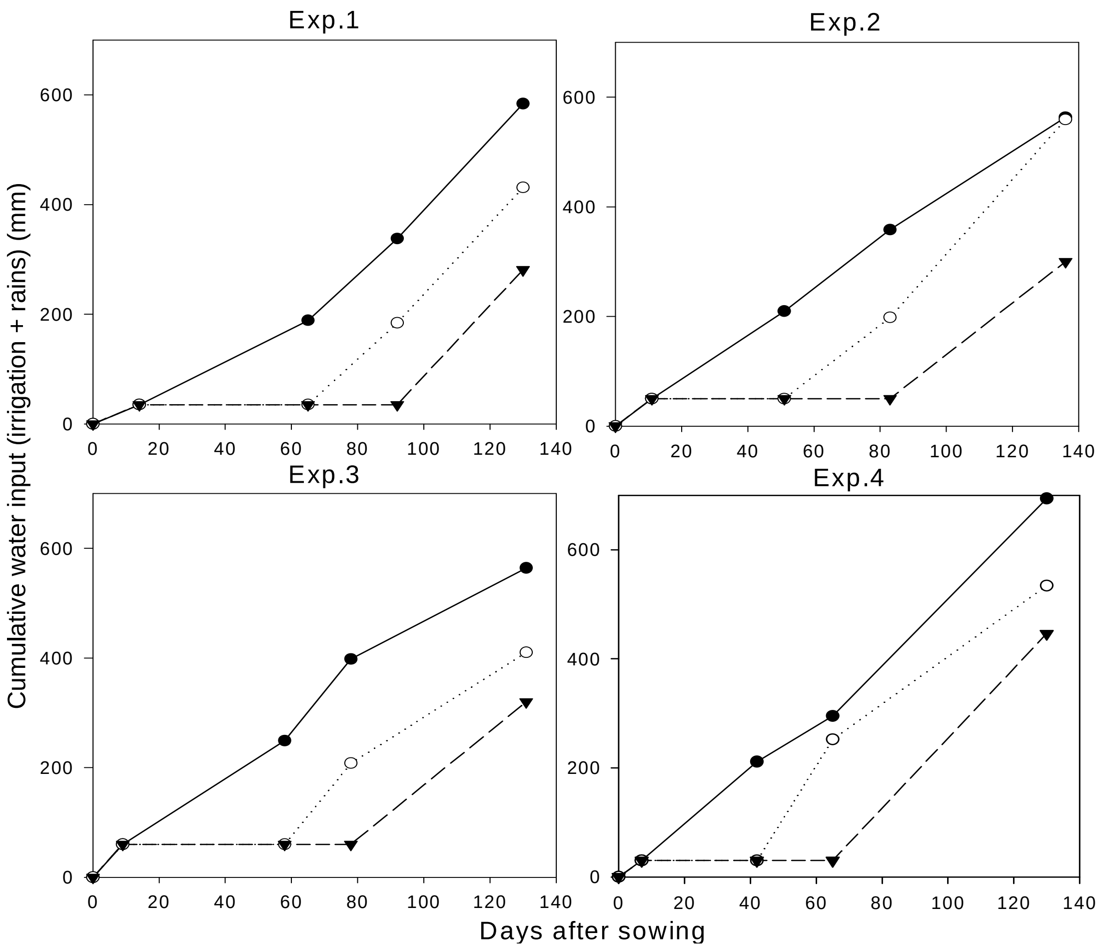

Figure 3.2 Cumulative water input (irrigation and rainfall) for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) during 2006/07 (Exp. 1, 2 & 3) and 2007/08 (Exp. 4) at Gatton, SE Queensland

During the second season (2007/2008), most of the water applied to the crop was in the form of rain (614 mm), with the total water supply being 694 mm in the I treatment.

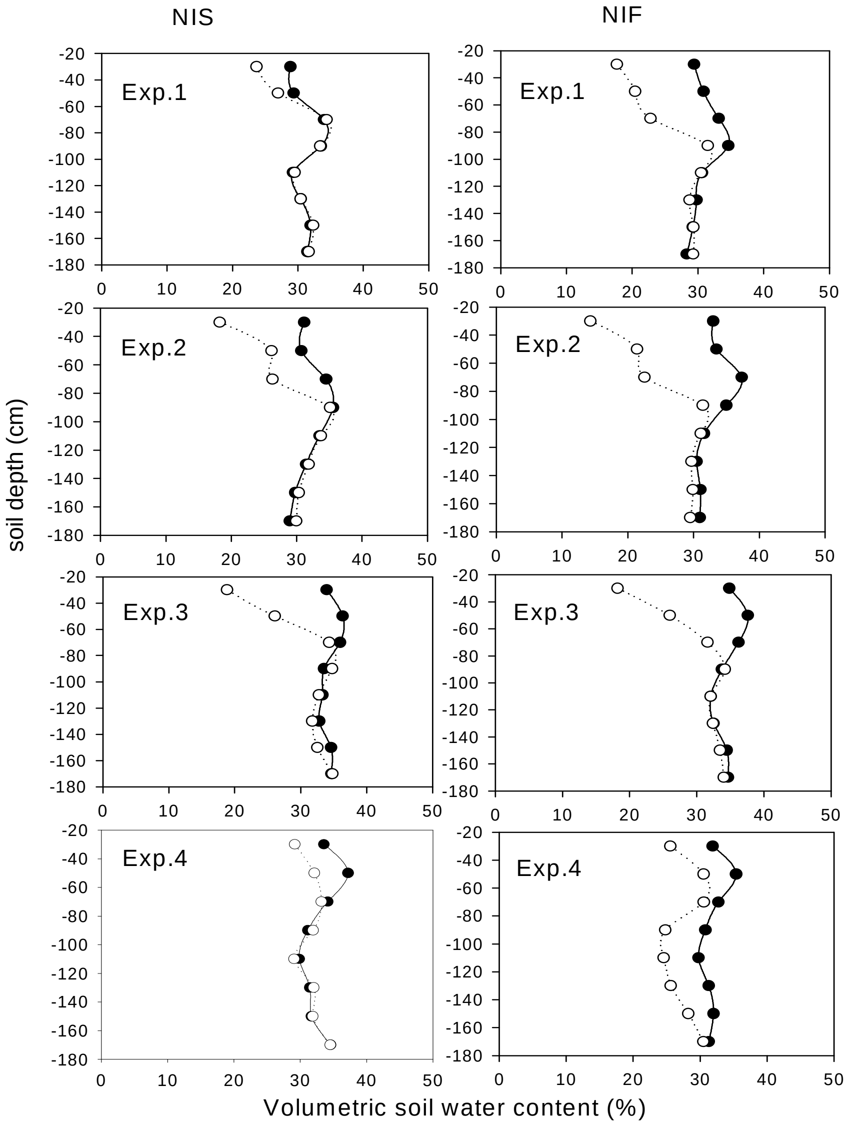

Figure 3.3 Volumetric soil water content at different depths at the beginning (●) and at the end (○) of the stress period for NIS (no irrigation until squaring) and NIF (no irrigation until flowering) during 2006/07 (Exp. 1, 2 & 3) and 2007/08 (Exp. 4) at Gatton, SE Queensland.

Volumetric soil water content at the beginning and the end of the stress periods are shown in Fig.3.3. At the beginning of the stress periods, all the treatments started with a similar volumetric water content, which then declined with the increasing duration of stress, to reach deficits of about 44% and 50% of estimated total crop available water (for 20-180cm soil layer) in NIS and NIF treatments, respectively, when compared with the irrigated treatment (I) (considered as

100%). At the beginning of the treatment period, the soil moisture content was close to field capacity at all depths. During the first season (2006/2007), in the NIS treatment the top layers (top 30 cm depth) dropped below wilting point (WP) in Exp.2 and Exp.3, while in the NIF treatment it dropped below WP to a depth of 70 cm in Exp.1 and Exp.2. However, below a depth of 90 cm, the soil moisture content was similar at the beginning and the end of the stress period, indicating that water extraction at these depths was relatively small and that the root system had developed mainly above this level. During the second season (2007/2008; Exp.4) the extraction in soil moisture content between beginning and end of the stress period took place at depths below 70 cm in NIF.

3.4.3 Squares, flowers and bolls number

Total number of squares plus flowers, green bolls and open bolls were determined at key stages of the crop development in each experiment (Fig. 3.4 and Fig.3.5).

In Exp.1, the full irrigation treatment (I) developed a significantly higher number of squares and flowers than NIS and NIF at 91 ($P <0.001$) and 120 DAS ($P = 0.013$). Similarly in Exp.2 and Exp.3, the total squares and flowers was higher for I relative to the water stress treatments between 89 and 120 DAS, while in Exp.4 the higher production for I was only around 80 DAS, with differences later in the season not being significant among the treatments. In all the experiments, the significantly higher production of squares and flowers was translated into a greater peak number of green bolls around 120 DAS for I, when compared with NIS and NIF. However, in all the experiments, early boll production was smaller in I, particularly in Exp.4 (80DAS). For all the experiments, the green boll load commenced earlier for NIF, followed by NIS and I. The number of open bolls was similar until about 140DAS in Exp.1-3, and was slightly less in I than in the stress treatments at 155DAS in Exp.4. However, as the crops approached maturity, I produced more open bolls than NIS and NIF did in all experiments.

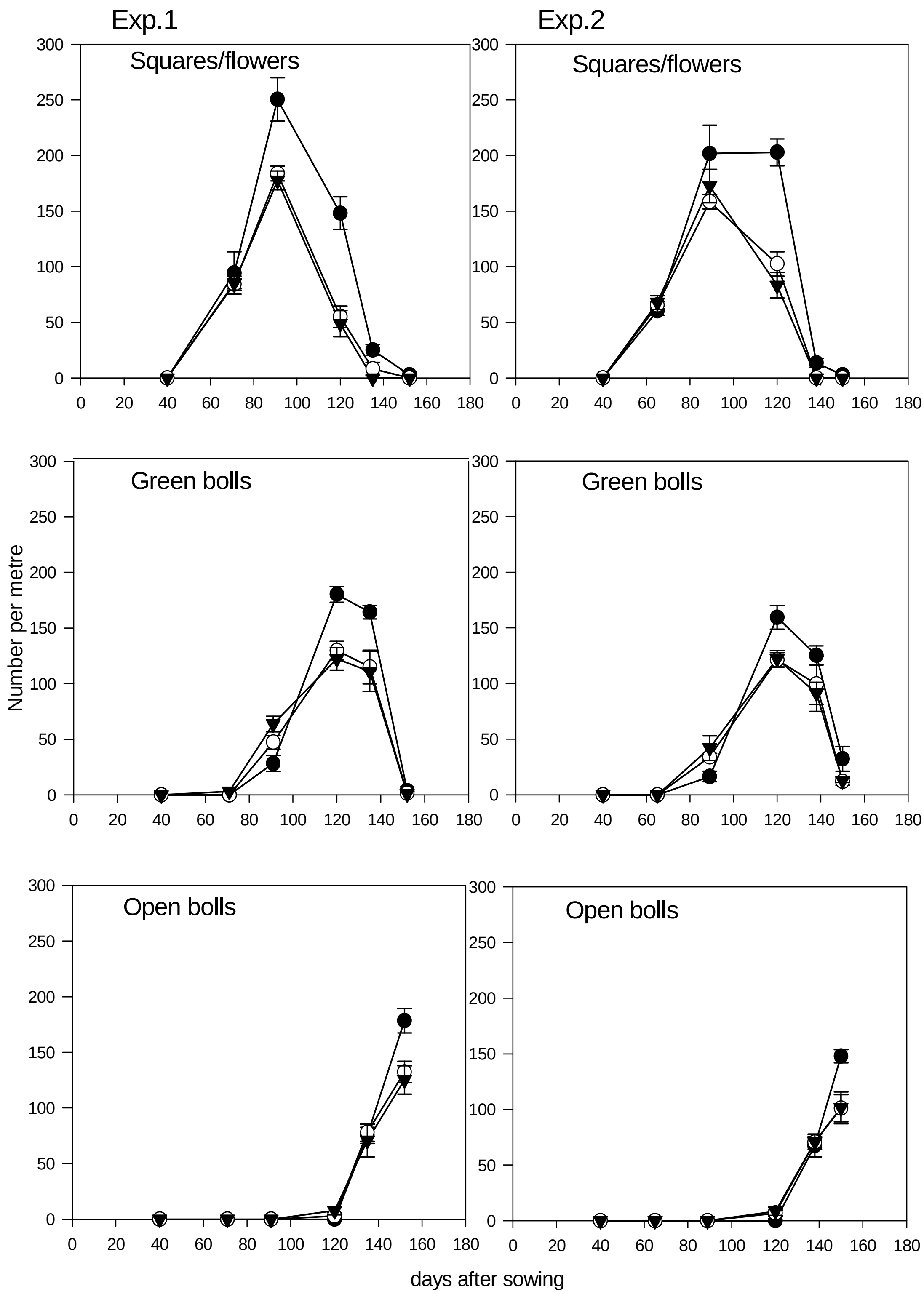

Figure 3.4 Change in number of squares and flowers, number of bolls and number of open bolls for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) for Exp. 1 and Exp. 2. Bars are two standard errors of the mean.

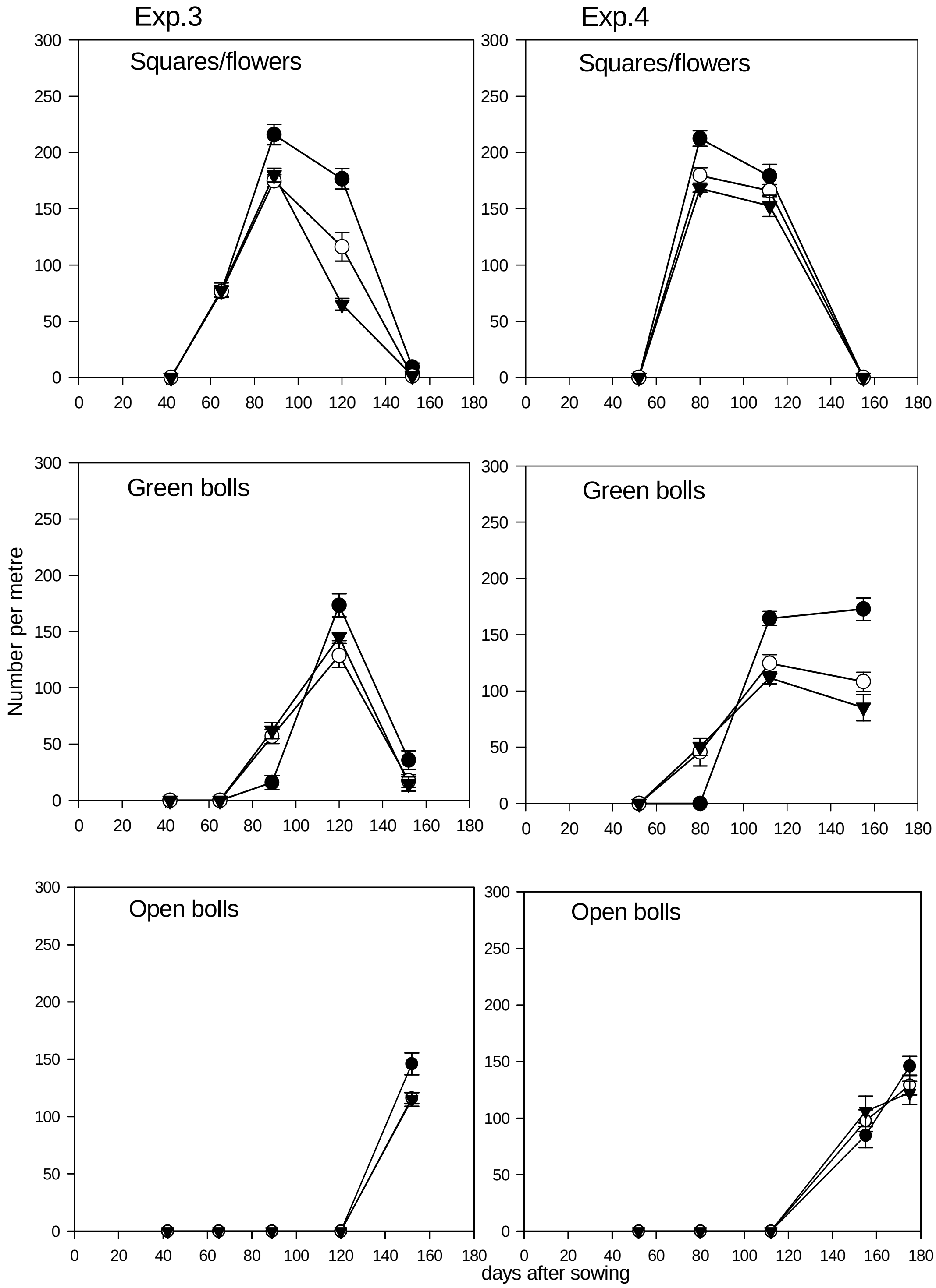

Figure 3.5 Change in number of squares and flowers, number of bolls and number of open bolls for I (irrigated)(●), NIS (no irrigation until squaring)(○) and NIF (no irrigation until flowering)(▼) for Exp. 3 and Exp. 4. Bars are two standard errors of the mean.

3.4.4 Height - node production and total fruit retention

Changes in plant height and main stem node production (i.e. potential site production) during growth are summarized in Table 3.2.

In Exp.1 there was no significant difference between the treatments in plant height early in the growth (to about 51 DAS), but significant differences in plant height were measured by 71 DAS when compared with NIS and NIF. The number of nodes produced was only different late in the growth. The NIS and NIF treatments stopped the production of new nodes with fruiting branches on the main stem, earlier than was the case for I.

A similar trend was recorded in Exp.2 and Exp.3, in which the production of nodes on the main stem, height and H/N ratio was significantly higher for I than for the water stress treatments later in the growth.

During the second year of experimentation (Exp.4), with higher rainfall, plants were taller and had more nodes particularly later in the growth. The trend in production of potential fruiting sites was also greater in the I treatment, relative to the early stress treatments.

The height to node ratio is used to define the balance between vegetative and reproductive structures. The height to node ratios (H/NR) were significantly higher throughout the season in the I treatment, followed by NIS and NIF, respectively. Water stress developed during the early stages significantly reduced internodes elongation during the later stages.

The fraction of flower/fruit retained decreased as the crop matured (Fig. 3.6). In Exp.1, there were no significant differences in flower retention between treatments at early flowering stage, but higher retention was recorded in the I treatment for mid (105 DAS) and late (130 DAS) phases of crop growth. Similar trends were found in Exp.2 and Exp.3, with significantly higher retention rates for I at 127 and 138 DAS, respectively. In Exp.4, the fruit retention showed a significant decrease in response to the stress treatments relative to the I treatment at 112 (P<0.001) and 145 (P<0.001) DAS.

Table 3.2 Changes with time in plant height (H), number of nodes (N) and their ratio (H/N) determined for three treatments in each of four experiments (Exp.1, 2, 3 and 4) (I-irrigated, NIS-no irrigation until squaring and NIF-no irrigation until flowering).

Exp1				**Exp2**		
	H (cm)	**N**	**H/N**	**H (cm)**	**N**	**H/N**
	51 DAS			*43 DAS*		
I	51.4	11.0	4.70	30.0	8.50	3.53
NIS	41.0	11.4	3.60	28.5	9.50	2.97
NIF	41.8	11.4	3.66	27.7	9.50	2.93
Significance	NS	NS	**	NS	NS	**
	71 DAS			*64 DAS*		
I	101.3	14.5	6.95	89.5	15.7	5.68
NIS	65.0	13.7	4.72	71.8	16.2	4.41
NIF	53.3	13.0	4.09	56.0	14.2	3.93
Significance	**	NS	**	**	*	*
	130 DAS			*127 DAS*		
I	128.0	24.0	5.33	108.0	20.5	5.26
NIS	81.0	21.5	3.76	74.8	17.2	4.33
NIF	67.5	19.0	3.55	61.8	15.7	3.92
Significance	**	**	**	**	**	**

Exp3				**Exp4**		
	H (cm)	**N**	**H/N**	**H (cm)**	**N**	**H/N**
Treatment	*51 DAS*			*51 DAS*		
I	29.0	8.7	3.29	37.0	11.1	3.31
NIS	25.8	9.2	2.79	30.6	11.0	2.78
NIF	25.5	9.2	2.76	27.0	11.0	2.45
Significance	NS	NS	NS	**	NS	*
	84 DAS			*82 DAS*		
I	85.4	14.6	5.82	117.5	17.1	6.84
NIS	69.8	13.5	5.22	106.7	15.6	6.80
NIF	66.2	13.5	4.91	96.7	15.0	6.41
Significance	**	NS	*	*	*	*
	138 DAS			*145 DAS*		
I	113.8	21.2	5.35	142.5	20.2	7.03
NIS	87.2	18.0	4.85	123.7	20.0	6.18
NIF	76.8	16.5	4.65	110.6	20	5.53
Significance	**	**	**	**	*	**
				175 DAS		
				166.4	29.7	5.59
				134.2	28.2	4.75
				124.2	26.0	4.77
				**	*	*

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

Fruit retention at the time of final harvest in each experiment was used for analysis of reproductive responses in the following section.

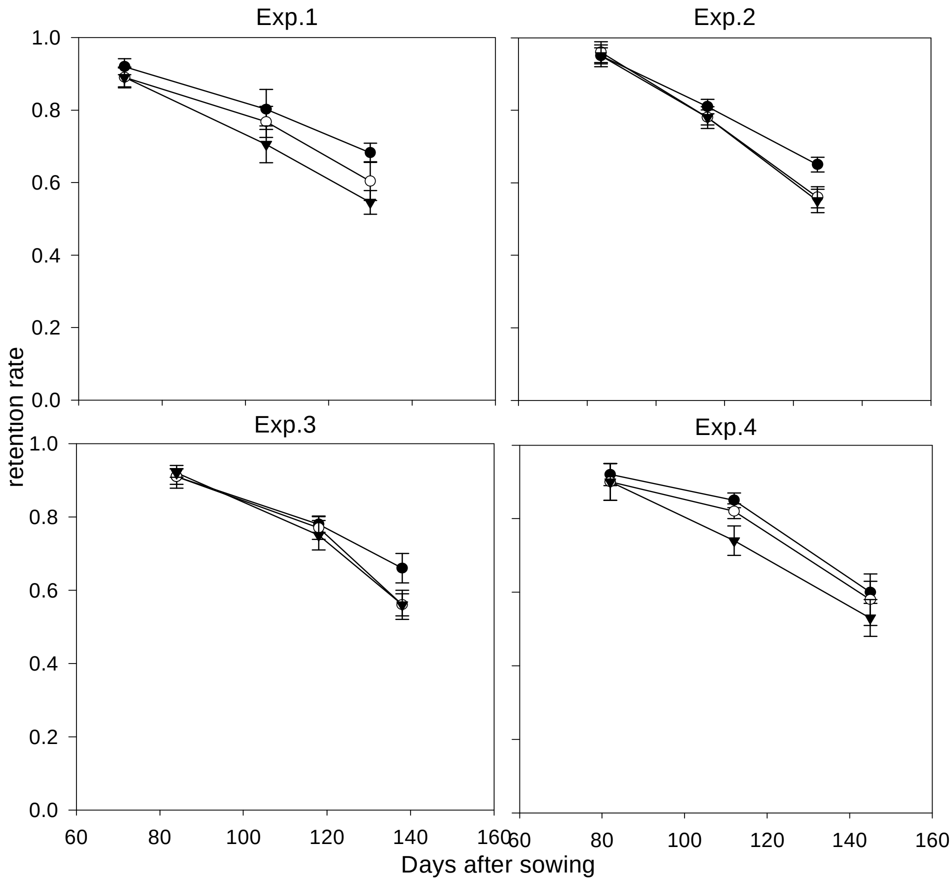

Figure 3.6 Change in total retention rates over the growing season in I (irrigated)(●), NIS (no irrigation until squaring)(○) and NIF (no irrigation until flowering)(▼) during 2006/07 (Exp.1, 2 & 3) and 2007/08 (Exp.4). Bars are two standard errors of the mean.

3.4.5 Dynamics of reproductive organ development

3.4.5.1 Total fruiting sites and final retention at different lateral fruiting positions.

The total number of fruits (TFN) was consistently higher for I, relative to the NIS and NIF treatments, in all experiments (Table 3.3).

Fruit retention rate was higher in the FS1 sites than FS2 and FS3+, for all water treatments in all four experiments. In FS1 sites, early water stress reduced the retention. However, fruit retention for position FS2 was greater in the water stressed treatments (NIS and NIF) than in the I treatment, although this difference was not significant in Exp.2 and 3. Retention rate at FS3+ was significantly higher in I than in the stress treatments in Exp.1, 3 and 4.

Table 3.3 Final fruit retention rates in three different lateral fruiting sites (FS 1, 2 and 3+) and total fruits number (TFN) per plant for I (irrigation), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) at maturity for four experiments during 2006/07 (Exp.1, 2 & 3) and 2007/08 (Exp.4).

		Retention					**Retention**		
Exp1	**TFN**	**FS 1**	**FS 2**	**FS 3+**	**Exp2**	**TFN**	**FS 1**	**FS 2**	**FS 3+**
I	21.0	0.752	0.603	0.422	**I**	17.6	0.721	0.452	0.322
NIS	16.3	0.633	0.663	0.355	**NIS**	14.6	0.630	0.461	0.222
NIF	14.4	0.612	0.688	0.241	**NIF**	12.1	0.628	0.469	0.288
Significance	**	**	*	*	**Significance**	**	*	NS	NS
		Retention					**Retention**		
Exp3	**TFN**	**FS 1**	**FS 2**	**FS 3+**	**Exp4**	**TFN**	**FS 1**	**FS 2**	**FS 3+**
I	19.5	0.730	0.411	0.406	**I**	23.8	0.775	0.485	0.399
NIS	16.7	0.677	0.417	0.260	**NIS**	14.5	0.680	0.496	0.460
NIF	16.0	0.653	0.415	0.263	**NIF**	12.5	0.682	0.546	0.219
Significance	*	*	NS	*	**Significance**	*	**	*	*

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

3.4.5.2 Retention at different vertical fruiting positions

The distribution of retention rates for fruiting sites on the vertical positions of the plant for Exp.4 is illustrated in Figure 3.7.

For I, the highest rate of fruit retention of 0.6 - 0.7 occurred between node 10 to node 23, with lower rates of retention on the lower (7 to 9) and upper (above 23) nodes.

For the NIS treatment, the retention rate increased from node 6 to node 12, with a retention rate of 0.6 or greater being maintained until node 18, after which there was a decline in the retention rate from nodes 19 to 21. The retention rate from lower fruiting nodes in the NIF treatment was higher than in the I and NIS treatments, but the NIF plants stopped the retention of fruit at lower node positions (around node 16) than for the I and NIS treatments.

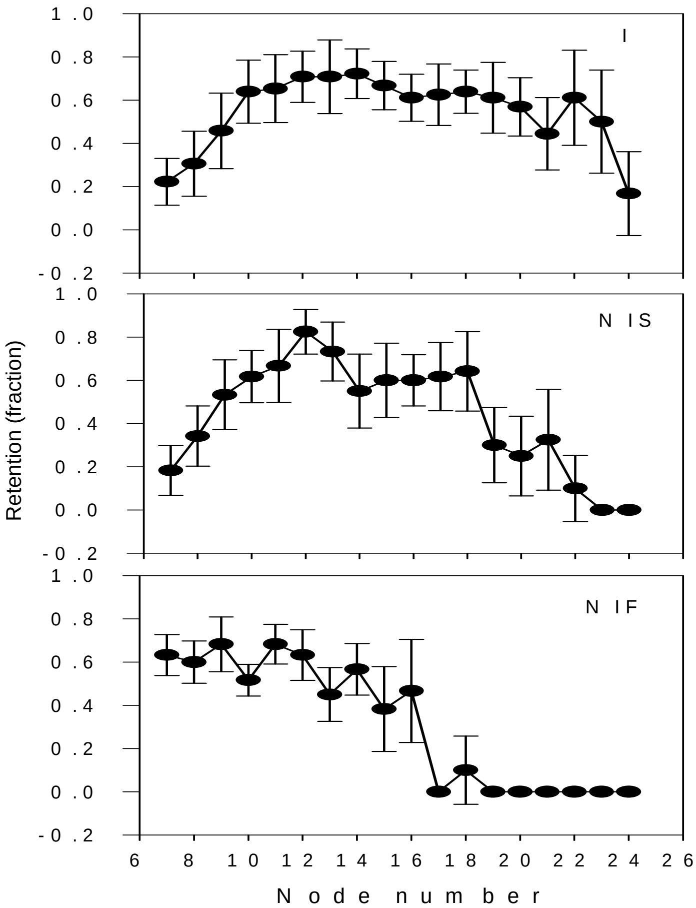

Figure 3.7 Retention fraction per fruiting node in I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) in Exp.4 (2007/08). Bars are two standard errors of the mean

3.4.6 Cotton yield and quality

3.4.6.1 Seed cotton and lint yield

Final seed cotton and lint yields are summarised in Table 3.4.

Seed cotton yield increased in response to early irrigation (I) in all four experiments, and NIF produced the lowest yield, although the difference with NIS was not significant in three out of four experiments. However, ginning percentage was significantly higher in the stress treatments than in I for Exp.1 and Exp.4. In Exp.1, seed cotton yield in NIS and NIF was reduced by 12% and 20 %, respectively when compared with the I. In Exp.2, the cotton lint yield from the NIF and NIS treatments was reduced by 42% and 39%, respectively of that achieved from the I treatment. In Exp.3, the lint yield was significantly higher ($P<0.001$) for I than for NIS and NIF by 36% and 44%, respectively.

Table 3.4 Seed cotton yield, gin-out and lint yield for I (irrigation), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) treatments during 2006/07 (Exp.1, 2 & 3) and 2007/08 (Exp.4).

Exp.1	Seed cotton yield (g m^{-2})	Gin-out (%)	Lint yield (g m^{-2})
I	580a	43.8b	254a
NIS	508b	46.9a	238b
NIF	462b	47.1a	218c
Significance	*	*	*
Exp.2			
I	472a	44.3	209a
NIS	286b	43.8	125b
NIF	270b	44.0	119b
Significance	**	NS	*
Exp.3			
I	507a	43.6	221a
NIS	320b	43.9	141b
NIF	281b	43.9	123b
Significance	**	NS	*
Exp.4			
I	542a	40.9b	221a
NIS	450b	40.4b	182b
NIF	327c	44.2a	145c
Significance	**	*	*

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

For Exp.4, a reduction in cotton lint yield of about 41% was recorded for NIF compared to I, similar to the result recorded in Exp.1 and Exp.2. However, the reduction in both final seed cotton and lint yields in the NIS treatment relative to I, was only about 9%, the stress impact being much less than recorded in Exp.2 and Exp.3.

Weekly seed cotton harvesting (Fig.3.8) was undertaken during the first season (Exps.1, 2 and 3 in 2006/2007), with cumulative yield data showing the weights being less in I during early periods. Maturity was delayed in the treatment receiving early irrigation (I), relative to the stress treatments (NIS and NIF) particularly in Exp.2 and 3, and the final yield was greater in I than in NIS and NIF.

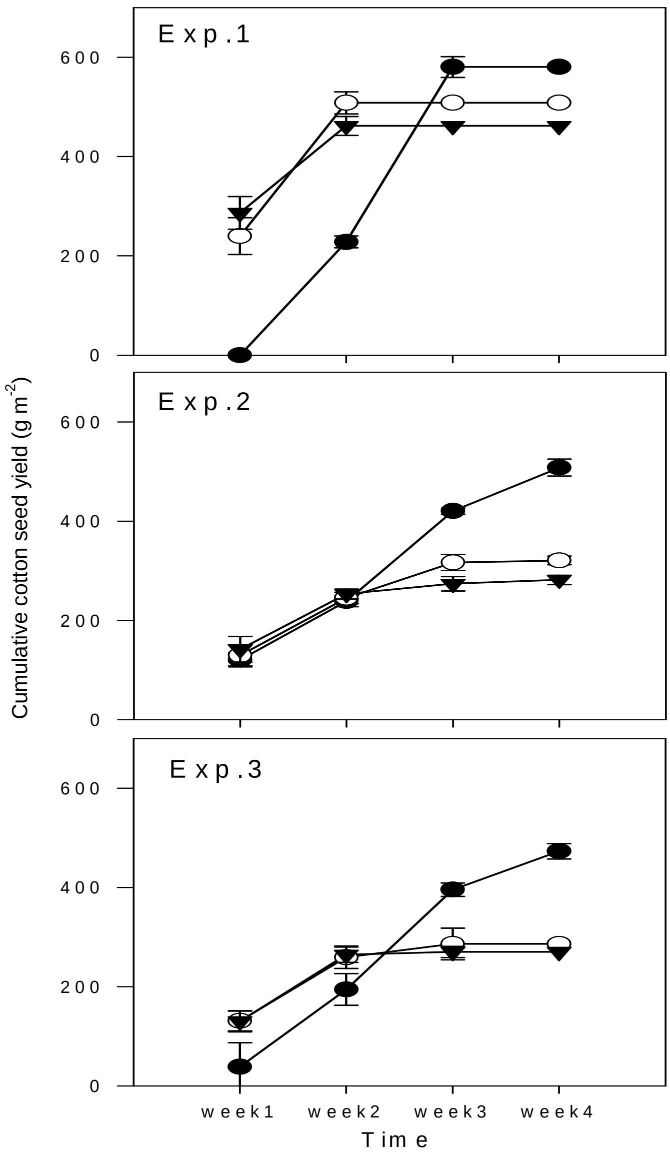

Figure 3.8 Cumulative seed cotton pick evolution per week in I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) treatments in Exp.1, 2 and 3 (2006/07) at Gatton, SE Queensland. Bars are two standard errors of the mean

3.4.6.2 Cotton quality fibre

The cotton fibre quality determined by HVI is shown in Table 3.5.

Length (UHM), Uniformity, Short fibre index (SFI) and strength were not significantly different between the treatments in all four experiments. Micronaire was not significantly different among the treatments in Exps.1, 2 and 3. However, for Exp. 4 micronaire was significantly higher for

NIF than the others. No major differences among the water treatments were found in fibre quality over the two years of studies.

Table 3.5 Cotton fibre quality determined by High Volume Instrumentation (HVI) for I (irrigation), NIS (no irrigation until square) and NIF (no irrigation until flower) treatments during 2006/07 (Exp. 1, 2 & 3) and 2007/08 (Exp. 4)

Treatments	Length UHM (in)	Uniformity (%)	Short Fiber Index SFI (%)	Strength (g/tex)	Micro-naire
Exp.1					
I	1.113	83.70	8.07	28.0	4.67
NIS	1.133	82.95	9.75	29.5	4.45
NIF	1.107	82.83	8.93	31.0	4.60
Significance	NS	NS	NS	NS	NS
Exp.2					
I	1.165	85.05	6.35	32.8	4.83
NIS	1.167	85.12	5.55	33.9	5.30
NIF	1.163	83.38	8.35	33.0	4.72
Significance	NS	NS	NS	NS	NS
Exp.3					
I	1.207	84.33	6.95	32.6	4.92
NIS	1.165	83.35	8.70	30.5	4.75
NIF	1.182	84.50	6.62	29.1	4.85
Significance	NS	NS	NS	NS	NS
Exp.4					
I	1.196	83.64	6.76	29.5	4.18
NIS	1.193	83.84	6.92	29.8	4.17
NIF	1.192	84.80	6.92	31.1	4.47
Significance	NS	NS	NS	NS	*

*Significant at P=0.05
NS = not significant

3.5 Discussion

The results of the four experiments reported indicate that even modest soil water deficits early in the growth of the crop can reduce lint yield in high retention Bt cotton. The results support the general hypothesis that insufficient early vegetative growth can have an impact on the high assimilate demands needed for boll development associated with a large number of bolls produced in high retention cotton. Larger number of fruiting sites and higher fruit retention rate were found with irrigation at pre-flowering.

Seed cotton yield increased in response to early irrigation (I) in all four experiments, and NIF produced the lowest yield, although the difference with NIS was not significant in three out of four experiments. However, ginning percentage was significantly higher in the stress treatments than in I for Exp.1 and Exp.4. Seed cotton yield increased in those plants with higher levels of assimilates produced earlier in the season in response to early inputs of water (Table 3.4). As expected, lint yield declined in response to increasing severity of water stress (Grimes and Yamada, 1982; Turner et al., 1986). In reference to the NIS and NIF treatments, the differences in reduction of final yield when compared with the irrigated (I) treatment, were smaller for early (October) sown experiments (Exp. 1 and Exp. 4) compared with later (November) sown crops (Exp. 2 and Exp. 3). A possible explanation is associated with less opportunity for the crop to develop new reproductive organs in late sowing compared with early sowing date because insufficient time remaining after flowering with ideal environmental conditions like temperature. The yield measurements reported for these studies as a result of hand-harvesting, were about 10% higher than those reported for machine harvesting under Australian conditions (Yeates, 2009, personal com). No major differences among the water treatments were found in fiber quality over the two years of studies.

Greater fruit numbers were produced in response to full irrigation prior to flowering in all four experiments conducted over the two growing seasons (2006/2007 and 2007/2008) (Fig.3.4 and Fig.3.5). A significantly higher number of squares and flowers produced in response to early irrigation resulted in a higher number of green bolls during the peak fruiting period towards the end, when compared with the stress treatments.

The increase in the size of the production sink of cotton plants is reflected in terms of a significant increase in the number of nodes with fruiting branches which have the potential to become production sites for future bolls. The ratio H/N (Table 3.2) also shows increases in plant vigor as an indicator of a better balance between the vegetative and reproductive structures (Bourland et al., 1992). The H/N ratio was always higher for I, indicating that increased boll load with a higher number of nodes will be supported by its larger canopy size. Often a high H/N is seen as an indicator of lower retention rates on a plant. Steger (1998) found that a high height to node ratio is indicative of a vegetative tendency and often associated with lower retention levels in conventional cotton varieties. However, in these studies, the higher H/N associated with I, was associated with a higher retention rate than for stressed plants.

The vertical retention recorded in Exp.4 also shows the impact of earliness of new site production and boll retention in the water stress treatments, with I continuing to produce more bolls concentrated in the middle and top of the plant (Fig 3.7). The stressed plants mainly retained bolls in the lower part of the plant, with the level of boll retention declining markedly once the plant reached a balance in the supply of assimilate relative to the retained boll load. This can be explained in terms of the organs closest to the source taking priority when water is in short supply (Hearn, 1994; Oosterhuis and Wullschleger, 1987).

Water limitation in early stages of growth, with resultant sink-source imbalances, affected the dynamics of reproductive organ development. The lateral retention fraction showed different trends, depending on the treatment. In all the cases, retention in FS1 decreased significantly with increases in water stress severity (Table 3.3). The number of aborted fruit in FS1 was higher in the stress treatments, while the production of new fruiting (nodes) sites stopped earlier in these treatments, when compared with the fully irrigated (I) treatment. In the experiments with early sowing dates (Exp.1 and Exp.4), the stress treatments with lower FS1 retention were compensated by retaining significantly more bolls in FS2 than in the I treatment. This compensation by increasing boll load in FS2 was not significant in late sown experiments (Exp.2 and Exp.3). Other studies have concluded that the first fruiting positions on the main stem produce the largest fruit in terms of both size and number (Heitholt and Schmidt, 1994) under good environmental conditions, increasing their contribution to final yield (Jenkins et al., 1990a) relative to other fruiting positions on the plant. This advantage of the first fruiting positions reflects the opportunities for competition for assimilates, relative to other fruiting positions (Constable and Rawson, 1980b; Wullschleger and Oosterhuis, 1990a; Wullschleger and Oosterhuis, 1990b).

Conclusions

Greater pre-flowering water availability in high retention cotton increased the number of fruiting sites, plant vigor, boll retention, and combined with changes in boll distribution and increased lint yield. The variation in number of reproductive organs was associated with duration and severity of the stress period. NIF with longer stress period than NIS produced fewer reproductive organs in all experiments. After the stress period, recovery in the production of reproductive organs and retention was insufficient.

These variations in components of yield, affected final seed cotton yield. NIS and NIF at an early sowing date was better recovered in terms of yield (decreased 7 and 20%) compared with late sowing date in stress treatments (41 and 44%).

These observations demonstrate the advantages of early water availability in high yielding cotton and relevant to the initial hypothesis that insufficient early growth limits supply of assimilates to meet a high boll demand later in growth.

In the next Chapter, the effect of pre-flowering soil water deficits on the phenology, biomass production and partitioning in high retention cotton will be discussed.

Chapter 4 The effect of pre-flowering soil water deficits on the phenology, biomass production and partitioning in high retention cotton

4.1 Abstract

Bt cotton has the potential for high fruit retention, but restricted water availability before flowering may limit the vegetative biomass, leading to imbalances between the demands for assimilates and the plant's capacity to supply the requirements during the reproductive stages of crop development. In Chapter 3 it was reported that even modest early soil water deficits can reduce lint yield in high retention cotton. Four experiments conducted over 2 years using Bt cotton producing two insecticidal Cry proteins, at Gatton, SE Queensland, Australia, examined the effects of pre-flowering soil water deficits of varying severity on phenological development, total dry matter (TDM) production, and assimilate partitioning. The water treatments included - irrigation (I) over the whole crop season, and two levels of water stress, no irrigation until squaring (NIS) and no irrigation until flowering (NIF), followed by irrigation until the end of the season.

Irrigation (I) extended the time to cut-out and maturity as a result of larger canopy biomass that was able to support a greater number of reproductive organs. Significant differences in biomass were recorded between years, while differences between sowing dates within a year were minor. The effect of water availability on TDM production during the stress period depended on soil moisture content of NIS and NIF relative to that of I. Recovery growth after the stress period differed between the two years, with differences in dry matter production among treatments being greater at harvest than at the end of the stress period in the first year; however, the recovery after the stress period was better in the second year, resulting in almost similar TDM at maturity

between the I and stress treatments. The differences between years reflected the fact that total rainfall and irrigation after stress period, was greater in the second year. The production of reproductive dry matter recovered after the stress period only in the second year. The partitioning to reproductive organs was lower in the I treatment, the exception being during the period close to plant maturity, when partitioning was also high in I. Crop growth and development was not only affected by the duration of the stress period, and the severity of the stress but also the inputs of water from rainfall and irrigation during the post-stress period.

4.2 Introduction

In Chapter 3, it was found that even modest early soil water deficits reduced lint yield in high retention cotton. The results supported the general hypothesis that insufficient early vegetative growth will not meet the high assimilate demands needed for boll development associated with a large number of bolls produced in high retention cotton. Increased pre-flowering water availability had a significant impact on the crop, increasing boll production and retention with associated changes in boll distribution and plant architecture, and resulting in increases in final yield, relative to the water stress treatments. These responses to early water availability during pre-flowering may be explained in terms of the result of different patterns of biomass production and partitioning, and phenological development.

The Bollgard II cotton varieties which contain two genes from *Bacillus thuringiensis* var *kurstaki* (Bt) that express proteins that are toxic to *Helicoverpa* spp., were recently released in Australia, and they have increased insect protection when compared with conventional (non-Bt) cotton varieties with similar genetic backgrounds. The net effect has been increased early boll retention and hence boll load, faster accumulation of boll weight, and a lower leaf area than their conventional equivalents (Yeates et al., 2006). The higher sink demand of smaller plants may risk early termination of flowering and reduce yields in Bollgard II cotton crops. However, it may be possible to manipulate water supply before flowering, to increase the canopy size for the enhanced provision of assimilates to be used in the development and maturation of the early bolls, and thereby increase the yield potential of Bt crops. In Australia, cotton crops are traditionally irrigated to ensure germination, but follow-up irrigation may not commence until 40 to 60 days after sowing (CRDC, 2003). The earlier provision of water in the post-germination growth phase may encourage more vigorous growth and increased leaf area that can assist in meeting the demands of the high early boll load.

The first response of cotton to a soil water deficit is to reduce leaf area expansion (Constable, 1981; Gerik et al., 1996; Hearn, 1979; Turner et al., 1986). However, the magnitude of this response depends of the timing, duration and severity of the soil water deficit. For example, Constable (1981) reported the results of four years of studies, that leaf expansion was affected only after 60% available moisture is depleted. Hearn (1979), found that cotton processes dependent on cell expansion, such as expansion of leaf area and increase in height, are more sensitive to water deficits than those associated with stomata closure, such as photosynthesis and transpiration. The effect of water stress on leaf area is to reduce interception of photosynthetically active radiation (PAR), hence canopy photosynthesis is also reduced (Ball et al., 1994; Ennahli and Earl, 2005; Turner et al., 1986). Radiation interception is a major determinant of crop growth and yield (Monteith, 1976), and directly affects the production of photo assimilates by leaves. Light penetration and interception are important in cotton because the early fruit production takes place at the lower branches of the plant in the bottom half of the canopy (Constable, 1986).

Hence depending on stage of growth there is an optimum balance between too much and too little radiation interception and penetration.

While there is substantial evidence that soil water deficit after first flower can significantly affect growth and crop yield (Kaur and Singh, 1992; Kock et al., 1990; Marur, 1991; Rosenthal et al., 1987; Turner et al., 1986), less is known about the effects of early water availability, particularly for high retention cotton varieties. A yield impact may occur in high retention cotton if there is a reduction in canopy size and available assimilates to meet the early fruiting demands. Early stress can also affect production of flowers and hence reduce sink capacity, and even if the plants have a good water supply later in growth, the reduced sink may become a limitation to higher yield**.** The approach in this study has been to increase canopy size with early water availability and to determine its effect on source supply and sink development.

The main objective of the work was to study the effects of soil water deficits of varying severity during the pre-flowering stages of Bt cotton, on phenological development, dry matter production and partitioning, so that high yield in well watered cotton prior to flowering (Chapter 3) is explained in terms of the source supply and sink capacity.

4.3 Materials and Methods

4.3.1 Experimental sites and growth conditions

The experimental methods have been described in detail in Chapter 3. Four experiments were conducted over a two year period; Exp.1 sown 6 October 2006; Exp.2 sown 16 November 2006; Exp.3 sown 21 November 2006; and Exp.4 sown 16 October 2007 at the Gatton campus of the

University of Queensland, (91m, 27°33'S, 152°20'E) in the Lockyer Valley, southeast Queensland, Australia. The soil type in the area of the study is a Lawes clay loam (Powel, 1982). The average annual rainfall is 760 mm with a summer dominance, whilst evaporation rate is high, about twice the annual average rainfall. However, during 2007/08, more rain was recorded in terms of amount and frequency, than during 2006/07.

4.3.2 Cultural practices

The Bt transgenic Bollgard ll®™ variety Sicot 71BR (producing the Monsanto Cry1Ac and Cry2AB proteins) was sown in all the experiments. The row spacing was 1 m and final plant density was 140,000 plants ha^{-1} (12-15 plants m^{-1}). The land was prepared a month before sowing using conventional tillage practices. The plots were fertilized with 100 kg ha^{-1} of N spread on surface at sowing.

4.3.3 Experimental design and water deficit treatments

Irrigation water was applied using overhead sprinklers, based on the following schedules for the different treatments:

I (Irrigation throughout the growth): Irrigation was applied to meet the crop water requirements that is 100% deficit replacement, calculated as the product of daily class "A" pan evaporation by a crop coefficient depending on the phenological stage of the crop (CRDC, 2003).

NIS (No irrigation until squaring): No water applied from establishment to squaring (water stress period), followed by irrigation through to maturity.

NIF (No irrigation until flowering): No water from establishment to flowering (water stress period) and then irrigation through to maturity.

For Exp.1, 3 and 4, water stress was achieved in the non-watered treatments by intercepting rainfall with the use of plastic covers which were placed on the ground between the rows within

1cm of the plant stems, with the covers then being secured using wire pegs. The water stress treatments were covered from the two first true leaves up to beginning of squaring (NIS) and beginning of flowering (NIF). The covers were removed when the treatments periods were finished and irrigation commenced.

For Exp.2, an automatic rainout shelter was used to ensure the exclusion of rainfall in water stress treatments (NIS and NIF).

4.3.4 Measurements

Volumetric soil water content was measured periodically using a neutron probe as described in Chapter 3.

The date of first squaring was defined as when 50% of plants in a plot had one square; a square was considered 'present' when the subtending leaf was unfolded. Dates of first flower and first open boll were defined as when 50% of plants had one flower or an open boll. The nodes above the uppermost first position white flower (NAWF) were counted on the same five plants in each plot at approximately weekly intervals from the time of first flowering. Cut-out or 'last effective flower' was defined as when NAWF < 4 (Bourland et al., 1992). Maturity was defined as the time with 60% open bolls.

Total dry matter production and partitioning were measured at 51, 75, 105 and 135 DAS in Exp.1; at 43, 64, 95 and 127 DAS in Exp.2; at 51, 84, 118 and 138 DAS in Exp.3; and at 51, 82, 112 and 145 DAS in Exp.4. These periods equated approximately with - 1st square, 1st flower, cut-out and physiological maturity-60% open bolls (defined open bolls when two sutures had dehisced), respectively. Plants from a 1 m^2 area in each plot were harvested for total fresh weight determination. A sub-sample of 3 plants was used to determine fresh weight, leaf area, dry matter and partitioning of DM into leaves, stems, petioles, squares, flowers, green bolls and open bolls (two sutures on the boll dehisced). Samples were dried at 80ºC for three days to determine dry matter content. Leaf area was measured using a LiCor planimeter (Model LI-3100, LiCor Inc., Lincoln, NB, USA). Specific leaf area (SLA) was calculated, and then leaf area index (LAI) was calculated as the product of SLA and the amount of leaf dry matter in the 1m^2 area.

Using a line quantum sensor, solar radiation interception was measured around midday 3-4 times for each experiment. Incident radiation was recorded above each plot. Three readings of transmitted radiation were recorded at ground level in each plot. The proportion of intercepted photosynthetically active radiation (PAR) was calculated as: (incident radiation – transmitted radiation)/ incident radiation.

Stomata conductance was measured on the youngest fully expanded leaf between 11 and 13 hrs using a calibrated portable porometer LICOR 1600.

A pressure chamber Model 1000 was used to measure leaf water potential at the end of each water stress period, immediately before irrigation was resumed in all experiments. For these measurements, the youngest fully expanded leaf was used, with the measurements being made between 9 and 10.30 am on non-cloudy days. Also predawn data was collected for Exp.1.

4.4 Results

4.4.1 Soil water content

Changes in total soil water content between 20 and 180 cm are shown in Fig. 4.1 for all experiments. For I, a total soil water content of between 500 and 550 mm (85 to 93% of field capacity) was maintained during the first season (Exp.1, 2 and 3), but it was slightly higher during the second season (Exp.4). For I in Exp.1 and Exp.4, the soil water content was closer to field capacity (586 mm) compared with I in Exp.2 and Exp.3.

For NIF, the rate of change in soil water content reflected the size of the plants being slower at the beginning of the monitoring period due to lower levels of extraction by younger plants, and faster towards the end of the stress period as the older plants flowering. The decrease in soil water content for NIS followed a similar pattern of extraction to NIF, but the stress period was shorter. The lowest soil water content in the NIF treatment was found in Exp.1, Exp.2 and Exp.4, while slightly higher water content prevailed in Exp.3 for the NIF treatment.

In both stress treatments in Exp.1, 2 and 3 during the first year, when the stress period finished and irrigation commenced, the soil water content increased slowly in response to the amount of water applied. The water applied was calculated from the evapotranspiration rate estimated for the phenological stage of development, without re-filling the full profile. For the NIS treatment in Exp.4, the soil water content after the stress period reached similar values to I. For both NIF and NIF, the water content increased rapidly after the stress period in this experiment, with the final value exceeding those in NIS and NIF in Exp.1 and 2.

The two methods used to prevent rainfall infiltration, that is, rainout shelters and covering the soil with plastic sheeting, were of similar effectiveness, with the soil water contents in Exp.2 and Exp.3 being similar for the same dates (Fig 4.1); the only difference was at the end of the stress period in NIF and after the stress period where Exp.3 had lower soil water content. I had a higher soil water at the beginning of the cropping period in Exp.3, but the soil water content of I in both experiments decreased later in the season possibly due to insufficient irrigation. In a comparison of results from Exp.1 from the first season, and Exp.4 in the second season where both experiments used plastics to exclude rainfall impact and planted at the same time, the irrigated (I) treatment in Exp.4 maintained a higher soil water content throughout the season, than for Exp.1. The second season was wetter, with higher water input from rain bringing the soil closer to field capacity in the I treatment in Exp.4 than in Exp.1. NIS also showed a better recovery after the end of stress period in Exp.4 compared with Exp.1. For the NIF treatment, similar water deficits were reached in both experiments. The duration of the NIS and NIF treatments was similar for both Exp.1 and Exp.4, with the severity of NIS being similar during the stress period but there was better recovery in Exp.4. The severity in NIF was also similar in both experiments, and both showed similar levels of water recovery later in the season.

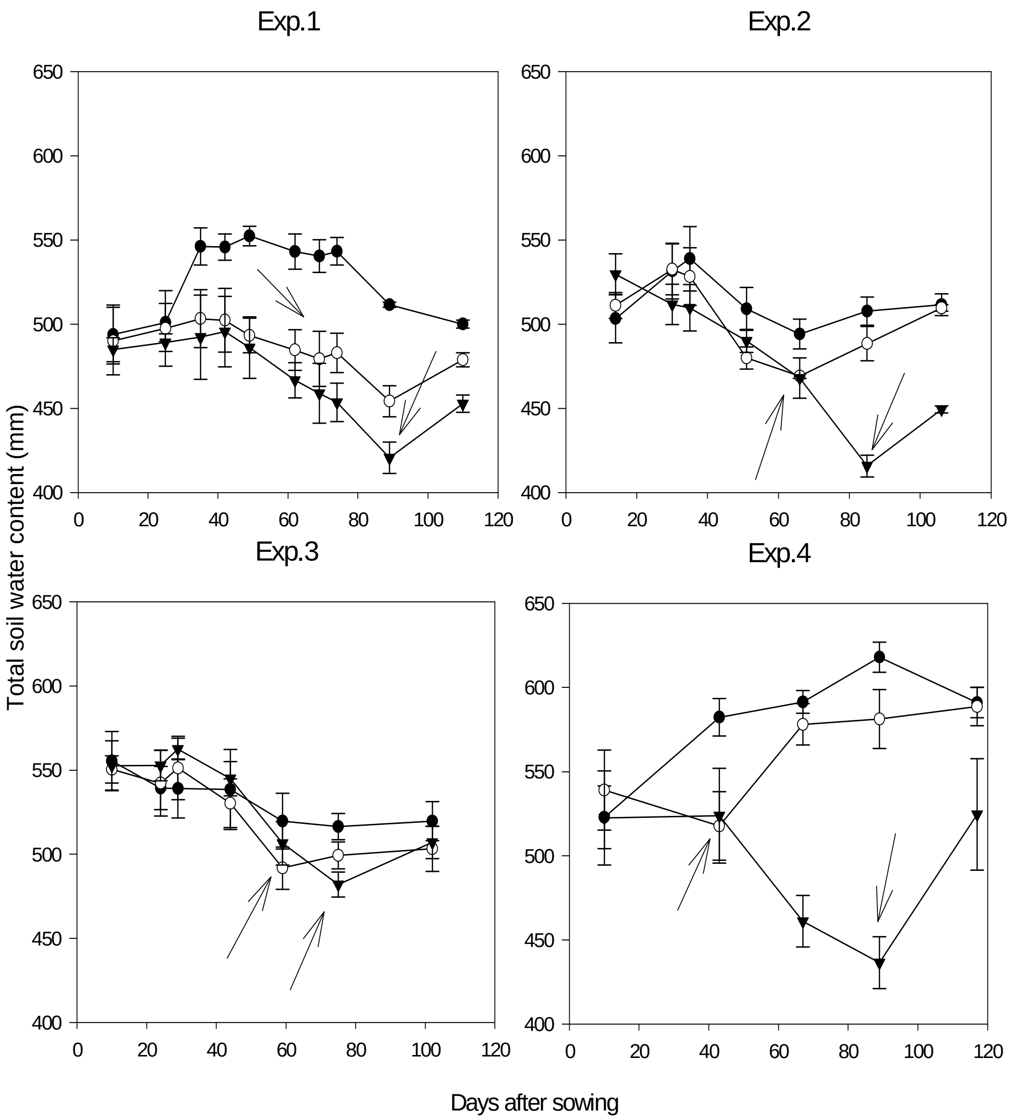

Figure 4.1 The effect of early water availability on changes in total soil water content (20-180 cm depth) for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2006/07 (Exp.1, 2 and 3) and 2007/08 (Exp.4) at Gatton, SE Queensland. Arrows indicate the end of the stress period. Bars are two standard errors of the mean.

4.4.2 Leaf water potential and stomata conductance

Changes in leaf water potential at 9 - 10.30 am are shown in Table 4.1. At the end of the stress period, just before commencing irrigation in NIS, LWP differed significantly between the I and stress treatments in Exp.1. For NIF, the LWP dropped to -2.29MPa at the end of the stress period, while for I it was maintained at -0.28MPa, and for NIS went from -1.29 to -0.54MPa (as the treatment was irrigated).

For Exp.2, at the end of the squaring stress period LWP was high in all treatments without significant differences among them, after which the LWP decreased for NIF. Treatments effects in Exp.3 were similar to those in Exp.1. During the second season (Exp.4), the results were similar to those obtained during the first season.

Predawn data was also collected during the first season for Exp.1, with higher LWP values in all the treatments during the night (I -0.20, NIS -0.59 and NIF -0.59 MPa) compared with daytime at the end of the NIS period (P=0.003).

Table 4.1 Changes in leaf water potential (MPa) at the end of the stress period around squaring (NIS) and flowering (NIF) in all three treatments I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) during 2006/07 and 2007/08 at Gatton, SE Queensland.

Exp 1	**End of squaring stress period**	**End of flowering stress period**	**Exp 2**	**End of squaring stress period**	**End of flowering stress period**
I	-0.25a	-0.28a	**I**	-0.15a	-0.15a
NIS	-1.29b	-0.54a	**NIS**	-0.25a	-0.25a
NIF	-1.34b	-2.29b	**NIF**	-0.20a	-1.79b
Significance	*	*	**Significance**	NS	*
Exp 3	**End of squaring stress period**	**End of flowering stress period**	**Exp 4**	**End of squaring stress period**	**End of flowering stress period**
I	-0.11a	-0.16a	**I**	-	-0.07a
NIS	-1.03b	-0.36a	**NIS**	-	-0.11a
NIF	-0.98b	-1.24b	**NIF**	-	-1.74b
Significance	*	*	**Significance**	-	*

In linking the soil water content to plant growth, any decrease in soil water content had a direct effect on plant water status. For example, in Exp.1 and Exp.2, the soil water content of NIF dropped to 410 mm at the end of the stress period, coinciding with the lowest value of LWP (-2.29 and -1.79MPa respectively). In another comparison, for the I treatment during the second season, the period of highest soil water content was linked to the highest LWP value.

Table 4.2 Changes in stomata conductance (cm s^{-1}) at the end of stress period around squaring (NIS) and flowering (NIF) in all three treatments I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) in 2006/07 (Exp.1, 2 and 3) and 2007/08 (Exp.4) at Gatton, SE Queensland.

Exp. 1	End of mild stress period	End of severe stress period	Exp. 2	End of mild stress period	End of severe stress period
I	1.44a	1.95a	**I**	1.12a	1.76a
NIS	0.94b	0.25b	**NIS**	1.00a	1.67a
NIF	0.94b	0.23b	**NIF**	1.11a	0.34b
Significance	*	*	**Significance**	NS	*

Exp. 3	End of mild stress period	End of severe stress period	Exp. 4	End of mild stress period	End of severe stress period
I	1.13a	1.42a	**I**	-	2.08a
NIS	0.94a	1.39a	**NIS**	-	2.02a
NIF	1.13a	1.41a	**NIF**	-	0.54b
Significance	NS	NS	**Significance**	-	*

For Exp 1, the stomata conductance (SC) measured about midday on the same day as LWP, followed a similar trend to changes in LWP (Table 4.2). In I, SC showed a slight increase in all experiments (1 to 3) as the plants got older. There were no significant differences between NIS and NIF, either at the end of the period of mild stress or end of severe stress in Exp.1, although the stomata conductance in the stress treatments were lower than in I. There were no significant differences at the end of the NIS stress period for Exp.2 and Exp.3, and the end of the NIF stress period in Exp.3. However, there were significant differences at the end of NIF stress period in Exp.2, there being a decline in stomata conductance associated with increasing soil water deficits. For Exp.4 in the second season, stomata conductance in the I treatment was higher than for the I treatments in the 3 experiments undertaken in the first season, and also differed significantly from that of NIF at the end of the stress period.

4.4.3 Phenology

The timing of all reproductive development stages was delayed in the I treatments (Table 4.3). This delay was generally small for first square (2-9 days), but increased with time to maturity (9-20 days), when compared with the water stress treatments, NIS and NIF. Differences between seasons and sowing dates in the days to the different reproductive development stages reflected temperature differences.

Table 4.3 Phenological development: days from sowing to 1st square, 1st flower, 1st open boll and maturity, for I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) in 2006/07 (Exp.1, 2 and 3) and 2007/08 (Exp.4) at Gatton, SE Queensland.

Exp1	1st Square	1st Flower	1stOpen Boll	Maturity
I	46.0a	75.0a	128.2a	154.5a
NIS	42.2b	71.5a	119.2b	142.7b
NIF	42.5b	65.5b	116.8b	141.2b
Significance	*	*	*	*
Exp2	1st Square	1st Flower	1stOpen Boll	Maturity
I	44.7a	69.5	127.2a	150.2a
NIS	40.0b	68.7	120.7b	141.5b
NIF	40.5b	68.2	120.5b	141.0b
Significance	*	NS	*	*
Exp3	1st Square	1st Flower	1stOpen Boll	Maturity
I	44.2	72.5	133.5a	156.2a
NIS	44.0	72.5	128.5b	144.7b
NIF	42.2	70.2	128.0b	144.5b
Significance	NS	NS	*	*
Exp4	1st Square	1st Flower	1stOpen Boll	Maturity
I	50.5a	80.8a	145.0a	186.2a
NIS	47.0b	79.1a	142.0a	181.0b
NIF	41.6c	71.5b	129.7b	166.7c
Significance	*	*	*	*

The differences in phenological development between water treatments were higher in 2007/08 (Exp.4) than in 2006/07 (Exp.1). The boll growth period (from anthesis to maturity) was significantly longer in the second season (Exp.4) compared with the first season (Exp.1). For I, the boll growth period in Exp.1 was 79 days, while in Exp.4 it was 106 days. For NIS the periods were 71 and 102 days, for Exp.1 and Exp.4, respectively. Soil water content during the second season (2007/08) was higher in all treatments compared with the first season (2006/07), which may have affected the vegetative and reproductive periods. For NIF, the boll growth period was also shorter in Exp.1 than in Exp.4, 75 days in Exp.1 and 95 days in Exp.4.

4.4.4 Dry matter production

In Exp.1, the accumulation of total dry matter was slightly higher for I at 51 DAS ($P=0.019$), then becoming much greater at 75 DAS (<0.001), 105 DAS (<0.001) and 135 DAS (<0.001), when compared with the soil water deficit treatments (Fig 4.2). TDM production in all the treatments in Exp.1 reflected differences in soil moisture content, with I producing the greatest TDM. NIS with an early deficit was followed by a recovery in TDM production, while NIF with the greatest water deficit had the lowest TDM and had not recovered by the end of the season. For NIF, TDM declined earlier on maturity when compared with NIS and I, with a correspondingly earlier (by about 12 days) maturity.

In Exp.2 with controlled conditions under the rainout shelter, TDM production was lower in all treatments when compared with Exp.1. TDM in Exp.2 was greater for I than for NIS and NIF, especially at 95 DAS (<0.001) and 127 DAS (<0.001). For Exp.3, there were highly significant differences (<0.001) between treatments in TDM at all measurements occasions, despite small differences in soil water content. For Exp.4, there was significantly higher TDM values in I, while NIS recovered in response to improved soil water content following the stress treatment, as did NIF at a still later stage of growth. The relative differences in final TDM among the irrigation treatments were less in Exp.4, particularly at the last measurement occasion.

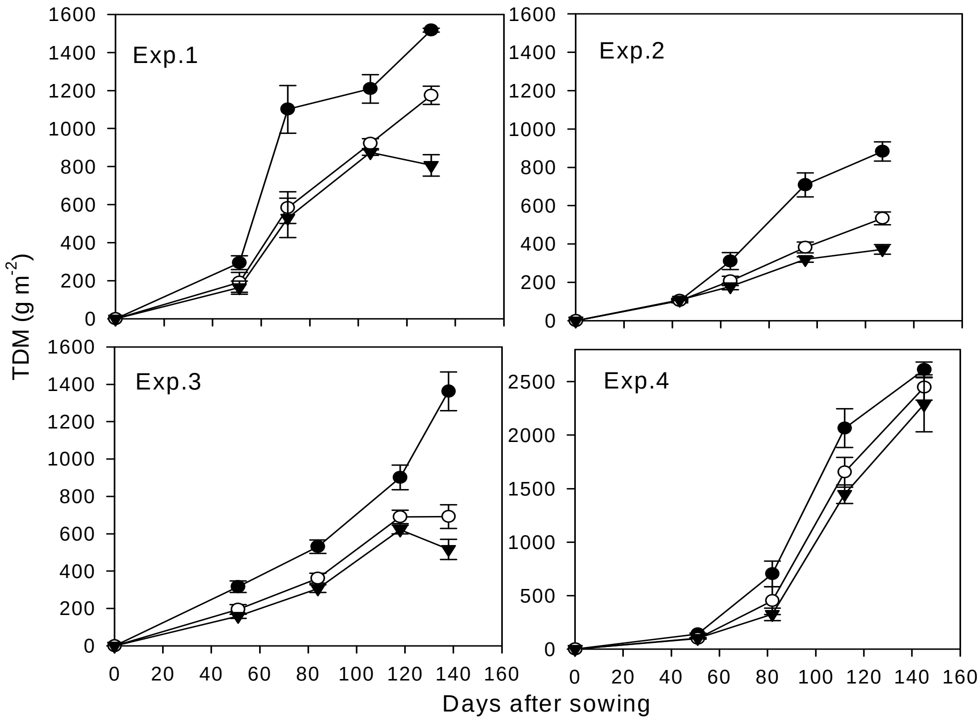

Figure 4.2 Changes in total dry matter for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2006/07 (Exp.1, 2 and 3) and 2007/08 (Exp.4) at Gatton, SE Queensland. Bars are two standard errors of the mean. Note: Exp 4 has a different scale.

In a comparison across years (Exp.1 in 2006/07 and Exp.4 in 2007/08), TDM was higher for the full period of experimentation in the second year; this result was not unexpected, due to the drier conditions in the first year. The pattern of TDM production was similar in both years for I, while for NIS and NIF, TDM recovered after the period of stress in the second year.

In a comparison of Exp.2 and Exp.3 which used a rainout shelter and plastic soil covering, respectively and were sown on similar dates, TDM in both cases did not recover following the end of the stress period, even for Exp.3 in which there were no high water deficit differences between the treatments. For the irrigated treatment (I) in Exp.2, TDM production was not as great as for the same treatment in Exp.3; this may reflect a failure in achieving complete irrigation as illustrated in Fig. 4.1. The NIF treatment in Exp.2 had the large stress effect on TDM, associated with a severe soil water deficit.

4.4.5 Leaf area index and solar radiation interception

In all the experiments, leaf area index (LAI) was greater in response to full irrigation prior to first flower, relative to no irrigation up to first square or first flower (Fig. 4.3). I developed its canopy sooner than NIF and NIS. Peak LAI was produced near cut-out (120 DAS) for all the treatments, then decreasing to maturity. In all experiments, I reached a peak LAI in excess of 4, the one exception being in Exp.2, which also showed lower values in soil water content. Treatments NIS and NIF in Exp.4 recovered in response to irrigation more than in other experiments, with the LAI reaching 4.1 and 3.1 respectively, associated with higher inputs of water after the period of stress ended.

In Exp.1, the differences between the I and stress treatments were significant during the season. However, in Exp.2 the differences in LAI between the treatments were not significant at 43 days, after which there was a significantly larger LAI in I, followed by NIS. NIF had the lowest LAI during the growing season in Exp.2.

For Exp.3 and Exp.4, the LAI followed a similar trend among the treatments with I> NIF>NIS, which reflected the duration and timing of moisture stress.

Comparing LAI across both years (Exp.1 and Exp.4), some differences were also found. In the first year, in the NIS and NIF treatments, LAI did not improve after the stress period, while in the second year when weather conditions were wetter, LAI showed more improvement following the end of the stress period.

The increase in the proportion of solar radiation intercepted (Fig.4.4) by the crop followed a similar trend to LAI (Fig.4.3). In Exp.1, I intercepted a higher proportion of solar radiation sooner than the stress treatments. None of the NIS and NIF plots in all experiments reached 95% interception levels. The percentage of radiation interception in Exp.4 was higher and earlier for I, reaching 95% interception at about 80-95 DAS, while for NIF the highest interception was achieved at the last measurement occasion.

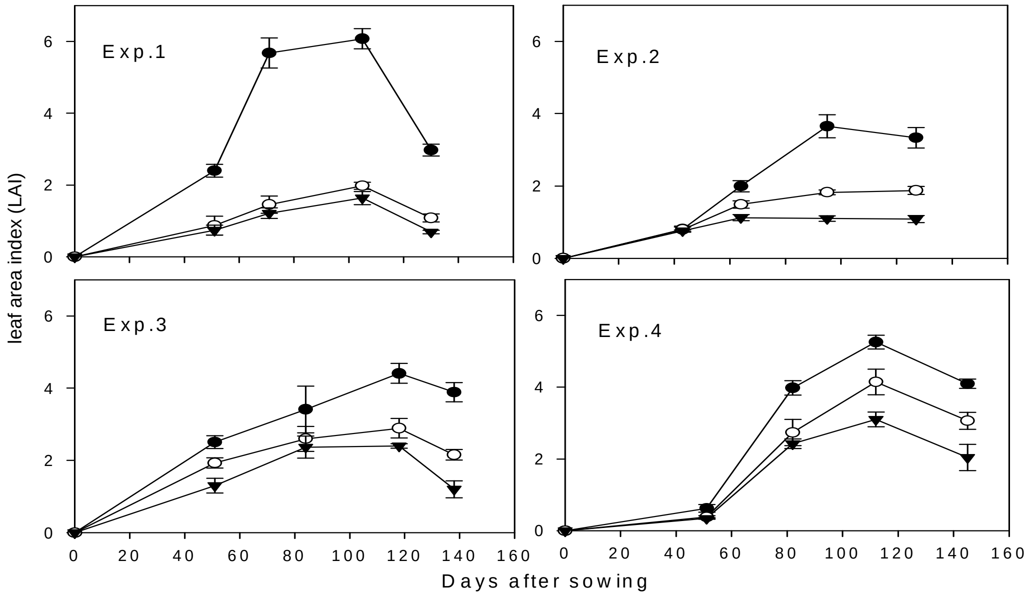

Figure 4.3 Changes in leaf area index for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2006/07 (Exp.1, 2 and 3) and 2007/08 (Exp.4) at Gatton, SE Queensland. Bars are two standard errors of the mean.

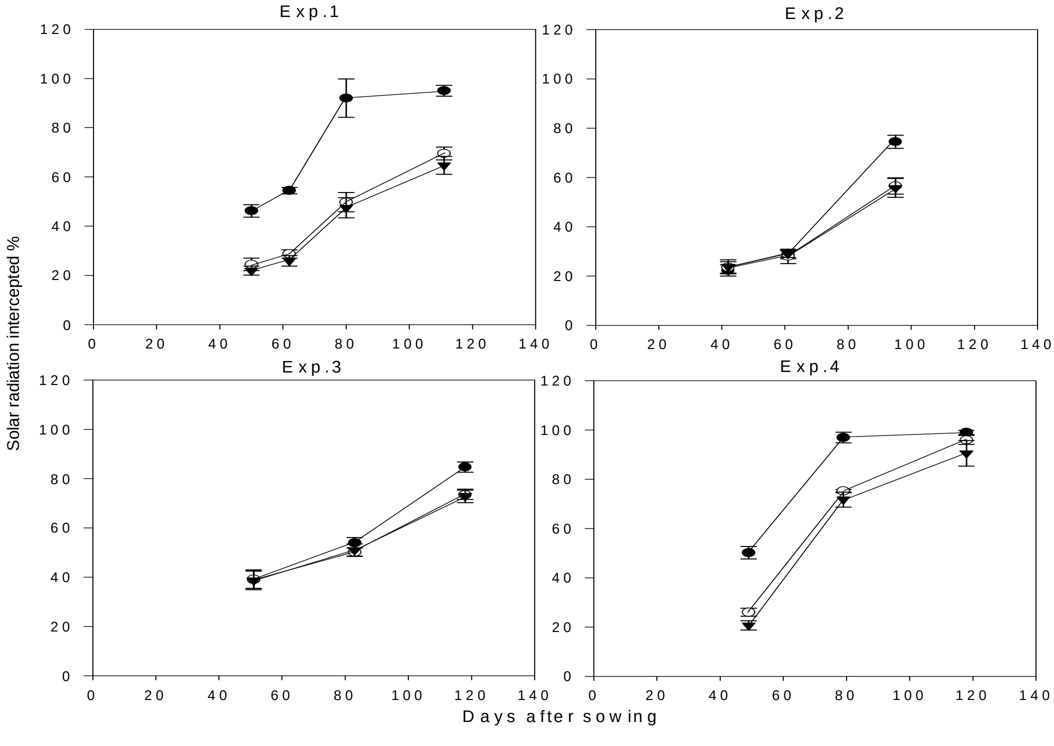

Figure 4.4 Percentage of photosynthetically active radiation (PAR) intercepted for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2006/07 (Exp.1, 2 and 3) and 2007/08 (Exp.4) at Gatton, SE Queensland. Bars are two standard errors of the mean.

4.4.6 Dry matter partitioning

The distribution of TDM into vegetative and reproductive components over the period of crop development is shown in Fig. 4.5 and Fig. 4.6. Vegetative dry matter production in the I treatments was significantly greater than in the water deficit treatments in the early stages of crop growth for all the experiments in both seasons.

There were no significant differences among the treatments around first flower at 71 (Exp.1), 64 (Exp.2), 84 (Exp.3) and 82 (Exp.4) DAS for reproductive dry matter production (squares, flowers or green bolls), but the production of vegetative matter by I was significantly higher in all experiments.

Significant differences among the three treatments were found around cut-out in production of vegetative and reproductive biomass (flowers and squares) at 105 (Exp.1), 95 (Exp.2), 118 (Exp.3) and 112 (Exp.4) DAS, with significantly higher vegetative biomass in the I treatments.

Exp.1 showed lower production of vegetative biomass in I, NIS and NIF than in Exp.4. In the latter experiment, the production of reproductive growth resumed in all the stress treatments following the period of stress. Exp.4 had a longer time-to-maturity than the other experiments.

Fig. 4.7 shows the relationship between total dry matter (TDM) production and total reproductive dry matter during the crop growth (the final harvest where cotton seed yield was determined was not included in the figure because of loss of leaves). During the stress period, TDM production was affected by the stress but the partitioning at reproductive organs was greater, so that reproductive DM was similar among the treatments. However, the I treatment, with higher TDM, was able to increase partitioning to reproductive organs later, resulting in a higher reproductive yield at the last measurement occasion, in Exp.1-3.

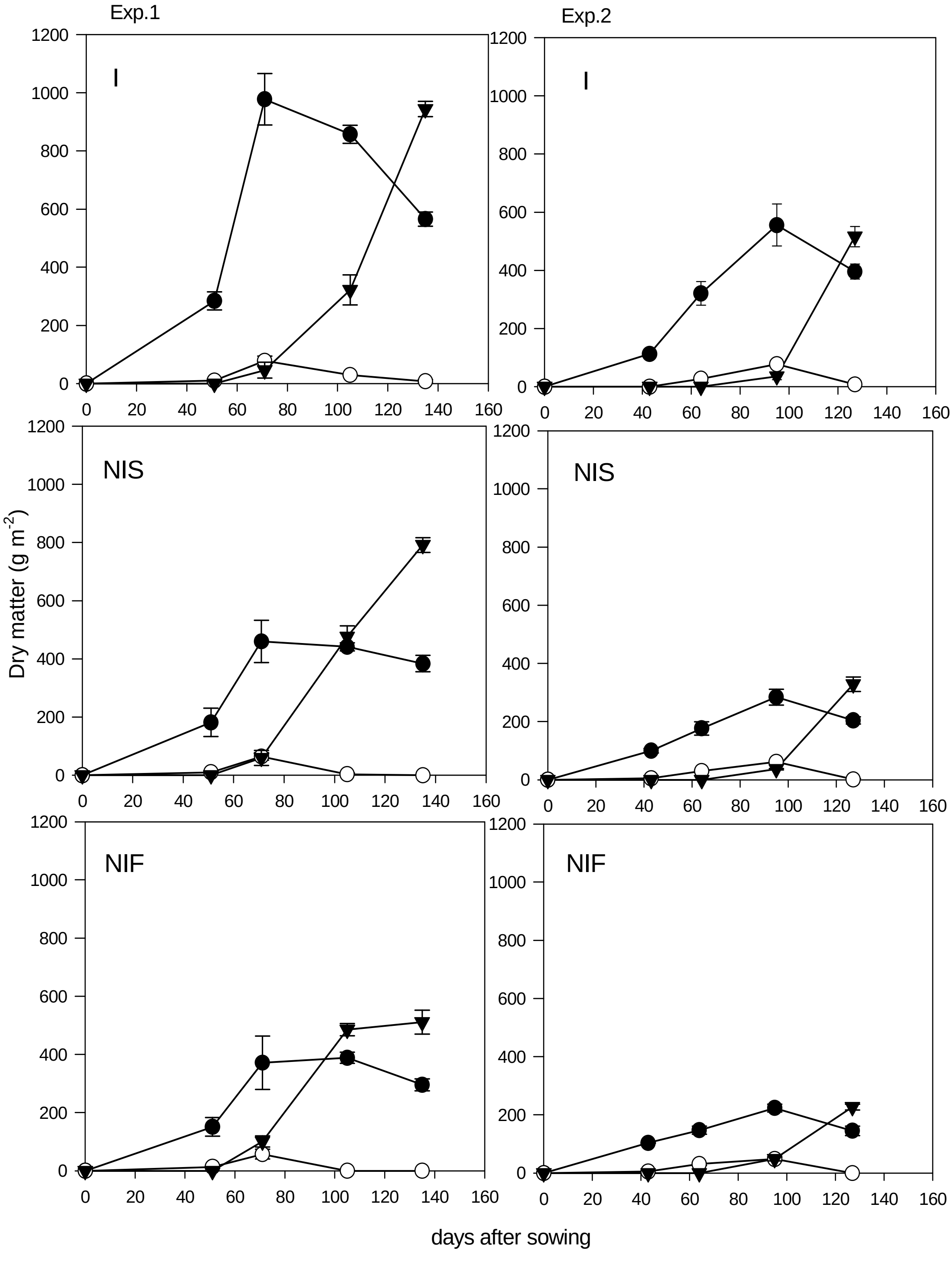

Figure 4.5 Changes in dry matter production of vegetative organs (leaf, stem and petiole) (•), square and flower (○) and bolls (▼) for I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) for Experiments 1 and 2. Bars are two standard errors of the mean.

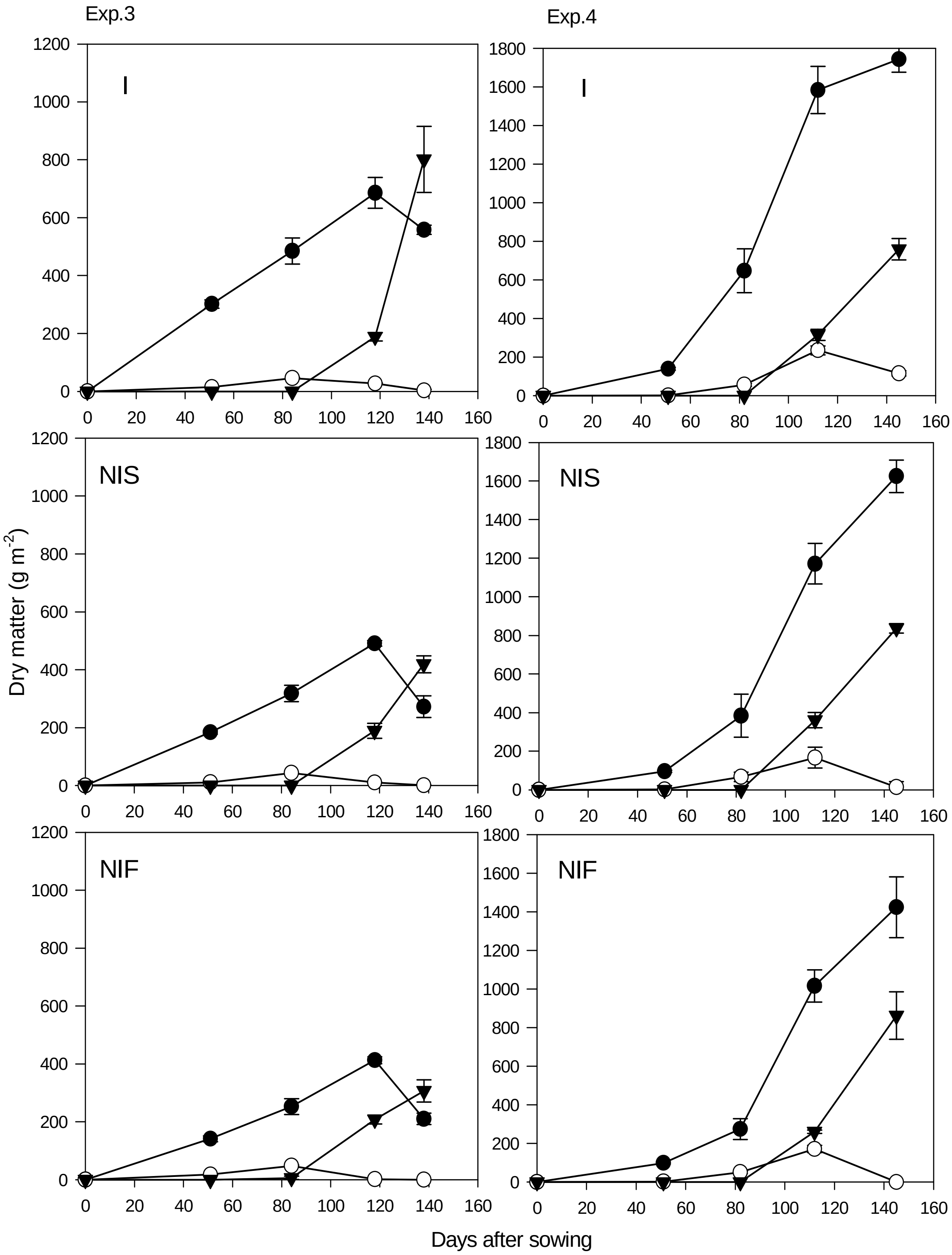

Figure 4.6 Changes in dry matter production of vegetative organs (leaf, stem and petiole) (•), square and flower (○) and bolls (▼) for I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) for Experiments 1 and 2. Bars are two standard errors of the mean. Note: Exp 4 has a different scale.

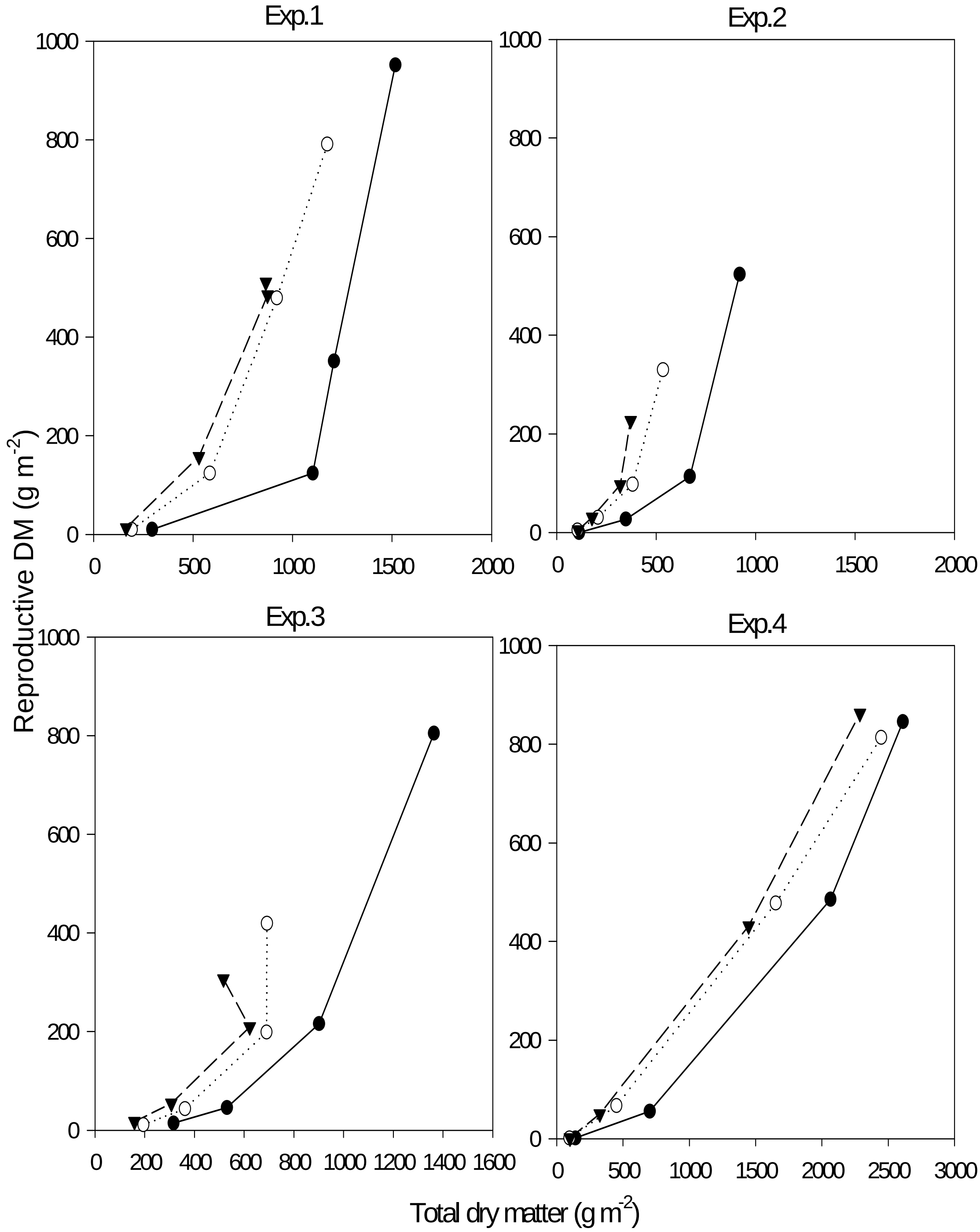

Figure 4.7 Relationship between total dry matter production and total reproductive dry matter during the period of crop growth for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in all experiments. Lines are shown for Irrigation (solid line), NIS (dotted) and NIF (long dash). Note: Exp. 4 is on a different scale.

4.5 Discussion

The aim of this research was to study the effects of soil water deficits of varying severity (varying duration and amounts of water available) during the pre-flowering stages of Bt high retention cotton, on the phenological development, dry matter production and assimilate partitioning, so that yield advantages of minimizing early soil water deficits (Chapter 3) can be fully understood.

Development and growth was affected under the different regimes of different water availability during the early stages of crop growth. The early irrigated treatment was able to continue the production of new vegetative growth and fruiting sites thus the longer reproductive phase and later maturity. Cut-out and maturity was delayed in early irrigated, high retention cotton, affecting the production of assimilates and their partitioning into reproductive retained organs. This is consistent with the nutritional hypothesis of Mason (1922) and later studies of Hearn (1972).

Phenological development

Water availability affected the time to reach different key crop growth stages, with cut-out and maturity occurring earlier with a decline in soil water content. Irrigation (I) produced a greater source of assimilates during the early stages of crop growth, delaying the time to cut-out and maturity, compared with NIF and NIS. A longer period to maturity associated with early irrigation was translated into a higher number of open bolls and a high boll retention rate by the end of the crop. This may be explained as a reflection of more assimilates being available from a larger canopy to meet a higher demand from the growing and developing fruit. It is not only temperatures and solar radiation that has the potential to affect maturity time, but also the balance of supply and demand for assimilates for the developing bolls and growing points (Bange and

Milroy, 2000). Therefore, the balance of assimilates available for boll production determines lint yield and time to maturity (Hearn, 1972; Hearn, 1994).

Across both seasons (2006/07 and 2007/08) the differences in DAS to reach various growth stages were larger than within seasons. The boll period (from anthesis to maturity) was significantly longer in the second season (Exp.4) compared with the first season (Exp.1). For I, the boll period in Exp.1 was 79 days, while in Exp.4 it was 106 days; for NIS it was 71 (Exp.1) and 102 (Exp.4) days, respectively. This difference was not related to boll load or cotton seed yield as they were higher in the first season (Exp.1) (Chapter 3). Soil water content during the second season was higher in I and NIS when compared with the first season, a factor which have affected the vegetative and reproductive periods. For NIF, the soil water deficit was similar in both Exp.1 and Exp.4, but in the second year water input for recovery was higher, resulting in a boll period of 95 days compared with 75 days in the first season (Exp.1).

Dry matter production and partitioning

Many significant differences in plant growth components were found in the course of the field studies, most of which were related to the impact of differences in soil water deficits among the treatments and between seasons. TDM production differed among I, NIS and NIF treatments in the early stages of plant growth, and increased with time after the end of the stress period in the first season. This was particularly the case for final DM harvest in the first season (Exp.1 to 3); while in the second season (Exp.4) the differences, while maintained, were smaller at the time of final DM harvest (which was earlier than crop maturity harvest).

In a comparison across years (Exp.1 in the first season and Exp.4 in the second), TDM production was higher in the second year for the whole period, this difference reflecting the wetter conditions, lower evaporative demand and cooler temperatures in the second year. The pattern of TDM production was similar in both years for I. However, the recovery after the stress period in both NIS and NIF was only in the second year, coinciding with higher inputs of water in the form of rainfall, after the stress period, with soil water content approaching close to field capacity. This happened despite similar soil moisture deficits for the NIF treatment and similar duration of NIS and NIF in both years.

Determination of LWP and stomata conductance indicates the plants were severely affected by prolonged stress period. As the water stress duration increased LWP declined, and in most cases NIS and NIF reached LWP values of -1.9 to -3.5MPa, at which photosynthesis starts to decline (Turner et al., 1986). Previous studies of Turner (1979) concluded that processes dependent on cell expansion such us leaf area development**,** is very sensitive to water deficits. This study also found a large response in LAI to the stress treatments particularly in NIF. However, processes associated with stomata closure were also affected by the end of the stress period in NIF.

During the stress period, TDM production was affected by stress, but partitioning was higher, so that reproductive DM was similar among the different treatments. However, the I treatments with higher TDM were able to increase partitioning to reproductive organs later, resulting in higher reproductive yield at maturity.

Leaf area index and light interception

The irrigated (I) treatment developed its canopy sooner and light interception reached 95% in most of the irrigated plots earlier than in the stress treatments. LAI differed significantly between the irrigated conditions and soil water deficit treatments. I in all the experiments reached a peak LAI higher than 4, the only exception being in Exp.2 which also had lower soil water content. A high LAI is usually associated with a higher number of fruiting sites. This association is well studied and is due to the assimilate supply by the leaves being primary determinant of yield, and essential to support vegetative and reproductive growth. Thus the radiation interception by the canopy is a major factor affecting crop growth and yield (Monteith, 1977).

Bt cotton with additional mechanisms for the plant to retain earlier fruiting structures with an earlier cut-out, may improve the retention of organs by an earlier increase of assimilates and longer vegetative stage. Early season water management in such cotton, should encourage the development of sufficient vegetative biomass and large canopy to produce more assimilates later in the season to support a higher number of retained reproductive organs in high retention cotton.

In summary, a delay in maturity for I may be explained as the result of a higher biomass production, available per plant, to supply a greater sink capacity or greater number of reproductive organs increasing final seed cotton yield (Chapter 3). Major differences were found between years (Exp.1 and Exp.4), while there were minor differences between early and late sowing dates (Exp.1 and Exp.3). There were no differences between the use of plastic covering and rainout shelters (Exp.2 and Exp.3) for excluding the effects of natural rainfall. TDM was affected during the stress period, as well as the recovery after the stress period, with greatest differences between treatments during the first season. A better TDM recovery came after the stress treatments in the second season (Exp.4), and was associated with the recovery in soil water content. I in all the experiments reached a peak LAI higher than 4, the only exception being in Exp.2 which had lower values associated with lower soil water content. The production of reproductive dry matter recovered after the period of stress only in Exp.4 in the second season, while none of the stress treatments in the first season showed such a recovery.

The assimilate source supply associated with a larger plant size may explain the differences between I and stress treatments in relation to the rate of reproductive site production, organs retention in cotton yield, which have been reported separately (Chapter 3).

Chapter 5 Sink-source relations in high retention cotton: effects of early irrigation, flower removal and canopy exposure after flowering on boll distribution, fruit retention and yield

5.1 Abstract

The low assimilate availability after flowering in high retention cotton may risk early cut-out and reduce final yield. Two years of experiments with Bt cotton producing 2 insecticidal Cry proteins (variety Sicot 71BR) were conducted at Gatton, Southeast Queensland, Australia, to study the effects of early water availability on source supply to fill in developing bolls and dynamics of fruit development, distribution and retention, and final yield in high and low fruit retention. Bollgard II, a high fruit retention cotton variety, grown without interference, was compared with the same variety but with early flower removal to generate lower retention (the simulation of conventional varieties). The water treatments included - irrigation (I) over the whole cropping season; water stress until squaring (NIS) followed by full irrigation to maturity; and water stress until flowering (NIF) followed by full irrigation until maturity. A further experiment was conducted with light exposure to the lower parts of the canopy under well irrigated conditions, the aim of which was to determine if increased source availability can increase yield.

The number of fruits increased under the irrigated (I) conditions (high availability of resources), with these fruits being mainly in first lateral position and concentrated in the middle and upper parts of the canopy. The absolute number of flower buds and bolls, and the percentage fruit retention, were higher in I than in the stress treatments in high retention cotton. Without flower removal (Bt), the effect of early water stress was about 20-25% reduction in seed cotton yield. However, with flower removal (simulation of conventional varieties), the yield reduction in

response to the stress was about 5-8%. This suggests that early irrigation of Bt cotton increased the supply of assimilates (before flowering) which was important for high retention cotton, whereas for conventional varieties (low retention) where the source-supply is relatively large, compensation can take place following the period of stress.

Light exposure to the lower parts of the canopy to increase assimilate source supply for the periods longer than 42 days from the time of flowering were associated with increased fruit retention and seed cotton yield by about 10%. These studies show the advantages of improving the canopy development in Bt cotton at pre-flowering to supply increased assimilate source to support a higher sink demand resulting in increased lint yield.

5.2 Introduction

The recent release of Bollgard II cotton varieties, which contain two genes from Bacillus turigensis (Bt) that express proteins toxic to *Helicoverpa spp*, has reduced the impact of such pests. Bollgard II has higher early fruit retention, faster accumulation of boll weight and lower leaf area than their conventional variety equivalents (Yeates et al., 2006). Compared with non-Bt varieties, Bt varieties has a shorter vegetative cycle and higher early fruit retention rates at the first and second positions of fruiting branches with high availability of resources to support boll growth (Ahuja, 2006; Hofs et al., 2006). The early fruit retention and growth may not be so critical in non-Bt cotton, due to cyclic compensatory growth of vegetative biomass and fruit, in response to early loss of fruit caused by biotic or abiotic factors, such as water deficits or insect attack (Sadras, 1996). Sadras (1995) based mainly on the plant carbon partitioning and the dynamics of resource allocation defined some plant responses to the loss of reproductive organs. Four types of compensatory responses have been studied by many people (Brook et al., 1992b;

Hearn and Room, 1979; Kletter and Wallach, 1982a; Sadras, 1995). One response is passive and instantaneous, in which the reproductive structures which are damaged, are shed. A second response is passive and time dependant, in which the reproductive organs were supposed to be aborted but, instead, are retained and replace those damaged previously, resulting in a delay in fruit setting. A third response is active and instantaneous, in which resources are partitioned to undamaged organs instead of damaged ones, increasing fruiting weight but without an increase in the number of fruiting sites. A fourth response is active and time dependent, in which the loss of reproductive organs prolongs flower bud production, increasing the rate of late flowering and number of fruiting sites. These four responses are not mutually exclusive and not easy to separate, but may provide the key to some agronomic parameters, such as time-to-maturity (Sadras, 1995) and to understand the responses of flower buds removal under different watering conditions used in this study. Removal treatments were used to simulate conventional varieties with low fruit retention, to provide a comparison with Bollgard II cotton, with high fruit retention.

The manual removal of squares has been successfully used to simulate pest damage in conventional cotton (Brook et al., 1992a; Sadras, 1996). Many studies using flower bud removal in conventional cotton (Kletter and Wallach, 1982b) showed that cotton plants are able to compensate final yield after severe damage levels early in the season with good growing conditions later in the season. Artificial flower buds removal causes many factors to be affected such as time to cut-out (Guinn, 1985) and boll retention (Guinn, 1982; Kletter and Wallach, 1982b). Some studies showed that compensation after flower bud removal in conventional cotton included increases in vegetative growth (Brook et al., 1992b), increases in flower production and boll retention (Guinn, 1985).

The results of previous experiments showed in earlier Chapters support the general hypothesis that insufficient early growth as a result of soil water deficit during the pre-flowering phase of development, reduces the supply of assimilates for large number of bolls retained in Bt cotton. Even modest early soil water deficits were found to affect seed cotton yield in high retention cotton. Measures aimed at improving pre-flowering water availability had a significant impact on the crop, with changes in boll distribution pattern, an increase in boll retention and increased final yield, when compared with water stressed cotton plants. In the previous studies (Chapters 3 and 4) the irrigation applied after the stress periods did not refill the soil profile, whereas in these current experiments the amount of water that crops received after the water stress periods finished was greater and refilled the soil profile.

For this study it was assumed that the higher sink demand on a smaller plant in Bollgard II cotton, may risk early cut-out and reduce yield when full irrigation is not supplied prior to flowering as it is the current practice in the cotton belt of NSW, Australia. However, it may be possible to increase water supply before flowering, and thereby increase the vegetative biomass to enhance the supply of assimilates for the development and maturation of bolls. For conventional cotton varieties, a soil water deficit at pre-flowering is usually maintained within recommended limits, for optimizing growth (Constable and Hearn, 1981; Hearn and Constable, 1984). However, for Bollgard II Bt cotton, the earlier provision of water may encourage more vigorous growth and thereby increase final yield.

The main objective of this work was to study the effect of early water availability on the dynamics of fruit development, fruit distribution and retention, and final yield in high and low fruit retention cotton. A hypothesis is that early irrigation should help increase boll number and yield in Bt cotton, but this may not be the case when flower number is artificially reduced to simulate traditional cotton varieties. A related study examined the effects of light exposure of the lower part of the crop canopy to increase source supply and its impact on boll retention and final yield under irrigated conditions.

5.3 Materials and Methods

5.3.1 Experimental sites and growth conditions

Four experiments were conducted over a two year period (Exp.4 from early October 2007 to April 2008; Exp.5 from mid October 2007 to April 2008; Exp.6 from mid October 2008 to April 2009 and Exp.7 from late October 2008 to April 2009). The experiments were undertaken at Gatton (91m, 27^{o}33'S, 152^{o}20'E) in the Lockyer Valley of Southeast Queensland, Australia. The soil type in the experimental area was a Lawes clay loam (Powell, 1982), with heavy dark cracking clays. Average annual rainfall is 760 mm with a summer dominance; evaporation rates are high, almost double the average rainfall. Some of the treatments from Exp.4 were used in previous Chapters.

5.3.2 Cultural practices

The Bt transgenic Bollgard ll®™ variety Sicot 71BR producing the Monsanto Cry1Ac and Cry2AB proteins) was sown in all the experiments. Exp.4, 6 and 7 were sown using a Nodet Gougis vacuum planter, while Exp.5 was sown by hand. High seeding rates were used at sowing with seedling numbers being later reduced to obtain a population of 140,000 plants ha^{-1} (12-15 plants in 1 m rows). The experimental area was prepared one month before sowing using conventional tillage practices. N fertilizer at a rate of 100 kg ha^{-1} was applied at sowing. Herbicides were used to control weeds pre-planting (pendimethelin), and post emergence (glyphosate). Insects were regularly controlled through monitoring the presence of insects in the crop and applying insecticide sprays when thresholds were reached for temperate Australia (Farrell, 2006).

5.3.3 Experimental design, water deficit and flower buds removal treatments

In all experiments, overhead sprinklers were used to provide the following irrigation treatments:

I (Irrigation): Irrigation was applied to meet the water requirements for a cotton crop, calculated as the product of daily class "A" pan evaporation by a crop coefficient depending on the phenological stage of the crop (CRDC, 2003).

NIS (No irrigation until squaring = mild water stress): No water was applied from establishment to squaring (water stress period), followed by fully refilling the soil profile and further irrigation as for I through to maturity.

NIF (No irrigation until flowering = severe water stress): No water from establishment to flowering (water stress period) followed by fully refilling the soil profile and further irrigation as for I through to maturity.

In these experiments, the soil profile was fully refilled after the stress period finished which differed from stress treatments in previous Chapters where the water applied was not enough to refill the soil profile after the stress period finished.

Flower removal. In each water treatment there were two levels of flower removal starting from the time of early flowering, (i) non-removal (NR) Bollgard II representing high retention cotton and, (ii) Bollgard II with 30 flowers removed per metre (30R). This second level of removal simulated conventional low retention cotton. The flower buds were removed three times a week over a two week period from early flowering at the first positions on the lower fruiting nodes of the plant.

5.3.3.1 Experiment 4

Sowing was done on 16th October 2007. The total area of the experiment was 2,400 m^2. The water treatments were randomized as main plots and then sub-plots were NR and 30R with four replications on split-plot design. The buffer areas were sufficient to ensure that there was no lateral water movement between plots. Water stress was achieved in the non-watered treatments by intercepting rainfall with the use of plastic covers which were placed on the ground between the rows within 1cm of the plant stems, with the covers then being secured using wire pegs. The water stress treatments were covered from the two first true leaves up to beginning of squaring (**NIS**) and beginning of flowering (**NIF**). The covers were removed when the treatments periods were finished and irrigation commenced. This experiment was fully described earlier under NR conditions (Chapter 3).

5.3.3.2 Experiment 5

Sowing was undertaken on 3rd October 2007 with NIS and NIF under rainout shelters and I under normal field conditions. Removal treatments (NR and 30R) were used in all three levels of watering. The rainout shelters were used to create the water stress treatments. The rainout shelters were 12 m by 15 m in area, while the experimental area under normal field conditions was 24 m by 30 m.

5.3.3.3 Experiment 6

Sowing was done on 15th October 2008. The total area of the experiment was 1,600 m^2. The treatments were laid out in a randomized complete block design with four replications. This experiment was conducted under well irrigated conditions, with the following treatments:

CE0 = no lower canopy exposure to sunlight (control); **CE20 =** lower canopy exposure for 20 days after first flower; **CE40** = lower canopy exposure for 40 days after first flower; **CE90** = canopy exposure from first flower for 90 days when final harvest took place. Lower canopy light exposure was achieved by pushing the plants in the rows immediately adjacent to the 'test' row (the row to be harvested) to a 45 degree inclination and then holding the plants in position using wires tied to steel posts (Fukai et al., 1991). At the end of the lower canopy exposure treatment period, the wire was removed and the plants allowed returning to their original canopy structure.

5.3.3.4 Experiment 7

Sowing was done on 27th October 2008. The total area of the whole experiment was 1,600 m^2. The design was the same as described for Exp.5 but in a different season with water treatments as main plots and removal treatments as sub-plots. Plastic covering of the whole plot area was used to ensure the water stress treatments as described for Exp.5. The plastic was removed when the treatment period was completed and full irrigation commenced.

5.3.4 Measurements

5.3.4.1 Meteorological conditions and soil water

Daily temperature, relative humidity, precipitation, pan evaporation and solar radiation were measured in a weather station adjacent to the experimental field.

5.3.4.2 Mapping

The dynamics of reproductive organ development in cotton plants was studied in the experiments. One of the most important components is fruits number as well as total scars or abortions and retention of fruits. Mapping of fruit retention was undertaken for the different phenological stages of crop development (flowering, cut-out and maturity) on a 1 m row (Kerby and Hake, 1996). Vegetative branches were not included in the study. Plant height and number of nodes were also collected. The retention rates in three different lateral fruiting positions on branches were studied - FS1, the first position closest to the main stem; FS2, the position adjacent to FS1; and FS3+, FS3 and beyond, a position further out on the branch. The distribution of retention rates for fruiting positions on the vertical positions (nodes) of the plant was also collected. In the case of 30R treatment, the flower buds removed were counted as aborted or shed as all others caused by other factors (insect, hormonal, etc). Height, number of nodes and retention was also measured around key stages of the crop.

5.3.4.3 Lint yield

To measure final yield, in both seasons, 2007/08 and 2008/09, all open bolls in a 4 m^2 section from the central rows of each plot were hand picked.

5.4 Results

5.4.1 Meteorological conditions

Daily maximum and minimum temperatures, solar radiation and evaporation during the experimental period are shown in Fig. 5.1 (a b & c). Cumulative solar radiation, cumulative degree days and total rainfall during the two seasons are summarised in Table 5.1. Total solar radiation was similar during both seasons (2007/2008 and 2008/2009).

Fig. 5.1b shows the levels of evaporation measured near the experimental site, based on Australian tank evaporation during both seasons. Total pan evaporation in 2007/2008 was 942 mm in Exp.4 and 1,042 mm in Exp.5, for the whole season, with daily averages of 5.1 and 5.2 mm respectively. The total evaporation during the second season (2008/09) was 1,090 mm in Exp.6 and 1,018 mm in Exp.7, with a daily average of 6.5 mm in both experiments. During the second season of these experiments the temperature and the pan evaporation was higher than in the first season.

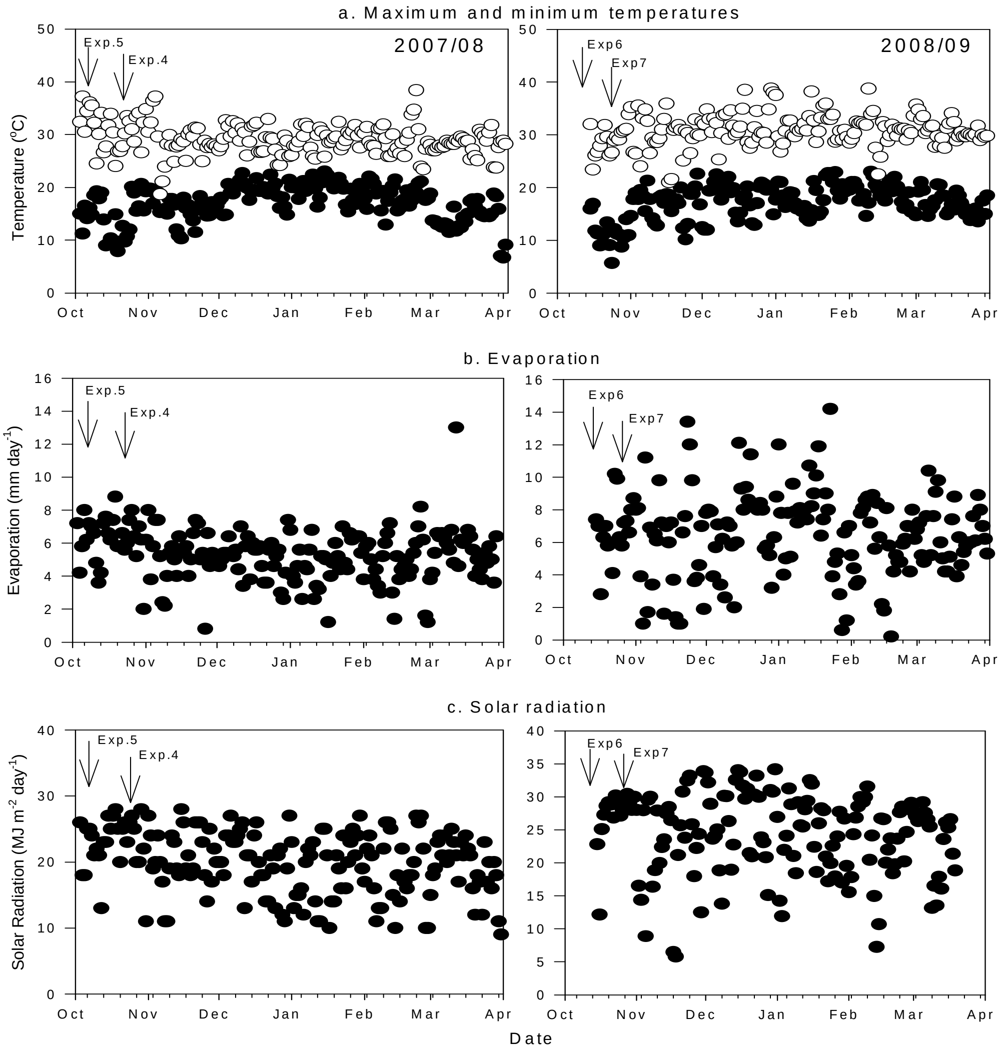

Figure 5.1 (a) Daily minimum and maximum temperatures (°C); (b) daily evaporation (mm); and (c) daily incident solar radiation (MJ m^{-2} day^{-1}) during 2007-08 (left) and 2008-09 (right). Arrows indicate sowing date in all experiments at Gatton, SE Queensland

Table 5.1 Cumulative degree days, mean maximum and minimum temperatures, total rainfall and cumulative solar radiation during the period the four experiments (2007/08 and 2008/09) at Gatton, SE QLD. Base temperature of 12°C is used (Constable and Shaw, 1988)

Variable	Exp4	Exp5	Exp6	Exp7
Cumulative degree day (base 12°C)	2163	2236	2026	1924
Average maximum temperature (°C)	28.7	29.5	30.8	31.0
Average minimum temperature (°C)	15.5	15.1	17.1	17.6
Total rainfall (mm)	606	582	631	616
Cumulative solar radiation (MJ m^{-2})	3544	3910	3896	3581

5.4.2 Water received from irrigation and rainfall.

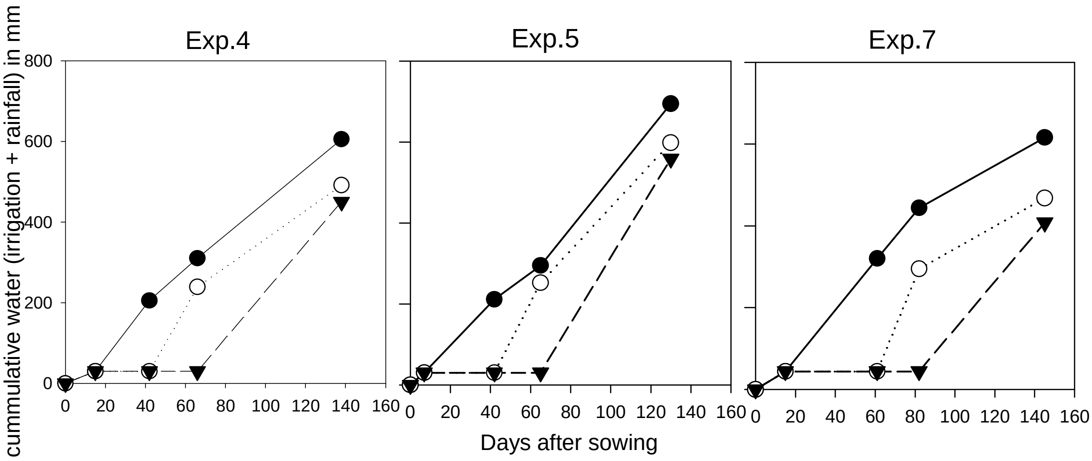

Figure 5.2 Cumulative water input (irrigation and rainfall) for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) during 2007/08 (Exp. 4 & 5) and 2008/09 (Exp. 7) at Gatton, SE Queensland

During 2008/09, about 68% of rainfall during crop growth was received during the pre-flowering stages of development, while in 2007/08 only 41% was received in the same period. The remaining 32% and 59%, respectively, for the two seasons, was received after flowering towards the end of growing season. During the early phase of crop growth, the use of the rainout shelters and plastic covering prevented the rainfall from having any impact on the soil water deficit treatments, however, later in the season all treatments received the benefit of the rainfall and supplementary irrigation as required (Fig. 5.2). Irrigation water after the stress periods was greater in the stress treatments than in I in the corresponding time and hence the difference in total amount of water supplied between the irrigation treatments at the end of the growing season was smaller than at the end of the stress period.

5.4.3 Squares, flowers and boll number

The components of fruit production subject to analysis included numbers of squares and flowers, green bolls and open bolls. These components were determined during key stages of crop growth (Fig. 5.3).

In Exp.5 (2007/08) at 48 DAS the number of squares per plant was not significantly different among I, NIS and NIF. This stage (48 DAS) coincided with commencement of squaring, and the end of the water stress period of NIS. Significant differences were exhibited at 77 DAS, with higher numbers of squares and flowers for I relative to NIS and NIF. At this stage of crop growth, the water stress period in NIF had ended with the commencement of flowering. At 110 DAS, in squares/flowers number was significantly greater for I and NIS than NIF. At 156 DAS there were significant differences in green boll number among the treatments, with the largest number being in I; however, the number of open bolls at this stage was higher in the stress treatments (NIS and NIF) due to the accelerated phenological development. I had a higher number of open bolls by 181 DAS when compared with the stress treatments.

During the second season (2008/09) the trend was similar to that observed during the first season. There were no significant differences among the treatments for squares and flower number at the first measurement occasion around 50 DAS (Exp.4 and 7). At 82 DAS a significantly higher number of squares and flowers ($P = 0.013$) was recorded in I in Exp.4 but the number of green bolls was higher ($P = 0.001$) in the stress treatments than I, probably due to their accelerated phenological development. No significant differences were found in squares and flowers in Exp.7 at 75 DAS. At 121 DAS (Exp.7), squares/flowers number decreased significantly in all the treatments, but I had a significantly higher number of green bolls compared with the stress treatments. At 155 (Exp.4) and 140 (Exp.7) DAS, there were significant differences in green boll number among the treatments, with the greatest numbers being in I. However, the number of open bolls at this stage was higher in the stress treatments. I had a higher number of open bolls by 175 (Exp.4) and 165 (Exp.7) DAS when compared with the stress treatments.

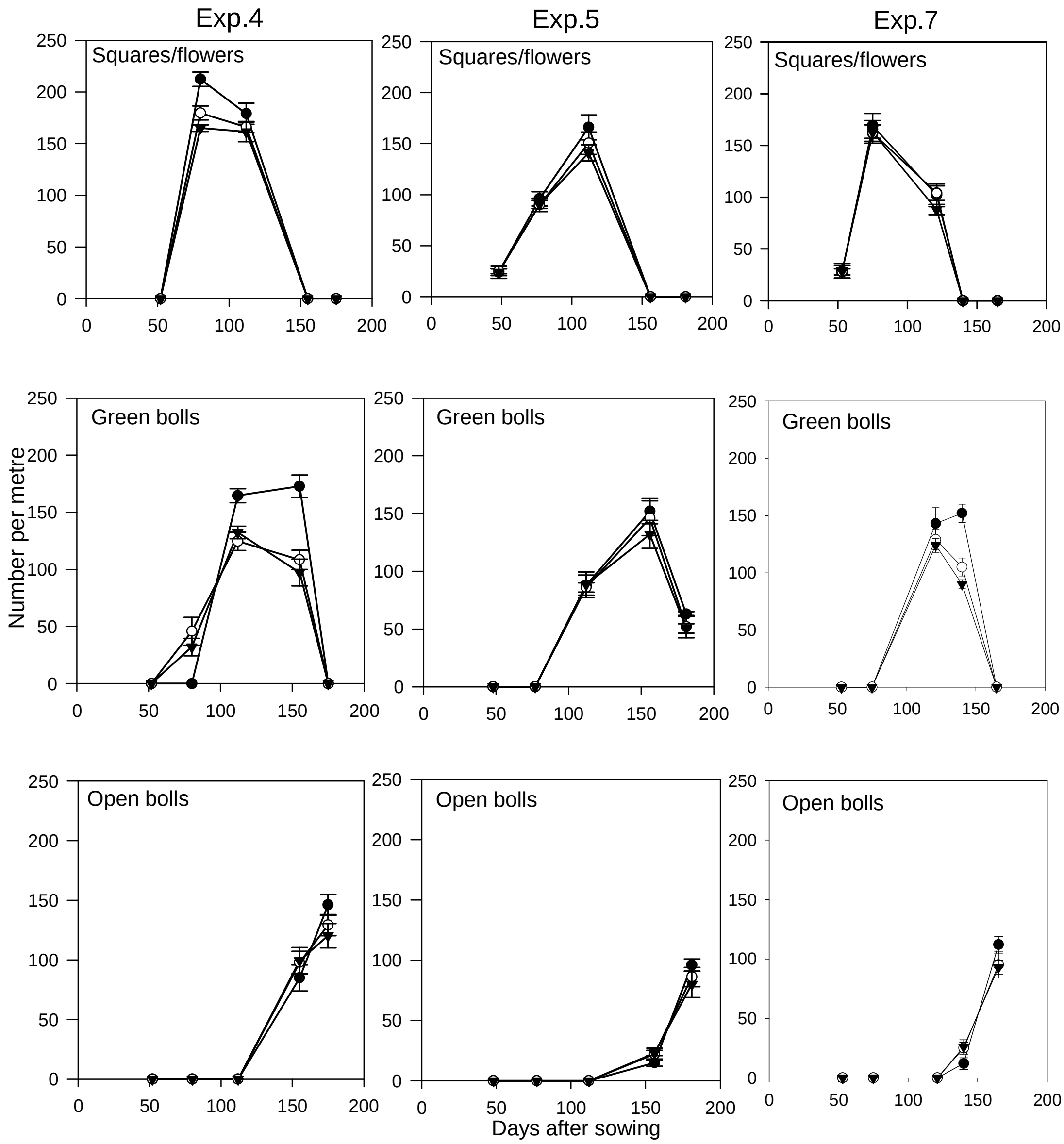

Figure 5.3 Change in number of squares and flowers, number of bolls and number of open bolls for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) for Exp. 4, 5 and 7. Bars are two standard errors of the mean.

5.4.4 Fruit production and retention

5.4.4.1 Plant height and number of nodes

Plant height and number of nodes were recorded at the time of each harvest. Plant height, main stem node production (as potential production sites) and fruit retention during early stages of growth are summarised in Table 5.2.

Table 5.2 Plant height (H), number of nodes (N) and retention fraction (Ret fr.) at all positions for I, NIS and NIF, early and mid season in three experiments (Exp.4, 5 and 7) at Gatton, SE Queensland during 2007/08 and 2008/09.

Treatment	**H (cm)**	**N**	**Ret fr.**	**H (cm)**	**N**	**Ret fr.**
Exp.4	***51DAS***			***82DAS***		
I	37.0	11.1	0.917	117.5	17.1	0.878
NIS	30.6	11.0	0.903	106.7	15.6	0.862
NIF	27.0	11.2	0.911	96.8	15.1	0.828
Significance	**	NS	NS	**	*	NS
Exp.5	***48DAS***			***77DAS***		
I	36.3	10.5	0.875	97.7	15.5	0.839
NIS	32.5	10.0	0.878	75.2	14.7	0.794
NIF	27.6	10.1	0.842	55.3	14.3	0.788
Significance	*	NS	NS	**	*	NS
Exp.7	***53DAS***			***75DAS***		
I	35.3	10.2	0.902	75.3	15.1	0.853
NIS	33.0	10.5	0.905	68.7	14.2	0.830
NIF	43.7	10.2	0.901	67.2	14.2	0.832
Significance	NS	NS	NS	*	*	NS

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

In terms of plant height, in Exp.5, plants in the I treatment were significantly taller (P <0.001) than in the water stress treatments early and mid-growth stages (48 and 77 DAS); there were also significant differences between NIS and NIF (P <0.001) in plant height. Node production increased throughout the season, with some differences between I and the stress treatments at 77 DAS. Even when considering differences in node number as representing in the production of reproductive sites, there were no differences in percentage fruit retention among the treatments at 48 and 77 DAS.

Similar results were obtained in the other experiments (Exp.4 and 7), although the height difference was not significant at the first measurement occasion in Exp.7.

Table 5.3 Height (H cm), number of nodes (N) and retention fraction (Ret fr.) at all positions for I, NIS and NIF combined with NR and R30 during mid-late season of the crop in three experiments (Exp.4, 5 and 7) at Gatton, SE Queensland during 2007/08 and 2008/09. WT-water treatment. RT-removal treatment.

Mid-late season	**Exp.4** *145DAS*			**Exp.5** *145DAS*			**Exp.7** *140DAS*		
Treatments	**H (cm)**	**N**	**Ret fr.**	**H (cm)**	**N**	**Ret fr.**	**H (cm)**	**N**	**Ret fr.**
I + NR	142.5	20.2	0.848	147.2	20.7	0.807	139.2	18.2	0.833
I + 30R	139.2	19.5	0.733	149.5	20.7	0.761	131.4	18.3	0.802
NIS + NR	123.7	20.0	0.815	137.0	20.5	0.790	122.3	16.3	0.777
NIS + 30R	130.0	18.7	0.731	131.7	21.2	0.748	121.9	17.2	0.701
NIF + NR	122.7	18.2	0.795	100.0	18.7	0.775	117.2	15.5	0.712
NIF + 30R	132.0	18.7	0.675	113.5	19.5	0.726	117.8	16.9	0.691
Signif. WT	**	*	*	**	**	*	**	*	*
Signif. RT	*	*	*	NS	NS	*	NS	NS	*
Signif. WT*RT	0.046	0.032	NS	NS	NS	NS	NS	NS	NS

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

Statistical analysis was conducted with water treatments as the main plot and removal as the subplot for mid to late growth stages (Table 5.3). In Exp.4, significant responses to water treatments were found in relation to plant height, number of nodes and retention fraction, with higher levels in the I treatments. Similar responses were found in response to the removal treatments. The water x removal interaction was significant for height and number of node (P = 0.046, P = 0.032, respectively), but not significant for the retention fraction.

For Exp.5, significant differences were found for height and number of nodes among I, NIS and NIF (P <0.001), with I having significantly higher levels of both than in the NIS and NIF treatments. Significant responses in terms of the retention fraction were found for the removal treatments (P = 0.024), with lower values in R30 (as expected) being associated with the early removal of fruit. There were no significant interactions between flower removal and irrigation in relation to plant height (P = 0.295) and node number (P = 0.236).

In Exp.7 results were similar to those obtained in Exp.5. None of interactions between irrigation and removal treatments were significant for any variable (P = 0.312, P = 0.311, P = 0.801), however significant responses to the water treatments were found in terms of height (P = 0.001) and number of node (P = 0.021). Retention fraction was significantly higher in I treatments compared with stressed treatments. There were also significant responses to the removal treatments in relation to the retention fraction (P = 0.012) with higher values in NR than 30R.

5.4.5 Dynamics of reproductive organ development

5.4.5.1 Lateral fruiting positions and retention

Table 5.4 shows the number of fruit retained (fruits that still existed at maturity) on a plant as well as the proportion of the total number of fruiting sites retained. Fruit number as well as the retention fraction, increased in response to the pre-flowering irrigation treatments (I), relative to the water stress treatments. Percentage retention decreased from the first to third positions within the sympodial branch for all treatments.

In Exp.4, the total fruit number (TFN) was significantly higher in I than in stress treatments. Total retention was only significantly affected by WT being higher for I. FS1 was significantly higher in I than in stressed plants. The responses in FS1 were significant for WT*RT (P = 0.026), while Ret1 responded significantly (P = 0.014) to WT by increasing in the higher water availability treatments. At the second fruiting position, WT significantly affected FS2 and Ret2. At the third fruiting position, FS3 and Ret3 were significantly affected only by WT and not by RT being higher under irrigated conditions.

Table 5.4 Total fruit number (TFN) and retention fraction (TRet) per plant at maturity and different fruiting sites (FS 1, 2, 3+ and total) for I, NIS and NIF combined with NR and R30 in three experiments (Exp.4, 5 and 7) at Gatton, SE Queensland during 2007/08 and 2008/09.

Exp.4	**T FN**	**T Ret**	**FS1**	**Ret1**	**FS2**	**Ret2**	**FS 3+**	**Ret 3+**
I + NR	40.1	0.595	17.1	0.797	14.9	0.456	7.9	0.445
I + 30R	39.4	0.574	16.0	0.753	14.7	0.515	8.6	0.355
NIS + NR	34.3	0.584	14.9	0.697	12.5	0.536	6.9	0.417
NIS + 30R	33.0	0.558	14.4	0.663	10.7	0.457	7.0	0.504
NIF + NR	28.9	0.523	12.1	0.712	11.7	0.356	6.4	0.433
NIF + 30R	29.4	0.465	12.1	0.642	11.9	0.376	6.6	0.294
Signif. WT	*	*	**	*	*	*	*	*
Signif. RT	NS	NS	NS	NS	NS	NS	NS	NS
Signif. WT*RT	*	NS	*	NS	NS	NS	*	NS
Exp.5	**T FN**	**T Ret**	**FS1**	**Ret1**	**FS2**	**Ret2**	**FS 3+**	**Ret 3+**
I + NR	36.7	0.620	14.9	0.816	13.9	0.640	7.7	0.409
I + 30R	35.5	0.575	14.9	0.680	14.0	0.505	6.4	0.472
NIS + NR	23.0	0.585	11.4	0.670	10.9	0.531	4.5	0.416
NIS + 30R	33.2	0.563	14.7	0.640	11.5	0.520	6.9	0.467
NIF + NR	31.7	0.552	12.7	0.647	11.4	0.588	7.5	0.326
NIF + 30R	29.2	0.488	12.0	0.585	11.0	0.458	6.2	0.361
Signif. WT	*	*	**	**	NS	NS	NS	NS
Signif. RT	NS	*	NS	*	NS	NS	NS	NS
Signif. WT*RT	*	NS	*	NS	NS	NS	NS	NS
Exp.7	**T FN**	**T Ret**	**FS1**	**Ret1**	**FS2**	**Ret2**	**FS 3+**	**Ret 3+**
I + NR	37.0	0.651	14.7	0.769	13.9	0.627	10.9	0.476
I + 30R	36.9	0.624	15.2	0.691	14.7	0.560	10.7	0.578
NIS + NR	32.5	0.584	13.0	0.619	12.5	0.566	7.0	0.492
NIS + 30R	33.0	0.563	13.7	0.614	11.9	0.532	9.9	0.506
NIF + NR	29.5	0.568	12.9	0.663	10.9	0.580	11.2	0.404
NIF + 30R	30.4	0.583	12.7	0.675	11.0	0.489	8.5	0.506
Signif. WT	*	*	*	NS	*	NS	*	NS
Signif. RT	NS	NS	NS	NS	NS	NS	NS	*
Signif. WT*RT	NS	NS	NS	NS	NS	NS	NS	NS

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

In Exp.5, the differences in total fruit number (TFN) across water treatments (WT) were significant, while there were no significant differences across removal treatments (RT). The WT and RT interaction was also significant as NIS + NR produced much lower fruit number. Percentage total retention was significantly higher in I than the stress and in NR than 30R; however, there was no significant interaction between WT and RT ($P = 0.376$). For FS1 there were significant differences among the water treatments. I had a higher number of FS in position 1 compared to NIS and NIF. There was an interaction between WT*RT ($P = 0.024$) for FS1 as NIS + NR produced much lower fruit number. In FS2 and FS3+, there were no significant differences in fruit retention. The higher percentage of retention was mainly concentrated in first position rather than the second position.

In Exp.7, the total fruit number (TFN) and total fruit retention responded significantly to WT with higher values for I than stress conditions. FS1 was significantly higher in I than stressed treatments. In the first position there were no responses to RT and WT*RT for FS1. In the second and third fruit positions the number of FS was increased under irrigation conditions, with no responses to RT and WT*RT.

5.4.5.2 Vertical fruiting positions and retention

The distribution of fruits per node per plant gives an idea of the vertical retention at maturity under the different water treatments. The number of fruits per node and number of abortions are shown in Fig. 5.4, 5.5 and 5.6 and the vertical retention in the different treatments is illustrated in Fig. 5.7, 5.8 and 5.9.

The I treatments produced more fruits at the top positions in both Bt and conventional (flower removal) than in stressed treatments. I 30R produced more total fruits positions (including aborted ones) in the middle but lower retention at the lower positions.

For Exp.4 (Fig. 5.4 and 5.7), at the lower nodes in I the retention was higher in NR than 30R but with a similar distribution on mid-upper nodes of the plant. From node 20 to the top of the plant, 30R showed a higher fruit retention compared with NR. In the NIS treatments, the vertical distribution among the removal treatments was different for the lower nodes, with decreases in 30R. As in Exp.4 NIF showed a similar trend between NR and R30, with a decline in fruit retention in the upper nodes when compared with I and NIS.

In Exp.5 (Fig. 5.5 and 5.8), the vertical distribution of retention at different fruiting nodes exhibited different trends, reflecting the level of water availability (I, NIS or NIF). In the case of I, high retention was concentrated in the middle part of the canopy with 10 nodes showing around 60-70% of retention. There were also a few more fruiting sites at higher levels (up to node 23) with lower levels of retention. Although NIS showed a similar trend in the lower positions as I, the number of fruiting nodes in the middle part of the canopy with high retention was reduced (around 8 nodes) when compared with I. NIF had a different distribution, with higher retention in lower fruiting nodes, and more variability for the upper nodes, followed by a decrease to zero retention on node 20. Earlier cut-out and maturity further reduced the production of fruiting branches in NIF, followed by NIS and I. 30R showed a decline in fruit retention in the lower nodes for all the water treatments due to earlier manual flower bud removal; however, fruit retention increased significantly in the upper fruiting nodes when compared with NR.

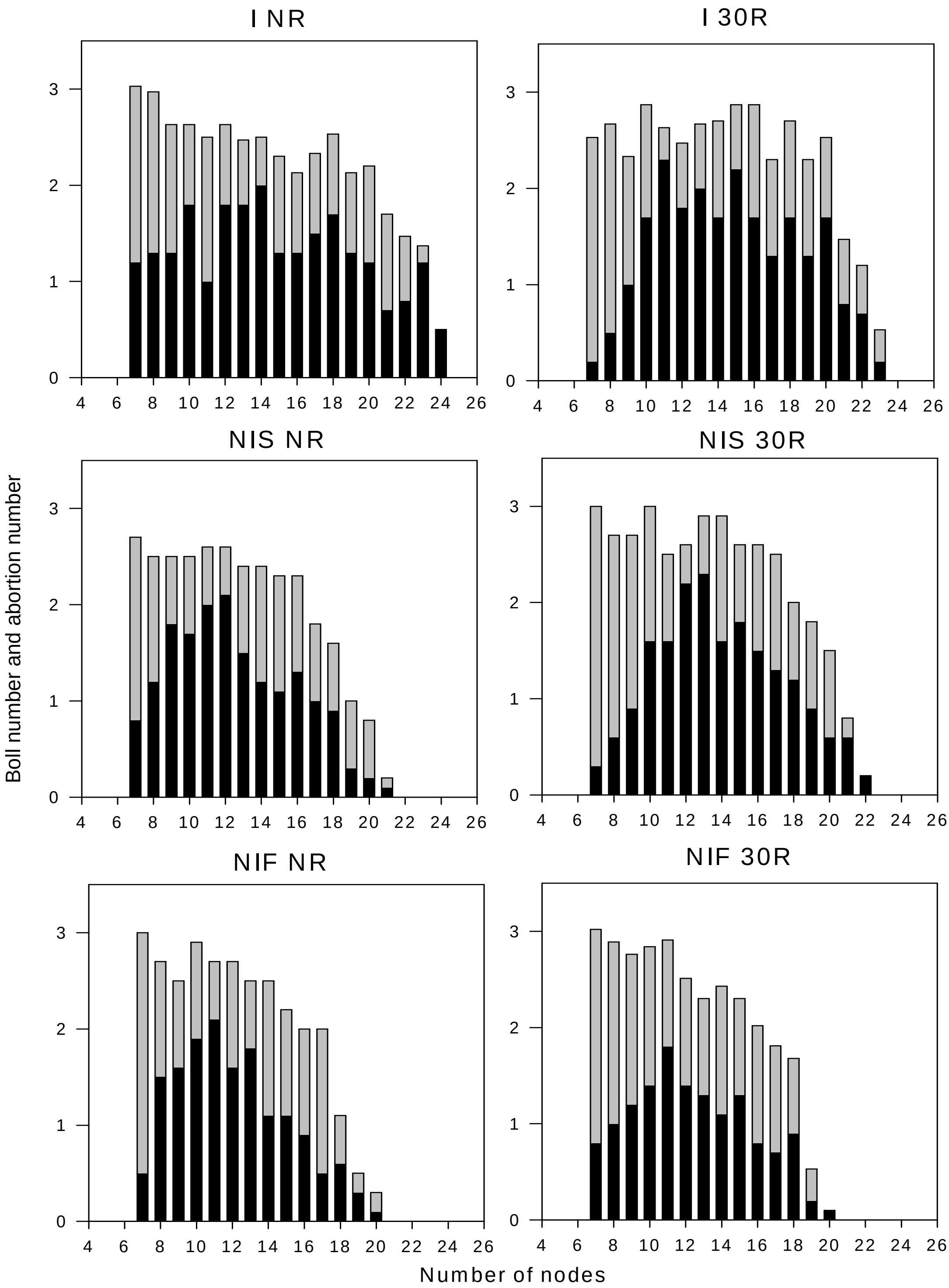

Figure 5.4 Number of fruits retained (black) and aborted (grey) per fruiting node in Exp.4 for I, NIS and NIF in combination with NR and 30R

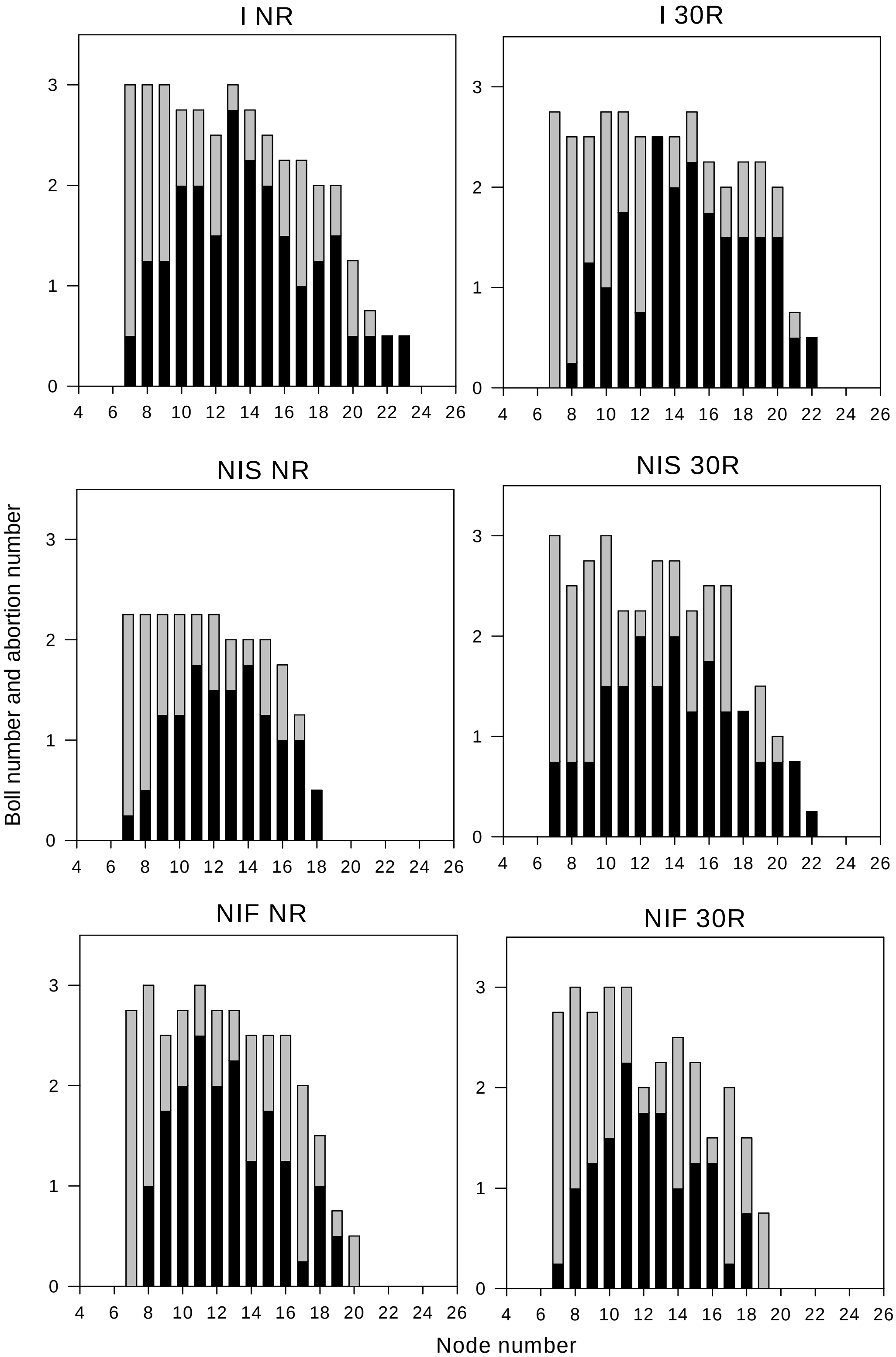

Figure 5.5 Number of fruits retained (black) and aborted (grey) per fruiting node in Exp.5 for I, NIS and NIF in combination with NR and 30R

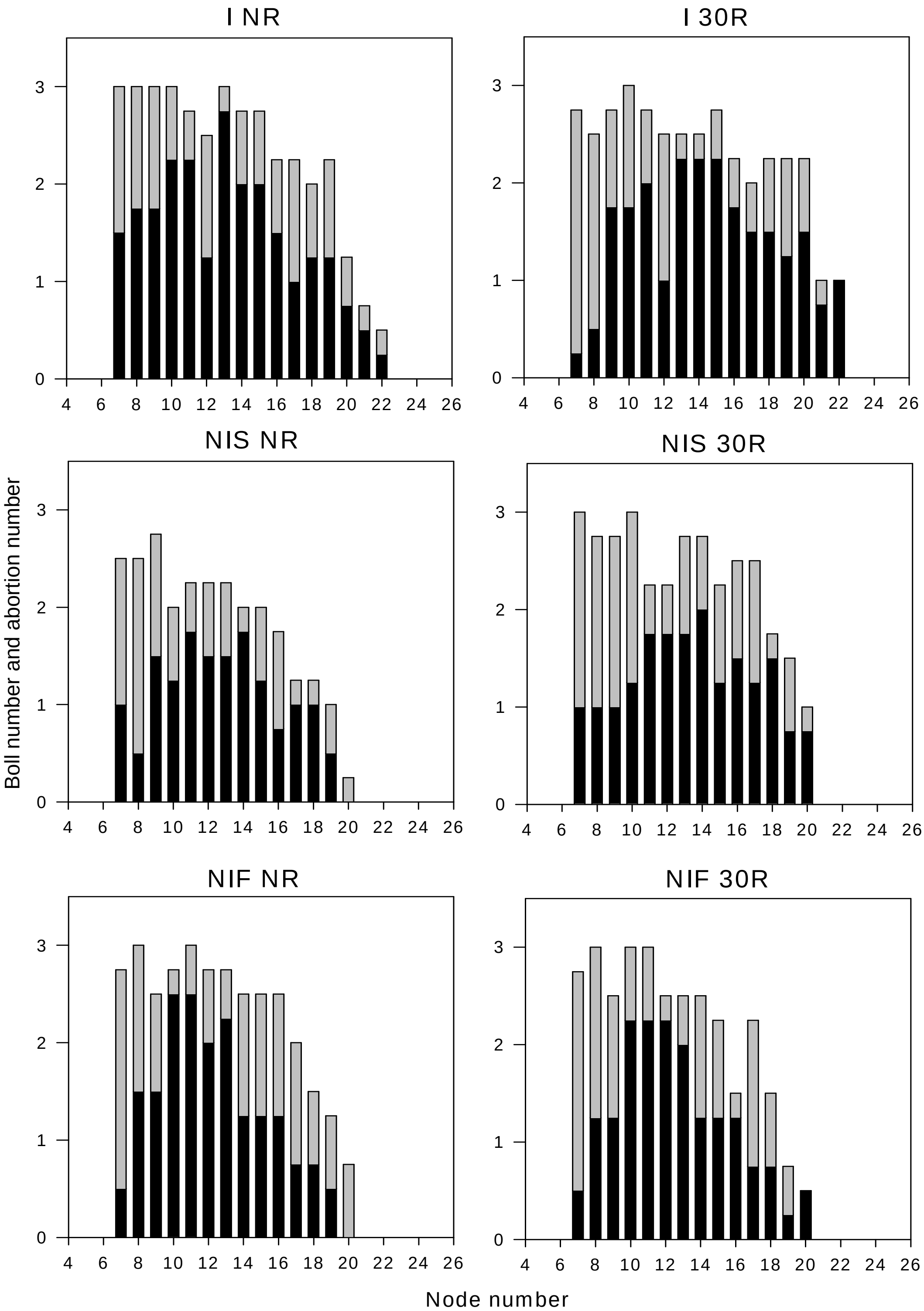

Figure 5.6 Number of fruits retained (black) and aborted (grey) per fruiting node in Exp.7 for I, NIS and NIF in combination with NR and 30R

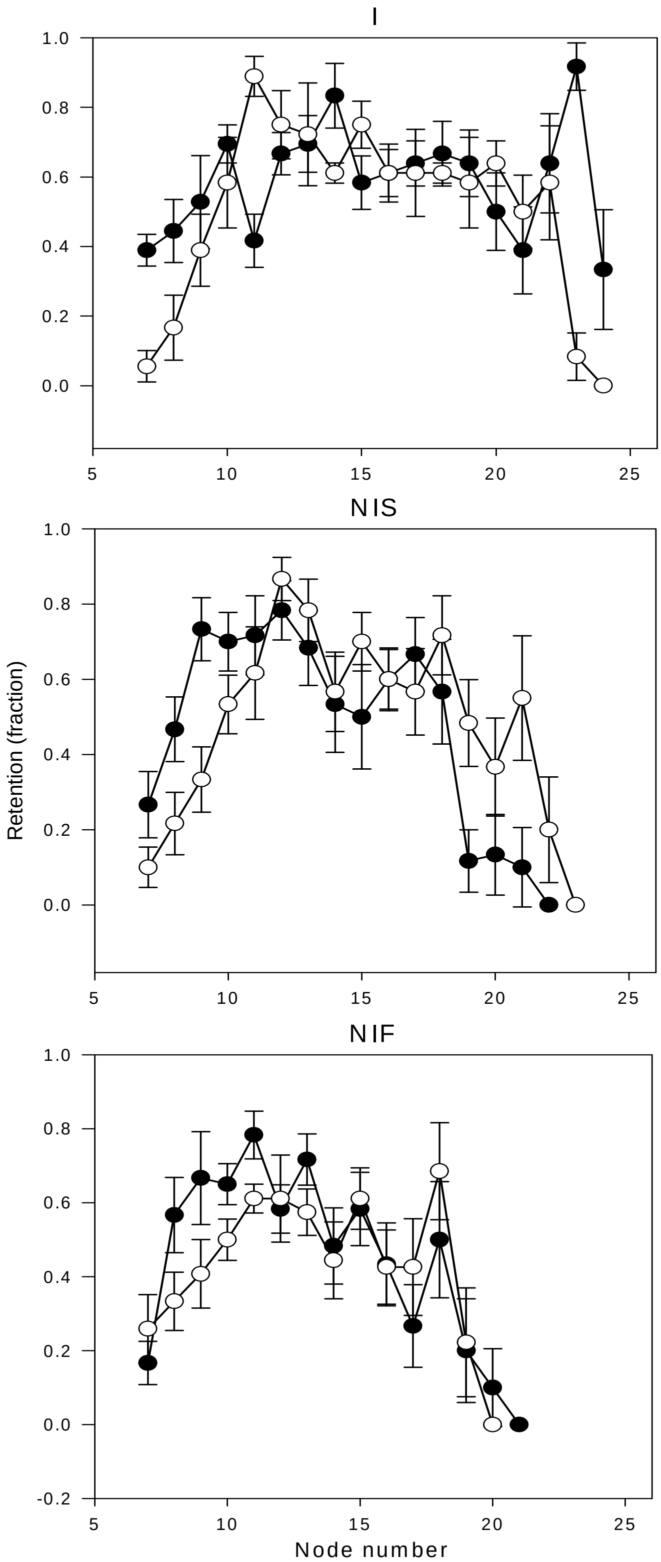

Figure 5.7 Retention fraction per fruiting node in Exp.4 for I, NIS and NIF in combination with NR (●) and (○) 30R. Bars are two standard errors of the mean.

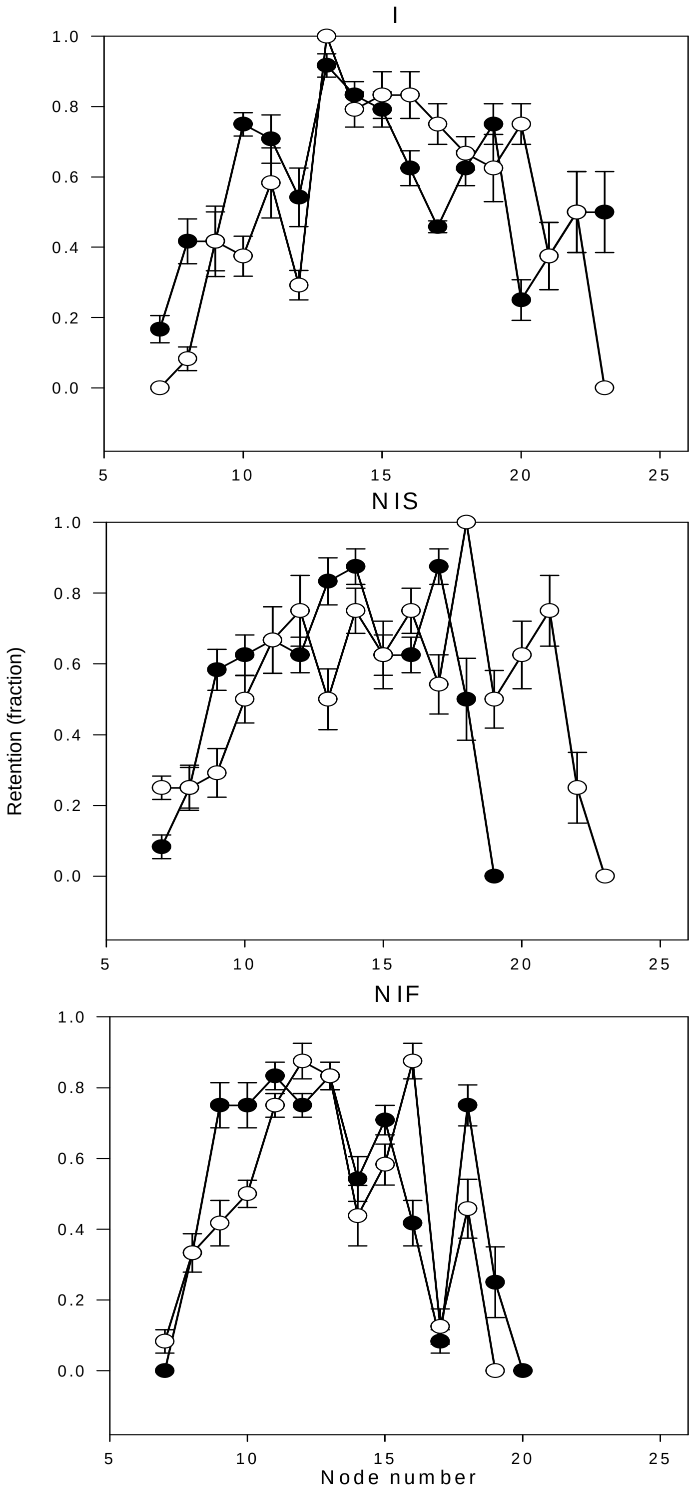

Figure 5.8 Retention fraction per fruiting node in Exp.5 for I, NIS and NIF in combination with NR (●) and (○) 30R. Bars are two standard errors of the mean.

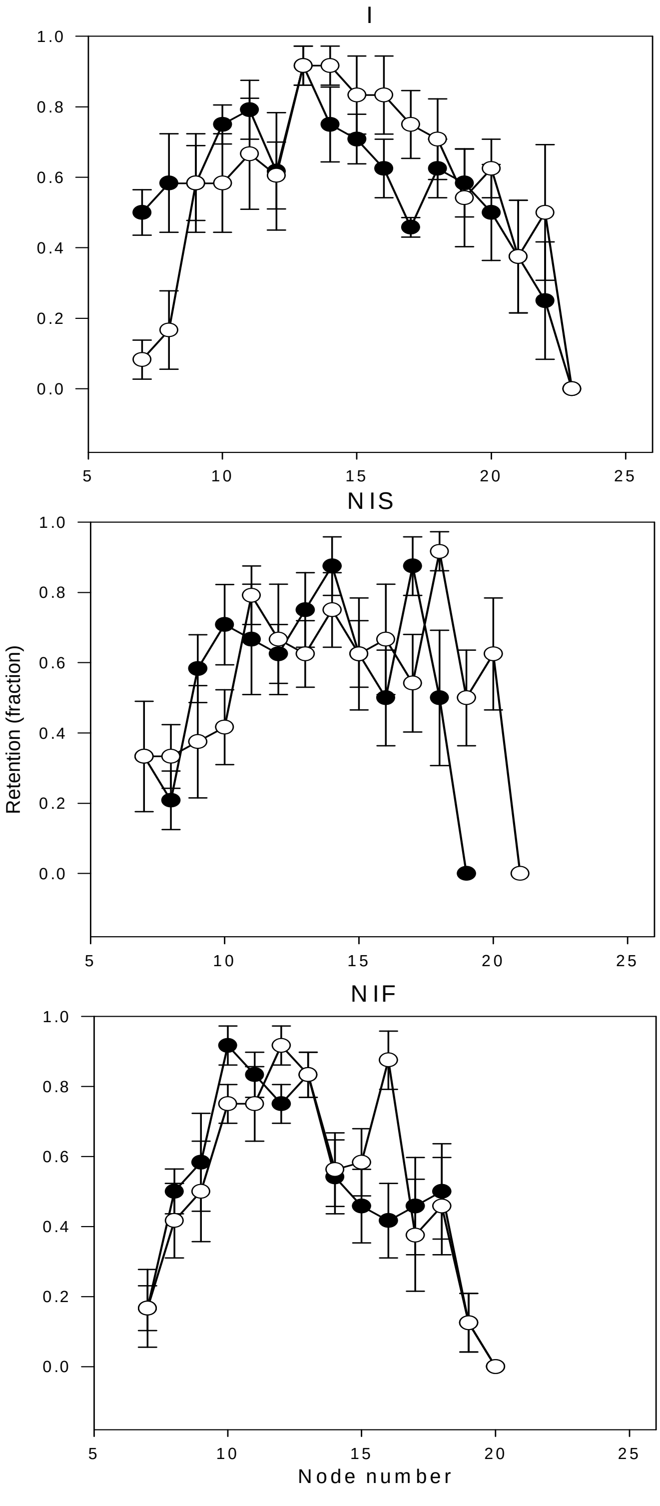

Figure 5.9 Retention fraction per fruiting node in Exp.7 for I, NIS and NIF in combination with NR (●) and (○) 30R. Bars are two standard errors of the mean.

Fig. 5.6 and 5.9 shows the levels of retention in Exp.7. Differences in retention at the lower nodes were found between NR and 30R under I conditions, while a compensatory higher level of fruit retention was recorded on the upper nodes for I + 30R. For NIS, the differences between the removal treatments were lower than for I. A decreased retention was found in the lower nodes in 30R. NIF produced fewer nodes with fruiting sites compared with I and NIS. Most of the retained fruit was concentrated on the lower nodes (between 9 and 14), with gradual decline in levels of retention towards the top of the plant. For NIF, the differences between NR and 30R were lower than for I and NIS.

5.3.6. Seed cotton yield

Seed cotton yields are shown in Fig. 5.10.

Statistical analysis in Exp.5 showed significant differences in seed cotton yield in response to the water treatments (<0.001) with higher values in I than stressed treatments; however, there were no significant differences in response to the removal treatments (P = 0.196). For Exp.4 and 7, seed cotton yield was also increased in I than in stressed treatments and the results were similar in those in Exp.5. Interaction WT*RT was also not significant in all three experiments, but the effects of irrigation tended to be larger in NR (Bt cotton) than in 30R (about 19% difference between NR and 30R under irrigation, while the differences between NR and 30R was about 7% under stress conditions).

Gin-out % was significantly different among the irrigation treatments only in Exp.5 (P = 0.003) with 43.66% in I, 41.89% in NIS and 41.56% in NIF. There were no significant differences between the treatments in Exp.4 and Exp.7 (P = 0.710 and P = 0.312, respectively). No significant responses to removal treatments were found for gin-out % (data not presented).

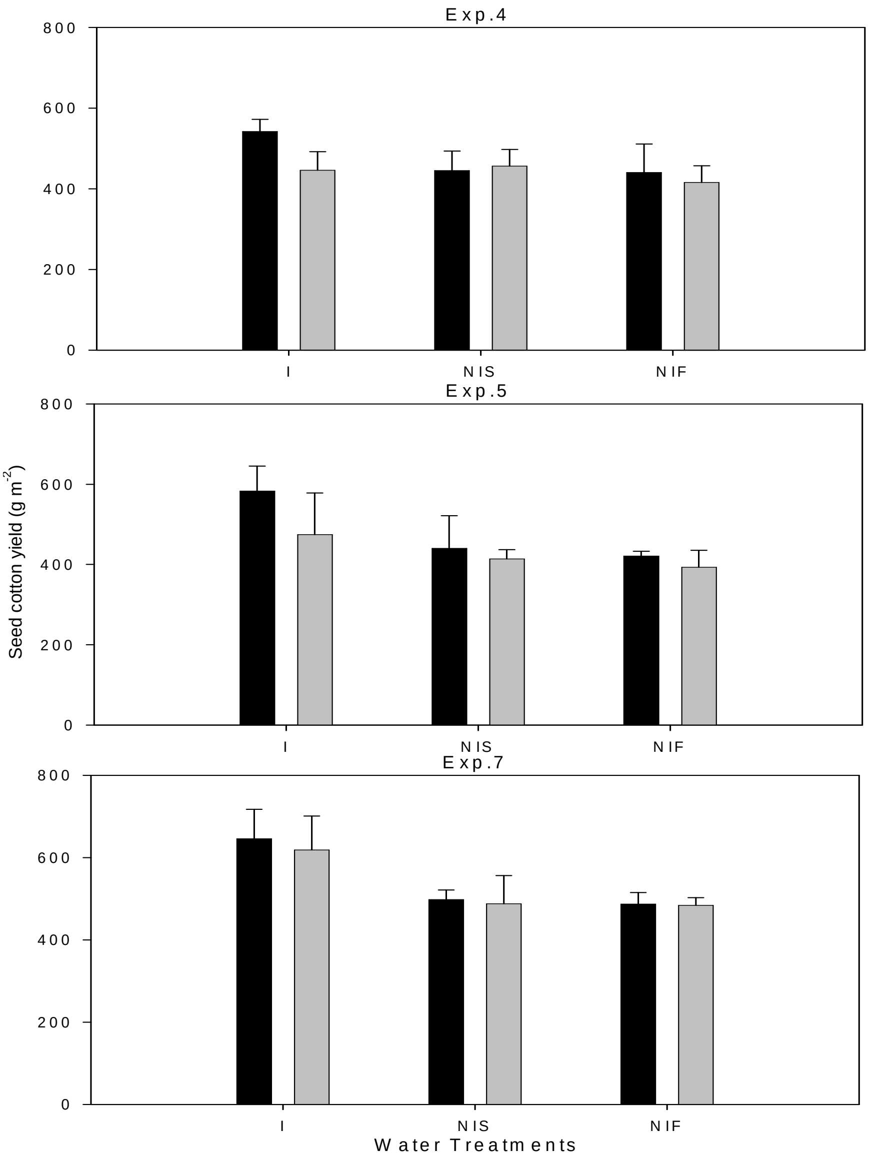

Figure 5.10 Seed cotton yield (g m-2) for I, NIS and NIF combined with NR (black) and 30R (grey) for each case in Exp.4 and 5 in 2007/08, and Exp.7 in 2008/09. Standard errors of the mean are also showed.

5.4.6 Canopy light exposure experiment (Exp.6)

5.4.6.1 Fruit number and lateral retention

Fruit number and retention at the first position (FS1 and Ret1) and the third position (FS3 and Ret3+) were not significantly different among the treatments (Table 5.5). However, FS2 showed a significant increase in CE40 and CE90 when compared with CE0 and CE20, but there were no significant differences in Ret2. Total fruit number (TFN) was increased from 22 to 27 and total retention (Tot.Ret) was also increased from 0.60 to 0.70 in CE40 and CE90 compared with CE0 and CE20.

Table 5.5 The effect of lower canopy light exposure on total fruit number (TFN) and retention fraction (Ret) in different fruiting sites (FS 1, 2, 3+ and total) per plant at maturity at Gatton, SE Queensland during 2008/09

Exp.6	TFN	Tot. Ret	FS1	Ret1	FS2	Ret2	FS 3+	Ret 3+
CE0	22.2a	0.604a	9.7	0.711	8.2a	0.606	4.2	0.428
CE20	22.0a	0.604a	9.5	0.705	7.7a	0.553	4.7	0.481
CE40	27.2b	0.709b	10.5	0.737	10.0b	0.733	6.7	0.628
CE90	27.0b	0.698b	11.2	0.774	10.2b	0.718	5.5	0.545
Significance	*	**	NS	NS	*	NS	NS	NS

* = significant at P=0.05
** = significant at P=0.01
NS = non- significant

5.4.6.2 Retention at different node positions

The vertical retention illustrated in Fig.5.11 shows the pattern of distribution and competition throughout the plant in the different canopy exposure treatments. Increases in retention of between 0.20 and 0.25% were found in the first 4 to 5 reproductive nodes in CE40 and CE90 when compared with CE0 and CE20.

5.4.6.3 Seed cotton yield

CE40 and CE90 produced significantly higher seed cotton yields (P = 0.014) than CE0 and CE20 (Fig.5.12). Yields increased by about 11% in response to 42 days exposure after flowering, as well as canopy exposure until maturity. However, 20 days of exposure did not have any significant effect on final yield.

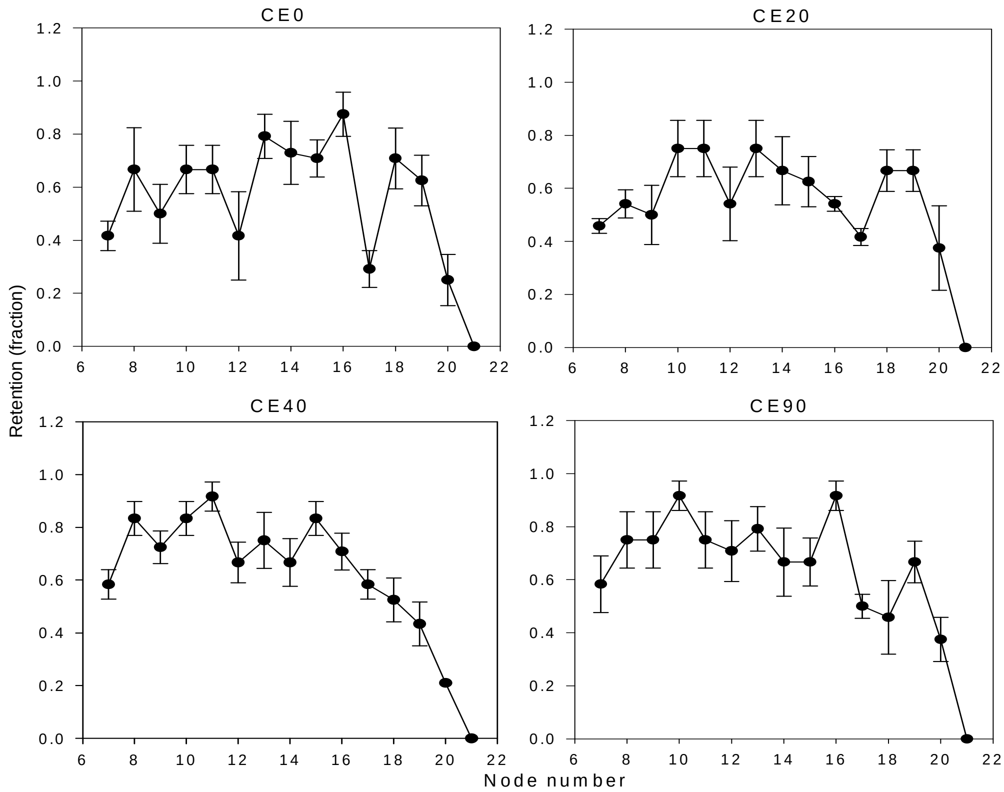

Figure 5.11 Retention fraction per fruiting node in Exp.6 for CE0, CE20, CE40 and CE90 at Gatton, SE Queensland during 2008/09. Bars are two standard errors of the mean.

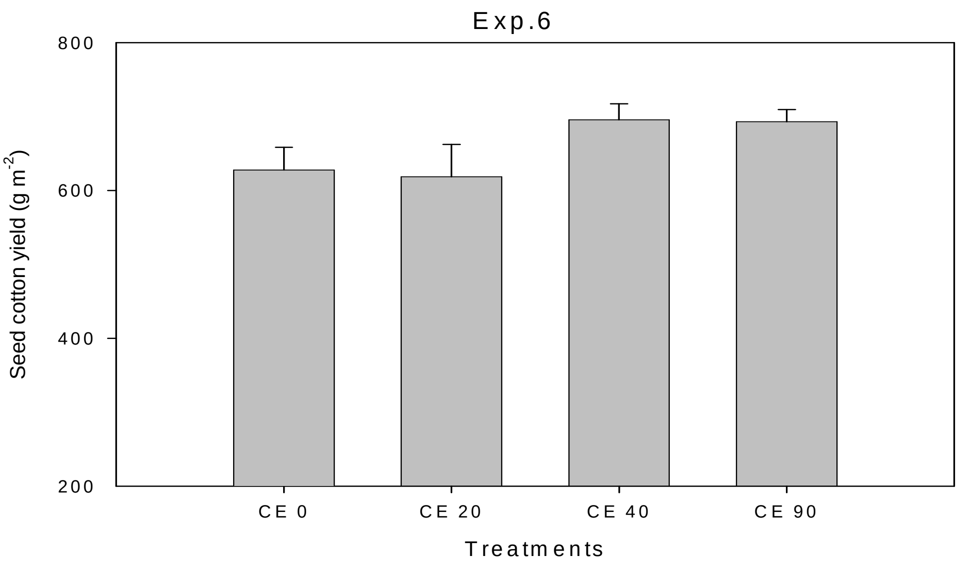

Figure 5.12 Seed cotton yield (g m^{-2}) for CE0, CE20, CE40 and CE90 in Exp.6 at Gatton, SE Queensland during 2008/09. Bars are standard errors of the mean.

5.5 Discussion

This research reported in this Chapter established that early soil water availability increased lint yield in high retention cotton when compared with pre-flowering water stress treatments. This result is consistent with the previous findings (Chapter 3) and supports the general hypothesis that insufficient early growth as a result of early pre-flowering soil water deficits, reduces the assimilates supply needed to meet a higher boll demand in high retention cotton. Seed cotton yield was reduced by 41-44% in late sowing dates and 7-20% in early sowing dates under water stress conditions at pre-flowering during 2006/07 – 2007/08. In the present experiments during 2007/08 – 2008/09 which were planted early in the season, early water stress reduced the seed cotton yield of Bt cotton by about 20% compared with pre-flowering irrigated cotton. Under water stress conditions (NIS and NIF), the differences in seed cotton yield between high retention (Bt) and low retention (flower removal) cotton were smaller, but in well irrigated conditions, high retention Bt cotton tended to produce higher yield. These results support the common agronomical practice in Australia that use a long period of water stress until squaring and flowering followed by irrigation that was developed for conventional varieties, may not be ideal for Bt cotton. Some of the variables studied in these experiments are described in the followings paragraphs to better understand these differences in yield between water treatments for high retention (Bt) and low retention (flower removal) cotton.

Comparing both sets of experiments (Chapter 3 vs. Chapter 5), the differences produced in terms of seed cotton yield between the two stress treatments are small (7-10% in Chapter 3 and 4-7% in Chapter 5) considering the different amount of water applied after the stress period finished, being greater in the second set of experiments. However, these differences in water applied for recovery were not reflected in differences in yield, but in TDM for all the cases (About 37% of increment of TDM in the second set of experiments compared with the first one).

Three different levels of water availability were used in these studies to help the manipulation of early biomass in Bt cotton. It was hypothesized that a bigger plant from the early pre-flowering stages, would potentially produce assimilates after flowering that are sufficient to meet the large sink demand in Bt cotton, resulting in higher fruit retention and higher seed cotton yield. I (Exp.5) produced a higher number of flower buds at 77 DAS compared with the soil moisture stress treatments (a result similar to that in Exp.4). These increases in flower buds were translated later in the season into a higher number of green bolls retained for all experiments. The stress treatments on the other hand resulted in earlier flowering, producing bolls earlier than I. Similar trend was found in previous studies in Chapter 3 with soil water deficits at pre-flowering.

The relationship between plant height and number of nodes was considered as part of the reproductive site production analysis. When considering fruit removal treatments, different responses to pre-flowering water stress were found. Flower bud removal, which simulated low retention cotton, significantly reduced fruit retention in Exp.4 and 5. The removal treatments did not affect internode elongation in Exp.4 and 7. However, in Exp.5, significant responses were found in relation to plant height and node number, with the low retention cotton showing an increase in plant height and number of nodes in those treatments associated with low levels of available resources (NIS and NIF). However, with high availability of resources (I), Bt cotton showed an increase in plant height and node number when compared with conventional cotton (i.e. treatments with fruit removal). Other researchers have reported that bud removal treatments have resulted in longer internodes, and more internodes and branches (Holman and Oosterhuis, 1999; Sadras, 1996).

During the mid-late season, all the experiments showed significant differences in fruit retention, with the I treatment being associated with higher levels of retention, compared with the water stress treatments. The flower removal treatments also significantly affected fruit retention, with levels of retention higher in Bt cotton compared with low retention cotton. Most of the removed fruits were in fruiting position 1, and contributed to the differences in final seed cotton yield between water treatments.

The distribution of fruits on lateral branches and retention of these fruits showed effects of the water and removal treatments. The retention decreased from the first to third positions within the sympodial branch for all treatments as was also found in previous Chapters. This may reflect the effects of lateral competition for assimilates between the fruiting sites, with the sites closer to the main stem having an advantage over those further from it. This advantage of the first fruiting position in competition for assimilates has also been reported by several other authors (Constable and Rawson, 1980b; Kerby and Buxton, 1981; Wullschleger and Oosterhuis, 1990a; Wullschleger and Oosterhuis, 1990b).This competition for assimilates is also reflected in the age of bolls in FS1 and FS2. Bolls at FS 1 were usually 6 to 12 days older than those at SF 2 and SF 3+, respectively.

Not surprisingly total fruit number was higher under fully irrigated prior to flowering. Final levels of retention were mainly affected by water treatments. Differences between conventional and Bt cotton in retention were smaller in treatments with limited levels of water (NIS and NIF), than in treatments with higher levels (I). Cotton seed yields followed similar trends. In Exp.4 and 7, for example, without flower removal, early water stress reduced the seed yield of high retention cotton by about 20%; however, with flower removal in low retention cotton, there was only a 5-8% yield reduction in the water stress treatment. This suggests that early irrigation resulted in the development of a larger canopy, which was important for high retention (Bt) cotton, whereas plants can be stressed during the early stages in low retention cotton where source-supply is relatively large and compensation can occur.

Manipulating the crop biomass through lower canopy light exposure under fully irrigated conditions, showed some responses in terms of fruit retention, boll distribution and yield. Long period of exposure for 40 days or longer after flowering increased fruit number in the second position by 20%, with an associated 18% increase in total fruit number. The increases were associated with a marked increase in the number of fruit retained in the lower part of the plant canopy, relative to the control and the 20 days exposure treatment. It is likely that solar radiation and photosynthesis in low position fruiting sites become a limitation, with a bigger plant and complete canopy closure resulting in fruit abortions and decreasing the yield potential in conventional cropping systems. The experimental result is consistent with the results of studies reported by Constable (1981), who concluded that shedding of young bolls happens when the radiation levels decrease, despite the plant having enough assimilates to support growing bolls. The light penetration and interception within the canopy changes as the crop grows, with the new leaves higher in the canopy shading the older leaves. The older leaves in the bottom of the canopy then reduce the production and supply of assimilates for growing bolls (Constable and Rawson, 1980a; Constable and Rawson, 1980b). This result indicates that high retention cotton has a capacity to respond to increased source supply even after flowering and that yield is limited by source availability.

In summary, the number of fruiting sites increased under conditions of high availability of resources in the I treatments relative to the stressed treatments (NIS and NIF), mainly in first lateral position and concentrated in the middle and upper parts of the canopy. The absolute number of flower buds and bolls, and percentage fruit retention, were higher in I compared with stress treatments in high retention cotton. Without flower removal (eg. Bt), early water stress reduced seed cotton yield by about 20%; however with flower removal (eg. Non Bt) the stress treatments reduced seed yield by only between 5 and 8%, relative to the I treatments. This suggests that early irrigation increased the supply of assimilates (before flowering), which was important for the high retention Bt cotton, whereas plants can be stressed during the early stages development in low retention cotton varieties in which source-supply is relatively large, potential yield is rather low and where post-stress compensation is possible. These studies show the advantages of improving source development in Bt cotton compared with low retention (fruits removal) during the pre-flowering phase of crop growth, to support a higher sink demand for assimilates which can result in higher lint yields under field conditions.

Chapter 6 Sink-source relations in high retention cotton: effects of early irrigation, flower removal and canopy exposure after flowering on biomass production and assimilate partitioning

6.1 Abstract

Compared to non-Bt varieties, Bt cotton has a shorter vegetative cycle due to higher early fruit retention rates. In Chapter 5 it was reported that early water availability increased final yield and fruit retention rates particularly in high retention cotton. Four experiments over 2 years using Bt cotton producing 2 insecticidal Cry proteins, were conducted at Gatton, SE Queensland, Australia, to study the effects of early water availability on the dynamics of biomass accumulation and partitioning, and development of phenological stages in high and low fruit retention situations (the latter was simulated with removal of 30 flowers in 1 m row). A related study examined the effects of light exposure of the lower part of the crop canopy to increase source supply and its impact on biomass accumulation and partitioning.

Water availability affected the time taken to reach different key crop growth stages. Cut-out and maturity occurred earlier in the stress treatments. Total biomass, vegetative production and LAI were significantly higher under irrigated (I) conditions. The total biomass production was higher in the I treatment in all 3 experiments when compared with treatments with soil water deficits during the early stages of plant growth. However, in both the water stress treatments (NIS and NIF) there was a recovery in total biomass production after the stress period in response to refilling of the soil profile. The various vegetative dry matter production components showed an increase in simulated low retention cotton (R30) when compared with Bt (NR). However, the differences between low retention and high retention Bt cotton in the water stress treatments (NIS

and NIF) were smaller than those in the I treatments during the second year (Exp.7). In reference to the lower canopy light exposure study, significant differences were found at maturity in vegetative dry matter production following long periods of exposure (about 40 days after flowering and until the end of the crop), when compared with control treatment and a short period of exposure (20 days). Boll dry matter and TDM were also higher in the long exposure treatments compared with the non-exposed control.

The higher assimilate source supply of larger plants with longer period after flowering in response to early irrigation may explain the yield differences recorded between the irrigated and stress treatments, particularly in high retention (Bt) cotton when compared with low retention cotton. The higher assimilate supply was reflected in higher rates of production of reproductive sites, higher organ retention and higher final cotton seed yield, as described in Chapter 5.

6.2 Introduction

In Chapter 5 it was reported that early water availability increased lint yield in high retention cotton, which supports the general hypothesis that early growth of the crop is critical to meeting assimilate demands for developing bolls from early flowering. The responses to early irrigation in terms of final yield tended to less in low (conventional cultivars simulated by early flower removal) than in high retention (Bt) cotton. These results support the view that early fruit retention and growth may not be so critical in non-Bt cotton cultivars due to the cyclical compensatory growth of vegetative shoot and fruits, in response to the early loss of fruit. However, this compensatory mechanism seems to be weak in Bollgard (Bt) cotton varieties. Also it was found that the yield was increased when the lower canopy was exposured to sunlight suggesting that the assimilate availability for bolls at lower canopy is also important for high yield in high retention cotton. This Chapter aims to explain the basis for differences in final yield and fruit retention and distribution reported in Chapter 5 through an analysis of the physiological parameters involved in growth and development.

During vegetative growth, the production of carbohydrates as a result of photosynthesis increases. Correspondingly, as plants grow, the demand for carbohydrates by the different plant organs increases. In this way, a balance is achieved between carbohydrate supply and demand. The initiation of reproductive growth and its timing with respect to vegetative development may also have a large effect on root development. Once the reproductive stage has been initiated with the development of flower buds or squares, several factors affect the processes involved in the control of flower bud number and boll retention, with a potential significant impact on lint yield (Guinn et al., 1981; Heitholt et al., 1992). During the squaring stage, it is more likely that small flower buds will be shed than larger and fully expanded squares, especially in the ten day period immediately before anthesis. Shedding during the early stages of squaring is be explained by two possible and conflicting hypotheses (Heitholt, 1999a). The first hypothesis is that shedding of small squares is strictly due to biotic stresses like insect damage, rather than in response to physiological causes. Supporting this hypothesis is the fact that small squares require a small supply of assimilates, which is not a resource limitation at this early stage of development. The second hypothesis is that either physiological, abiotic (Ungar et al., 1989) or biotic stresses (Sadras, 1996) can cause shedding of small flower buds. Constable (1981) concluded that older squares and flowers are less likely to be shed due to the fact that 50% of their assimilate requirements can be produced from the bracts of the flower buds. A similar conclusion was made for bolls older than 10 days.

Boll retention and distribution within a plant play an important role in determining final yield, and is linked to the allocation of assimilates produced during the vegetative growth by the plant.

If the level of available assimilates is adequate to support the developing bolls, then these bolls will be retained (Constable, 1991; Jenkins et al., 1990a; Jenkins et al., 1990b). However, if the demand from growing bolls exceeds the supply of assimilates from the current photosynthesis and some stored carbohydrates in the vegetative structures, the retention of bolls will decline on account of an increase in the number of boll abortions or shedding (Guinn, 1998; Mason, 1922).

In the first series of experiments (Chapter 3 and 4), at the end of water stress period, the soil water deficit was not fully replaced with irrigation water and controlled deficit irrigation schedule followed. This may be the reason for TDM production not fully recovering after the stress period in these experiments. While in the current series of experiments, the soil water deficit was fully replaced to the drained upper limit after the stress period, then irrigation was applied to replace the daily crop water use. However, the effect of pre-flowering water stress on cotton yield was similar in the two sets of experiments. Thus, dry matter growth of different organs will be investigated to explain the variation in growth and partitioning of assimilates. The objective of the work reported here was to study the effects of early water availability on the dynamics of biomass accumulation and partitioning, and development of phenological stages in high and low fruit retention cotton (the latter simulated by removal of 30 flowers per m row). A second objective was an examination of the effects of light exposure of the lower part of the crop canopy to increase source supply on biomass accumulation and partitioning, under irrigated conditions.

6.3 Material and Methods

6.3.1 Experimental sites and growth conditions

The experiments were described in details in Chapter 5. To summarise, four experiments were conducted over a two year period (Exp.4 and Exp.5 from October 2007 to April 2008; Exp.6 and Exp.7 from October 2008 to April 2009). The experiments were undertaken at the Gatton campus of the University of Queensland (91m, 27^{o}33’S, 152^{o}20’E) in the Lockyer Valley of Southeast Queensland, Australia. The soil type in the experimental area was a Lawes clay loam (Powell, 1982), with heavy dark cracking clays. The average rainfall is 760 mm with a summer dominance, whilst evaporation rate is high, about twice the annual average rainfall.

6.3.2 Cultural practices

The Bt transgenic Bollgard ll®™ variety Sicot 71BR) producing the Monsanto Cry1Ac and Cry2AB proteins) was sown in all the experiments. High seeding rates were used at sowing with seedling numbers then being reduced to obtain a population of 140,000 plants ha^{-1} (12-15 plants in 1 m rows).

6.3.3 Experimental design and water deficits, flower bud removal and canopy exposure treatments

For Exp.4, 5 and 7, overhead sprinklers were used to provide the following irrigation treatments:

I (Full irrigation): Irrigation was applied to meet the water requirements for a cotton crop, calculated as the product of daily class “A” pan evaporation by a crop coefficient depending on the phenological stage of the crop (CRDC, 2003).

NIS (No irrigation until squaring = mild water stress): No water was applied from establishment to squaring (water stress period), followed by fully refilling the soil profile and further irrigation as per the I treatment through to maturity.

NIF (No irrigation until flowering = severe water stress): No water from establishment to flowering (water stress period) followed by fully refilling the soil profile and further irrigation as per the I treatment through to maturity.

Flower bud removal In each water treatment there were two levels of flower removal, starting from the time of early flowering (i) non-removal (NR) Bollgard II representing high retention cotton and, (ii) Bollgard II with 30 flowers removed per meter (30R) or about 4 flowers per plant. This second level of removal simulated conventional low retention cotton. The flower buds were removed three times a week over a two week period from early flowering.

6.3.3.1 Experiment 4

Sowing was done on 16th October 2007. The water treatments were laid out in a randomized complete block design with four replications. The buffer areas were sufficient to ensure that there was no lateral water movement between plots.

6.3.3.2 Experiment 5

Sowing was undertaken on 3rd October 2007, using a split-plot design layout with four replications in three different environments (two under a rainout shelters and the third under normal field conditions). The rainout shelters were used to create the water stress treatments.

6.3.3.3 Experiment 6

Sowing was done on 15th October 2008. The treatments were laid out in a randomized complete block design with four replications. This experiment was conducted under well irrigated conditions, with the following treatments:

CE0 = no lower canopy exposure to sunlight; **CE20 =** lower canopy exposure for 20 days after first flower; **CE40** = lower canopy exposure for 40 days after first flower; **CE90** = canopy exposure from first flower until final harvest (which was approximately 90 days after first flower). Lower canopy light exposure was achieved by pushing the plants in the rows immediately adjacent to the 'test' row (the row to be harvested) to a 45 degree inclination and then holding the plants in position using wires tied to steel posts (Fukai et al., 1991). At the end of the canopy exposure treatment period, the wire was removed and the plants allowed to return to their original canopy structure.

6.3.3.4 Experiment 7

Sowing was done on 27th October 2008. The water (irrigation) treatments were laid out in a randomized complete block design with four replications. The stress period (NIS and NIF) started 10 days later than the experiments of the first season due to rainfall at early stages.

6.3.4 Measurements

Volumetric soil water content was measured periodically using a neutron probe calibrated in the fields where the experiments were being conducted. A 2 m long x 50 mm diameter access tube was placed within a row at the center of each plot. Measurements were made at soil depths of 30, 50, 70, 90, 110, 130, 150 and 170 cm. The calibration was done after crop establishment and emergence, when the soil profile was near field capacity in every plot. The bulk density at each depth was used to convert gravimetric soil water content into volumetric water content.

Total dry matter and partitioning were measured at 1st square (48-53 DAS), 1st flower (75-82 DAS depending on experiment), cut-out (110-121 DAS) and physiological maturity-60% open bolls (145-161 DAS) (open bolls defined as such when two sutures on the boll dehisced). Plants in 1 m^2 area were harvested from each plot. Total fresh biomass was measured and a-sub sample of 3 plants were used to determine leaf area, dry matter and partitioning of DM into leaves, stems, petioles, squares, flowers, green bolls and open bolls. Samples were dried at 80ºC over three days to determine dry matter content.

Leaf area was measured using a LiCor planimeter (Model LI-3100, LiCor Inc., Lincoln, NB, USA) and then drying the leaves at 80ºC for three days. Specific leaf area (SLA) was calculated and the product of SLA and the amount of leaf dry matter in the 1m^2 area was the leaf area index (LAI).

6.4 Results

6.4.1 Soil water content

Changes in total soil water content between 20 and 180 cm are shown in Fig. 6.1.

In Exp.4, the duration of the stress period and the severity of NIS and NIF were similar to Exp.5, but the soil water content of I treatment was higher in Exp.4. In both stress treatments, full irrigation after the period stress brought the soil moisture content levels back to values similar to I.

Exp.5 was conducted under rainout shelter conditions to produce the early stress period. Soil water content in NIS decreased slowly until 55 DAS and NIF until 69 DAS, after which full irrigation brought the soil moisture content back to the values of the I treatment.

During the second season (Exp.7, 2008/2009), the end of NIS was 10 days beyond 1st square due to the higher soil water content in the profile, in the early stages of crop growth. The end of NIF was also extended by 12 days after 1st flower, unlikely the same treatment in the first season's experiments. The severity of water stress in year 2 was similar to year 1. After the end of the stress period, full irrigation restored the soil water content to levels close to that of the I treatment.

In all experiments, the lowest soil water content was reached at the end of the stress period in NIF. The recovery from the soil water deficit came from the combined water inputs from irrigation and rainfall.

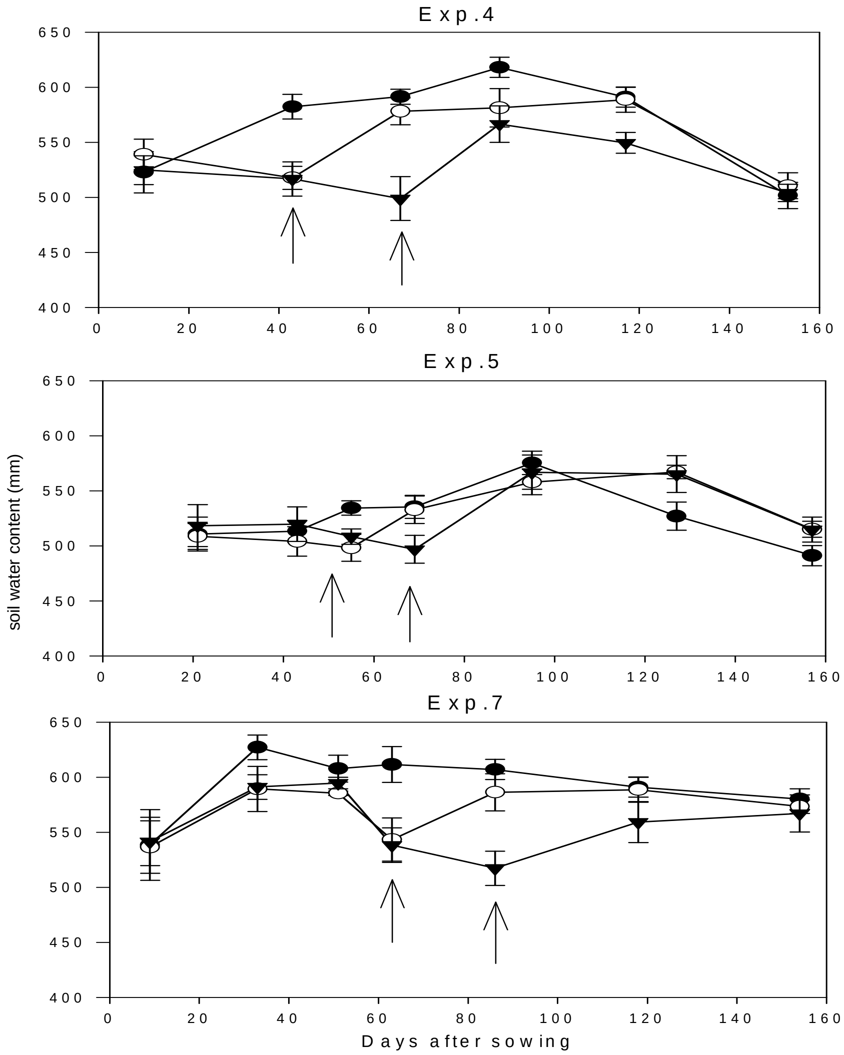

Figure 6.1 The effect of early water availability on changes in total soil water content (20-180 cm depth) for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2007/08 (Exp.4 and 5) and 2008/09 (Exp.7) at Gatton, SE Queensland. Arrows indicate the end of the stress period. Bars are two standard errors of the mean.

6.4.2 Phenological development

Periodic mapping and visual inspections were used to determine the date each phenological stage of development was achieved. The effect of flower removal on phenological development was not significant and hence not included in Table 6.1. The timing of all reproductive development stages was delayed in I when compared with the stress treatments, particularly in Exp.5.

Table 6.1 Phenological development: number of days (DAS) from sowing to 1st square, 1st flower, 1st open boll and 60%open bolls, for I (irrigated), NIS (no irrigation until squaring) and NIF (no irrigation until flowering) in 2007/08 (Exp.4 and 5) and 2008/09 (Exp.7) at Gatton, SE Queensland.

	1st Square	1st Flower	1st Open Boll	60% Open Bolls
Exp.4 Treatment				
I	50.5	80.8	145	186
NIS	47.0	79.1	142	181
NIF	46.0	78.0	141	180
Significance	*	NS	NS	*
Exp.5 Treatment				
I	48.6	77.7	15	182
NIS	43.0	75.1	142	175
NIF	44.1	71.6	138	172
Significance	*	*	**	**
Exp.7 Treatment				
I	49.0	69.1	130	161
NIS	49.1	69.3	127	154
NIF	49.1	69.1	126	152
Significance	NS	NS	*	**

For Exp.4, significant differences were recorded in response to the treatments at 1st square and maturity; a delay of 6 days was recorded at maturity in I compared with NIF. In Exp.5, a delay of 4-6 days was recorded at 1st square and 1st flower, but the delay was increased to 15 days by 1st open bolls. NIF was associated with earlier maturity (172 DAS), followed by NIS (175 DAS) and then I (182 DAS). No differences were found between the treatments in Exp.7 for 1st square and 1st flower. This may have reflected high soil water content even in water stressed treatments during early growth stages, although delays in I were recorded for 1st open boll and maturity, with a 9 day difference for the latter parameter between I and NIF.

Considering the boll period from first flower to maturity, in Exp.7 it was 9 days longer in the I treatment when compared with NIF, while in Exp.4 and Exp.5 the difference was only 5 and 4 days, respectively.

6.4.3 Dry matter production and partitioning

6.4.3.1 Total dry matter production

The effect of flower removal on total dry matter (TDM) was rather small, and hence means TDM across the flower removal treatments is shown in Fig.6.2. The trend of TDM accumulation during the season was similar in all the treatments with higher means in I compared with NIS and NIF. In Exp.4, at 82 DAS, significant differences were found among the treatments, with higher values for I, followed by NIS, and significantly lower for NIF. In Exp.4, after the stress period NIS and NIF showed a recovery in TDM in response to full irrigation. Although by 112 and 145 DAS, there were significant differences ($P = <0.001$ and $P = <0.001$) between I and stress treatments, TDM in the NIF treatment had responded to the irrigation post-stress and was not significantly different from in the NIS treatment at 112 and 145 DAS. In Exp.5, TDM for I was the highest followed by NIS and then NIF at 77 DAS about 1st flower stage. At this stage, the NIS stress period had ended by about three weeks ago and the soil water profile had been restored through irrigation, allowing time for some recovery in dry matter in the NIF treatment. TDM growth recovered well after 77 DAS in response to full irrigation in both stress treatments, and the difference in TDM from I became smaller. There were no significant differences in TDM between NIS and NIF at 110 and 158 DAS, but it was significantly higher for I ($P = <0.001$ and $P = 0.022$, respectively).

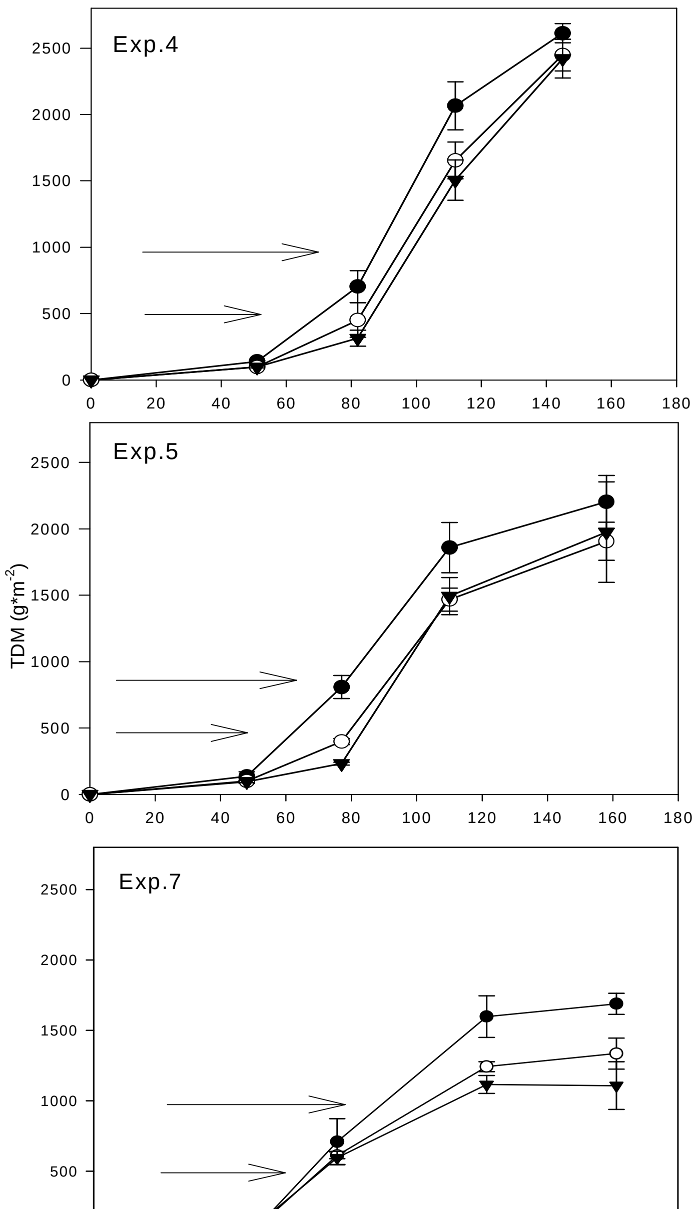

Figure 6.2 Total dry matter versus days-after-sowing for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2007/08 (Exp.4 and 5) and 2008/09 (Exp.7) at Gatton, SE Queensland. Bars are two standard errors of the mean. Stress period: long arrow- NIF and short arrow- NIS

In Exp.7 significant differences were not found for TDM at 53 and 75 DAS between I and the stress treatments. This may be linked to high soil water content in NIS and NIF during the early stages of crop growth with high rainfalls. However, significant differences were found at 121 and 161 DAS with higher TDM accumulation by I in comparison with the stress treatments. Unlikely other experiments where the difference in TDM among irrigation treatments decreased as the crop approached maturity, in Exp.7 the difference in TDM increased to maturity.

6.4.3.2 Dry matter partitioning

The partitioning of TDM into vegetative and reproductive components over the period of crop development for high retention cotton and simulated low retention cotton is shown in Fig.6.3.

Vegetative dry matter produced in the early stages of crop growth in the I treatments was significantly greater than in the water deficit treatments, over all experiments in both seasons.

For most organs DM at any measurement occasions in all experiments, water treatments had significant effect, but not flower removal treatments not water and removal interaction. Significant removal effects were found for boll DM at 112 DAS in Exp.4 (RT $P < 0.001$) and 121DAS for Exp.7 (RT $P=0.038$). At the stage of cut-out (112DAS Exp.4, 110DAS Exp.5 and 121DAS Exp.7) NR produced significantly higher boll DM than 30R. This reflected the impact of the earlier flower bud removal. At 145 DAS (Exp.4), 156 DAS (Exp.5) and 161DAS (Exp.7), boll dry matter production was significantly different in the water treatments ($P = 0.011$) and removal treatments ($P = 0.010$), but there were no significant interactions. For all the water treatments, R30 produced lower bolls dry matter than NR, but the differences between NR and R30 in boll dry matter production in the water stress treatments was lower than in the I treatments (No significant interaction WT*RT). The Fig.6.4 shows the dry matter production of different vegetative organs under different water conditions (Exp.5), with higher values of leaf and stem DM for the I treatment compared with NIS and NIF.

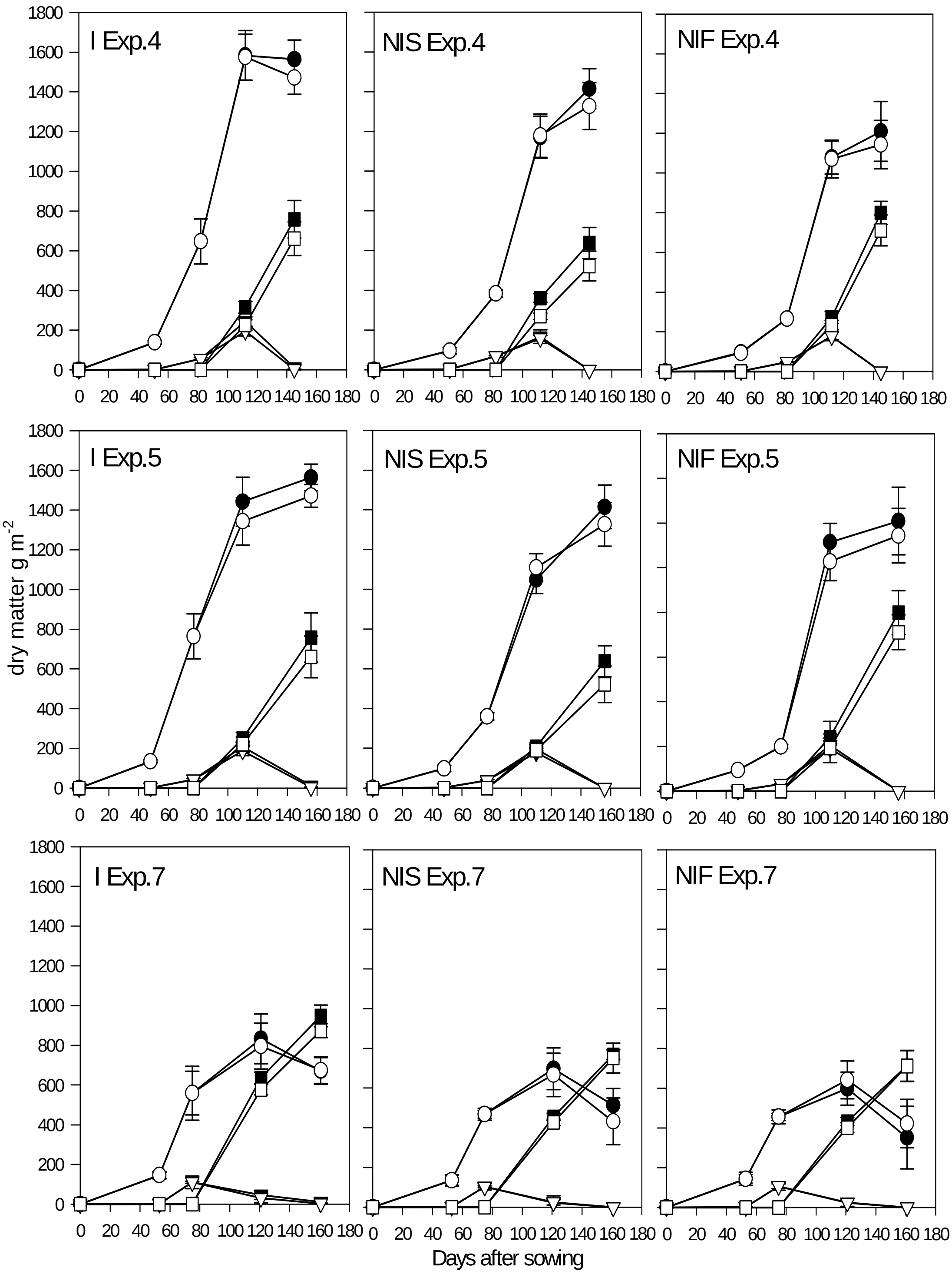

Figure 6.3 Dry weight of vegetative organs (leaf, stem and petiole), early reproductive organs (squares and flowers) and late reproductive organs (bolls) for NR (■) and R30 (□) versus days-after-sowing for I, NIS and NIF for Exp. 4, 5 and 7. Error bars are two standart of the mean.

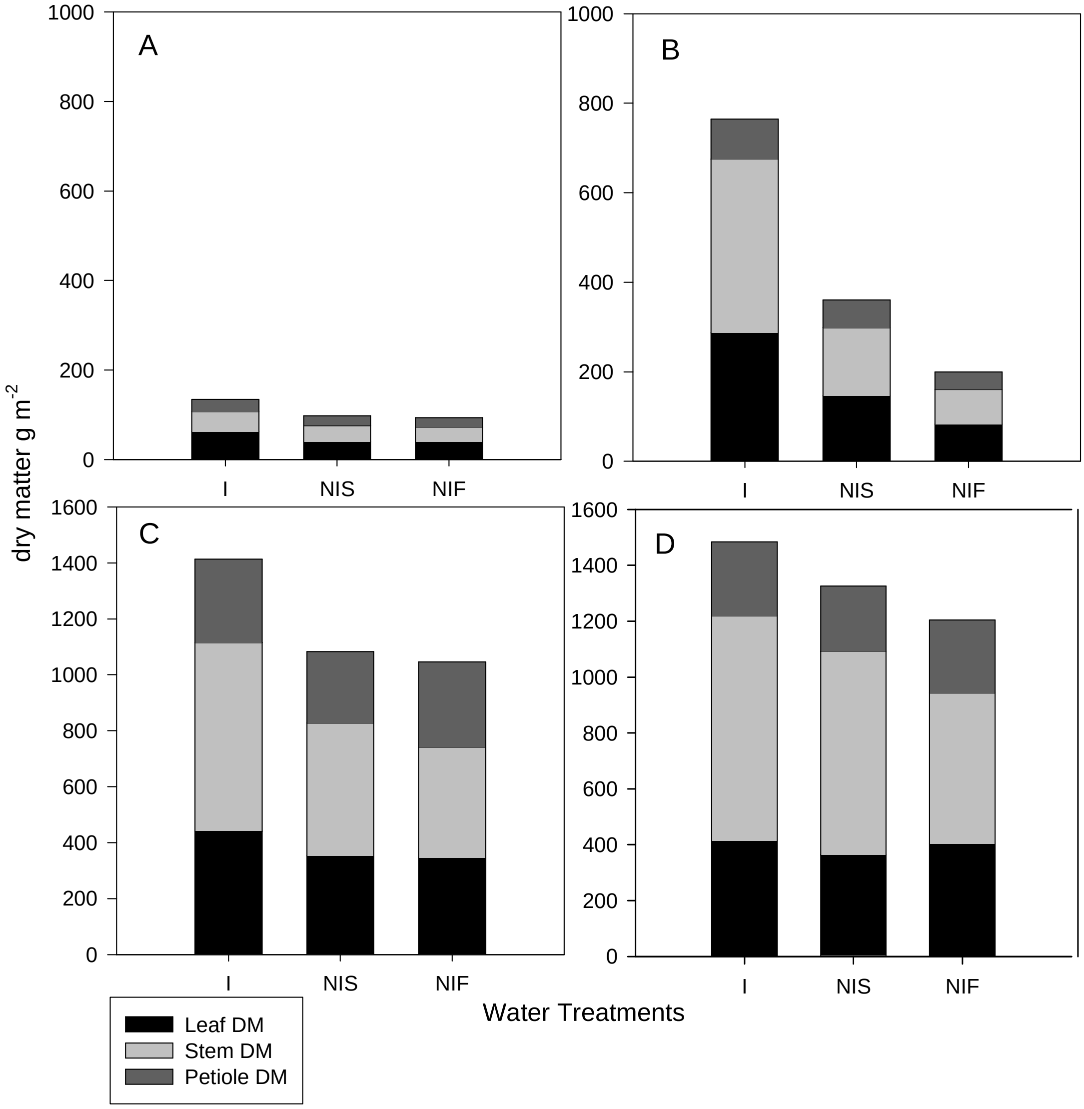

Figure 6.4 Dry matter production of different vegetative organs (leaf, stem and petiole) over the season: 48 (A), 77 (B), 110 (C) and 156 (D) DAS for I, NIS and NIF in Exp.5. Note: Scales differ between A-B and C-D.

6.4.3.3 Relationship between leaf dry matter and total dry matter production

Significant differences between treatments were recorded for leaf dry matter production over the whole season. Assimilate use for the production of leaves was related to total dry matter production during the cropping season, and was used to calculate the distribution ratio shown in Fig. 6.5. I treatments had a higher leaf/TDM ratio at the beginning of the cropping season over squaring. No significant differences for the leaf/TDM ratio were found between the stages of flowering and cut-out. However, as the season advanced, differences were found, with a higher ratio produced by NIF relative to I and NIS.

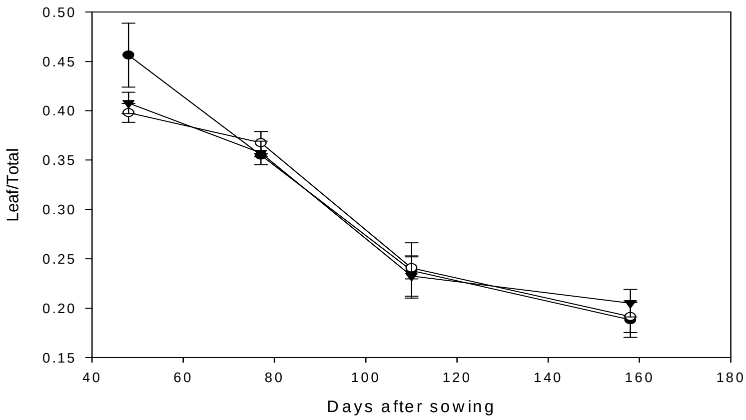

Figure 6.5 Changes in leaf DM/TDM for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2007/08 (Exp.5) at Gatton, SE Queensland. Bars are two standard errors of the mean.

6.4.3.4 Relationship between reproductive dry matter and total dry matter production

Fig.6.6 shows the relationship between total dry matter (TDM) production and reproductive dry matter (RepDM). During the stress period, TDM production was affected by the stress treatments but the partitioning was higher so that reproductive DM was similar among the treatments. However, the I treatments with higher TDM were able to increase partitioning to reproductive organs at a later stage. The trend during the first season's (2007/08) experiments (Exp.4 and Exp.5) was quite different to that during the second season (2008/09). In Exp.7 the effect of treatments was small during the stress period, but I produced more TDM and reproductive DM at maturity.

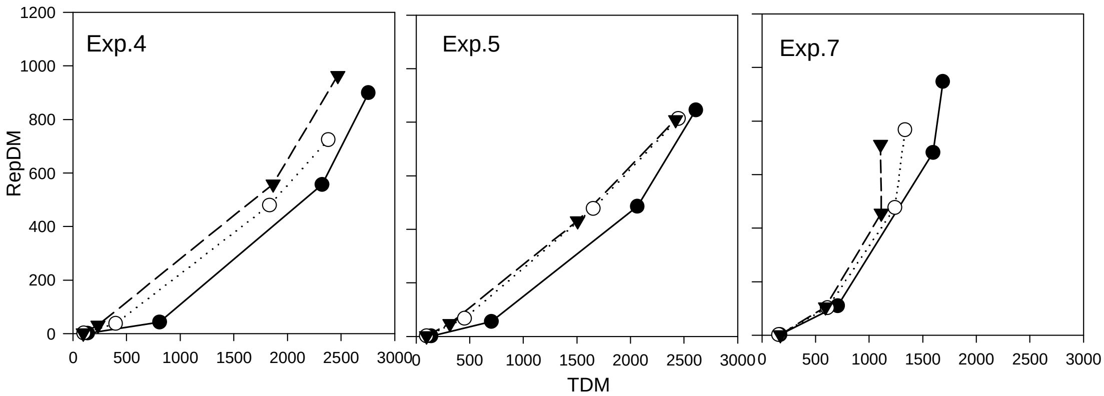

Figure 6.6 Relationship between reproductive dry matter (RepDM) and total dry matter (TDM) for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2007/08 (Exp.4 and 5) and 2008/09 (Exp.7) at Gatton, SE Queensland

6.4.4 Leaf area index

Fig. 6.7 shows the change in the mean LAI across flower removal treatments for each water treatment. Responses of LAI to water levels (I, NIS and NIF) were consistent across the seasons. In Exp.4 LAI was significant higher for I relative to the water stress treatments. At about 80DAS LAI in NIS and NIF were similar and much lower than in I. At 53 and 77 DAS (Exp.7), there were no significant differences between the treatments ($P = 0.935$ and $P = 0.097$, respectively), the responses being similar to those obtained for dry matter production. However, significant differences were found at 121 (<0.001) and 161 DAS (<0.001) between water treatments.

In Exp.5, significantly higher LAI values for I were measured at all measurement occasions when compared with NIS and NIF. At 77 DAS, LAI for NIS increased, showing a recovery from the water stress period. At 110 DAS, LAI had also recovered for NIF, in response to the end of the water stress period and the provision of full irrigation. Peak LAI was recorded at 110 DAS, which was near cut-out for all the treatments, after which it then declined to maturity.

The results of statistical analysis of LAI for the effects of water treatments and flower removal treatments are summarised in Table 6.2. In all the cases, I produced significant higher LAI compared with stressed treatments. No effects were found by removal treatment except for 154 DAS in Exp.4, where all the 30R produced higher LAI than NR (P = 0.010).

Table 6.2 Mean leaf area index (LAI) during the season for I, NIS and NIF and NR and R30 in 2007/08 (Exp.4 and 5) and 2008/09 (Exp.7) at Gatton, SE Queensland.

Exp.4	**112 DAS**	**154 DAS**
I + NR	5.18	4.14
I + 30R	5.32	4.23
NIS + NR	4.02	2.88
NIS + 30R	4.25	3.23
NIF + NR	3.88	2.74
NIF + 30R	3.80	3.23
Signif. WT	<0.001	<0.001
Signif. RT	0.119	0.010
Signif. WAT*REM	0.219	0.040
Exp.5	**110 DAS**	**158 DAS**
I + NR	4.53	3.06
I + 30R	4.34	3.26
NIS + NR	3.43	2.76
NIS + 30R	3.20	2.54
NIF + NR	3.19	2.72
NIF + 30R	3.27	2.49
Signif. WT	0.001	0.009
Signif. RT	0.181	0.753
Signif. WAT*REM	0.457	0.498
Exp.7	**121DAS**	**161 DAS**
I + NR	4.36	2.16
I + 30R	4.22	2.43
NIS + NR	3.75	1.87
NIS + 30R	3.65	1.48
NIF + NR	3.15	1.12
NIF + 30R	3.25	1.66
Signif. WT	0.001	0.001
Signif. RT	0.743	0.490
Signif. WAT*REM	0.771	0.040

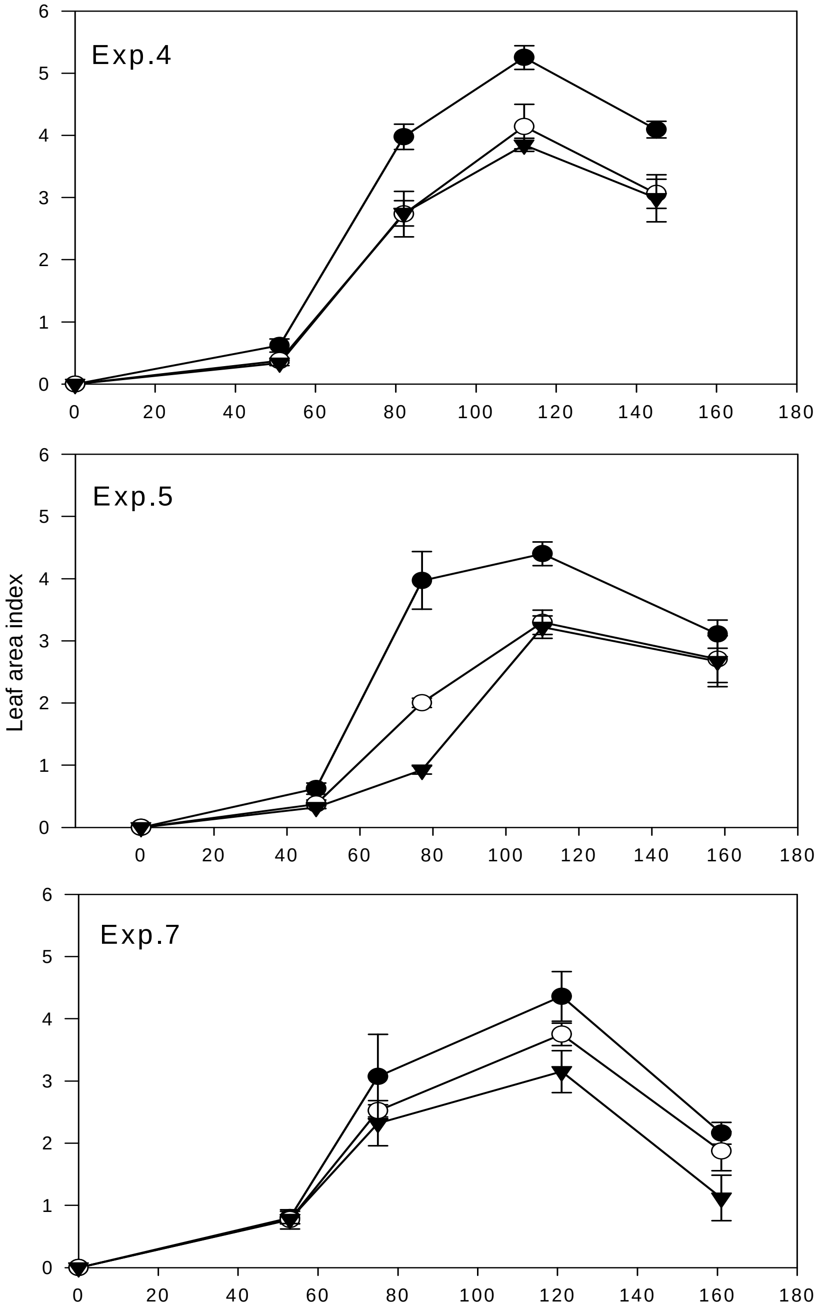

Figure 6.7 The effects of early water stress on leaf area index for I (irrigated) (●), NIS (no irrigation until squaring) (○) and NIF (no irrigation until flowering) (▼) in 2007/08 (Exp.4 and 5) and 2008/09 (Exp.7) at Gatton, SE Queensland. Bars are two standard errors of the mean

6.4.5 Response to lower canopy light exposure (Exp.6)

The results of the lower canopy light exposure treatments in Exp.6 are summarised in Table 6.3. First flower occurred at 78 DAS in Exp.6. The canopy exposure treatments (CE20, CE40, and CE90) commenced at this time. At 120 DAS when CE40 exposure treatment was just completed, leaf dry matter production was significantly higher ($P = 0.028$) in CE40 and CE90 compared with CE0 (the control) and CE20. However, there were no significant differences between treatments for stem ($P = 0.271$), petiole ($P = 0.221$) and total vegetative dry matter ($P = 0.908$). Significant differences were found in boll reproductive dry matter, with higher values in CE40 and CE90, but no differences for squares and flowers dry matter ($P = 0.845$).

At 152 DAS, vegetative dry matter and its components declined in all treatments. Leaf dry matter was significantly higher in CE40 and CE90 compared with CE20 and CE0. Stem dry matter and total vegetative biomass were also significantly different between the treatments, with higher values in CE40 and CE90. Boll dry matter production was also higher in long exposure treatments (CE40 and CE90) compared to CE0 and CE20.

Table 6.3 Leaf, stem and petiole dry matter and vegetative and reproductive (squares + flowers and bolls) dry matter per m-2 produced by CE0, CE20, CE40 and CE90 treatments at 120 and 152DAS in Exp.6 during 2008/09 at Gatton, SE Queensland.

120DAS Treatment	**Leaf ($g\ m^{-2}$)**	**Stem ($g\ m^{-2}$)**	**Petiole ($g\ m^{-2}$)**	**Vegetative ($g\ m^{-2}$)**	**Sq/Flo ($g\ m^{-2}$)**	**Bolls ($g\ m^{-2}$)**
CE0	338	440	115	894	51	660
CE20	341	434	121	897	61	642
CE40	363	404	132	901	62	763
CE90	365	404	147	917	64	781
Significance	*	NS	NS	NS	NS	*
152DAS Treatment	**Leaf ($g\ m^{-2}$)**	**Stem ($g\ m^{-2}$)**	**Petiole ($g\ m^{-2}$)**	**Vegetative ($g\ m^{-2}$)**	**Sq/Flo ($g\ m^{-2}$)**	**Bolls ($g\ m^{-2}$)**
CE0	229	271	72	573	0	824
CE20	230	263	83	576	0	768
CE40	258	333	90	682	0	898
CE90	272	342	86	701	0	900
Significance	*	*	NS	*	-	*

Figure 6.8 shows TDM accumulation and LAI for all the treatments in Exp.6. At 120 and 152DAS, CE20 did not produce significant differences in TDM and LAI, when compared with the control. However the responses to longer canopy exposure in CE40 and until maturity in CE90 were significant, and increased biomass production and LAI.

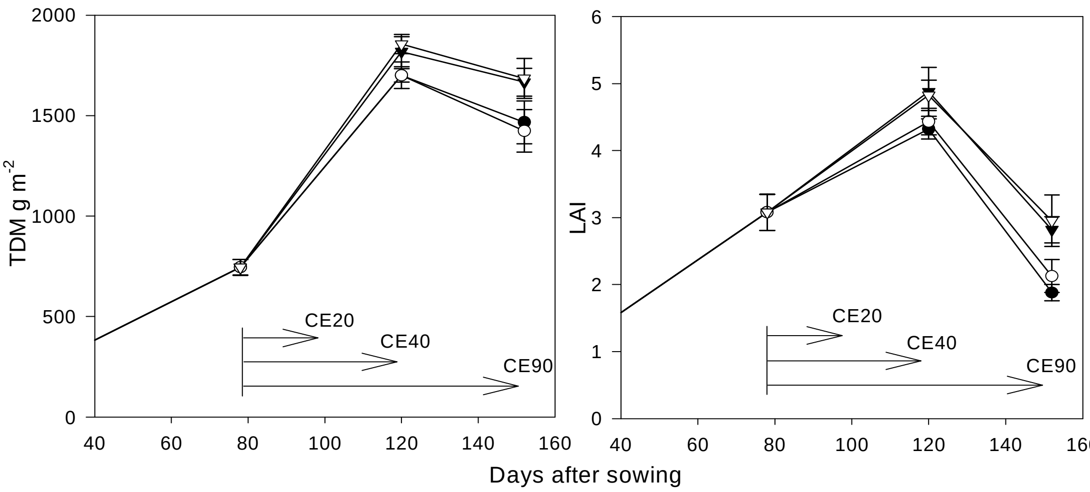

Figure 6.8 Changes in total dry matter and LAI versus days after sowing for different canopy exposure treatments CE0 (●), CE20 (○), CE40 (▼) and CE90 in Exp.6. Bars are two standard errors of the mean. The arrows show the period of the canopy exposure in each treatment

6.5 Discussion

The objective of this work was to study the effects of early water availability on the dynamics of biomass accumulation and partitioning, and crop development of phenological stages in high and lower fruit retention conditions, so that yield advantages of minimizing early soil water deficits at pre-flowering particularly in high retention cotton shown in Chapter 5, can be fully understood. A second objective was an examination of the effects of light exposure of the lower part of the crop canopy to increase source supply and its impact on biomass accumulation and partitioning, under irrigated conditions.

Comparison of different sets of experiments

In Chapter 5, early availability of water produced differences in responses in terms of seed cotton yield, fruit distribution and fruit retention, compared with water stress treatments for both high (Bt cotton) and low retention (conventional cotton). The increased yield and improved fruit retention associated with early irrigation may be due to greater biomass production and increased assimilate available to assist development of more bolls (Fig.6.2 and Fig.6.3). Comparing both sets of experiments (Chapter 3 vs. Chapter 5), differences in yield increase due to early irrigation were small considering the different amount of water applied after the stress period finished, which was greater in the second set of experiments. However, these differences in water applied for recovery were not reflected in differences of yield (20 to 25%), but in TDM (about 45%) for all the cases. In this study the differences in response between early irrigated and water stress treatments prior to flowering for high and low retention cotton were examined, using a framework based on the physiological determinants of crop growth, as conducted earlier in Chapter 4.

In the current experiments, total biomass production was significantly higher in I treatments when compared with soil water deficits during the early stages of crop growth. However, after the stress period was finished and moisture levels in the soil profile were restored through irrigation and rainfall, both stress treatments (NIS and NIF) recovered, increasing total biomass production in Exp.7. This trend was different from the results in Chapter 3 and 4 due to the amount of water applied after the stress period finished. In the first set of experiments (Chapter 3 and 4) the TDM in stressed treatments did not recover as much as in the second set of experiments (Chapter 5 and 6), because the soil profile was not refilled after water stress. In the first set of experiments (Chapter 3 and 4), biomass recovery was not complete and this reduced biomass partitioning to reproductive organs, boll retention and final yield. In the same experiments differences in DAS to reach specific phenological stages of development were significantly more pronounced in comparison with the current work (about 6 days vs. 20 days delayed to maturity in I, comparing both sets of experiments), where there was no water deficit after the stress period. This may have reflected in reduced early growth with fewer fruit positions in response to the marked pre-flowering water deficit, with a resulting shortened boll period and earlier crop maturity in NIS and NIF in earlier Exp.1-4 (first set of experiments) compared with the current set of experiments. This interpretation of the demand determining phenology is consistent with the nutritional hypothesis of Mason (1922) and later studies of Hearn (1972, 1994).

In the current experiments, LAI increased after the stress period to a level of 3-4 which would be sufficient to intercept most incident solar radiation. Then, this contributed to increased DM production which was similar to that in I, except in Exp.7 where LAI declined sharply towards maturity and the DM production was reduced in NIS and NIF.

The aim of increasing water inputs early in the season was to increase the production of vegetative biomass to achieve a bigger canopy with potential source of assimilates supply (vegetative shoots) to meet the high sink demand after flowering. The I treatments developed a significantly higher proportion of vegetative biomass in the early stages of crop development, than was the case for the stress treatments (Fig.6.2). These differences were more pronounced during the first season experiments (2007/08) when compared with the second season (2008/09). This difference between seasons may have reflected differences in profile soil water content, with higher rainfall in the second year delaying the onset of the period of moisture stress, and thereby delaying the period when reduced availability of resources affected crop growth.

Later in the growth (110 DAS Exp.4, 112 DAS Exp.5 and 121 DAS Exp7), all the treatments were under full irrigation (continuing irrigation in I since early growth, and recovery from stress in NIS and NIF as a result of full irrigation following the end of the stress period), increasing the soil water content in all the cases.

Effects of flower removal

In terms of DM production, removal treatments only produced significant effects on boll DM. In 30R the production of boll dry matter (reproductive biomass) was reduced compared with NR under the different water treatments. However, in Exp.7 the differences between NR and R30 were lower in the stress treatments (NIS and NIF) than the I treatment, although there was no significant interaction between removal and water treatments. Sadras (1996) found that under favorable growing conditions (low plant density and high nitrogen), the manual removal of fruit resulted in increases in dry matter production, including the tap root. However under unfavorable conditions (high plant density, low nitrogen), fruit removal did not increase dry matter production. Although plants in this study received the recommended nitrogen inputs and the density was the same in all the cases, the water stress treatments may have constrained the plant capacity to recover from early flower bud loss.

Leaf area is one major variable affected by fruit loss (Brook et al., 1992b) through an extension of the period of canopy expansion and growth. This was the case in all stress treatments for the three experiments (Exp.4, 5 and 7) where leaf dry matter production increased following fruit removal (R30) compared with fruit retained (NR), while in the fully irrigated treatment (I), leaf dry matter always was slightly higher in NR than in R30.The other vegetative components were slightly higher in NR than R30 for all the water treatments in all experiments. The removal of flowers or simulated low retention cotton induced those assimilates which were otherwise used for developing bolls were used for canopy development.

Canopy exposure

In reference to the canopy exposure experiment, TDM and reproductive dry matter increased in response to long term exposure (CE40 and CE90) compared with short exposure (20 days and the control). It is likely that solar radiation may become limiting in I cotton due to the larger canopy, earlier closure, and an increase in the proportion of the lower canopy that is shaded, leading to a decrease in of fruit retention and final seed cotton yield, as was reported in Chapter 5. The higher reproductive DM as a result of lower canopy exposure may have resulted in greater fruit retention. This increased source supply at lower canopy position, increased boll number and seed cotton yield, indicating the cotton yield is commonly limited by assimilate availability at lower positions of the canopy.

In summary, the assimilate supply of larger plants with longer vegetative cycle in response to early irrigation, may explain the differences between I and stress treatments in relation to the rate of reproductive site production, fruit retention and increased cotton yield, which were reported in Chapter 5. Water availability affected the time to reach the different key crop growth stages: cut-out and maturity occurred earlier in the stress treatments due to the associated decline in soil water content and assimilate availability. Total biomass production was higher in the I treatments in all experiments, compared with those treatments with a soil water deficit in the early growth stages. However, both NIS and NIF recovered and increased total biomass production after the period of stress ended and the soil profile was restored. Considering the first set of experiments (Chapters 3 and 4), the TDM production was smaller compared with the current results due to differences in water applied after the stress period finished.

In relation to the lower canopy exposure treatments, at maturity significant differences were found in vegetative, boll and total dry matter production in response to long exposure (42 days after flowering, and until the end of the cropping period) compared to the control (CE0) and short exposure.

Chapter 7 Responses of high retention cotton to pre-flowering water deficit using furrow irrigation

7.1 Introduction

Seventy per cent of Australian's cotton is grown in New South Wales and the rest in Queensland (CRDC, 2005), and the growing area extends from Emerald in Queensland to Hay in New South Wales (Fitt, 1994). In Australia, less than 20% of the crop is rainfed, and the rest is irrigated (CRDC, 2005). The environmental conditions of the cotton area in New South Wales are quite different to those in Queensland with tropical conditions. Cooler temperatures are found further south in the Australian cotton belt, as well as differences in rainfall.

Narrabri is the center of cotton research in Australia, and it is located in the main Australian cotton growing area next to the Naomi Valley in New South Wales. Cotton is planted around mid-October and most farmers use furrow irrigation to provide water to the crop during the cotton season. Furrow irrigation is the most popular and widely investigated in Australian cotton systems compared with alternative irrigations such us sprinkler or drip. It has been associated with high yield and some great returns per megalitre and machinery is designed and built around the system and many others.

In previous Chapters it was found with high retention cotton that increased early water availability increased early biomass production and a large canopy supported a higher number of fruits retained resulting in increases in final lint yield compared with water stress treatments until squaring and flowering. All the experiments were conducted with sprinkler irrigation at Gatton, Southeast Queensland. The aim of this chapter was to study the responses of pre-flowering irrigation in high and low retention cotton under furrow irrigation at Narrabri, New South Wales. The results are compared with those obtained at Gatton, Southeast Queensland.

7.2 Material and Methods

7.2.1 Experimental sites and growth conditions

A field experiment was conducted from October 2008 to April 2009 at the Australian Cotton Research Institute (ACRI) (30^{o}13’S 149^{o}47’E) 24 km west of Narrabri, New South Wales, Australia. The annual rainfall is 650 mm with a mean maximum temperature of 26.5^{o}C and mean minimum of 11.7^{o}C (BOM, 2008). The soil type is a Grey Vertosol (Isbell, 2002).

7.2.2 Cultural practices

The Bt transgenic Bollgard ll®™ variety Sicot 71BR producing the Monsanto Cry1Ac and Cry2AB proteins) was sown by machine on the 15th of October 2008. Row spacing was 1 m and plant density was 70,000 plants ha^{-1} (6-8 plants m^{-1}). Land preparation and fertilization rate was done one month before sowing using conventional tillage. Fertilizer was consistent with cotton on this soil type, that is, with 200 kg N ha^{-1} as anhydrous ammonia and 9 kg P ha^{-1} as single superphosphate. Herbicide to control weeds was applied during pre planting (pendimethelin), and post emergence (glyphosate). Insects were regularly controlled through monitoring the presence of insects in the crop and insecticide spray decisions were made according to thresholds derived in temperate Australia (Farrell 2006).

7.2.3 Experimental design and water deficit treatments

The experiment was furrow irrigated on single row hills using a split plot design with four replications and had the treatments shown below: Plots were the length of the field (165m) by 18 rows (1m) wide. Measurements were made in a 25m long by 5 row (centre rows) wide area within these plots where rain was excluded using plastic sheeting (described below).

I (Full Irrigation): Irrigation was applied when the plant available water content (PAWC) was 65 to 75% of the drained upper limit, or a 50mm soil water deficit at maximum rooting depth. Irrigation was applied nine times: 12/12/08, 24/12/08, 7/01/09, 15/01/09, 25/01/09, 2/02/09, 10/02/09, 3/03/09 and 16/03/09 and the soil profile refilled to 100%±5% of PAWC.

NIF (No water until flowering – 90DAS): No irrigation was applied from establishment to flowering (water stress period), when the soil water deficit was 100mm or 48% of PAWC remained in the profile and then the crop was irrigated five times: 16/01/09, 25/01/09, 2/02/09, 10/02/09 and 3/03/09 using the same deficit as the I treatment and the soil profile refilled to 100%±5% of PAWC. Water stress was achieved by preventing rain falling in the plots using plastic covers placed on the ground between the rows within 1cm of the plant stem and secured with wire pegs. The covers were removed at the end of the stress period. However, the plastic covers were not as efficient in excluding rainfall as in Gatton experiments; due to strong wind conditions and heavy rains during the early stages of the crop lifting and ripping the covers. The stress period was delayed as the soil water at first squaring was 90% of PAWC. In addition the full irrigation treatment received rainfall within 48 hrs of the first irrigation and water logging symptoms were observed.

In each water treatment there were two levels of flower removal, starting from the time of early flowering (i) no-removal (NR) representing high retention cotton and, (ii) Lower retention cotton where 30 flowers removed per metre (30R) or about 4 flowers per plant. This second level of removal simulated lower retention cotton. The flower buds were removed from early flowering at the first positions on the lower fruiting nodes of the plant.

7.2.4 Measurements

Meteorological conditions during the crop season were recorded. Plant height, main-stem node number, number of squares, flowers and bolls, crop maturity and number of nodes above white flower (NAWF) were recorded. Total dry matter and partitioning were measured at about 1st square, 1st flower, cut-out and physiological maturity (defined open bolls when two sutures had dehisced), respectively. Plants from a 1 m^2 area in each plot were harvested for total fresh weight determination. A sub-sample of 4 plants was used to determine fresh weight, leaf area, dry matter and partitioning of DM into leaves, stems, petioles, squares, flowers, green bolls and open bolls (two sutures on the boll dehisced). Samples were dried at 80ºC for three days to determine dry matter content. Leaf area was measured using a LiCor planimeter (Model LI-3100, LiCor Inc., Lincoln, NB, USA). Specific leaf area (SLA) was calculated, and then Leaf Area Index (LAI) was calculated as the product of SLA and the amount of leaf dry matter in the 1m^2 area (g m^2). Using a line quantum sensor, solar radiation interception was measured around midday Incident radiation was recorded above each plot. Three readings of transmitted radiation were recorded at ground level in each plot. The proportion of intercepted photosynthetically active radiation (PAR) was calculated as: (incident radiation – transmitted radiation)/ incident radiation.

Maturity picking for seed cotton yield commenced when about 20 to 40% of bolls had opened (bolls were defined as having opened when two sutures on the boll had dehisced) and continued weekly from 3m^2 from the center rows of each plot until the last boll had opened. Seed cotton yield was measured by hand picking 2 by 5m lengths of row in each plot (10 m^{-2}).

7.3 Results

7.3.1 Meteorological conditions

Daily average maximum and minimum temperatures, solar radiation, rainfall and evaporation during the experimental period are shown in Fig.7.1. For the 2008/09 season comparing with Gatton experiments, the average maximum temperature was 31.6^{o}C (Narrabri) and 31.0^{o}C (Gatton) and the average minimum temperature was 16.7^{o}C (Narrabri) and 17.6^{o}C (Gatton). There were no major differences between the experimental sites in terms of temperature in the current study, except slightly colder nights at Narrabri during the first 50-60DAS. The rainfall recorded was 416 mm at Narrabri and 616 mm at Gatton.

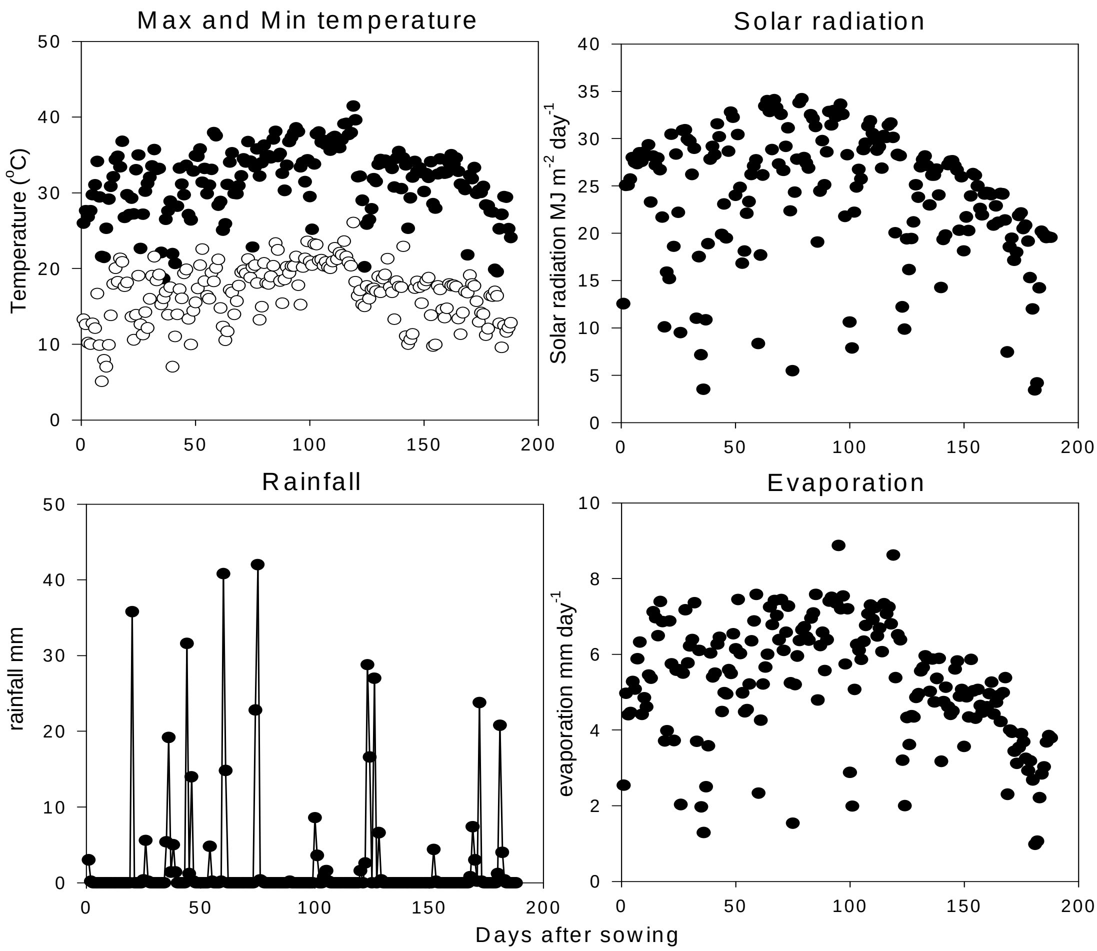

Figure 7.1 (a) Daily minimum and maximum temperature (^{o}C), (b) daily incident solar radiation (MJ m-2 day-1), (c) rainfall (mm) and (d) daily evaporation at Narrabri NSW during 2008/09.

7.3.2 Dry matter production

The production of total biomass is shown in Fig.7.2. At early stages of the crop growth, there were no significant differences in total biomass production between treatments. At 118DAS, I NR produced significantly higher total biomass than the rest of the treatments. At 168DAS, the early irrigated treatments (I NR and I 30R) produced significantly higher biomass compared with early water stress treatments (NIF NR and NIF 30R), but there was no significant effect of flower removal.

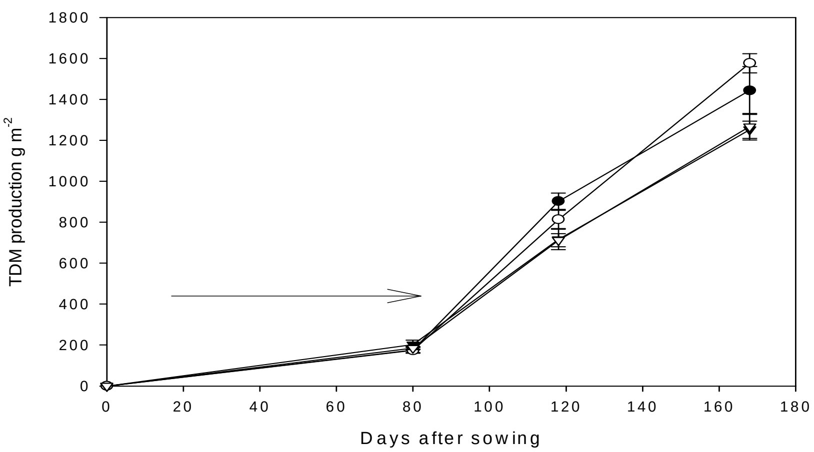

Figure 7.2 Total dry matter production for I NR (●), I 30R (○) and NIF NR (▼), NIF 30R during 2008/09 at Narrabri, NSW. Error bars are two standard errors of the mean. Arrow shows the period of water stress.

7.3.3 Leaf area index and radiation interception

The changes in LAI during the crop growth is shown in Fig.7.3. No significant differences were found at early stages of the crop growth. At 118 DAS, early irrigated treatments produced a slightly higher LAI than stress treatments, but the difference was not significant. The peak LAI reached by I NR was 3.4. At 168 DAS, LAI decreased in NR treatments under both early irrigated and water stress conditions, while there was an increase in 30R under both water treatments. The differences in LAI were not significant.

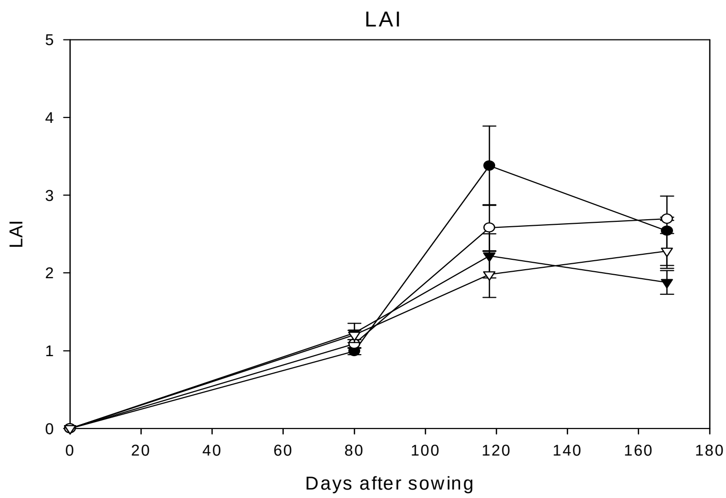

Figure 7.3 Changes in leaf area index for I NR (●), I 30R (○) and NIF NR (▼), NIF 30R during 2008/09 at Narrabri, NSW. Error bars are two standard errors of the mean

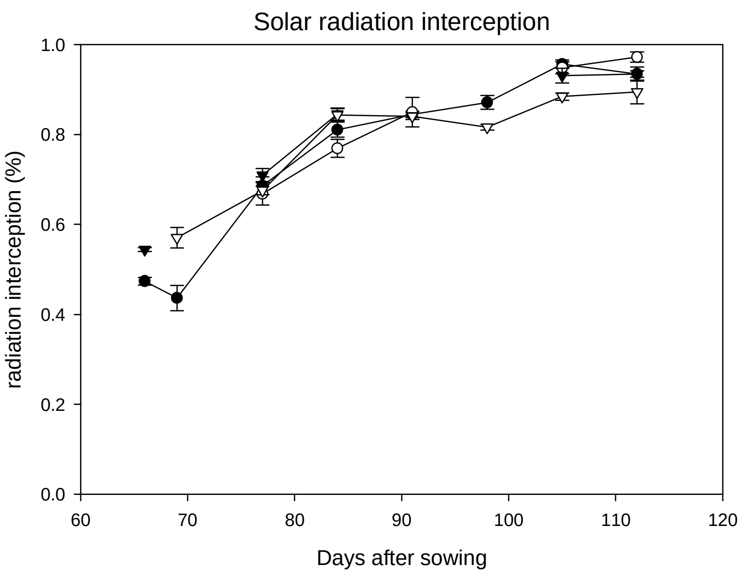

Figure 7.4 Percentage of photosynthetically active radiation (PAR) intercepted for I NR (●), I 30R (○) and NIF NR (▼), NIF 30R during 2008/09 at Narrabri, NSW. Error bars are two standard errors of the mean.

The solar radiation interception is shown in Fig.7.4. At about 70DAS, the full irrigated treatment intercepted less solar radiation than the stressed treatment, coinciding with a high rainfall period that may have produced water logging in the irrigated treatment. At 84 DAS, most of the treatments intercepted more than 80% of solar radiation. At 98, 105 and 112 DAS, the irrigated treatments increased the light interception associated with higher LAI and canopy closure compared with stress crops but there were no effects of removal treatments.

7.3.4 Dry matter partitioning

The distribution of TDM into vegetative and reproductive weight over the whole crop growth is shown in Fig.7.5. Vegetative dry matter production in the I treatments was significantly greater than in the water deficit treatments mainly at 118 and 168 DAS. Significant differences were found for green bolls dry weight, being higher in NR treatments (irrigated and stressed) compared with 30R. At 168 DAS, the green bolls dry weight was significantly higher under full irrigation treatments (I NR and I 30R) compared with water stress ones (NIF NR and NIF 30R). In terms of open bolls, there were no significant differences between treatments at 168 DAS, however it was slightly higher under water stress conditions compared with full irrigated treatments due to earliness of stressed crops associated with a higher green boll weight produced in full irrigated treatment at the last measurement occasion.

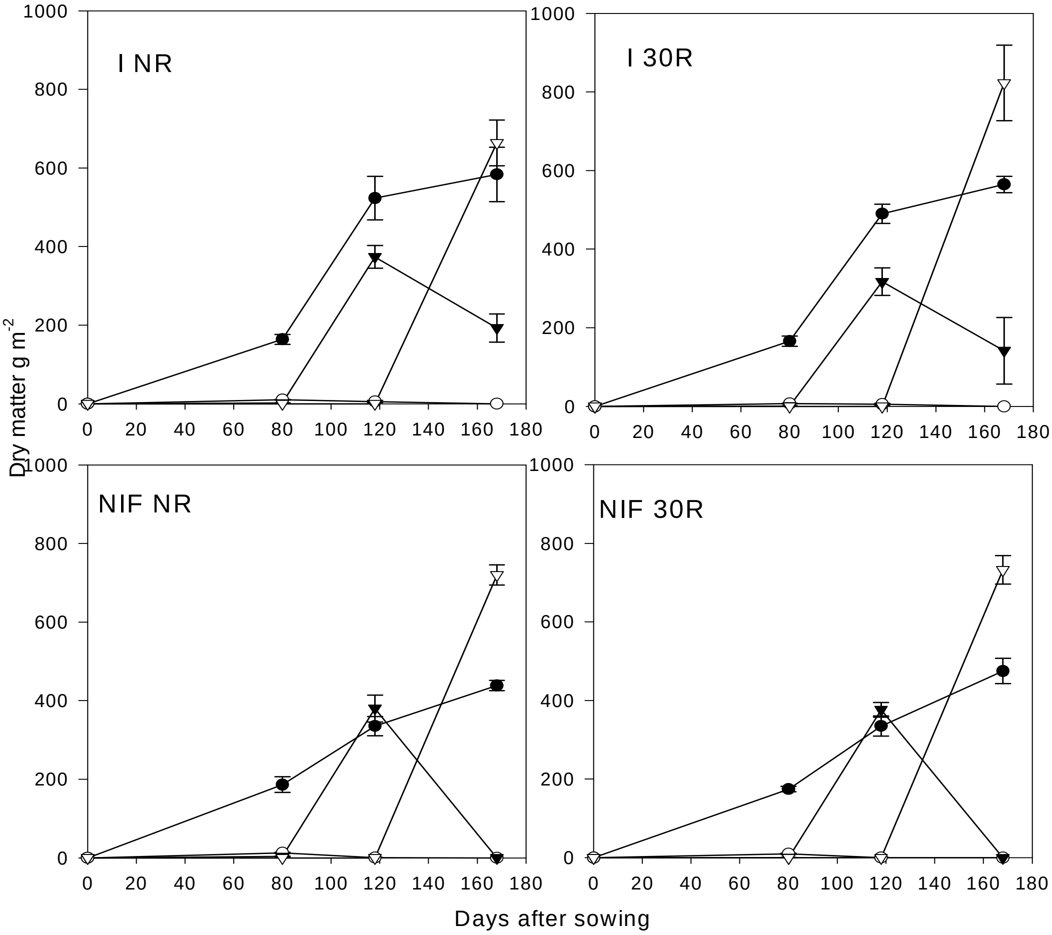

Figure 7.5 Dry matter production of vegetative organs (leaf, stem and petiole) (●), early reproductive organs (flower) (○), green bolls (▼) and open bolls versus days after sowing for I NR, I 30R, NIF NR and NIF 30R at Narrabri. Error bars are two standard error of the mean

7.3.5 Squares, flowers and bolls number

The total fruit production was segregated into squares and flowers, green boll and open boll number, during key stages of the crop (Fig.7.6). The number of squares and flowers were not significantly different between treatments, however it was slightly lower at 80DAS in those treatments that flowers have been manually removed at early flowering (30R) compared with NR treatments under irrigated and stress conditions.

The number of green bolls at 118 DAS was significantly higher in early irrigated treatments (I NR and I 30R) than under early stress (NIF NR and NIF 30R). At 168 DAS, there were still green bolls to be mature in I treatments, while none in stressed ones due to their earliness. No significant differences for number of open bolls were found at 168 DAS.

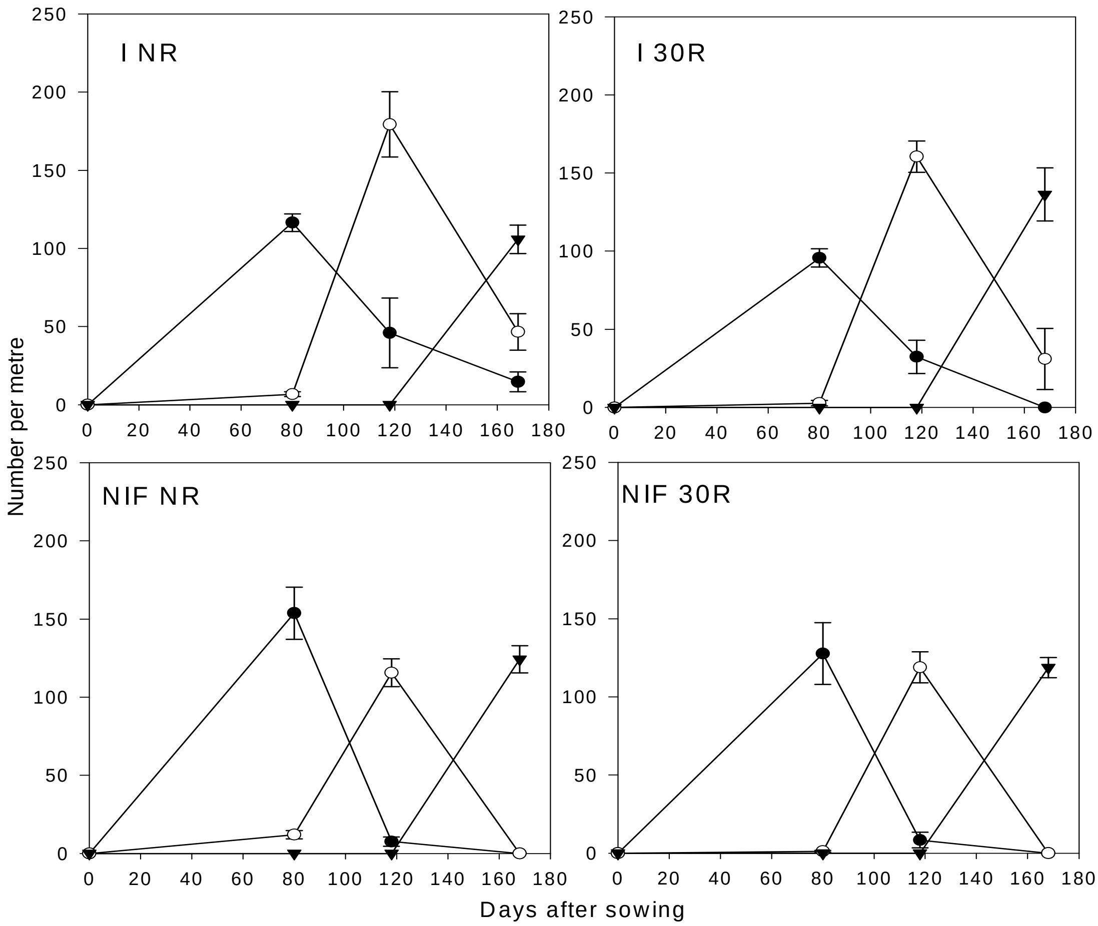

Figure 7.6 Number of flowers (●), green bolls (○) and open bolls (▼) versus days after sowing for I NR, I 30R, NIF NR and NIF 30R at Narrabri. Error bars are two standard errors of the mean

7.3.6 Height - node production and total fruit retention.

Measurements of plant height and main stem node production (as a potential site production) are shown in Fig.7.7. No significant differences were found between treatments in terms of height and number of nodes over the time. The stress treatments had significantly lower NAWF compared with I (Fig.7.8).

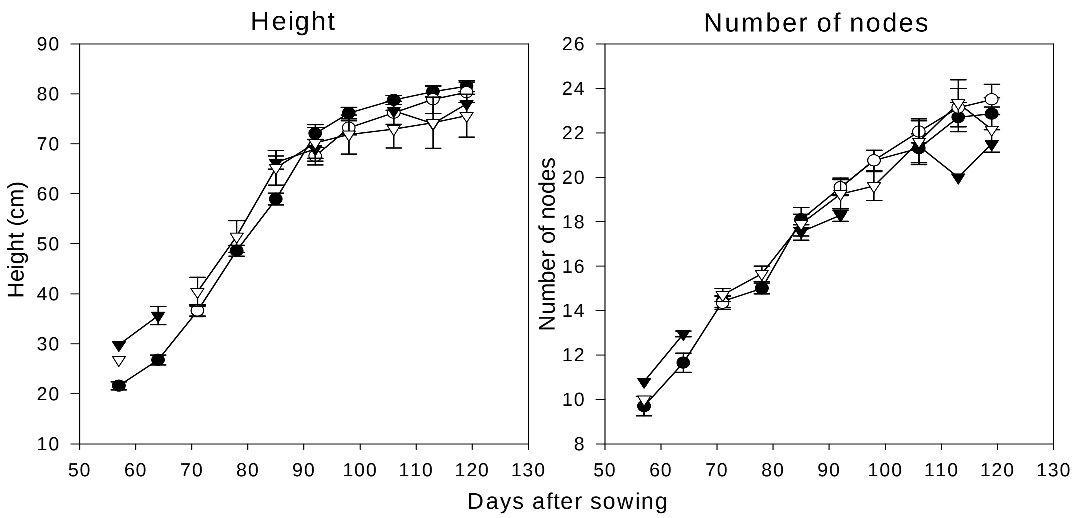

Figure 7.7 Changes in height and number of nodes for I NR (●), I 30R (○) and NIF NR (▼), NIF 30R during 2008/09 at Narrabri, NSW. Error bars are two standard errors of the mean.

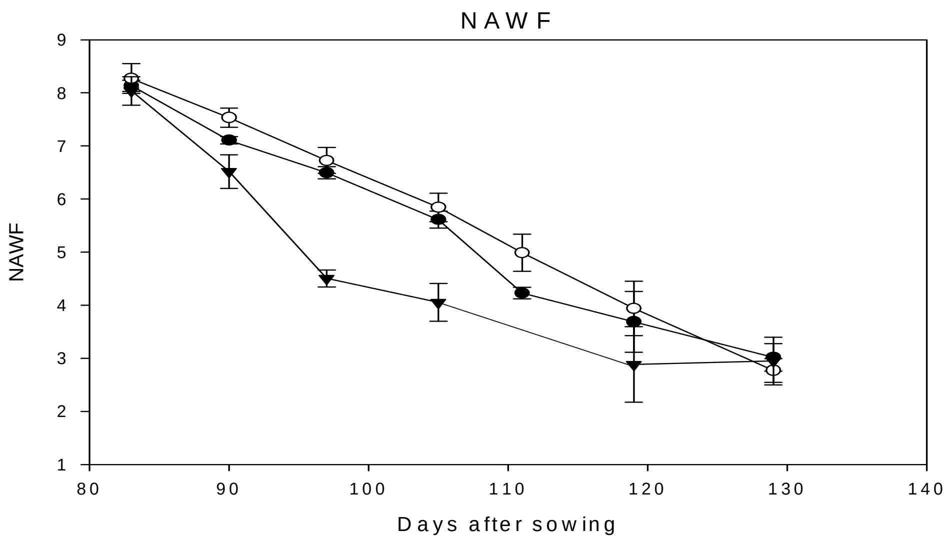

Figure 7.8 Changes in the number of nodes above white flower versus DAS for I NR (●), I 30R (○) and NIF NR (▼) during 2008/09 at Narrabri, NSW. Error bars are two standard errors of the mean.

7.3.7 Seed cotton yield and quality

The time change to maturity is shown in Fig.7.9. The percentage of cotton picked at each time was slightly higher under stress conditions than full irrigated conditions, and the time to maturity (60% of seed cotton pickable) occurred about 10 days earlier in stressed than the in full irrigated treatments.

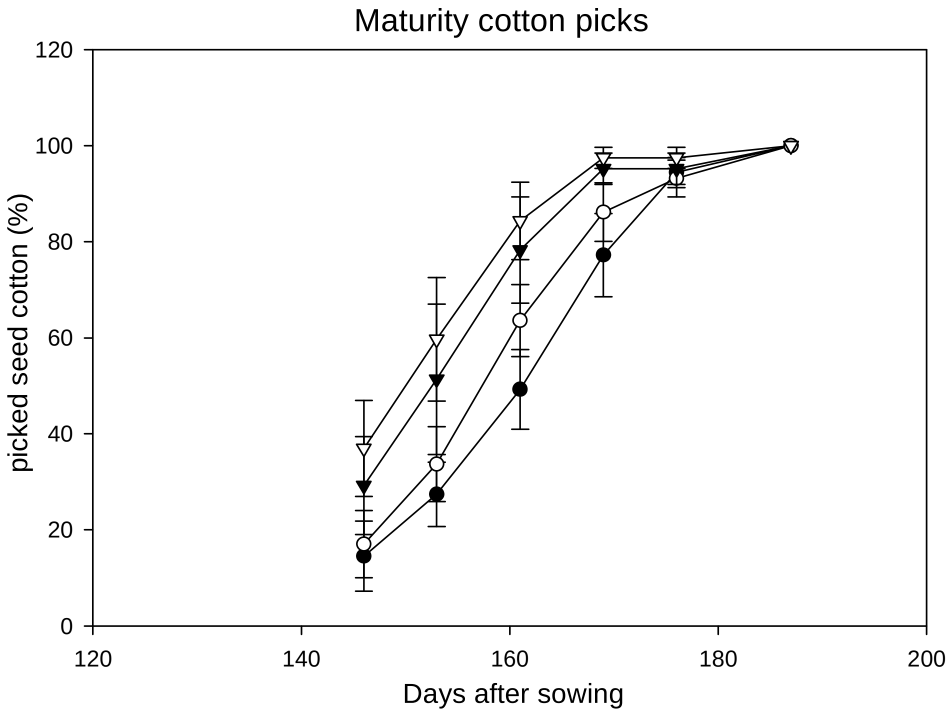

Figure 7.9 Picked seed cotton (%) at different days after sowing (DAS) for I NR (●), I 30R (○) and NIF NR (▼), NIF 30R during 2008/09 at Narrabri, NSW. Error bars are two standard errors of the mean.

There was a significant increase in seed cotton yield under full irrigated conditions compared with stressed treatments (Fig. 7.10). For the different parameters of cotton quality, no significant differences were found between treatments.

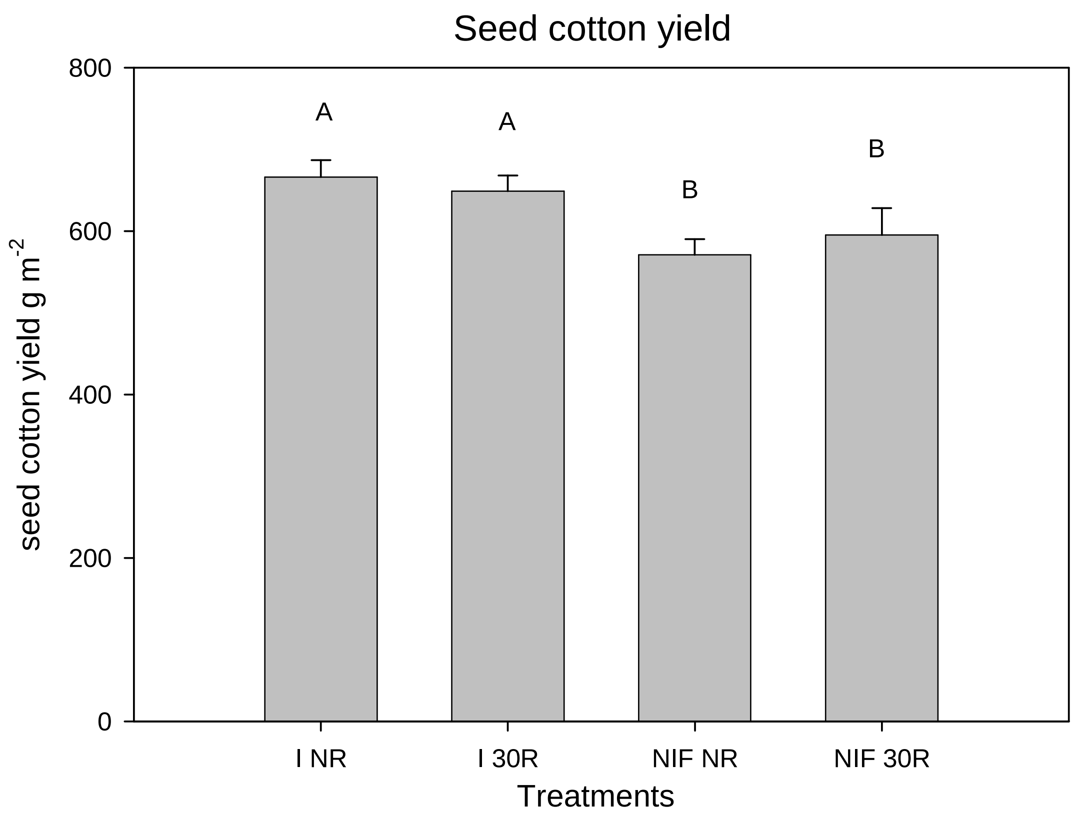

Figure 7.10 Seed cotton yield for I NR, I 30R, NIF NR and NIF 30R during 2008/09 at Narrabri, NSW. Treatment means with the same letter are not significantly different, Lsd0.05=39.8 g m^{-2}.

7.4 Discussion

The results of this study indicate that early soil water availability impacted positively on crop growth and development in high retention Bt cotton when furrow irrigated on a heavy clay soil, compared with water stress that commenced prior to flowering. The results support the same approach that was found during three years experiments at SE Queensland which refers that insufficient early vegetative growth can have a negative impact on the high assimilate demands needed for boll development associated with a large number of bolls developed in high retention cotton.

In the previous chapter the fully irrigated treatment (**I**) at Gatton (Exp.7, sowed about the same date) produced an increase of 22% in seed cotton yield compared with stressed treatment. The differences between water treatments at Narrabri using furrow irrigation were also significant and increased yield by 9 to 16% compared with the pre-flowering water stress treatments. As was the case at Gatton using sprinkler irrigation early furrow irrigation increased boll number at maturity. With rather a small effect of irrigation treatment, there was also no significant effect of flower removal in this experiment.

However, this experiment highlighted some of the trade-offs with increased early season furrow irrigation in the sub-humid climate with highly variable rainfall where about 75% of Australia's cotton is grown. The risk of water logging, either from poorly applied irrigation or by rainfall, is greater with more frequent irrigation and waterlogging can significantly reduce yield (Hodgson and Chan, 1982) and could negate any benefits of early irrigation. More frequent early irrigation will also reduce the effectiveness of any in-crop rainfall that occurs prior to flowering and reduce water use efficiency. The risk of soil borne disease is greater where rain is associated with cool temperatures and an already wet soil from irrigation. Further research is required to evaluate these risks.

Biomass production after flowering was increased with early irrigation at pre-flowering. However, it is possible to mention that I treatments received excessive water from rainfall at early stages inducing waterlogging symptoms which may have had negative effects on crop growth. Comparing with Exp.7, with similar sowing date but at Gatton, SE QLD, the production of biomass was similar, increasing the TDM by 25% under irrigated conditions compared with stressed treatment. In both experimental sites, the maximum TDM was reached about 160-170 days after sowing. LAI was greater in Exp.7 compared with Exp.8. In addition, the irrigated conditions increased LAI to 4.3 and 3.5 for Exp.7 and Exp.8, respectively. In contrast, the LAI in stressed treatment was about 3.15 (Exp.7) and 2.2 (Exp.8). For both experiments, the I treatment increased the number of nodes and plant height compared with stressed ones. However, plants at Gatton tended to grow taller with longer internodes than those at Narrabri under both water conditions.

The irrigation system used in each place is an important factor to understand this study. Numerous research results concluded that furrow irrigation produce a different pattern of growth for roots and aerial biomass, as well as fruit retention and yield compared with sprinkler irrigation (Carmi et al., 1993; Cetin and Bilgel, 2002; Constable and Hodgson, 1990; Sagarka et al., 2002). Those differences are due to many variables involved such us: larger amount of water applied with less frequency under furrow irrigation compared with sprinkler, different type of soils with changes in dry-wet cycles, or in this case limitations related to meteorological conditions like excessive rainfall.

Chapter 8 General conclusion and future research direction

The introduction of high yielding GM cotton varieties to Australian farming systems is one of those technological advances that has improved tolerance to insect pests and better control of weeds. Nevertheless, issues related with water management at early stages of the crop to develop a bigger sized plant that would produce a larger amount of assimilates post flowering to meet a higher demand from an increased number of fruits retained in high yielding cotton are still not investigated due to its short history since it was released. Thus, there is strong interest in the Australian cotton industry to improve the efficient use of water as it is currently a limited resource for a better and sustainable production system with higher yields.

Over the last 30 years, the Australian cotton industry has grown dramatically increasing the potential yield with new varieties and intensive production systems compared with the 1970's cotton systems, becoming the highest yielding cotton producer in the world under intensive production systems (G A Constable, 1998). The Bollgard II cotton varieties, which containing two genes from *Bacillus thuringiensis* var *kurstaki* (Bt) that express proteins toxic to *Helicoverpa* spp., which were recently released in Australia, has increased insect protection compared with conventional (non-Bt) varieties with similar genetic backgrounds, leading to increased early retention and hence boll load, faster accumulation of boll weight, while they have lower leaf area than their conventional equivalents (Yeates et al., 2006). Using new GM varieties some issues such us management of water at pre-flowering is still relevant to be explored to improve the sustainability of cotton systems.

This study was the first step to understand the potential of pre-flowering irrigation, as a production practice in Australian systems, that develops higher biomass at early growth stages to support a higher rate of retained fruits produced by high retention Bt cotton compared with long periods of water stress at pre-flowering and long vegetative cycles in conventional cotton varieties, which have a lower fruit retention due to greater susceptibility to insect pests.

8.1 Conclusions

Seed cotton yield

This study found that even modest early soil water deficits affected lint and other components of seed cotton yield in high retention cotton (Table 8.1). The I treatment increased final seed cotton yield in all 8 experiments over the last three season compared with stressed treatments (increase calculated over NIF).

Considering the first set of experiments (2006/07-2007/08) the I treatments increased final cotton seed yield by 44% in November sowing dates and about 20% in early sowing dates, compared with stressed conditions (NIF). In the second set of experiments (2007/08 – 2008/09), I increased final seed cotton yield by 25-28% (Exp.4 and 7, respectively) compared with stressed treatments (NIF). These differences may be associated with differences in soil water content after the water stress period finished. In the first set of experiments at Gatton, SE Queensland (2006/07-2007/08), the amount of water applied after the stress period had finished was not enough to re-fill the soil profile and plant growth was not recovered compared with the second set of experiments Gatton, SE Queensland (2007/08-2008/09) where rainfall was higher and the soil profile was refilled after the stress period.

Finally, considering the experiment (Exp.8) at Narrabri, NSW, final yield was also affected by early water availability. However the increase in seed cotton yield of 15% was smaller than that in all the experiments at Gatton, SE Queensland. This was related to heavy storm rainfall resulting in soil water saturation in the I treatment and the failure of the plastic covers reducing the number of water stress days in NIF treatment.

Table 8.1 Comparison of seed cotton yield (g m-2) for all the experiments

	I	NIS	NIF	Increases (%)
Exp.1	580	508	462	20
Exp.2	472	286	270	44
Exp.3	507	320	281	44
Exp.4	583	440	421	28
Exp.5	542	450	327	40
Exp.6	627	-	-	-
Exp.7	645	497	486	25
Exp.8	666	-	571	15

Mechanisms for increased yield in high retention cotton

These variations in yield and components of yields are mainly explained through a better understanding of growth and development during the season. Increased pre-flowering water availability impacted significantly on the crop, increasing retention of boll load, with changes in boll distribution on lateral and vertical fruits positions increasing final yield.

The variation in number of reproductive organs was associated with duration and severity of the stress period. NIF with longer stress period than NIS produced fewer reproductive organs in all experiments. After the stress period, recovery in the production of reproductive organs, site production and retention was insufficient in the first set of experiments (Chapters 3-4) compared with a better recovery in the second set (Chapters 5-6). The level of fruit retention was 85 - 92% for all treatments at early flowering stage and decreased to 65 - 68% by the irrigated treatment and 53 - 59% in the stressed treatments at the time of crop maturity. The total of fruits number increased with the I treatments relative to the stressed treatments (NIS and NIF), mainly in first lateral position and concentrated in the middle and upper parts of the canopy. The absolute number of flower buds and bolls were higher in I compared with stress treatments in high retention cotton.

Total biomass and vegetative production, LAI and early canopy closure, were significantly higher under irrigated (I) conditions. The total biomass production was higher in the I treatments in all the experiments by 20-48% when compared with treatments with soil water deficits during the early stages of plant growth. Thus increased LAI as a result of early irrigation produced sufficient assimilates to fill in a larger number of bolls. However, in the water stress treatments (NIS and NIF) there was a recovery in total biomass production after the stress period in response to refilling of the soil profile in the second set of experiments compared with the first set of experiments. Nevertheless the yield of previously stressed plants was lower, indicating increased sink size as a result of early irrigation contributed to a higher yield in high retention cotton. Similar patterns of growth and development were found at using furrow irrigation at Narrabri, NSW.

These results support the general hypothesis that insufficient early growth as a result of early pre-flowering soil water deficits, reduces the assimilates supply needed to meet a higher boll demand in high retention cotton, producing reductions in seed cotton yield.

Phenology

Water availability affected the time taken to reach different key crop growth stages. Cut-out and maturity occurred earlier in the stress treatments, while time-to-maturity by I was significantly delayed in all experiments. The differences in phenological development between water treatments were higher in 2007/08 (Exp.4) than in 2006/07 (Exp.1). For example, the boll growth period was significantly longer in the second season (Exp.4) compared with the first season (Exp.1). For I, the boll growth period in Exp.1 was 79 days, while in Exp.4 it was 106 days. For NIS the periods were 71 and 102 days, for Exp.1 and Exp.4, respectively.

This increased boll growth period in I ensured to fill a larger number of bolls produced. However it should be noted that increased growth duration and extra irrigation prior to flowering requires increased water inputs, and hence this is significant cost to the growers.

Simulated low retention cotton

Under water stress conditions (NIS and NIF), the differences in seed cotton yield between high and low retention (simulated by removal of 30 flowers per meter at the early stage of flowering) cotton were smaller. However under irrigation conditions yield tended to be higher in high than in low retention cotton. In high retention cotton (Bt), early water stress reduced seed cotton yield by about 20%; however in low retention (flower removal) cotton the stress treatments reduced seed yield by between 5 and 8%, relative to the I treatments at Gatton experiments. This suggests that early irrigation increased the supply of assimilates (before flowering), which was important for the high retention Bt cotton, whereas plants can be stressed during the early stages development in low retention cotton.

In 30R the production of boll dry matter (reproductive biomass) was reduced compared with NR under the different water treatments. Leaf dry matter production increased in R30 compared with NR in all stress treatments for the three experiments (Exp.4, 5 and 7), while in I, NR always was slightly higher than R30. In some way, the removal of flowers or simulated low retention cotton utilized those assimilates, which were otherwise used for developing bolls, for canopy development. The various vegetative dry matter production increased in low retention cotton (R30) when compared with Bt (NR), in the second year of studies (Exp.4 and Exp.5). However, the differences between conventional and Bt cotton in the water stress treatments (NIS and NIF) were smaller than for conventional and Bt cultivars in the I treatments during the third year (Exp.7) at Gatton experiments.

Source availability after flowering limiting yield

The artificial canopy opening to exposure to higher light showed that the longest period of exposure of 42 days after flowering and until the end of the crop, increased vegetative dry matter production, boll dry matter and TDM, and fruit retention in second position by 15% and total fruit retention by 10%, with a much larger number of fruits being retained in the lower part of the plant. The treatments increased significantly final seed cotton yield compared with control (no canopy exposure). This result indicates that high retention cotton has a capacity to respond to increased source supply even after flowering, again indicating the importance of increased source supply to increase cotton yield.

8.2 Future research direction

These observations show the advantages of early water availability in high retention cotton under field conditions in order to improve final lint yield, and support the general hypothesis that insufficient early growth at pre-flowering, produced under soil water deficits, reduces the assimilates supply to a higher boll demand after flowering in high retention cotton.

Further research is needed to continue understanding the effects of early irrigation in high retention cotton. Most of these experiments used overhead sprinkler irrigation on a relatively well drained soil at Gatton, Southeast Queensland and it would be ideal to further test the concept in major cotton growing areas in Southwest Queensland and Northwest New South Wales where cotton is furrow irrigated on heavy clay soils. Also, most experiments were conducted under sprinkler irrigation, and because most growers use furrow irrigation system, the concept need to be tested under alternative irrigation systems such us furrow or drip irrigation.

Further studies to compare Bt cotton with conventional varieties where fruit removal is due to insect damage and not simulated by hand removal as in these studies. In addition non – Bt varieties are very susceptible to damage to the vegetative growth point and are often tipped-out, which if occurs early in growth stimulates the production of multiple fruiting branches that can change the timing of boll load and light interception pattern.

Cost benefit analysis also need to be investigated, whether increasing inputs of water for irrigation produce enough returns for farmers. This should consider not only the pre-flowering irrigation, but also the cost of growing crops for a longer period. As the Narrabri experiments demonstrated rain may fall after pre-flowering irrigation and negate the positive effect of irrigation to some extent. Thus a simulating study is required to determine the chance of success and the risk of extra irrigation before flowering.

Finally, further studies are needed for issues relating to physiological and morphological factors influencing time to maturity and cotton yield especially in terms of boll size, as this was not studied in the present work, but it may explain also some differences in yield found in the current study. Another important area of research would be root growth and development under high inputs of water at pre-flowering in relation to nutrient uptake in high retention cotton. Additional irrigation may reduce effective root depths, and this would have further effect on water and nutrient uptake during boll growth.

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Appendix

Plate 1. View of experiments using plastics between rows at Gatton, SE Queensland.

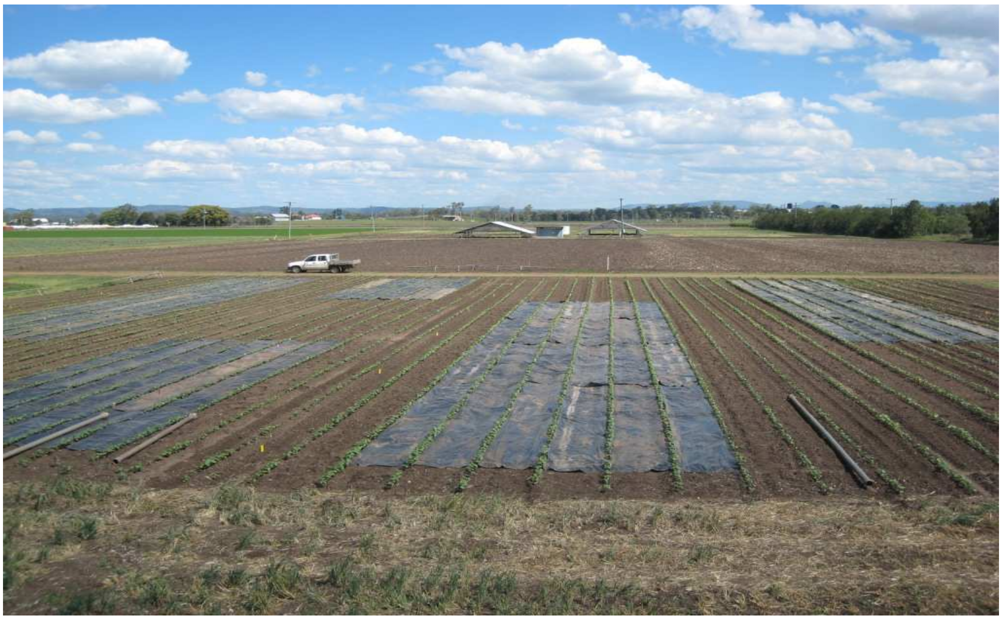

Plate 2. View of experiments using plastics between rows at Gatton, SE Queensland.

Plate 3: View of experiments under rainout shelter at Gatton, SE Queensland.

Plate 4: View of experiments at Narrabri, New South Wales.