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EVALUATION OF A TILLER INHIBITION (*tin*) GENE IN WHEAT

by

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Research School of Biological Sciences
Environmental Biology Group

&

CSIRO Plant Industry
Improvement of rainfed crops

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I am not afraid to confess that I am ignorant of what I do not know

Cicero

DECLARATION OF ORIGINALITY

This thesis reports the original work of the author except where otherwise stated. Specific contributions and cooperative work with others are referred to in the acknowledgements.

Brian Duggan

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ABSTRACT

Reduced tillering cereals have been proposed as being advantageous under terminal drought conditions through their presumed reduction in leaf area and increased partitioning of assimilate towards fertile stems. The reduced leaf area should reduce pre-anthesis transpiration and conserve soil water for grain filling while the partitioning of a greater proportion of biomass into fertile stems should subsequently result in more efficient partitioning of assimilate towards grain. The tiller inhibition (*tin*) gene reduces the number of tillers produced by spring wheat plants and this study was undertaken to assess the agronomic potential of this gene in addition to investigating the physiological changes associated with it on a plant and crop basis.

The inhibition of tiller bud growth by the *tin* gene and the phenomenon known as "stunting" are linked and the latter appears to be an extreme manifestation of the former whereby the development of the mainstem apex is retarded. High light intensity, long photoperiod and low minimum temperatures are required to induce stunting, while high CO_2 induces associated traits such as reduced leaf length.

The accumulation of biomass did not appear to be significantly affected by the presence of the *tin* gene. The reduction in tiller number was compensated for by more assimilate being partitioned into those tillers that were produced. The presence of the *tin* gene resulted in higher harvest index values, indicating more efficient partitioning of biomass into grain, as well as larger spikes with more kernels spike^{-1} at low densities. However, as spike densities of the *tin* lines approached 450 spike m^{-2}, the differences in the characteristics associated with the *tin* gene disappeared.

Leaf area index was not reduced by the presence of the *tin* gene as plants were able to produce longer and wider leaves. Using epidermal cells as an example, the increase in leaf length was due primarily to an increase in the number, rather than the length, of the leaf cells. The maximum rate of cellular division was also increased by the presence of the *tin* gene.

Yield was not significantly altered by the presence of the *tin* gene under most conditions where it was tested. There were however significant changes in the composition of yield with kernel weight increased by 5 to 6% for the spring lines containing the *tin* gene relative to the near-isogenic pairs, and although the number of kernels spike^{-1} was often greater for the *tin* lines, kernels m^{-2} was reduced due to lower spike densities. The application of nitrogen increased spike densities in the *tin* lines, but not to similar densities as those produced by the freely tillering near-isogenic pairs.

Water extraction was the same for the lines with and without the *tin* gene under field conditions, except at one site at maturity where the *tin* lines extracted more than their near-isogenic pairs. Root length density appeared to be unaffected by the *tin* gene.

The intrinsic benefits of the *tin* gene appeared to be higher harvest index values, shorter stems and higher kernel weights as these traits were exhibited in the *tin* lines when plants were grown to achieve the same spike densities as their near-isogenic pairs. Most other traits such as increased number of spikelets spike^{-1}, increased number of kernels spike^{-1} and increased stem length density appear to be contingent on spike densities being lower for the *tin* lines. Stem water soluble carbohydrates were also as high or higher for the *tin* lines under field conditions when spike densities were low, although when grown to achieve the same spike density the levels were the same or lower in the *tin* lines than their near-isogenic pairs.

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LIST OF ABBREVIATIONS

°C	degrees Celsius
aka	also known as
Alt	Altitude
DC	Decimal Code
DC 15	5th leaf more than half visible
DC 16	6th leaf more than half visible
DC 30	stem starts to elongate
DC 37	flag leaf visible
DC 39	flag leaf collar just visible
DC 65	50% of spikes flowering, mid flowering (i.e. anthesis)
DC 71	kernels watery ripe, clear liquid
DC 79	very late milk, half solids/half liquid
DC 92	harvest ripe
GA	gibberellic acid
hsr	higher sowing rate
HT	High Temperature
LAI	Leaf Area Index
LAR	Leaf Area Ratio
LD	Long Day
LSD	Least Significant Difference
LT	Low Temperature
LWR	Leaf Weight Ratio
PAR	Photosynthetically Active Radiation
PVC	Poly Vinyl Chloride
SD	Short Day
SLA	Specific Leaf Area (=1/SLW)
SLW	Specific Leaf weight
ssr	standard sowing rate

CHAPTER 1

REVIEW OF THE LITERATURE AND OBJECTIVES

The former Australian 2 dollar note with the portrait of pioneering Australian wheat breeder, William Farrer

1.1 INTRODUCTION

The importance of achieving correct spike densities in cereal crops was reported as early as 1898 when William Farrer wrote "I have invariably found that in seasons in which soil has been dry while grain was being formed, varieties which had stooled largely had also failed to fill their ears properly, and that when they came to be thrashed they only gave a poor yield of inferior shrivelled grain; while those which had stooled less and had looked less promising, generally yielded better grain and a greater weight of it. It looked, in fact, as if the varieties which had stooled largely had undertaken too much, and that having expended their strength in seeking preparation for a large crop, had failed to produce more than a small amount of grain, and that of little value; and that, when we come to consider the matter, is what we might reasonably expect to occur" (1898).

Although the number of tillers produced by a wheat plant can vary widely depending on the genetics and environmental conditions, under field conditions the number of fertile spikes per plant is usually much lower than the number of tillers produced (Puckridge and Donald, 1967; Ishag and Taha, 1974; Fischer *et al.* 1976; Innes *et al.* 1981; Whan *et al.* 1988; Davidson and Chevalier, 1990; El Alaoui *et al.* 1992; Yunusa and Sedgley, 1992; Sharma, 1994; van Sanford and Utomo, 1995). A lower plant density increases both the number of tillers and fertile spikes produced by a plant (Kasperbauer and Karlem, 1986) allowing plants to compensate for gaps in the crop.

While there is usually an optimum fertile spike density, a wide range of fertile spike densities will produce similar yields (Saini and Nanda, 1979) due to the plants ability to adjust yield components and reduce the number of kernels per spike at high densities (Puckridge and Donald, 1967; Holliday and Willey, 1969). At low plant densities however, the plants are unable to produce sufficient number of fertile spikes to compensate and yield is reduced (Puckridge and Donald, 1967; Holliday and Willey, 1969; Saini and Nanda, 1979). Likewise, if the number of fertile spikes is too high, the rate at which moisture is used by the crop is increased leading to a reduction in grain yield (Pelton, 1969; Kirby, 1970). This has serious implications for crops grown under water limited environments and, in particular, areas suffering from

terminal drought conditions as exhaustion of soil moisture can reduce both the kernel number and weight (Fischer and Maurer, 1978).

1.2 THE AUSTRALIAN PERSPECTIVE

The southern Australian wheat belt is situated in the south east, south central and south west corners of the continent and usually receives between 250 to 650mm of rainfall (Nix, 1987). It is a winter dominant rainfall area with 150 to 400mm of rain falling between May and October. Significant contributions to soil moisture from summer (November - April) rainfall are restricted to the northern section of the eastern wheat belt. Crops are sown between mid April and late July and harvested from late September through until early January. As a consequence, grain filling often occurs in situations of decreasing soil moisture availability (Nix, 1987). The need to develop strategies aimed at preserving soil moisture for the grain filling period can therefore not be understated. Reduced tillering lines were therefore proposed to be beneficial in water limited environments (Innes *et al.* 1981; Islam and Sedgley, 1981) as a crop of plants with restricted tillering should have a reduced leaf area prior to anthesis, thus lowering pre-anthesis transpiration and increasing the amount of soil moisture available to the plant during grain filling (Richards and Townley-Smith, 1987; Yunusa and Sedgley, 1992). Islam and Sedgley (1981) found that crops consisting of plants which had tillers removed yielded significantly more than freely tillering crops in a Mediterranean environment where yields were 2 t ha^{-1} or less and spike densities were less than 300m^{-2}.

Studies involving tiller removal (Kirby and Jones, 1977; Mohamed and Marshall, 1979; Kemp and Whingwiri, 1980; Islam and Sedgley, 1981; Gu and Marshall, 1988) and selection of lines with reduced tillering capacity (Innes *et al.* 1981) indicated that unproductive tillers compete with productive tillers and thereby limit grain yield. Several authors proposed that sterile tillers might be parasitic to the whole plant for nutrients (Engledow and Wadham, 1924; Smith, 1933; Sims, 1963). van Herwaarden *et al.* (1998c) suggested that the canopies of reduced tillering wheat crops would have improved light penetration which could result in an increase in the storage of water soluble carbohydrates in the stems which could inturn be utilised during grain filling. However, freely tillering varieties have the ability to compensate

for sub-optimal plant density when crop establishment is poor by producing more spikes plant^{-1} (Benbelkacem *et al.* 1984). Some authors have also reported benefits associated with tillers that fail to produce a head, claiming that they are able to translocate their assimilate and nutrients to productive tillers (Tincker and Jones, 1931; Palfi and Daszi, 1960; Bremner, 1969; Lauer and Simmons, 1988) or through their ability to prevent lodging (Rasmusson, 1987). However, Rawson and Donald (1969) claimed that the nutrient content of unproductive tillers was small (e.g. nitrogen was 3% of unproductive tiller biomass) and regardless of their ability to translocate nutrients to productive tillers, unproductive tillers were a total loss in their wastage of soil water.

1.3 ASSIMILATE SUPPLY AND ITS EFFECT ON TILLERING

High light intensities have been shown by many authors to be associated with high rates of tillering in ryegrass (Mitchell, 1953b), wheat (Khalil, 1956), oats (Wiggans, 1959) and barley (Aspinall and Paleg, 1963). Photoperiod however has a somewhat more confusing effect on tillering as some plants require minimum daylengths before they reach floral initiation (Evans *et al.* 1964). Temperature also has a varied effect on tillering with the optimum temperature for tillering varying with different light intensities (Friend, 1965). It is also confounded by vernalisation requirement, with varieties requiring vernalisation (winter lines) tillering more profusely than those that do not (spring lines) due to their longer vegetative phase (Bell and Kirby, 1966). As long as the requirements for floral initiation are met, increasing the temperature causes the plant to develop faster which means that plants invest more assimilate into growth of the mainstems and its leaves at the expense of tiller production. As a consequence the plants mature sooner (Rawson, 1988). The most important environmental stimulus for tillering appears to be the combination of light and temperature into a term known as photothermal quotient (PTQ). PTQ is a measure of the amount of radiation received by the plant per unit of temperature (Nix, 1976). A high PTQ is associated with high light intensity and/or long daylength and low temperatures. These conditions are favourable to photosynthesis and allow the plants to direct extra carbohydrates towards increasing tiller production. Rawson (1987) claimed that provided water and nutrients are non limiting, temperature had a

minimal effect on photosynthesis per unit leaf area. Carbohydrate production is therefore directly proportional to hours and intensity of sunlight as well as the leaf area present per unit of ground area (Rawson, 1987). Mitchell (1953b) claimed that increasing the light intensity increased the number of tillers produced due to the increase in photosynthesis while Jewiss (1972) claimed that this lead to an increase in carbohydrates in the plant that promoted tillering. Mitchell (1953b) also found that while low temperatures retarded the rate of tillering, plant development was retarded to an even greater extent, resulting in the number of tillers per plant at maturity being greater under low temperatures.

Aspinall (1961) claimed that the intra-plant competition and the ability of the tiller buds to withstand the associated nutrient stress controlled tillering. The application of nitrogen (Watson, 1936; Gardner, 1942; Aspinall, 1961; Barley and Naidu, 1964; Black, 1970; Power and Alessi, 1978, Garcia del Moral *et al.* 1984), phosphorous (Gardner, 1942; van Dillewijn, 1952) and magnesium (Gardner, 1942) have all been shown to increase tillering, although boron deficiency has also been shown to increase tillering (Warington, 1933). Many authors (Aspinall, 1961; Bremner, 1969; Cannell, 1969; Ishag and Taha, 1974; Power and Alessi, 1978) have demonstrated that the application of nitrogen greatly enhanced the chances of survival of later formed tillers, although under water limited conditions this does not necessarily result in an increase in fertile spikes per unit area at maturity (Fischer and Kohn, 1966b).

1.4 HORMONAL REGULATION OF TILLERING

Several hormones have been identified as having an effect on tillering, of which auxins, cytokinins and gibberellic acid are thought to act through regulation of nutrient supply. Auxins have been known for sometime to have an inhibitory effect on tiller bud development (Leopold, 1949). Auxins are the hormones associated with apical dominance, the control exerted by the apex over development of lateral buds (Cline, 1991). Produced in the apex, auxins suppress the growth of tiller buds. Working with common bean, Lim and Tamas (1989) found that auxins played a role in auxiliary bud regulation. The method by which this regulation occurred was

unresolved, but it appeared to be either regulation of the nutrient supply or through the concentration of other growth substances.

Cytokinins are hormones produced primarily in the roots of young plants and migrate to tiller buds where they have been shown to promote tillering (Langer *et al.* 1973; Johnston and Jeffcoat, 1977). Miller *et al.* (1955) identified cytokinins as being essential in cell development and growth, while Langer *et al.* (1973) suggested that they may increase the flow of assimilate to the tiller buds. Jewiss (1972) suggested that auxins and cytokinins were both important in controlling tillering and that it was in fact the ratio of these two hormones that regulated tillering.

Gibberellic acid (GA) is known to be involved in stem elongation in wheats that are sensitive to it. It has been shown to restrict tillering (Jewiss, 1972), although any reduction in tillering associated with GA is probably due to the elongating stems successfully competing with tiller buds for assimilate as GA insensitive lines. Semi-dwarf lines containing *Rht D1B (*aka *Rht2)* have been shown to tiller more profusely than those that are GA sensitive (Allan, 1989; Borrell *et al.* 1993).

1.5 THE ISOLATED PLANT

The rate of tiller appearance generally follows the Fibonacci series (Friend, 1965) and only deviates from this after double ridge formation (Gomez-MacPherson *et al.* 1998a). Tillering has usually ceased by terminal spikelet (Rawson, 1971; Baker and Gallagher, 1983; Thorne and Wood, 1988; Craufurd and Cartwright, 1989; Gomez-MacPherson *et al.* 1998a), although there have been reports of tillering continuing in wheat until ear emergence (Friend, 1965).

Tiller buds in wheat have been identified in the axil of the first leaf by the time the fifth leaf has started to develop (approximately five days after germination) (Williams *et al.* 1975). Although many buds never emerge from the leaf axil (Williams *et al.* 1975), they continue growing slowly until the mainstem has reached anthesis (Williams and Langer, 1975). Williams and Metcalf (1975) proposed that foliar development reduced the volume into which the tiller buds can expand. This physically constrains the growth of the bud, which must fight to escape and elongate.

Rawson (1971) found that the pattern of tillering was more or less synchronous between a variety of environments. He found that T1 (the tiller emerging

from the axil of the first true leaf) was usually the first to emerge, followed by C (the coleoptile node tiller), then T2, T1C and T3. A similar pattern was reported by Fraser *et al.* (1982). Tiller death by contrast occurs in the reverse order with smaller, later formed tillers which have failed to produce an ear senescing sooner than the earlier formed tillers (Rawson, 1971; Thorne and Wood, 1988).

1.6 THE CANOPY ENVIRONMENT

Both inter and intra plant competition for assimilate can restrict tillering when plants are growing in a canopy. Interplant competition involves the competition for light and nutrients between plants. Fraser *et al.* (1982) noticed a significant reduction in tillering for plants at a higher plant density by the time the fourth or fifth leaf had emerged. While the emergence of tillers may be affected by plant density, it does not alter the formation of tiller buds (Kirby and Faris, 1972).

The quality of light has also been found to be an important factor controlling tillering. A dense crop canopy causes a decrease in the red:far red ratio in light experienced at the base of the plant (Casal *et al.* 1985) as red light is absorbed by the leaves whereas far red is usually reflected (Kasperbauer, 1971; Woolley, 1971). This causes an increase in phytochrome red relative to phytochrome far red (Kasperbauer and Karlen, 1986) and subsequent reduction in the number of tiller buds initiated by the plant (Casal *et al.* 1985). Although the mode of action is not understood, the phytochrome ratio serves as a way for the plant to detect the degree of interplant competition for light (Holmes and Smith, 1975; Kasperbauer and Karlen, 1986).

Competition for nutrients can be observed at inter as well as at intra plant levels. Puckridge and Donald (1967) found that levels of nitrogen decreased across all plant parts as sowing density increased indicating increased competition for limited nutrients. If however nutrients can be supplied as the plants require them, then the number of tillers produced is increased due to reduced competition both between and within plants (Aspinall, 1961).

At an intra-plant level, the canopy is thought to affect assimilate partitioning to various plant organs. Gomez-MacPherson *et al.* (1998b) suggested that after reaching terminal spikelet tiller production stopped and canopy grown plants became source limited due to demands by reproductive organs and stem growth. By growing plants

with and without border rows, Davis and Simmons (1994) altered the competition experienced by plants by manipulating the red:far red ratio. Plants with borders, which experienced lower red:far red ratios, displayed longer leaves and longer stem internode lengths, tended to have longer leaf sheaths and flowered earlier. The authors claimed that this was an adaptive response to shading and increased the likelihood of the plants receiving adequate photosynthetic active radiation (PAR).

Apical dominance also plays a role in the partitioning of assimilate throughout the plant. Cline (1991) claimed that the growth of the mainstem deprives the lateral buds of nutrients and assimilates and thus inhibits their outgrowth. Leeky and Longman (1986) found that increasing the supply of nutrients (nitrogen, phosphorous and potassium) did not affect sprouting in decapitated plants, but it did promote the elongation of those buds that had already started to grow. Tomar (1983) found similar results, but added that auxin and gibberellin were also required for continuation of bud outgrowth.

1.7 GENETIC REGULATION OF TILLERING

Tillering usually continues until terminal spikelet formation in wheat, so genes that alter the time from sowing to terminal spikelet (i.e. photoperiod (ppd) and vernalisation (vrn) genes) must have an effect on tillering. Photoperiod genes respond to increasing daylengths by reducing the time until ear emergence (Scarth *et al.* 1985). While they do not affect the time from germination until floral initiation, they can extend the time from floral initiation until terminal spikelet if their long day requirement is not met (Scarth *et al.* 1985).

Vernalisation genes are often used to keep plants vegetative and subsequently avoid frost damage (Crofts, 1989). Unlike the photoperiod genes however, they delay floral initiation, but not the time from floral initiation until terminal spikelet (Crofts, 1989). Austin *et al.* (1974) found that the longer imbibed winter wheat seed was kept at cold temperatures (2.5°C), the fewer tillers were produced due to the reduction in time taken to reach terminal spikelet. Pinthus (1967) also found that the later initiation of unvernalised winter wheat seedlings, the higher the maximum number of tillers produced. After stem elongation had commenced, intra-plant competition for assimilate was increased, resulting in the death of many tillers to such an extent that

the number of spikes per unit area was similar to the number produced by spring wheats grown under the same conditions.

1.8 THE *tin* AND uc_2 GENES

In 1968, Donald described a wheat ideotype that would make a minimum demand on resources available per unit of dry matter produced. An important and somewhat controversial trait that he included in this ideotype was that the plant should have a single culm. He claimed that "a community of wheat plants with a single culm - that is, with a mainstem and no tillers - will give a greater production per unit area than is given by a variety which tillers freely or even sparsely".

Around the same time, Reid and Wiebe (1968) reported the existence of a uniculm barley. The result of a thermal neutron induced mutation, it was found to be caused by a single recessive gene called *uc2* which was located on chromosome 6 (Haus *et al.* 1971). A uniculm wheat line named 492 was later reported (Atsmon and Jacobs, 1977) which displayed "gigas" features i.e. large spike, thicker leaves and stems. In addition to the reduced tillering ability, it displayed other physiological characteristics proposed by Donald as being desirable i.e. erect ear and awns. Another trait demonstrated by line 492 was the phenomenon termed "stunting". Stunted plants were described as not developing beyond the 3 to 5 leaf stage and dying out with tillering or the mainstem elongating (Atsmon and Jacobs, 1977)

Using monosomic lines of Chinese Spring wheat, Christopher *et al.* (1985) identified what they believed were two major recessive genes on chromosomes 4B and 5B that were responsible for the stunting phenomenon in addition to several other minor genes associated with the condition. However, Richards (1988) found that a single major recessive gene controlled the inhibition of tillering in line 492, the locus for which was designated *tin* (tiller inhibition). He demonstrated that the tiller inhibition trait was closely linked to the loci for hairy glumes, which is found on the distal end of chromosome 1AS. Whereas Inbal and Atsmon (1983) claimed that stunting was a new form of dwarfism in wheat, Richards (1988) claimed that the stunting was an extreme manifestation of the tiller inhibition trait and that due to the linkage to the loci for hairy glumes, those genes on the chromosomes 4B and 5B could not be responsible for the stunting phenomenon.

1.9 THE PHYSIOLOGICAL EFECTS OF THE *tin* GENE AND STUNTING

The gigas characteristics of line 492 were thought to occur due to the plant's ability to utilise savings associated with the reduced tillering i.e. partition assimilate towards productive tillers (Atsmon *et al.* 1986a). Richards (1988) found that the mean kernel number of the mainstem of the plants containing the *tin* gene was almost twice that of their commercial wheat parents, and that kernel weight was also significantly greater in some instances, a result that was repeated in field studies (Yunusa and Sedgley, 1992). The thicker leaves of plants containing the *tin* gene may also be more transpiration efficient as specific leaf weight (SLW) is positively correlated with CO_2 exchange rate (Bhagsari and Brown, 1986; although see Rawson *et al.* 1987). The thicker stems may also act as storage organs for assimilate that can later be remobilised to the grain (Austin *et al.* 1977).

The stunting phenomenon was found to occur early in the development of line 492 and to be influenced by photoperiod and temperature (Atsmon and Jacobs, 1977). The combination of long days (16hrs) and cool nights (10°C) was found to induce 100% stunting in line 492 (Inbal and Atsmon, 1983). The apices of stunted plants were also found to be distorted and failed to elongate.

By transferring the plants between high and low temperature regimes under long day conditions, it was discovered that the period just prior to double ridge formation was the critical time for stunting (Atsmon *et al.* 1986b). Conditions before and after this stage had no effect. An oligoculm line named 380, developed from the same parents as line 492, was also observed to stunt when grown under long days and low temperatures, although not with the same frequency. Tillers of both lines were observed to stunt, even when the mainstem elongated. The total number of tillers, stunted or normal, produced when the plants were grown under low temperatures was however significantly less than the total number of tillers produced at high temperatures, leading to the conclusion that some tillers may be inhibited and their growth suppressed before they become visible. The authors proposed that a stunted plant may be the extreme expression of tiller suppression and that line 492 was simply more liable to this compared to line 380. They did however note the

paradox that as the critical time for stunting was immediately prior to when the mainstem apex reached double ridge, then the tiller buds would have normally emerged from the subtending leaf sheath before they were inhibited. However the tillers of stunted plants failed to develop beyond the bud stage. The *uc2* gene has never been reported to cause stunting in barley and tends to be more extreme than the *tin* gene with tillers other than the mainstem rarely observed. It seems unlikely then, due to their chromosomal locations and physiological effects, that they are equivalent genes.

1.10 CONCLUSION

The importance of correct spike density has long been recognised, particularly in areas when soil moisture becomes limiting towards then end of the season. Reduced tillering wheats have been proposed as being of benefit in these areas as they should have a reduced leaf area prior to anthesis that should lower pre-anthesis transpiration. This in turn should increase the amount of soil moisture available to the plant during grain filling. Several years ago, reduced tillering lines of wheat were developed and this trait was found to be under the control of a single recessive gene designated *tin*. This gene may have considerable agronomic potential in dry areas due to its ability to manipulate tiller numbers.

1.11 OBJECTIVES

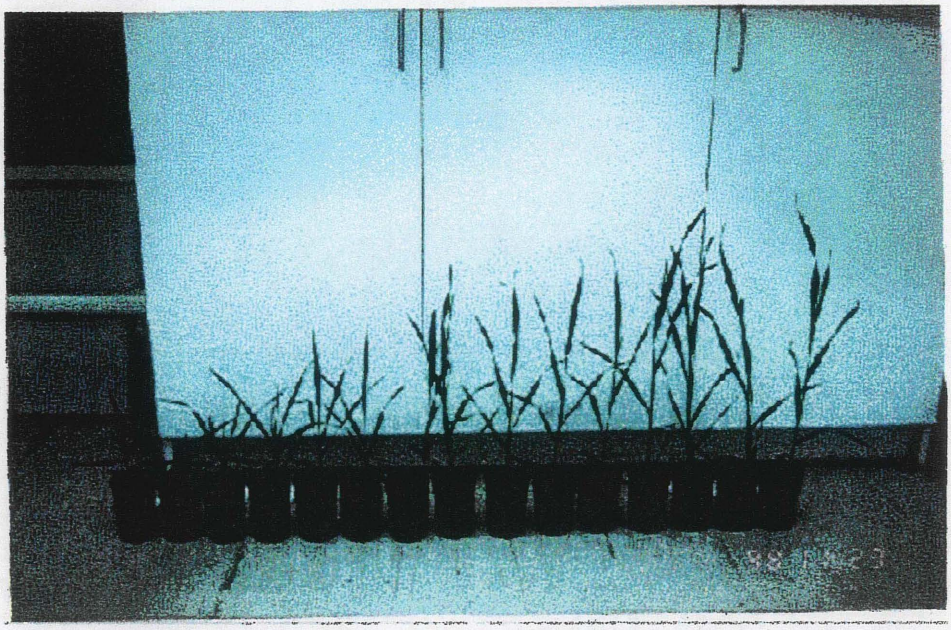
This study was undertaken to evaluate the *tin* gene in environments where it has been hypothesised it may increase yield. If it proves to be advantageous, lines that are near-isogenic with current cultivars can then be made available to Australian wheat breeders who can incorporate these lines into their breeding programs and then commercially release new varieties containing the *tin* gene to farmers.

An understanding of how the *tin* gene functions is also required to appreciate what functions and processes it affects. One of the most obvious is the process associated with the stunting phenomenon. Cultivars of wheat containing the *tin* gene could not be released to farmers if they stunted when grown under current management practices so the environmental cues that induce stunting need to be fully investigated and understood. The gigas traits associated with the *tin* gene also need to

be understood to determine whether they truly are advantageous and whether there are any deleterious effects which may in some way be overcome through plant breeding and/or management. Finally, an understanding of the effect of the *tin* gene on yield components and factors associated with yield (i.e. maturity biomass and harvest index values) are required to determine if these traits provide new methods for increasing grain yield or if they are in some way simply altered at the expense of one another (e.g. kernel size is increased at the expense of kernel set with no net effect on yield).

CHAPTER 2

THE EFFECT OF TEMPERATURE, DAYLENGTH, LIGHT INTENSITY AND CO_2 ON THE EXPRESSION OF THE *tin* GENE



Line 492 displaying the range in phenotype from stunted (left) to normal (right)

2.1 INTRODUCTION

Environmental conditions have long been known to affect the morphology and development of plants. Mitchell (1953a) found that increasing the light intensity increased tillering in ryegrass, while Friend (1965) reported that wheat plants produced the most spikes at maturity when grown at low temperatures and high light intensities. When grown at low temperatures during their vegetative phase, ryegrass (Mitchell, 1953b) and wheat (Rawson, 1971) plants were also found to produce more tillers than those grown at high temperatures for a given photoperiod. Wheat lines containing the *tin* gene, a gene that reduces the number of tiller produced by a wheat plant (Richards, 1988), are also affected by light and temperature, although quite differently compared to what normally occurs in cereals. Plants containing the *tin* gene may "stunt", a condition where the stems fail to elongate and the plant eventually dies after exposure to long days and low temperatures prior to double ridge formation (Atsmon and Jacobs, 1977; Atsmon *et al.* 1986b). Stunting was claimed to be an extreme manifestation of the reduction in tillering by Atsmon *et al.* (1986b).

If lines containing the *tin* gene are to be released commercially, its expression under various environmental conditions needs to be fully understood. In the Australian wheatbelt, daylengths are typically shorter and temperatures higher (Nix, 1987) than those found to be most conducive to stunting in line 492 by Atsmon *et al.* (1986b). However, stunting has been reported under short days and relatively high temperatures (Atsmon *et al.* 1986b) albeit at a lower frequency, and wheat is grown in other areas of the world where daylengths are long and temperatures low during the vegetative phase (Nix, 1987). Knowledge of the *tin* gene's effect on tiller dynamics is also required to determine if and how much commercial sowing rates need to be adjusted to cope with the reduction in tiller number.

The aim of experiments reported in this chapter are to determine what effect the *tin* gene has on tillering when near-isogenic lines differing in the presence of the *tin* gene are grown under different daylengths, temperatures and light intensities. Experiment 1 was designed to investigate the effect of daylength, temperature and light intensity on the expression of the *tin* gene in a variety of genetic backgrounds. Atsmon *et al.* (1986b) only reported that photoperiod and temperature affected the phenotype of line 492 and failed to comment on the effect of light intensity. The

purpose of Experiment 2 was to determine how the *tin* gene would be expressed in a variety of genetic backgrounds when sown outdoors at various times throughout the year. Experiment 3 was designed to determine whether it was the cumulative amount of light energy or the photoperiod that the plant was exposed to that determined whether a plant containing the *tin* gene stunted. Experiment 4 was designed to determine whether it was the minimum or average daily temperature that is critical in determining whether the plant stunts as well as confirming the light intensity effects observed in Experiment 1. Finally, Experiment 5 was planned to demonstrate whether or not it is the availability of assimilate is an important factor in determining whether plants containing the *tin* gene stunt.

2.2 MATERIALS AND METHODS

2.2.1 Experiment 1

Ten plants of the Israeli breeding lines 492 and 380 (sibling lines both containing the *tin* gene) and two spring wheat cultivars (Bodallin and Banks) along with their near-isogenic pairs differing in the presence of the *tin* gene (Bodallin+*tin* and Banks+*tin* respectively) were individually grown in pots (81mm diameter, 146mm deep) containing a 1:1 mixture of perlite and vermiculite. Plants were irrigated daily with full strength Hoagland's nutrient solution (see Table 1 in the Appendix) in the morning and de-ionised water in the late afternoon. Kernels were imbibed at 4°C for 24 hours and sown at a depth of 15mm on June 1, 1995 (from here on referred to as winter sowing) and January 22, 1996 (from here on referred to as summer sowing). The experiment took place in the CERES Phytotron in Canberra. Long day treatments of 16 hours were provided by extending the natural light as necessary into the evening with incandescent light (~0.9mol m^{-2} day^{-1} Photosynthetically Active Radiation (PAR)) while short days were provided in cabinets which closed automatically after experiencing 12 hours of daylight (08:00 to 20:00). For the winter experiment and part of the summer experiment, daylength was extended to 20:00 by the same incandescent lights that were used for the 16 hour treatment. The plants experienced natural variations in light quality and intensity, but the average natural daily PAR level inside the glasshouse for the winter sowing was 11.3mol m^{-2} day^{-1} while in the summer it was 32.9 mol m^{-2} day^{-1}. Low (17/9°C

day/night) or high (26/18°C day/night) temperatures were provided for both daylength regimes. The number of tillers and the incidence of stunting were determined from populations of 10 plants from each line. The presence of tillers was recorded every second day. The experiment was terminated after 49 days for the winter experiment (637 degree-days at low temperature and 1078 degree-days at high temperature) and 60 days (780 degree-days for the low temperature treatment and 1320 for the high temperature treatment) for the summer experiment by which time tillering had ceased for all lines under all conditions. Additional wheat plants were grown with these plants and dissected every second day to determine when double-ridge formation occurred in order to determine whether time to double ridge formation was related to the expression of the *tin* gene.

2.2.2 Experiment 2

Three repetitions of the spring wheat breeding lines 492 and 380 and two spring wheat cultivars (Bodallin and Banks) along with their near-isogenic pairs differing in the presence of the *tin* gene (Bodallin+*tin* and Banks+*tin* respectively) were grown three to a pot in cylindrical 200mm diameter by 200mm deep containing a soil:compost mix. Plants were grown outdoors in Canberra, ACT, Australia (149°06'E, 35°19'S, Alt 600m). Kernels were imbibed for 24 hours at 4°C before being sown at a depth of 15mm. Tiller numbers were recorded every second day until tillering had ceased. Ten kernels of each line were also sown under the same conditions and plants were dissected every second day to determine the date of double-ridge formation. Sowing took place on the following dates: August 18, 1995; September 27, 1995; November 2, 1995; December 21, 1995; February 9, 1996; March 18, 1996; April 23, 1996; May 30, 1996; July 8, 1996. Monthly photoperiods and average daily temperatures for this period in Canberra are given in Fig 2.13 while average monthly temperatures (maximum and minimum) are given in Fig 1 of the Appendix.

2.2.3 Experiment 3

Kernels of the spring wheat breeding lines 492 and 380 were imbibed for 24 hours at 4°C, then sown 15mm deep with two kernels per cylindrical PVC pot (81mm

diameter, 146mm deep) containing a complete nutrient potting mix before being placed in a Conviron CMP 2023 growth cabinet. Shortly after emergence plants were thinned to one plant per pot. Fifteen plants of each line were exposed to 16 hour (12 hours at 320μmol quanta m^{-2} s^{-1} immediately followed by 4 hours at 100μmol quanta m^{-2} s^{-1}) photoperiods while a further 15 pots of each line were exposed to 12 hour (at 320μmol quanta m^{-2} s^{-1}) photoperiods. Temperatures measured a constant 17°C and 9°C at the high and low light intensity periods respectively.

Forty two days after sowing (leaf 4 and 5 extending for the short and long day treatments respectively), plants were classified as normal, partly stunted or stunted, mainstem leaf lengths measured and the stage of development and number of spikelet primordia on the mainstem apex recorded.

2.2.4 Experiment 4

Kernels of line 492 were imbibed for 24 hours at 4°C then sown 15mm deep at two kernels per PVC pot (81mm diameter, 146 mm deep) containing a 1:1 mixture of perlite and vermiculite. Shortly after emergence plants were thinned to one plant per pot. Pots were then placed in CERES Phytotron growth cabinets at various combinations of temperature (30/8°C, 26/12°C or 19°C constant) and light intensity (350 or 700μmol quanta m^{-2} s^{-1} using mercury lamps), but all at a constant 16 hour photoperiod. There were fifty pots per treatment. Plants were irrigated daily with Hoagland's solution at the beginning of the light period and with de-ionised water near the end of the light period. Nineteen days after sowing (when mainstem leaf 3 was fully extended), plants were classified as normal, partially stunted and stunted and the length of mainstem leaves 1, 2 and 3 recorded.

2.2.5 Experiment 5

Kernels of the spring wheat breeding lines 492 and 380 were imbibed and sown in the same way as described in Experiment 4. On December 11 1998, kernels were sown and the pots placed in open glasshouses in the CERES Phytotron in Canberra at 21/16°C with natural photoperiod extended to 16 hours with incandescent light (~0.9 mol m^{-2} day^{-1} PAR) at elevated (700ppm) and ambient (350ppm) levels of

CO_2. The PAR level averaged 27.3mol m^{-2} day^{-1} for the duration of the experiment. Plants were irrigated daily with Hoagland's solution in the morning and de-ionised water in the late afternoon. On January 16 1999 (mainstem leaf 6 extending), plants were rated as normal, partially stunted or stunted and the lengths of mainstem leaves 1 to 5 were recorded.

2.3 RESULTS

2.3.1 Experiment 1

Under most conditions, lines containing the *tin* gene produced fewer tillers than their commercial parent (Table 2.1). For the cultivars, high light intensity levels tended to increase final tiller number. The combination of long days and low temperatures however either reduced the number of tillers produced or induced lethal stunting in lines containing the *tin* gene. Final tiller number was often higher when plants containing the *tin* gene that did not stunt were grown over the higher light intensity summer period.

Table 2.1 Average final number of tillers $plant^{-1}$ (including the mainstem) of non-stunted plants under the various combinations of temperature (17/9°C or 26/18°C) and daylength (12 or 16 hours) grown during winter and summer

	Winter sowing				Summer sowing				
	LD/LT	LD/HT	SD/LT	SD/HT	LD/LT	LD/HT	SD/LT	SD/HT	LSD (0.05)
Line	----------tillers $plant^{-1}$----------								
492	*	2.6	1.7	3.1	*	2.2	1.7	2.6	*1.0*
380	2.0	1.3	2.6	2.2	3.0	3.1	2.1	3.6	*1.3*
Bodallin	4.8	3.4	4.1	3.2	4.9	6.2	6.2	3.9	*1.8*
Bodallin+*tin*	1.1	1.9	2.0	2.2	2.3	2.6	1.8	2.4	*1.1*
Banks	4.4	3.1	4.7	3.8	6.1	9.0	7.9	7.0	*2.5*
Banks+*tin*	*	3.1	2.9	2.2	*	3.5	2.7	3.0	*0.9*
LSD (0.05)	*1.9*	*1.0*	*1.6*	*1.1*	*2.5*	*1.7*	*2.9*	*1.2*	

LD = Long Days (16 hour photoperiod); SD = Short Days (12 hour photoperiod)

HT = High Temperature (26/18°C); LT = Low Temperature (17/9°C)

* = 100% stunted plants

Stunting was most common when plants containing the *tin* gene were exposed to the combination of long days (16 hours) and low temperatures (17/9°C) (Table 2.2). Some lines had a greater tendency to stunt than others. The two reduced tillering lines from the Israeli program, lines 492 and 380, both stunted although 492 did so more frequently than 380. Bodallin+*tin* and Banks+*tin* also stunted although Banks+*tin* did so more often than Bodallin+*tin*.

Table 2.2 Percentage of stunted plants for lines with and without the *tin* gene grown under combinations of photoperiod (12 and 16 hour) and temperature (17/9°C and 26/18°C) during winter and summer

	Winter				Summer			
	LD/LT	LD/HT	SD/LT	SD/HT	LD/LT	LD/HT	SD/LT	SD/HT
Line	----------% stunted plants ----------							
492	100	50	10	40	100	0	30	0
380	10	0	0	0	50	0	10	0
Bodallin	0	0	0	0	0	0	0	0
Bodallin+*tin*	30	0	0	0	60	0	0	0
Banks	0	0	0	0	0	0	0	0
Banks+*tin*	100	0	10	10	100	0	10	0
LSD (0.05) (line)		7						
LSD (0.05) (season)		4						
LSD (0.05) (line*season)		9						

LD = Long Days (16 hour photoperiod); SD = Short Days (12 hour photoperiod)

HT = High Temperature (26/18°C); LT = Low Temperature (17/9°C)

The increase in light intensity over summer caused an increase in the frequency of stunting of plants grown under low temperatures. However no stunting was observed for any line when plants were grown under high temperatures regimes in summer.

The number of degree-days required to reach double-ridge formation did not appear to affect the tendency of a line to stunt (Table 2.3). Bodallin+*tin* and lines 492

and 380 initiated after experiencing a similar number of degree-days under all conditions tested, yet stunting occurred more frequently in line 492. Like line 492, 100% of Banks+*tin* plants stunted when it was grown under long day and low temperature conditions even though it required more degree-days before it reached double-ridge formation. The *tin* gene did not affect the time taken to reach double-ridge formation for either Bodallin or Banks lines for the winter sowing and had an inconsistent effect for the summer sowing.

Table 2.3 Number of degree-days from sowing until double-ridge formation when grown under combinations of photoperiod (12 and 16 hour) and temperature (17/9°C and 26/18°C) during winter and summer

	Winter				Summer			
	LD/LT	LD/HT	SD/LT	SD/HT	LD/LT	LD/HT	SD/LT	SD/HT
Line	----Number of degree-days from sowing to double ridge formation----							
492	NA	NA	NA	NA	*	297	351	440
380	NA	NA	NA	NA	338	330	351	440
Bodallin	364	308	429	462	286	286	338	418
Bodallin+*tin*	364	308	429	462	312	286	351	396
Banks	416	352	533	506	364	352	416	550
Banks+*tin*	*	352	559	550	*	352	416	528
LSD (0.05) (line)		4						
LSD (0.05) (season)		7						
LSD (0.05) (line*season)		6						

LD = Long Days (16 hour photoperiod); SD = Short Days (12 hour photoperiod)

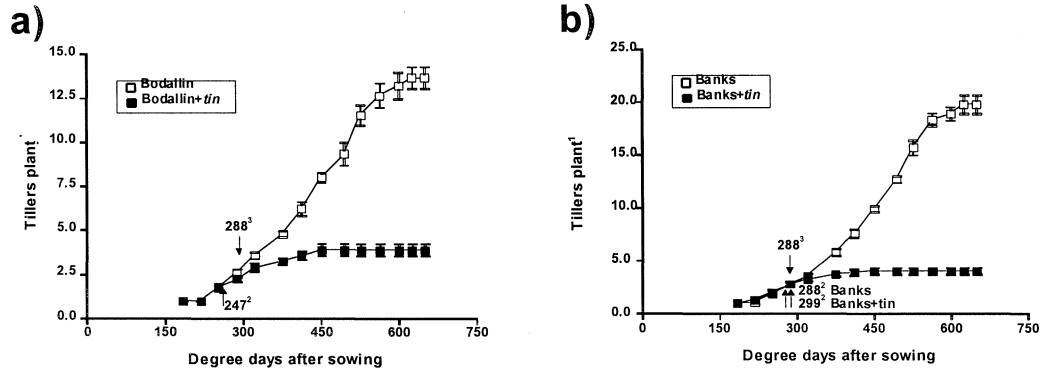
HT = High Temperature (26/18°C); LT = Low Temperature (17/9°C)

NA = Not Available; * = 100% of plants stunted

2.3.2 Experiment 2

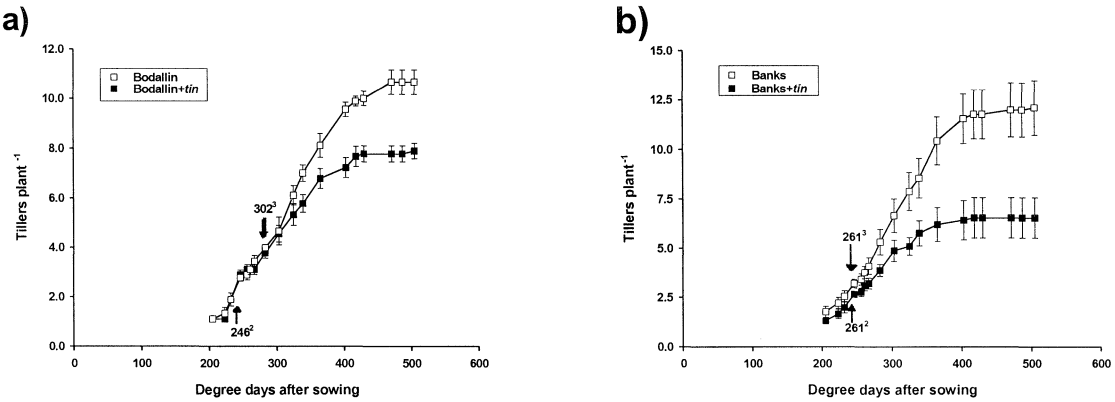
Lines containing the *tin* gene produced fewer tillers than their near-isogenic pair regardless of the sowing date (for examples, see Figs 2.1, 2.2 and 2.3).[1] For both Bodallin and Banks lines, this was the result of an early cessation rather than a reduced rate of tillering.

Figure 2.1 Tiller production of Bodallin±*tin* (a) and Banks±*tin* (b) sown in Canberra on February 9, 1996 (±standard errors)



[2] number of degree-days after sowing when double ridge was recorded
[3] number of degree-days after sowing from when tiller number of the near-isogenic lines became significantly different

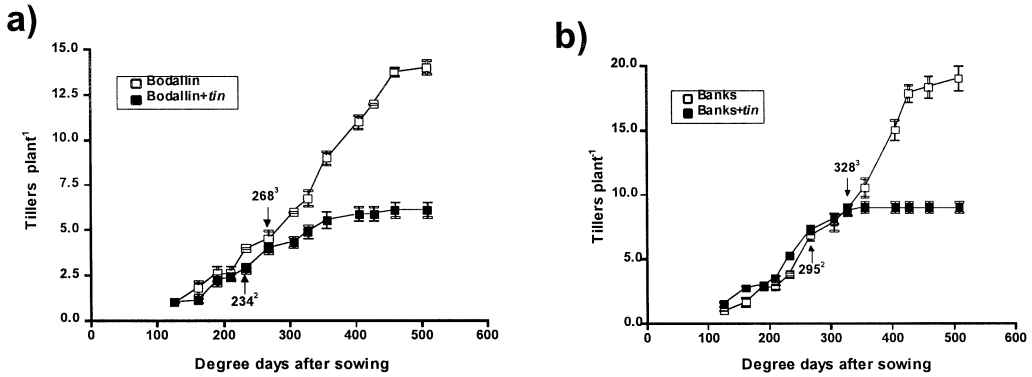
Figure 2.2 Tiller production of Bodallin±*tin* (a) and Banks±*tin* (b) sown in Canberra on May 30, 1996 (±standard errors)



[2] number of degree-days after sowing when double ridge was recorded
[3] number of degree-days after sowing from when tiller number of the near-isogenic lines became significantly different

[1] These sowing dates given as examples because of the difference in environmental conditions;
February - long days and high, but falling, average temperatures and light intensity levels
May - typical sowing date for commercial wheat crops in Australia with cool temperatures and relatively short days and low light intensity levels
September - increasing daylengths, temperatures and light intensity levels, similar to those experienced in high latitude Northern Hemisphere spring wheat growing zones

Figure 2.3 Tiller production of Bodallin±*tin* (a) and Banks±*tin* (b) sown in Canberra on September 27, 1995 (±standard errors)



[2] number of degree-days after sowing when double ridge was recorded
[3] number of degree-days after sowing from when tiller number of the near-isogenic lines became significantly different

The expression of the *tin* gene varied throughout the year. The % reduction in tillering resulting from the presence of the *tin* gene was greatest in late spring, late summer and early autumn in both the Bodallin and Banks lines (Fig 2.4). The incidence of stunting was also greater at this time in line 492, except over summer when it was absent (Fig 2.5). The final tiller number plant^{-1} for Bodallin varied from 6 to 15 (Fig 2.6) while for Bodallin+*tin* it varied from 2.5 to 7 (Fig 2.7). The maximum final tiller number was observed for Bodallin when sowing took place between spring and the beginning of autumn (September to March), with the exception of the December sowing when the rate of tillering was low. For Bodallin+*tin* however, the maximum final tiller number was observed for the sowing dates between May and August, the same period of time when the *tin* gene was least effective in reducing tiller number (Fig 2.4). Rates of tillering on the basis of degree-days were similar within the near-isogenic pairs initially (Fig 2.7), but decreased at or shortly after double-ridge formation for the lines containing the *tin* gene (for examples, see Figs 2.1, 2.2 and 2.3). The rates of tillering tended to be highest over winter when degree-days day^{-1} were lowest (i.e. May to September).

Regardless of when sowing occurred, the number of degree-days from sowing until double-ridge formation was not altered by the presence of the *tin* gene for the Bodallin or Banks lines, even though there was some variation between near-isogenic pairs throughout the year (Fig 2.8). The Bodallin pair usually reached double-ridge

formation after fewer degree-days followed by lines 492 and 380, and then the Banks pair.

Figure 2.4 Percentage reduction in tiller number brought about by the presence of the *tin* gene at various sowing dates throughout the year (±standard errors)

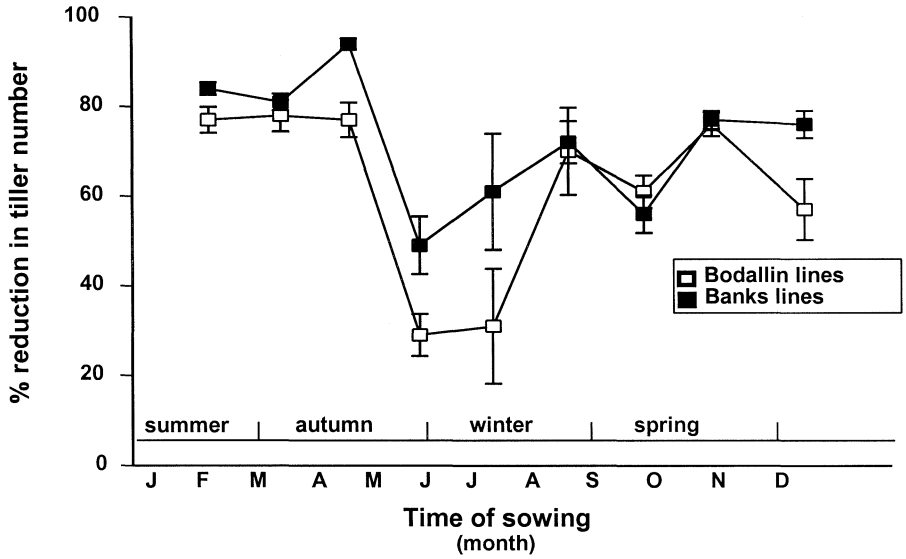


Figure 2.5 Percentage of stunted line 492 plants when sown at various dates throughout the year at Canberra, ACT

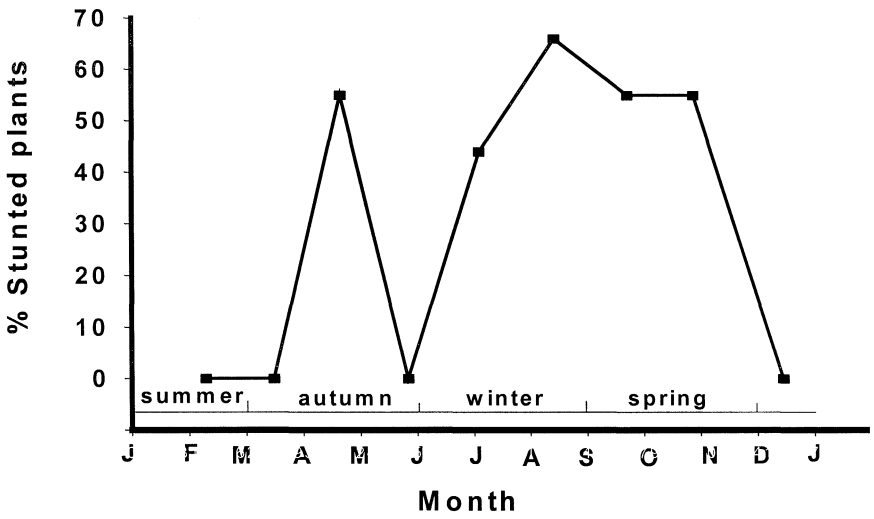


Figure 2.6 Tillering pattern of a) Bodallin and b) Bodallin+*tin* at nine different sowing dates throughout the year

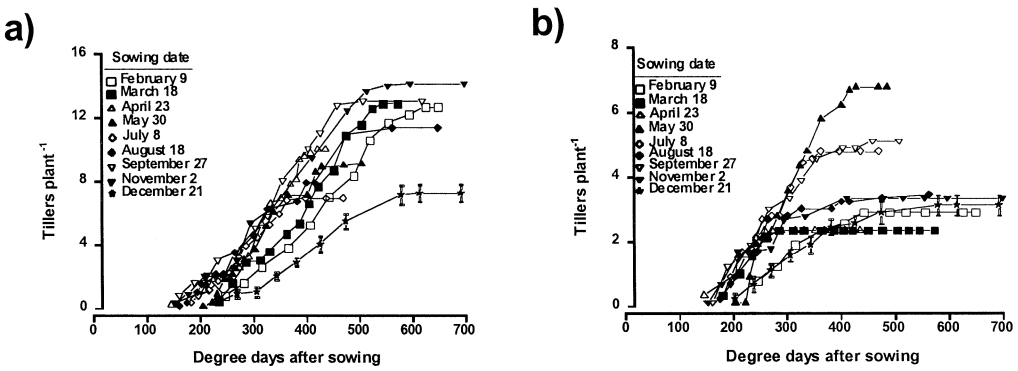
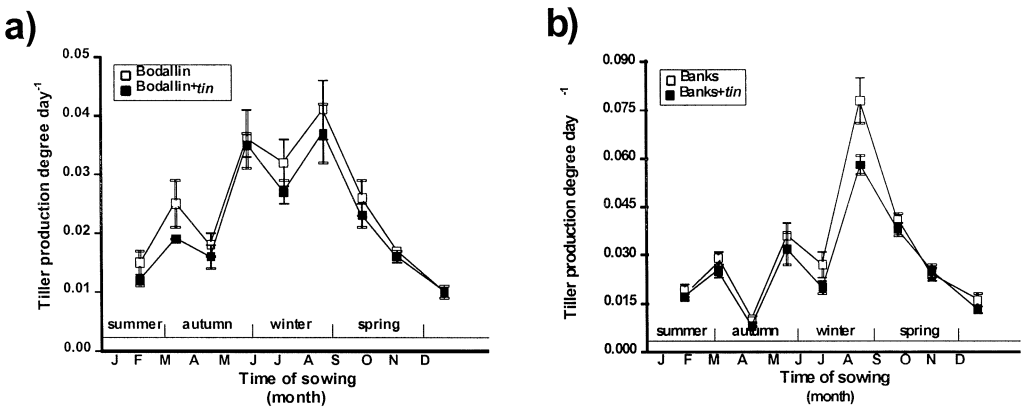


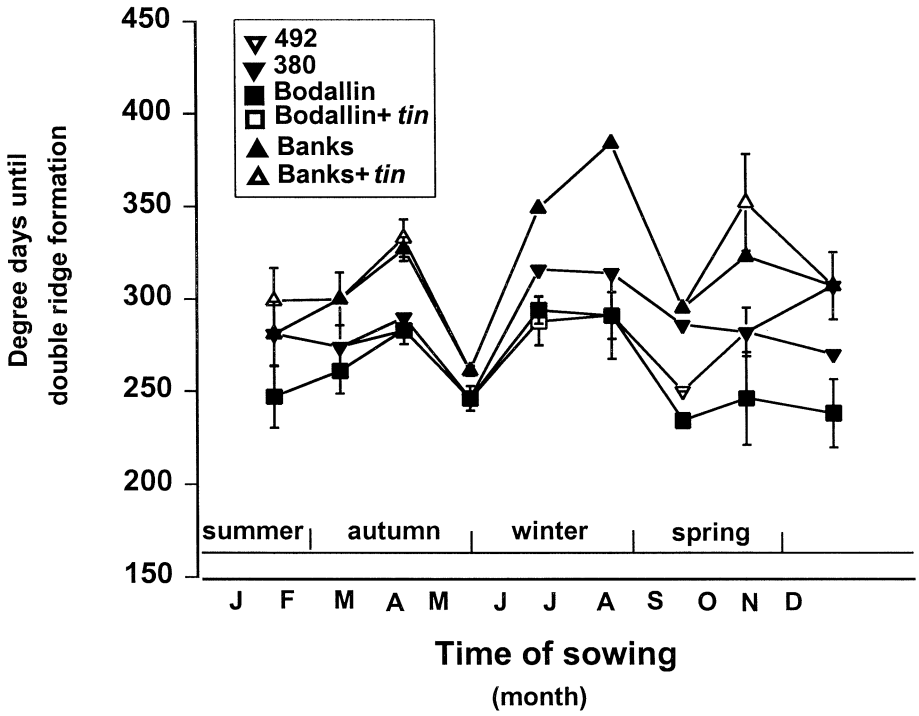
Figure 2.7 Initial tiller production (during the linear phase of tiller production) per degree-day (±standard error) for a) Bodallin±*tin* and b) Banks±*tin* for nine sowing dates throughout the year



As determined in Experiment 1, the conditions most conducive for the induction of stunting in lines containing the *tin* gene were long days and low temperatures prior to double-ridge formation. The incidence of stunting of line 492 was plotted against the average photoperiod and average minimum temperature for the seven days prior to double-ridge formation (Fig 2.9 - colour "contour lines" indicating the percentage stunting). As daylength increased and the average minimum temperature experienced prior to double-ridge formation decreased, the incidence of stunting rose. When the average minimum temperature was replaced with the cumulative number of degree-days (i.e. the average daily temperatures) experienced

for the seven days prior to double-ridge formation, the correlation obtained was lower (data not shown).

Figure 2.8 Number of degree-days from sowing until double-ridge formation for Bodallin±*tin*, Banks±*tin* and lines 492 and 380 sown at nine different sowing dates throughout the year (±maximum/minimum values)

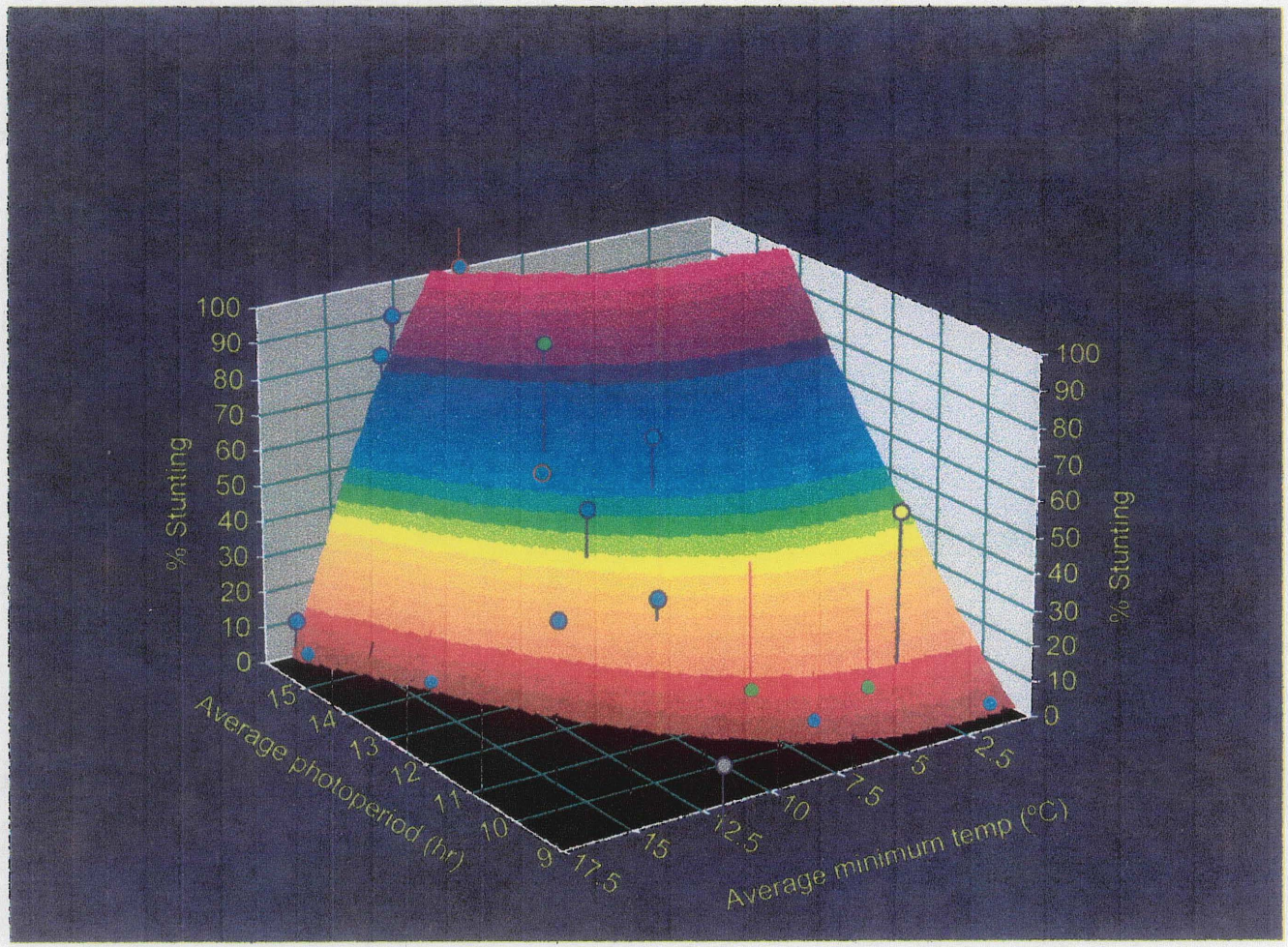


2.3.3 Experiment 3

Line 492 plants displayed a greater tendency to stunt than line 380 plants when grown at 16 hour photoperiods (Table 2.4). However, they did not display as great a tendency to stunt as when line 492 was grown under the natural (and higher intensity) light conditions used in Experiment 1 (Table 2.2). There was no stunting in either line 492 or 380 when grown under 12 hour photoperiods.

The line 492 and 380 plants that were classified as stunted produced fewer spikelet primordia than those plants classified as normal 42 days after sowing (Table 2.5). Under long photoperiod conditions the normal line 492 and 380 plants had passed the terminal spikelet development stage by this time while under short day conditions, glume primordia and lemma primordia were being produced respectively.

Figure 2.9 Percentage of stunted line 492 plants when grown at various combinations of photoperiod (average for the 7 days prior to double-ridge formation) and minimum temperatures (mean for the 7 days prior to double-ridge formation) from experiments 1 and 2, as well as data from Atsmon *et al.* 1986(b) (r^2=0.74)



The apices of stunted plants however varied in their stage of development between early double-ridge formation for the most stunted plants to lemma primordia formation for the least stunted.

Table 2.4 Percentage stunted, partially stunted and normal line 492 and 380 plants grown under conditions of 12 hours (at 320μmol quanta m^{-2} s^{-1}) or 16 hour photoperiod (12 hours at 320μmol quanta m^{-2} s^{-1} followed by 4 hours at 100μmol quanta m^{-2} s^{-1}) at 17/9°C

	Line 492		Line 380	
	12 hour photoperiod	16 hour photoperiod	12 hour photoperiod	16 hour photoperiod
normal	100	40	100	87
partially stunted	-	27	-	7
stunted	-	33	-	7

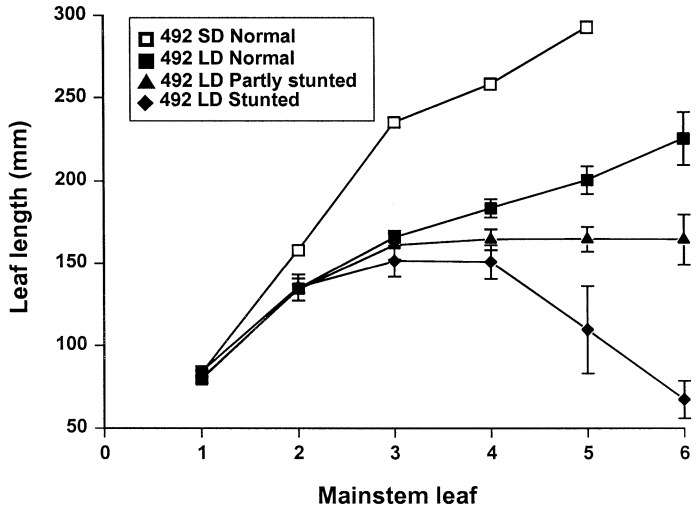
Table 2.5 Number of spikelet primordia (± standard error) on the mainstem apex of stunted, partially stunted and normal line 492 and 380 plants grown under conditions of 12 hours (at 320μmol quanta m^{-2} s^{-1}) or 16 hour photoperiod (12 hours at 320μmol quanta m^{-2} s^{-1} followed by 4 hours at 100μmol quanta m^{-2} s^{-1}) at 17/9°C, 42 days after sowing

	Line 492		Line 380	
	12 hour photoperiod	16 hour photoperiod	12 hour photoperiod	16 hour photoperiod
normal	19.9±0.4	18.5±0.7	21.7±0.3	19.5±0.1
partially stunted	-	17.8±0.6	-	20.0±0.0
stunted	-	12.5±2.0	-	15.0±0.0

The mainstem leaves of stunted plants became significantly shorter from the third leaf compared to the normal plants under the long photoperiod conditions, while

the length of leaves from partially stunted plants remained the same from leaf 3 onwards (Fig 2.10).

Figure 2.10 Mainstem leaf lengths (mm) (± standard errors) of line 492 plants grown under short day (SD) (12 hours at 320μmol quanta m^{-2} s^{-1}) or long days (LD) (12 hours at 320μmol quanta m^{-2} s^{-1} followed by 4 hours at 100μmol quanta m^{-2} s^{-1}) at 17/9°C. Under LD conditions, plants were segregated into normal, partly stunted and stunted phenotypes



2.3.4 Experiment 4

All plants grown at 30/8°C and 26/12°C were rated as stunted when exposed to the higher (700μmol quanta m^{-2} s^{-1}) irradiance levels, while they were rated as normal when exposed to the lower (350μmol quanta m^{-2} s^{-1}) irradiance levels (Table 2.6). Plants grown at a constant 19°C displayed only a slight degree of stunting when exposed to the higher irradiance levels, but failed to stunt when exposed to lower levels.

Table 2.6 Percentage stunted, partially stunted and normal line 492 plants grown under conditions of 16 hour photoperiod at 350μmol quanta m^{-2} s^{-1} or 700μmol quanta m^{-2} s^{-1} at 30/8°C, 26/12°C or 19°C constant

	30/8° 350μmol quanta m-2 s-1	30/8° 700μmol quanta m-2 s-1	26/12° 350μmol quanta m-2 s-1	26/12° 700μmol quanta m-2 s-1	19° 350μmol quanta m-2 s-1	19° 700μmol quanta m-2 s-1
normal	100	-	100	-	100	96
partially stunted	-	-	-	-	-	4
stunted	-	100	-	100	-	-

The lengths of the mainstem leaves were shorter for all plants grown at high light intensities relative to those grown at low light intensities at the same temperature conditions as displayed by stunted plants in Experiment 3 (Fig 2.11, leaf 3 as an example). It is interesting to note that while they were not classified as stunted, the length of mainstem leaf 3 of plants grown at a constant 19°C exposed to a light intensity of 700μmol quanta m^{-2} s^{-1} were shorter than those exposed to 350μmol quanta m^{-2} s^{-1} of light. The length of leaf 3 also tended to fall as the minimum temperature plants were exposed to fell.

At 30/8°C and 26/12°C, increasing the light intensity not only induced stunting, but it also reduced the number of tillers (Fig 2.12). At 19°C however increasing the light intensity increased tillering.

2.3.5 Experiment 5

All line 492 plants grown under both levels of CO_2 were rated as partly stunted while all line 380 plants were rated as normal. However the mainstem leaf lengths (Fig 2.13) of line 492 plants grown at high (700ppm) CO_2 were shorter than those grown at ambient (350ppm) CO_2. Line 380 plants displayed no difference in leaf length at the two levels of CO_2.

Figure 2.11 Mature leaf lengths (mm) (± standard errors) of mainstem leaf 3 of line 492 plants grown under conditions of 16 hour photoperiod with combinations of light intensity at 350μmol quanta m^{-2} s^{-1} or 700μmol quanta m^{-2} s^{-1} and temperatures at 30/8°C, 26/12°C or 19°C constant

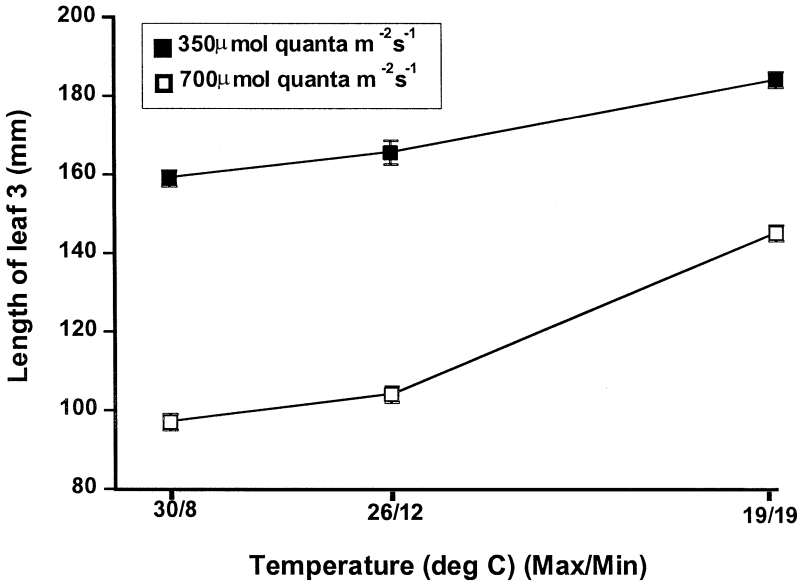


Figure 2.12 Number of tiller $plant^{-1}$ (including mainstem) (± standard errors) for line 492 plants 19 days after sowing when grown under conditions of 16 hour photoperiod with combinations of light intensity at 350μmol quanta m^{-2} s^{-1} or 700μmol quanta m^{-2} s^{-1} and temperatures at 30/8°C, 26/12°C or 19°C constant

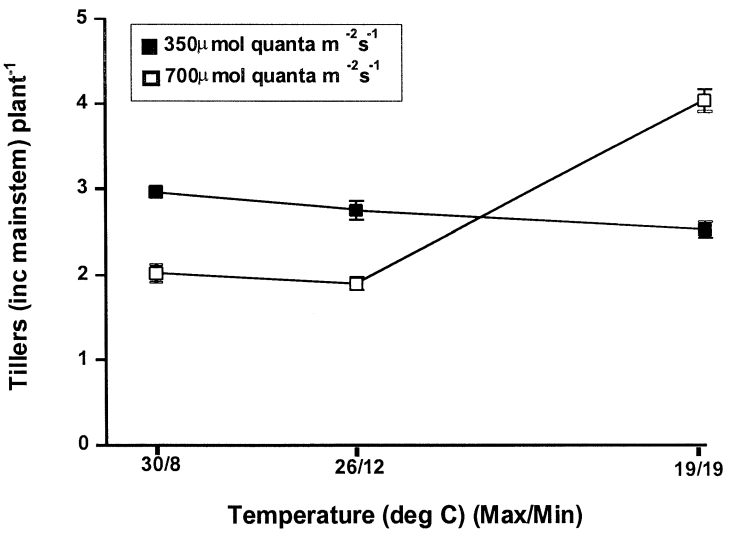
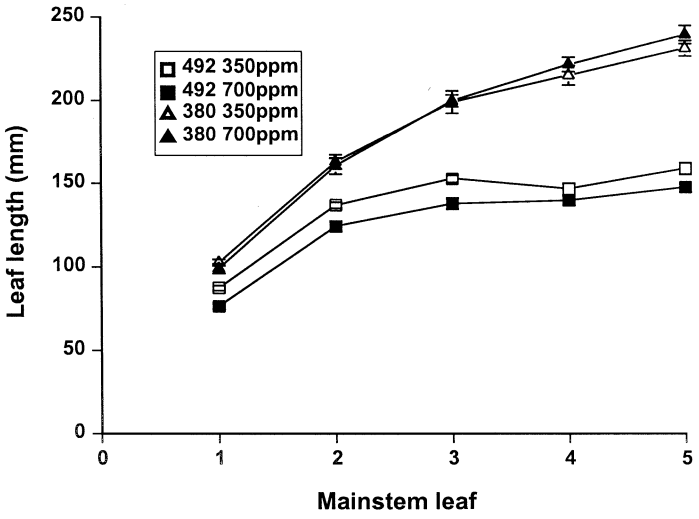


Figure 2.13 Mainstem leaf lengths (mm) (± standard errors) of lines 492 and 380 plants grown in open glasshouses with 16 hour photoperiods at 21/16°C at 350ppm and 700ppm CO_2



2.4 DISCUSSION

2.4.1 Apex development

The *tin* gene did not appear to affect the time taken for spring lines to reach double-ridge formation. The results from Experiment 1 demonstrated that both Bodallin and Banks reached double-ridge formation after experiencing fewer degree-days when exposed to longer days indicating that they have some sensitivity to photoperiod. Regardless of the conditions however, the lines containing the *tin* gene reached double-ridge formation at similar times compared to their commercial near-isogenic pair. Increased light intensity tended to hasten the onset of double-ridge formation in terms of degree-days but equally affected *tin* and non-*tin* lines. However when stunting took place, the apex was retarded at some point between double-ridge formation and terminal spikelet. The apex of the plants which displayed the most extreme expression of stunting in Experiment 3 were at early double-ridge formation at harvest while other plants which were classified as normal when grown under identical conditions had apices which had progressed past terminal spikelet. The

apices of those plants classified as partly stunted fell somewhere between these developmental stages and by harvest had produced significantly fewer spikelet primordia than the normal plants. This would indicate that stunting operates by retarding apex development.

2.4.2 Leaf length

Mainstem leaf length appeared to be good indicators as to the extent of stunting. Although some of the line 492 plants grown under long photoperiods in Experiment 3 were classified as normal, they produced leaves much shorter than those produced by plants grown under short days which were also classified as normal. This effect is unusual, as increasing the photoperiod does not affect leaf length in lines without the *tin* gene (Hotsonyame and Hunt, 1998). These plants may have appeared normal, but were infact under the influence of the same mechanism that caused stunting, albeit to a lesser extent compared to those plants which were classified as stunted.

2.4.3 Tillering

Mitchell (1953b) claimed that the tillers of plants grown at higher temperatures and short days were suppressed, while the same tillers developed when plants were grown under conditions of low temperatures and long days. The author also demonstrated a significant increase in the number of tillers produced with increasing light intensity (Mitchell, 1953a). This was generally the case for both cultivars and *tin* lines in Experiment 2 with the exception of the December sowing when the rate of tillering was unexpectedly low. This is most likely explained by the high average daily temperatures experienced that hastening floral initiation in calendar time. The plants may have been unable to produce and initiate tiller buds at the same rate on a degree-day basis during summer as at other times throughout the year (see Fig 2.7).

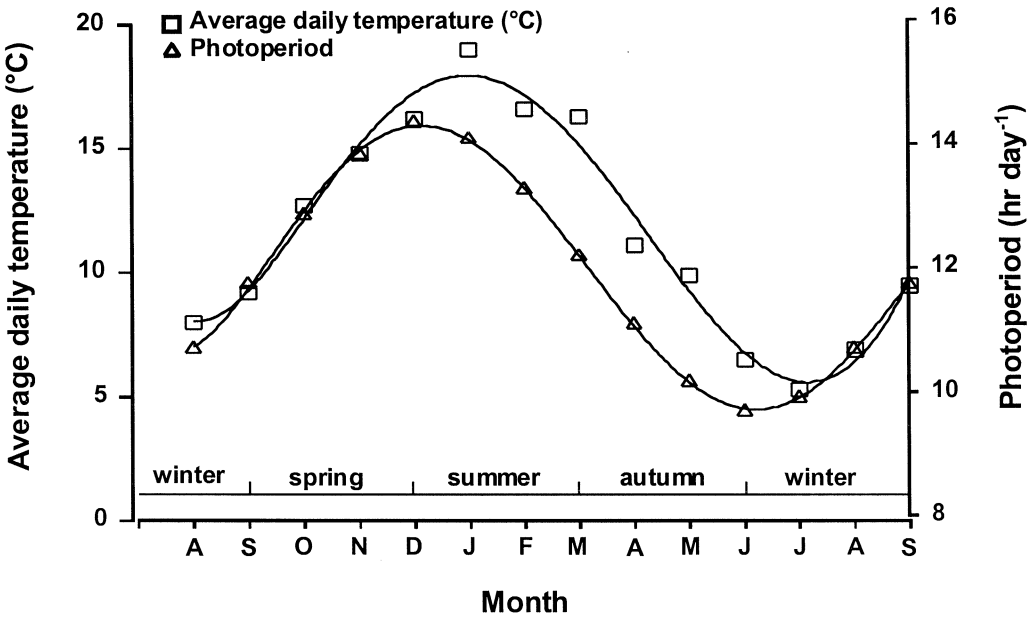
Lines without the *tin* gene produced more tillers as light intensity levels were increased, but there was minimal effect within an intensity level for Experiment 1 when they were grown at various temperatures and daylengths. This may have occurred due to the plants being grown in relatively small pots, because they were

grown in an artificial medium, or because of the sowing density (153 plants m^{-2}). While Mitchell (1953a) and Rawson (1971) noted cultivar differences in terms of tillering response with final tiller number not significantly different for some cultivars when grown at different temperatures when photoperiod was kept constant. Rawson found that one of the wheat cultivars he used, Gabo, produced the same number of tillers under high and low temperatures. This cultivar however is photoperiod responsive and initiates sooner when exposed to long days (Rawson, personal communication). The hypothesis of long days and low temperatures promoting tillering can therefore be confounded by photoperiod and vernalisation requirements of some lines. In Experiment 2, Bodallin and Banks, which respond only slightly to long photoperiods and have no vernalisation requirements, produced the most tillers when average daily temperatures were low relative to photoperiod (i.e. spring) (Fig 2.14). Lines containing the *tin* gene however often produced fewer tillers when grown under long days and low temperatures. This is of course providing that stunting did not occur in which case all tillers, as well as the mainstem, were suppressed. Line 492 was particularly prone to this reversal in expected pattern of tillering with most tillers being produced by plants grown under conditions of short days and high temperatures. Lines containing the *tin* gene also varied in their responses with some producing the most tillers under long days and high temperatures, while others produced the most under short days and low temperatures.

Increasing the light intensity reduced the number of tillers produced by line 492 under temperatures that resulted in stunting i.e. 30/8°C and 26/12°C, in conjunction with 16 hour photoperiods. When the temperature was changed to a constant 19°C however and the incidence of stunting fell and increasing the light intensity increased tillering. It seems then that tillering can be promoted in *tin* lines by an increase in light intensity, provided the other conditions conducive to stunting do not occur. Plants containing the *tin* gene still have an "intrinsic desire" to tiller more profusely as light intensity/photoperiod increases, but this is overridden by tiller suppression brought about by the *tin* gene. If much of this suppression can be overcome, as demonstrated by growing the plants at a constant temperature of 19°C in Experiment 4, *tin* lines will indeed tiller more profusely. This experiment also proves that stunting is the extreme manifestation of tiller inhibition. At the higher light

intensity at a constant temperature of 19°C, no stunting occurred and tillering was greater than at the low light intensity. However when 100% stunting was induced at 26/12°C and 30/8°C at the high light intensity, more tillers were produced than at the low light intensity.

Figure 2.14 Relationship between average daily temperature and photoperiod in Canberra from August 1995 until September 1996



2.4.4 Sensitivity to stunting and assimilate supply

Increasing the irradiance (Experiment 4) increased the incidence of stunting as well as some of the traits associated with stunting (i.e. leaf length). Even at 19°C constant where the incidence of stunting was low at high light levels, leaf length was less than at the low light levels. This experiment also demonstrated that it is the minimum and not the average temperature that impacts on the incidence of stunting and reduction in tillering. Although the average temperature was the same (19°C), the incidence of stunting was higher and the leaf lengths shorter as light intensity increased and minimum temperature fell.

When grown outdoors, tillering in the lines containing the *tin* gene was also suppressed to the greatest extent relative to the commercial parent when sowings took place from spring until the end of summer (September to March) in conditions of long days, but also still low night temperatures and high irradiance levels. Stunting also tended to occur more often in the spring as for a given photoperiod the night temperatures were lower than for late summer and autumn (February to May). While there is obviously some natural symmetry between average daily temperature and minimum temperature making elucidation of which is the important stimulus difficult under natural conditions, spring and summer night temperatures in Canberra appear to be cold enough to induce the mechanism which inhibits the development of tillers associated with the *tin* gene. This occurs despite average daily temperatures being quite high in late spring and summer and results in a reduction in the tillering of the *tin* lines. However, while the night temperatures during summer in Canberra may be low enough to inhibit most of the tillers being produced in lines containing the *tin* gene, they are not low enough to produce the extreme manifestation of the reduction in tillering, i.e. stunting. In June, while minimum temperatures are low, the days are generally too short to induce stunting and the degree of tiller inhibition is not as great as over late spring and early summer. By August and September however the minimum temperatures are still low despite the photoperiod increasing. The Australian wheat crop is sown from mid-autumn to mid-winter (April to July), depending on seasonal conditions and location. Daylengths in the wheat-belt would be similar to those experienced in Canberra at this time while minimum temperatures would typically be higher (Nix, 1987). Given that this period is when the lowest incidence of stunting occurred in Canberra and assuming that lines developed that contain the *tin* gene would be selected to be less likely to stunt (i.e. more like Bodallin+*tin* than Banks+*tin*), then stunting need not be a concern for Australian wheat farmers.

In many ways, the induction of stunting/tiller inhibition is similar to achieving vernalisation requirements in temperate cereals. Trione and Metzger (1970) identified that the optimum temperature for vernalisation was 7°C. As plants were exposed to higher minimum temperatures, the length of time needed to overcome the vernalisation requirement increased. In the same way, plants containing the *tin* gene

which have tendency to stunt are left under conditions which induce stunting for long periods will stunt or increasingly display traits that indicate a tendency towards stunting than those left under less inductive conditions or for shorter periods of time. Increasing the level of CO_2 available to plants containing the *tin* gene increased the severity of the symptoms of stunting in the same way as increasing photoperiod or increasing light intensity (i.e. reducing leaf length). This finding indicates that increasing the ability of the plant to assimilate carbon in unison with low minimum temperatures induces the stunting mechanism. In the same way that Mitchell (1953b) and Rawson (1971) found that tillering was promoted under conditions where assimilate availability was high (i.e. high light intensity and long photoperiod), the *tin* gene's effect also appears to be amplified under conditions which promoted high assimilation rates. Indeed, provided that the minimum temperature is low enough, increasing the assimilate availability appears to reduce tiller number in lines containing the *tin* gene and under extreme circumstances, results in stunting through repression of the mainstem apex and all other tiller apices.

The sensitivity to stunting and the reduction in tillering is almost certainly due to minor genes that do not affect floral initiation. Bodallin+*tin* and lines 492 and 380 all reach double-ridge formation at approximately the same number of degree-days, yet their patterns of tillering and tendency to stunt vary. Banks+*tin* requires more degree-days before it reaches double-ridge formation, yet all plants stunted when they were exposed to the long days and low temperatures conditions used in Experiment 1. It appears as though minor genes increase the sensitivity to stunting and more of these genes appear to be present in line 492 and Banks+*tin* compared to line 380 and Bodallin+*tin*.

2.5 CONCLUSION

The findings presented in this chapter prove that the inhibition of tillers and the stunting phenomenon associated with the *tin* gene are linked and that stunting is an extreme manifestation of tiller inhibition. While exposure to high light intensities and long photoperiods results in increased tillering in spring wheat lines without the *tin* gene, the combination of long photoperiods, low minimum temperatures and high light intensities inhibit tiller buds in spring wheat lines containing the *tin* gene. The

maximum suppression of tillers relative to freely tillering near-isogenic lines also occurs when daylengths are long and minimum temperatures are low. The time taken to reach double-ridge formation does not affect the tendency of a line containing the *tin* gene to stunt although stunting is associated with a retardation in the development of the apex between double ridge and terminal spikelet formation. The stunting/tiller inhibition phenomenon appears to be associated with conditions of low minimum temperature in combination with conditions that increase the ability to assimilate carbon as increasing the photoperiod, light intensity and CO_2 all induce stunting or symptoms associated with stunting.

CHAPTER 3

THE EFFECT OF THE *tin* GENE ON LEAF CELLULAR DEVELOPMENT

Bodallin, Bodallin+*tin*, Morex and Unimorex plants in growth cabinets at RSBS, Australian National University, Canberra

3.1 INTRODUCTION

The presence of the *tin* gene causes major structural changes to the wheat plant, namely a reduction in tiller number and 'gigas' features such as larger ears, an increase in kernel number per ear and thicker stems (Atsmon and Jacobs, 1977; Richards, 1988). One of the other gigas features noted was the larger leaves (Atsmon and Jacobs, 1977; Marshall and Boyd, 1985), however it is not known whether this was due to an increase in cell length or number.

Expanding monocotyledon leaves are useful for studying cellular changes as leaf growth is unidirectional as a result of parallel files of cells produced from meristematic tissue (Sharman, 1942). The growth of leaves in cereals commences with periclinal divisions in cells of the dermatogen and sub-epidermal layers of the apex (Sharman, 1942). These cells form a collar around the axis as cellular division continues laterally. The first few cells elongate and form a small hood over the tip of the apex. Cells at the base of the leaf continue to divide towards the periphery resulting in a widening in the young leaf. When the leaf is third or fourth from the apex it begins to elongate, with the growth originating at the extension zone which is found at the base of the leaf while it is enclosed by the sheath of the subtending leaf (Begg and Wright, 1962).

In cereals, the distance between any leaf cell and the apex from which it originated is a function of its age as well as its developmental stage. Growth is unidirectional in the zones of extension in leaves and thus can be suited to the study of cellular development (Boffey *et al.* 1980; Schnyder and Nelson, 1987, 1988, 1989; Gandar and Hall, 1988; Schnyder *et al.* 1988).

Cells in a grass leaf file are displaced as a result of cell expansion and production at the base of the leaf, both of which occur simultaneously (Green, 1976). The most common approach to the analysis of cellular elongation in the leaves of grasses is to analyse the length of the cells within the growth zone when it is in a steady state of growth (Erickson and Sax, 1956; Green, 1976; Silk *et al.* 1989). The end walls of cells (Volenec and Nelson, 1983; Schnyder *et al.* 1990; Beemster *et al.* 1996) and stomata (Paolillo *et al.* 1991) have been used as anatomical markers for leaves in kinematic

analysis. From this, the rate of cell expansion against time, cell number and distance can be determined.

The gigas traits associated with the *tin* gene are the result of a change in cellular dynamics which may in turn be the result of changes in partitioning brought about through the inhibition of tillers. The aims of the experiments described in this chapter are to determine whether the larger mature leaves of the *tin* wheat and *uc2* (uniculm) barley lines are the result of larger mature leaf cell size or an increase in cell number, as well as to understand the kinematic processes of cell production and elongation in these lines.

3.2 MATERIALS AND METHODS

3.2.1 Growth conditions

Wheat (Bodallin±*tin*) and barley (Morex and Unimorex) near-isogenic pairs differing in the presence of the *tin* and *uc2* genes respectively were grown in cylindrical pots (87mm diameter, 500 mm deep) in a controlled growth chamber with 17/9°C day/night temperatures, 870 μmol quanta m^{-2} s^{-1} irradiance and a 12 hour photoperiod. Kernel weights were calibrated (45-50mg), kernels imbibed for 24 hours at 4°C and sown 15mm deep into a 50/50 fine sand/perlite mix. Plants were watered with Hewitt's nutrient solution (see Table 2 in Appendix) shortly after lights came on and were flushed with tap water shortly before the lights were turned off.

3.2.2 Mature leaf dimensions

The mature leaf lengths of leaves 1 to 6 for wheat and leaves 1 to 7 for barley were determined when leaf 6 or 7 had ceased elongating. The lengths and widths of the leaves were determined, and the leaf area determined using a Licor LI-3000A portable area meter with a LI-3050A/4 transparent belt conveyer. Leaves were then dried at 70°C for 48 hours, weighed and the specific leaf area (SLA) values determined.

3.2.3 Morphometric measurements

The lengths of mainstem leaves 5 and 6 for the wheat lines and 6 and 7 for the barley lines were measured three times a day (onset, middle and end of the light period) as soon as the leaf tip became visible. The leaves of five plants were measured with whole plants harvested when the leaf of interest was elongating at a constant rate. Leaves blades were cut at the base with a razor blade, the length recorded, then cleared by immersing in boiling methanol until chlorophyll was removed and finally stored in lactic acid.

The length of epidermal cells in files on the abaxial surface of the leaf were measured by mounting the cleared leaves onto a light microscope (Zeiss axioscope) fitted with a Panasonic video camera (model WV-CL 702E). A file of cells that was continuous from the base through to mature zone of the blade was selected and cell lengths measured from video images using morphometric program MTV (Garr Updegraff/Datacrunch, 1991).

Each file could be divided into two distinct zones:

a) Zone of symmetrical division. This zone occurs at the base of the leaf where cells divide and give rise to two daughter cells of approximately the same size. This zone, typically 3 to 5mm in length, was identifiable by the new, thin cell walls of the daughter cells indicating recent divisions. The presence of these thin walls was recorded with the final daughter cell marking the end of the zone of symmetrical division (x_{sd}) as described in Beemster *et al.* (1996). Unlike Beemster *et al.* (1996), no trichomes were present on the leaves of the barley or wheat pair used in this experiment so there were no zones of asymmetrical division.

b) Zone of elongation. In this zone, cells only expanded and underwent no further division. The cells increased in length from the proximal to the distal end of the leaf. The end of this zone, x_{el}, occurred when cells reached their final, fully expanded length.

3.2.4 Kinematic analysis

a) Elongation zone

The individual length of successive cells were averaged over 0.5, 1.0 and 2.0 mm intervals in the basal (x_{sd}+2)mm, the next 8mm and the remaining length of the zone for each leaf. Parameters from these measurements were used in a Richards function to generate 0.5mm spaced data over the length of the growth zones of each individual leaf blade and is based on the work of Morris and Silk (1992). The function used to calculate these data that describes cell length distributions along the cell file is:

$$l(x)= l_f/(1+e^{-k(x-x')})^{1/n}$$

where $l(x)$ is the length of a given cell, l_f is the length of the mature cells, x is the location of the cell along the blade and n, k and x' are fitting parameters. Parameter n relates to the inflection point of the curve with a greater value indicating a higher inflection point. Parameter k determines the spread of the curve along the x-axis with lower k values indicating a greater spread (Morris and Silk, 1992). l_f was determined from the average cell length over the distance between x_0+25mm and x_0+35mm (by which time all cells had ceased elongating) where x_0 is the base of the leaf.

To calculate the relationship between time and location in the cell file, the following equations were used;

1) Cell density, $p(x)$, was determined as the inverse of the cell length:

$$p(x)=1/l(x)$$

2) The average cell density $p(x)$ between two locations, x-0.5dx and x+0.5dx was estimated by averaging the local densities calculated at these two locations

3) The number of cells between these points was then determined as $p(x)dx$

4) The flux (F) of cells past any point distal to x_{sd} was calculated using the equation:

$$F=E/l_f$$

where E is the rate of linear elongation rate as determined from measurements made prior to harvest.

5) The cellochron (c), the time for a cell to be displaced by one position is the inverse of the flux (Silk *et al.* 1989) so the time, $t(x)$ taken for a cell to move from x_{sd} to a more distal point, x, is determined from the equation:

$$t(x)=cn(x)$$

where $n(x)$ is the total number of cells between x_{sd} and x.

The relative elongation rate, $r(x)$, in the zone of elongation was calculated from the equation:

$$r(x)=d(\ln l(x))/dt$$

where t is the time related to location x.

N_{el}, the number of cells in the zone of elongation, was determined as the number of cells between x_{sd} and the location where the cell lengths were 95% of l_f as determined by the Richards function equation for each leaf blade. The length of time that a cell resided in the zone of elongation, T_{el}, was calculated as:

$$T_{el}=cN_{el}$$

The contribution of the elongation zone to the total elongation rate of the leaf blade is given by the equation:

$$E_{el}=F(l_f-l_{ad})$$

b) Zone of symmetrical division

The average partitioning rate, p_{sd}, is determined as the rate at which cells in the file are produced relative to the number of cells in the zone of symmetrical division, N_{sd}:

$$p_{sd}=F/N_{sd}$$

while $1/p_{sd}$ is the average cell cycling time (i.e. the time between successive cell divisions), tc:

$$tc=N_{sd}/F$$

The local symmetrical partitioning rate at any location within the division zone, $p_{sd}(x)$, in this study was calculated as the average partitioning rate over the interval of 21 cells around the cell at point x from the equation $T_{el}=cN_{el}$ with n_i substituted for N_{sd} and F corrected for the number of new cell walls in the interval of i cells with the equation:

$$p_{sd}(x,i)=(\varnothing p,i/n_i)*F$$

where $(\varnothing p,i)$ is the proportion of newly formed cell walls:

Local cell elongation rates in the zone of symmetrical division were calculated based on the equations used in Gandar and Rasmussen (1991). The cumulative rate of cell production up to point x is:

$$Y(x)=\int_{x0}^{x} y(s)ds$$

where s indicates the values between x_O and x. As Y is a flux:

$$Y(x)=v(x)/l(x)$$

as calculated by Silk *et al.* (1989). When $x=x_{sd}$, $Y_{sd}=F$, so that:

$$Y(x)/Y(x_{sd}) = (v(x)/l(x))/(1/F)$$

This means then that:

$$Y(x)/Y(x_{sd}) = \left(\int_{xo}^{x} newcellwalls * ds\right)/\left(\int_{xo}^{xsd} newcellwalls * ds\right)$$

which can be written as $\varnothing p,x$. It follows then that:

$$v(x)= \varnothing p,xFl(x)$$

where $l(x)$ is the average cell length of 11 cells around location x. $\varnothing p,x$ increases from 0 at x_0 to 1 at x_{sd}.

Local relative elongation rates were calculated over intervals of 20 cells around x according to Silk *et al.* (1989) as:

$$r(x) = dv/dx$$

where the total length of the 20 cells equals dx and dv is the difference in the velocities calculated at the extremities of those 20 cells. To reduce the noise associated with $r(x)$, running averages of the 20 cells was used. The total elongation generated from within the

zone of symmetrical division was calculated as:

$$E_{sd} = \int_{xo}^{xsd} r(x)dx$$

or put more simply as:

$$E_{sd} = Fl_{sd}$$

where l_{sd} is the cell length at x_{sd}.

Fitting of modified Richards functions were done using Microcal Origin (version 3.5; Microcal Software Inc).

3.3 LIST OF SYMBOLS

c	cellochron, i.e. time interval during which a new cell is added to a cell file in the elongation only zone (h)
E	leaf elongation rate (m s^{-1})
F	number of cells passing a given point of the elongation only zone per unit of time (cells h^{-1})
$l(x)$	local length of cells (μm)
l_f	length of mature cells (μm)
L_{sd}, L_{el}	length of the zones of symmetrical and elongation respectively (mm)
$n(x)$	number of cells along a file between location x_{sd} and a further location x
N_{sd}, N_{el}	number of cells in the zones of symmetrical and elongation respectively
p_{sd}	local relative rate of symmetrical partitioning (cells $cell^{-1}$ h^{-1})
$Øp,i$, $Øp,x$	proportion of fresh transverse walls within an interval, i, of the division zone, and between the base of the leaf and a location x, respectively
r_{max}	maximum local relative rate in the elongation only zone (h^{-1})
$r_{sd}(x)$, $r_{el}(x)$	local relative elongation rates in the zone of symmetrical division and in the elongation only zone respectively (h^{-1})
t_c	average cell cycling time in the zone of symmetrical division (h)
$t(x)$	time taken for a cell to be displaced from x_{sd} to a particular further location x
x	location along the growth zone
x_{sd},x_{el}	location from the base of the leaf (x_o) of the distal end of the zone of symmetrical division and elongation only respectively
$p(x)$	cell density at location x (cells m^{-1})
T_{el}	residence time of a cell in the zone of elongation only
$v(x)$	local velocity of displacement along a cell file (m h^{-1})

3.4 RESULTS

3.4.1 Mature leaf measurements

The leaf area of each mainstem leaf was greater for Bodallin+*tin* and Unimorex than their freely tillering near-isogenic pair where it was recorded (Fig 3.1). The lengths of leaves 1 to 5 were greater for Bodallin+*tin* relative to Bodallin although there was no significant difference between the lengths for leaf 6 (Fig 3.2). For the barley near-isogenic pair, the length of leaves 1 to 7 of Unimorex were greater than those of Morex.

Figure 3.1 Leaf area (±standard errors) of main stem leaves; a) Bodallin and Bodallin+*tin*; b) Morex and Unimorex

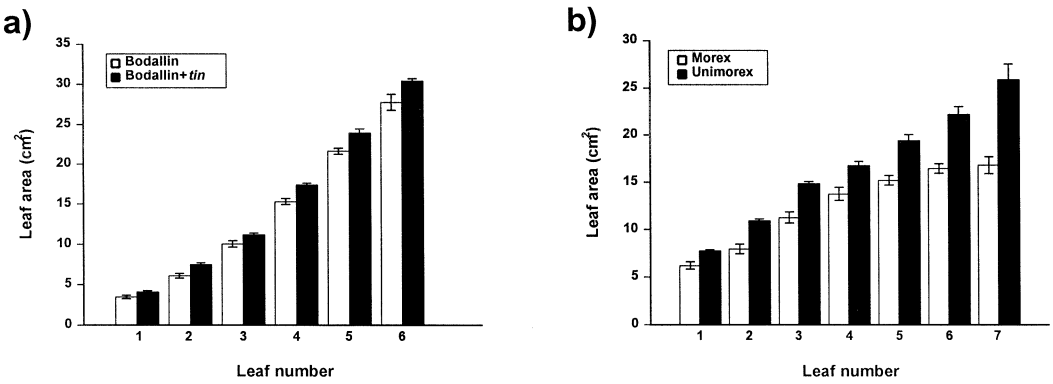
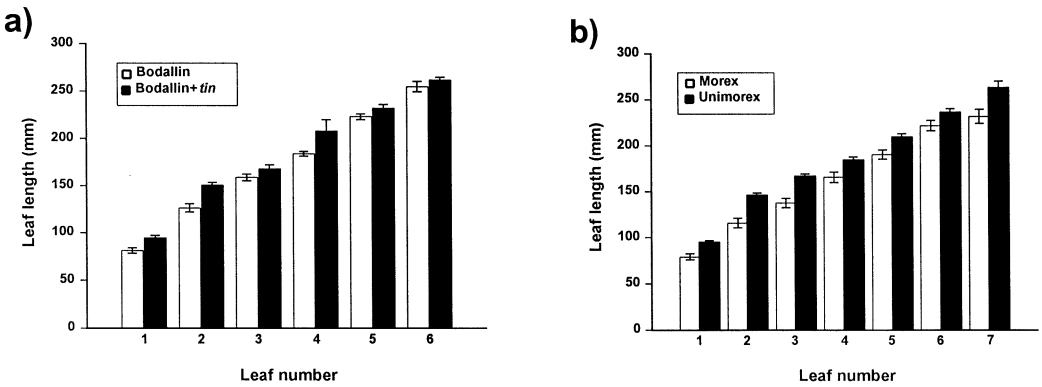


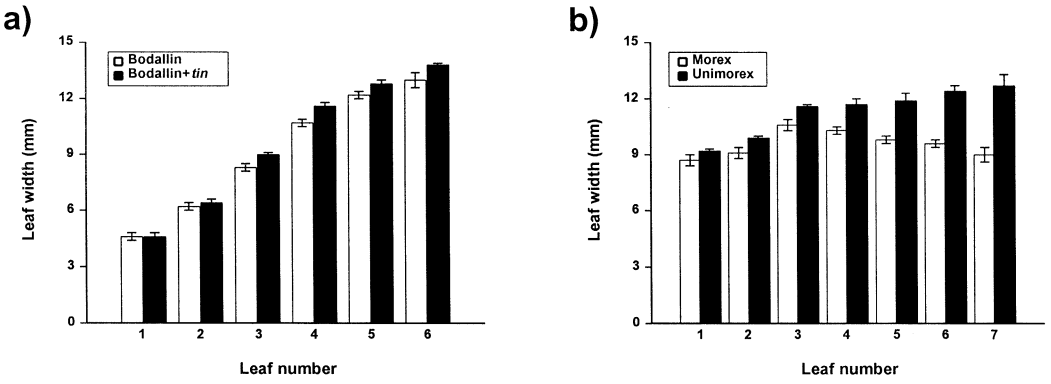
Figure 3.2 Leaf length (±standard errors) of main stem leaves; a) Bodallin and Bodallin+*tin*; b) Morex and Unimorex



The maximum widths of leaves 1 and 2 did not differ significantly for the wheat pair although they were greater for Bodallin+*tin* for leaves 3 to 6 (Fig 3.3). For the leaves

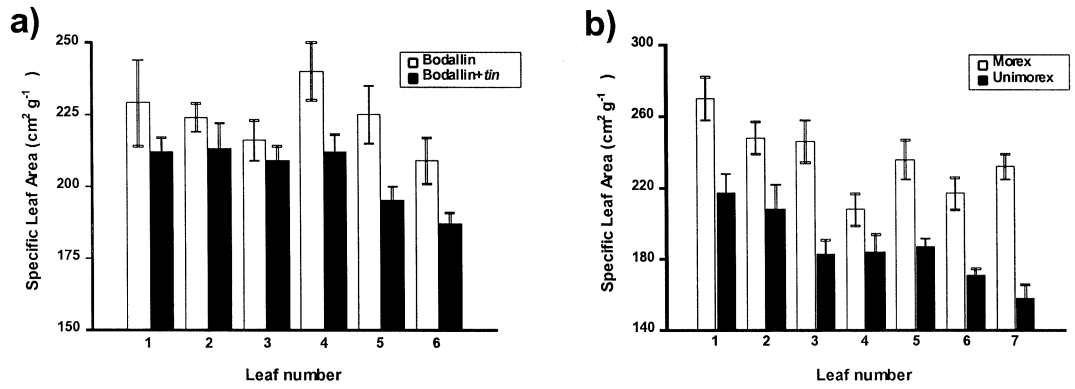
of the barley pair, the leaf widths were greater for Unimorex for leaves 1 to 7. Unlike Morex, which showed a continuous decrease in leaf width from leaf 3, the leaf widths of Unimorex tended to increase from leaf 1.

Figure 3.3 Maximum leaf width (±standard errors) of main stem leaves; a) Bodallin and Bodallin+*tin*; b) Morex and Unimorex



The specific leaf area (SLA) values were greater for Bodallin from leaf 4 onwards indicating that the *tin* gene resulted in thicker and/or more dense leaves (Fig 3.4). The values were greater for Morex relative to Unimorex from leaf 1 to 7 indicating that the *uc2* gene acted in a similar manner.

Figure 3.4 Specific Leaf Area (SLA) (±standard errors) of main stem leaves; a) Bodallin and Bodallin+*tin*; b) Morex and Unimorex



3.4.2 Cellular dynamics of leaf elongation

All leaves were sampled during the phase of linear elongation (i.e. when the rate of leaf elongation was constant), and the length of the leaves were not significantly different between either near-isogenic pair for either leaf (Table 3.1). There was also no difference in the number of tillers produced by the Bodallin and Bodallin+*tin* lines at the leaf 5 harvest although by leaf 6 Bodallin had produced significantly more tillers. The uc_2 gene was more extreme in its suppression of tillering than the *tin* gene and it is uncommon to see any tillers produced. By leaf 6, Morex had commenced tillering and had already demonstrated a significant difference in the number of tillers produced.

Table 3.1 Length of the leaves used for kinematic analysis in the wheat and barley near-isogenic pairs and corresponding number of tillers per plant

Leaf length (mm)					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	92.7	96.8	Morex	104.8	122.8
Bodallin+*tin*	110.8	93.8	Unimorex	97.4	158.2
LSD (0.05)	N.S.	N.S.		N.S.	N.S.
Tillers plant^{-1}					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	2.3	5.0	Morex	2.2	5.0
Bodallin+*tin*	2.0	2.8	Unimorex	0.0	0.0
LSD (0.05)	N.S.	1.0		0.6	0.0

In wheat, the *tin* gene significantly increased the elongation rate of leaves 5 and 6 (Table 3.2.) with leaf 5 of Bodallin+*tin* elongating 84% faster than Bodallin while leaf 6 elongated 43% faster. For both leaves this was due to an increase in the cell flux (number of cells moving out of the growth zone per unit of time) of Bodallin+*tin*. There was no apparent difference in the relationship between cell length in leaf 5 and the physical location in the elongation only zone with the maximum cell length being achieved at approximately the same distance from x_{sd} (Fig 3.5). For leaf 6 however, cells in the

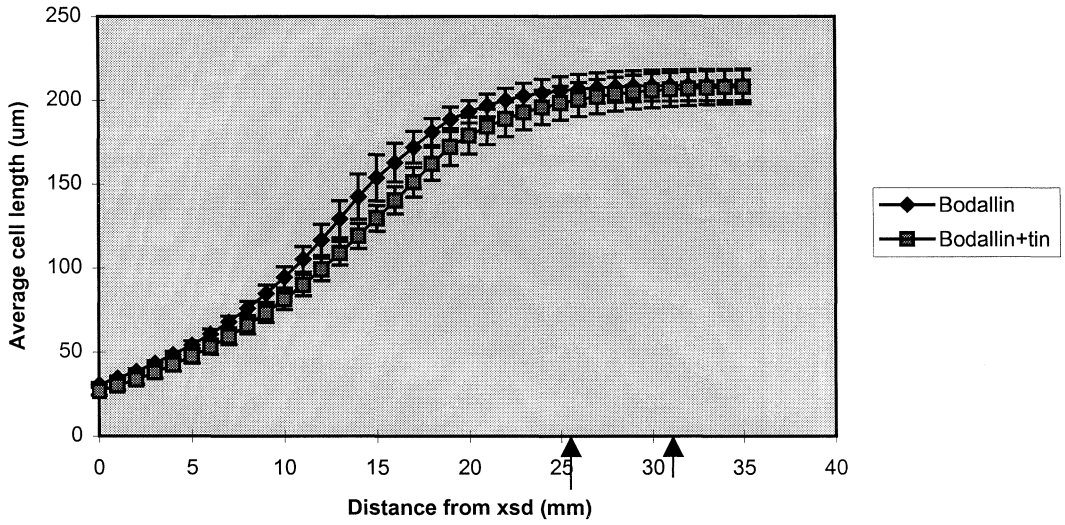
leaves of Bodallin were longer throughout the elongation zone compared to those in Bodallin+*tin* (Fig 3.6) although there was no significant difference in the length of the zone of symmetrical division (L_{sd}).

Table 3.2 Average leaf elongation rate during the linear phase of elongation and corresponding cell flux (number of cells produced per day) for the leaves of the wheat and barley near-isogenic pairs

Elongation (mm day^{-1})					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	33.6	43.5	Morex	43.5	45.3
Bodallin+*tin*	61.9	62.1	Unimorex	41.2	55.7
LSD (0.05)	18.4	7.2		N.S.	7.5
Flux (cells day^{-1})					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	175.1	221.5	Morex	219.6	259.2
Bodallin+*tin*	293.3	376.8	Unimorex	196.6	396.0
LSD (0.05)	41.1	75.0		N.S.	15.7

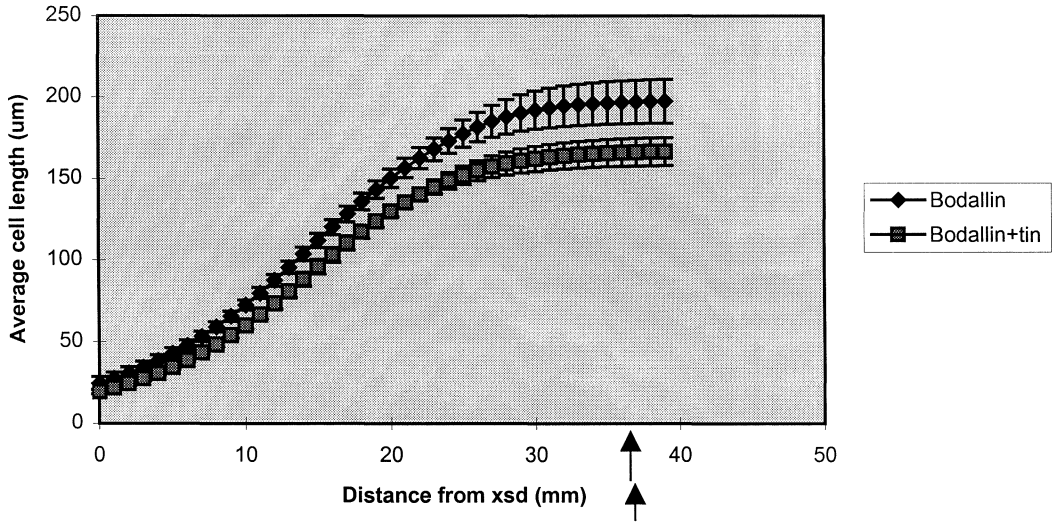
For leaf 6 of the barley near-isogenic lines, there was no significant difference in the elongation rate, flux (Table 3.2), mature cell length for leaf or the way in which the cells elongated in the elongation only zone (Fig 3.7). However leaf 7 of Unimorex elongated 19% faster than Morex. This was due primarily to a significant increase in cell flux rather than an increase in mature cell length, which was not significantly different between Morex and Unimorex. The cells of Morex however were longer than those of Unimorex over much of the elongation only zone. (Fig 3.8).

Figure 3.5 Relationship between the average cell length (±standard errors) and distance from x_{sd} for the leaf 5 of Bodallin and Bodallin+*tin*



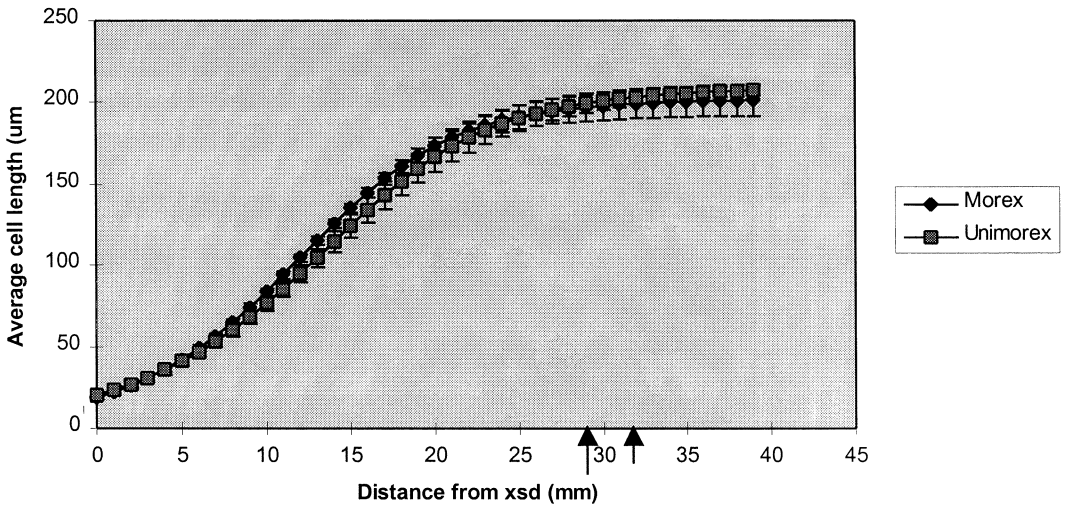
N.B. The long and short tailed arrows indicate the location of x_{el} of Bodallin and Bodallin+*tin* respectively.

Figure 3.6 Relationship between the average cell length (±standard errors) and distance from x_{sd} for the leaf 6 of Bodallin and Bodallin+*tin*



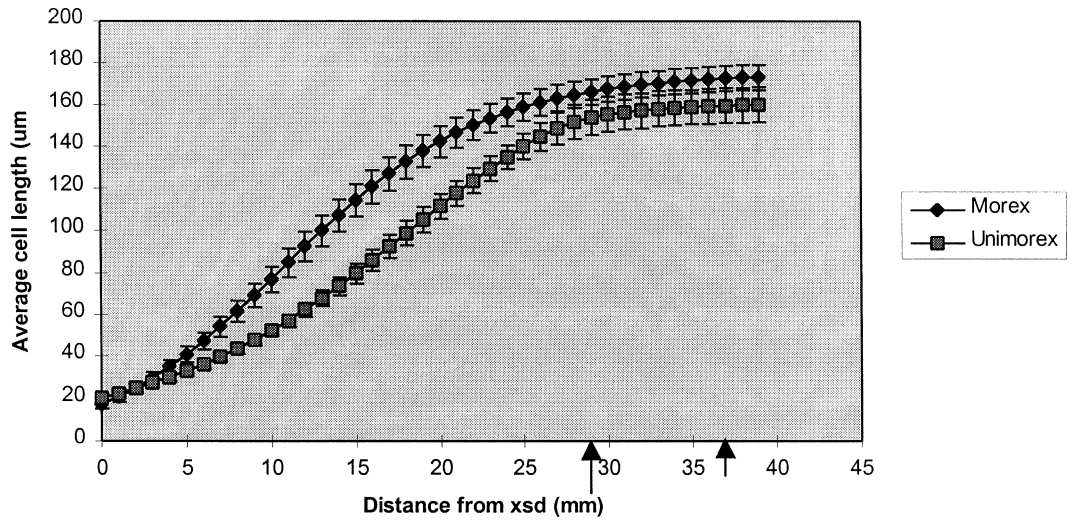
N.B. The long and short tailed arrows indicate the location of x_{el} of Bodallin and Bodallin+*tin* respectively.

Figure 3.7 Relationship between the average cell length (±standard errors) and distance from x_{sd} for the leaf 6 of Morex and Unimorex



N.B. The long and short tailed arrows indicate the location of x_{el} of Morex and Unimorex respectively.

Figure 3.8 Relationship between the average cell length (±standard errors) and distance from x_{sd} for the leaf 7 of Morex and Unimorex



N.B. The long and short tailed arrows indicate the location of x_{el} of Morex and Unimorex respectively.

There were no significant differences in the lengths of the zones of symmetrical division (L_{sd}), elongation (L_{el}) or for the entire growth zone (L_{gz}) for leaf 5 or 6 of the wheat near-isogenic pair or leaf 6 or 7 of the barley near-isogenic pair (Table 3.3).

Table 3.3 Average length of the zones of symmetrical division (L_{sd}), the zone of elongation (L_{el}) and the growth zone as a whole (L_{gz}) for the leaves of the wheat and barley near-isogenic pairs

L_{sd} (mm)					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	1.8	2.6	Morex	1.7	2.1
Bodallin+*tin*	2.0	2.5	Unimorex	1.6	2.4
LSD (0.05)	N.S.	N.S.		N.S.	N.S.
L_{el} (mm)					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	25.6	29.9	Morex	26.0	25.0
Bodallin+*tin*	26.3	29.9	Unimorex	28.8	32.3
LSD (0.05)	N.S.	N.S.		N.S.	N.S.
L_{gz} (mm)					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	27.4	32.6	Morex	27.7	27.1
Bodallin+*tin*	28.3	32.4	Unimorex	30.4	34.6
LSD (0.05)	N.S.	N.S.		N.S.	N.S.

There were no significant differences in the number of cells in the zone of symmetrical division (N_{sd}) for leaf 5 or 6 of the wheat near-isogenic pair, or leaf 6 or 7 of the barley near-isogenic pair (Table 3.4). There were however significantly more cells in the zone of elongation (N_{el}) of leaf 6 of Bodallin+*tin* compared to Bodallin. Overall, there were significantly more cells in the entire growth zone (N_{gz}) of leaves 5 and 6 of

Bodallin+*tin* compared to Bodallin. However there was no significant difference for either leaf of the barley near-isogenic pair.

Table 3.4 Average number of cells in the zones of symmetrical division (N_{sd}), the zone of elongation (N_{el}) and the growth zone as a whole (N_{gz}) for the leaves of the wheat and barley near-isogenic pairs

N_{sd}					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	63	126	Morex	108	141
Bodallin+*tin*	88	112	Unimorex	94	130
LSD (0.05)	N.S.	N.S.		N.S.	N.S.
N_{el}					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	313	407	Morex	381	419
Bodallin+*tin*	349	482	Unimorex	411	548
LSD (0.05)	N.S.	70		N.S.	N.S.
N_{gz}					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	376	473	Morex	490	559
Bodallin+*tin*	437	594	Unimorex	505	679
LSD (0.05)	57	79		N.S.	N.S.

There was a significant increase in the number of new cell walls (NCW) but no difference in the partitioning rate (p_{sd}) of Bodallin+*tin* compared to Bodallin for leaf 5. However there was no difference in both the number of NCW or p_{sd} for the wheat near-isogenic pair for leaf 6 (Table 3.5). There was a significant difference in the number of NCW for the barley near-isogenic pair with more produced by Morex relative to Unimorex. There was no difference in the number of NCW for leaf 7 of the barley near-isogenic pair. The partitioning rates for both leaves 6 and 7 for the barley near-isogenic pair were unaffected by the presence of the *uc2* gene.

The *tin* gene significantly increased the rate of extension in the zone of symmetrical division (r_{sd}) for leaf 6 but not leaf 5 of the wheat near-isogenic pair (Table 3.6). However, there was no difference in the rate of extension for the same zone for either leaf 6 or 7 for the barley near-isogenic pair. The *tin* gene increased the rate of extension of the elongation zone (r_{el}) for both leaves 5 and 6, almost doubling it in the case of the former. Both Morex and Unimorex had similar values for the rate of elongation in the elongation zone for leaf 7, although it was not significantly different for leaf 6.

Table 3.5 New cell walls (NCW) and partitioning rate (p_{sd}) for the leaves of the wheat and barley near-isogenic pairs

NCW					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	8.7	14.0	Morex	21.0	28.8
Bodallin+*tin*	13.6	20.6	Unimorex	13.2	23.2
LSD (0.05)	3.6	N.S.		N.S.	N.S.
p_{sd} (cells^{-1} cell^{-1} hr)					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	0.13	0.10	Morex	0.10	0.10
Bodallin+*tin*	0.17	0.15	Unimorex	0.10	0.14
LSD (0.05)	N.S.	N.S.		N.S.	N.S.

The maximum rates of extension of the cells in the zone of elongation of leaf 5 for the wheat near-isogenic pair were achieved at approximately 16mm past x_{sd} for Bodallin+*tin* and 17mm for Bodallin (Fig 3.9). This corresponded to cell 230 after x_{sd} for Bodallin+*tin* and cell 250 for Bodallin (Fig 3.10). The cells in the elongation zone of Bodallin+*tin* appeared to reach their maximum rate of extension 18 hours after passing through x_{sd}, while it took 32 hours for the maximum rate to be reached for cells in the elongation zone of Bodallin (Fig 3.11).

Table 3.6 Rate of elongation of the zone of symmetrical division (r_{sd}) and elongation zone (r_{el}) for the leaf cells in wheat and barley near-isogenic pairs

r_{sd} (μm hr^{-1})					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	196	220	Morex	156	187
Bodallin+*tin*	270	351	Unimorex	159	294
LSD (0.05)	N.S.	90		N.S.	N.S.
r_{el} (μm hr^{-1})					
Wheat	Leaf 5	Leaf 6	Barley	Leaf 6	Leaf 7
Bodallin	1514	1813	Morex	1815	1887
Bodallin+*tin*	2580	2587	Unimorex	1718	2335
LSD (0.05)	827	299		N.S.	313

Figure 3.9 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 5 of Bodallin and Bodallin+*tin* plotted as a function of distance from x_{sd}

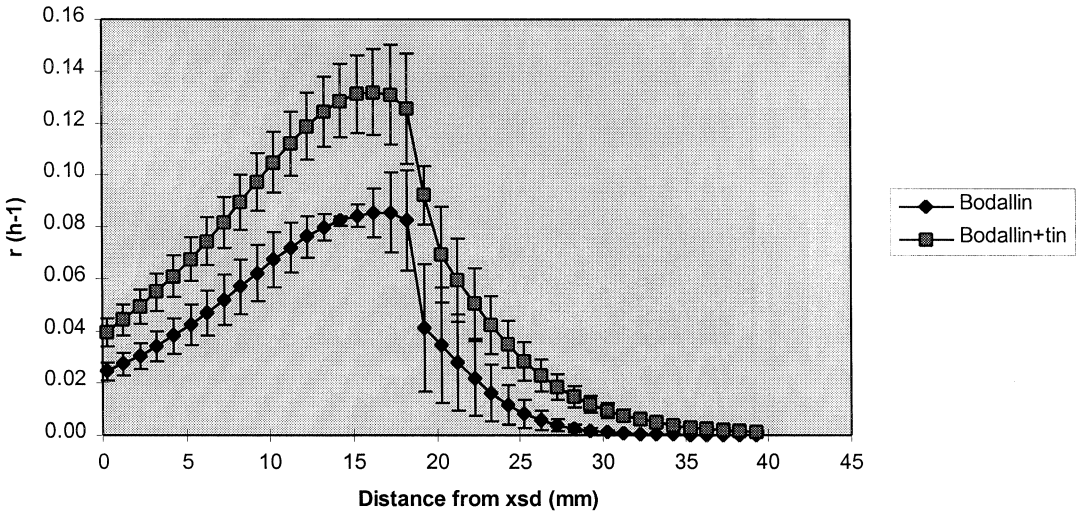


Figure 3.10 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 5 of Bodallin and Bodallin+*tin* plotted as a function of cell rank from x_{sd}.

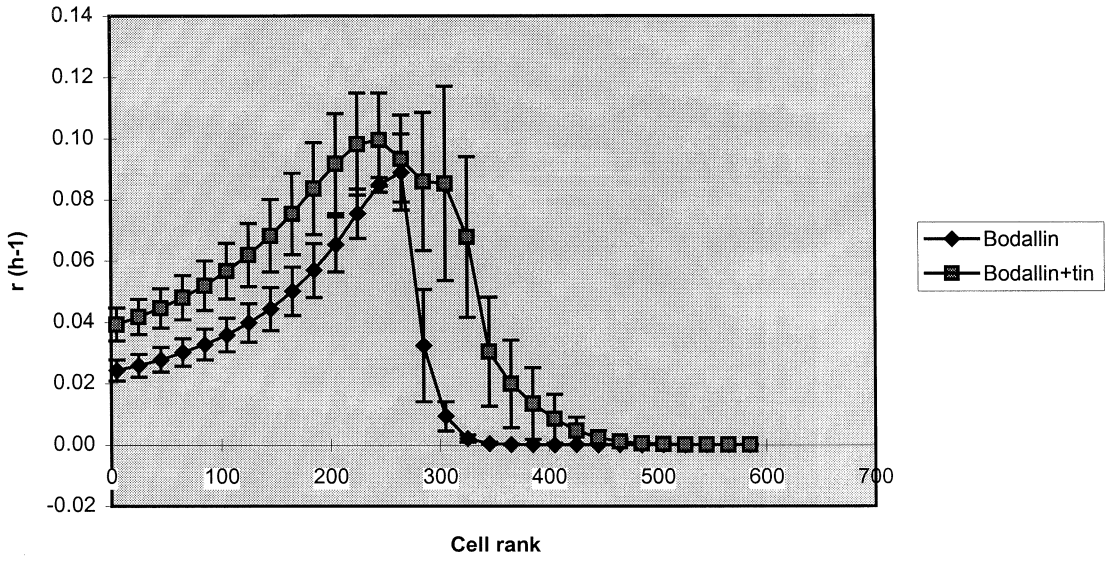
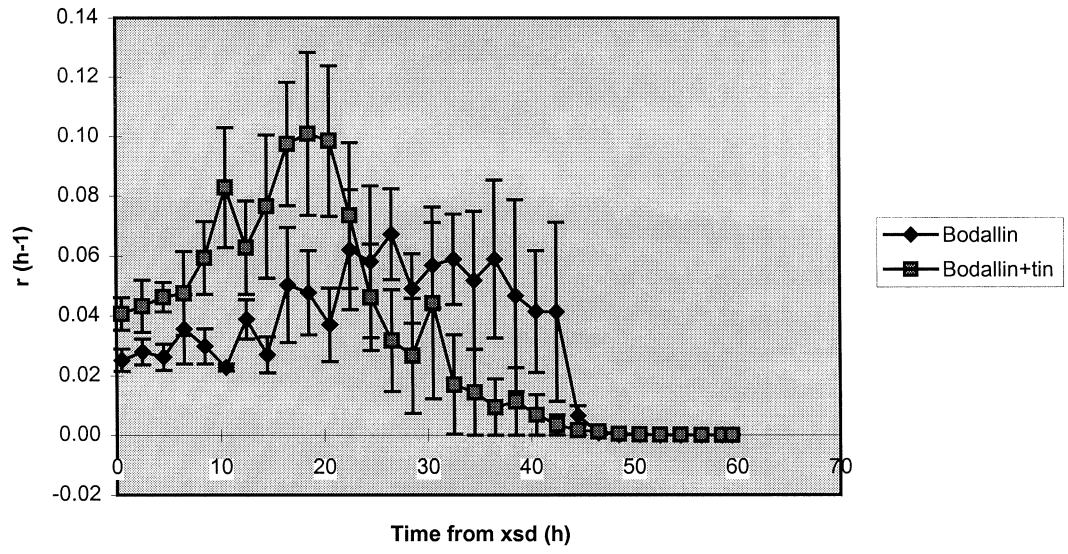
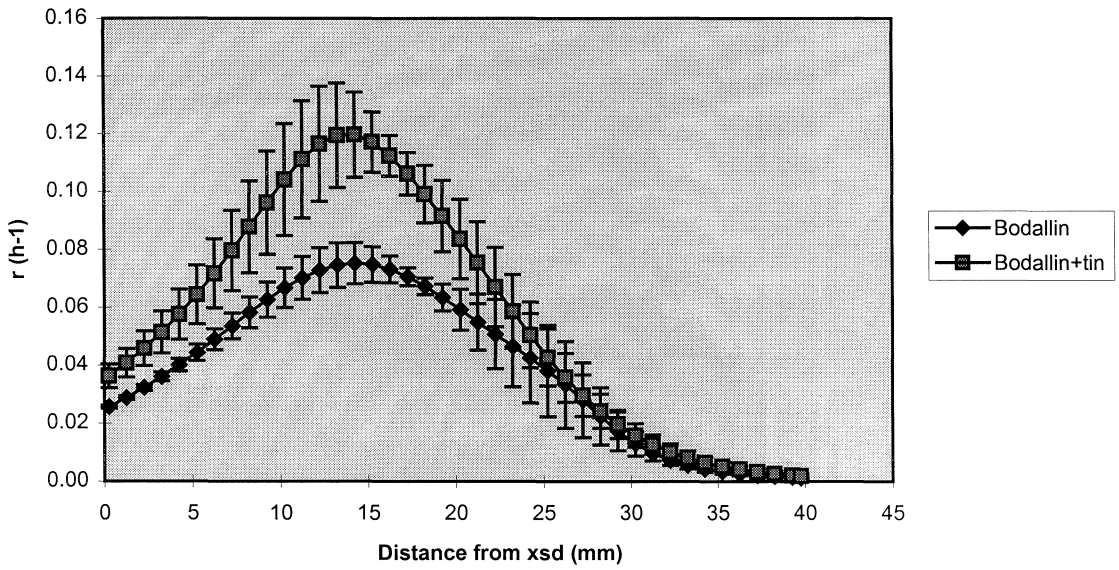


Figure 3.11 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 5 of Bodallin and Bodallin+*tin* plotted as a function of time since the cell moved out of the division zone.



As with leaf 5, the maximum rate of extension of cells in the zone of elongation occurred at similar distances, although it was only 14mm from x_{sd} for leaf 6 of the wheat near-isogenic pair (Fig 3.12). Unlike leaf 5 however, the cells of Bodallin+*tin* reached their maximum extension rate later in the cell rank than Bodallin at cells 380 and 310 respectively (Fig 3.13). The maximum rate of extension was reached sooner for Bodallin+*tin* at approximately 21 hours after the cells had passed through x_{sd} compared to Bodallin which reached their maximum 33 hours after passing the same point (Fig 3.14).

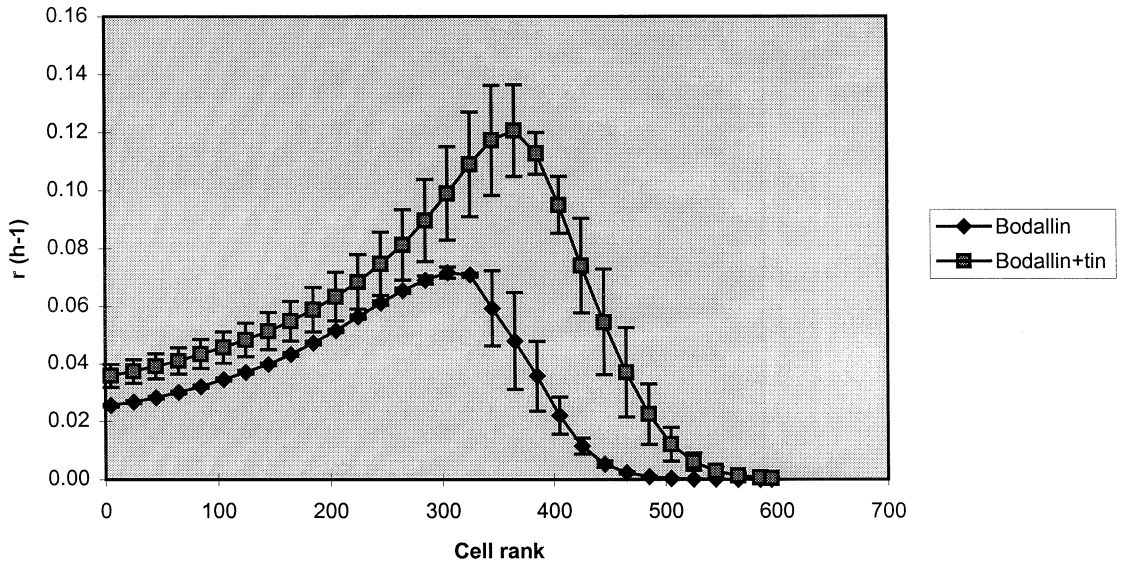
Figure 3.12 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 6 of Bodallin and Bodallin+*tin* plotted as a function of distance from x_{sd}



The cells in the elongation zone of leaf 6 of Morex reached their maximum rate of extension 12mm from x_{sd} compared to 15mm for Unimorex (Fig 3.15). When comparing on the basis of cell rank, the maximum rate of extension was reached at approximately cell 280 from x_{sd} for Unimorex and cell 300 for Morex (Fig 3.16), although the rate declined much faster for the later after achieving a higher maximum rate. The maximum

rates of extension were similar when comparing the two barley lines on the basis of time since passing through x_{sd} with the maximum rate occurring after 28 hours for Morex and 35 hours for Unimorex (Fig 3.17).

Figure 3.13 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 6 of Bodallin and Bodallin+*tin* plotted as a function of cell rank from x_{sd}



A lower maximum rate of cell extension in the elongation zone of leaf 7 of Morex occurred 11mm past x_{sd} compared to 18mm for Unimorex (Fig 3.18). When comparing on the basis of cell rank, Morex did not achieve as high a maximum rate of extension, but achieved it earlier at approximately cell 280 compared to cell 400 for Unimorex (Fig 3.19). There appeared to be little difference in the rate of cellular extension when comparing on the basis of time with both Morex and Unimorex achieving similar maximum rates of extension at approximately 23 hours after the cells had passed through x_{sd} (Fig 3.20).

Figure 3.14 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 6 of Bodallin and Bodallin+*tin* plotted as a function of time since the cell moved out of the division zone

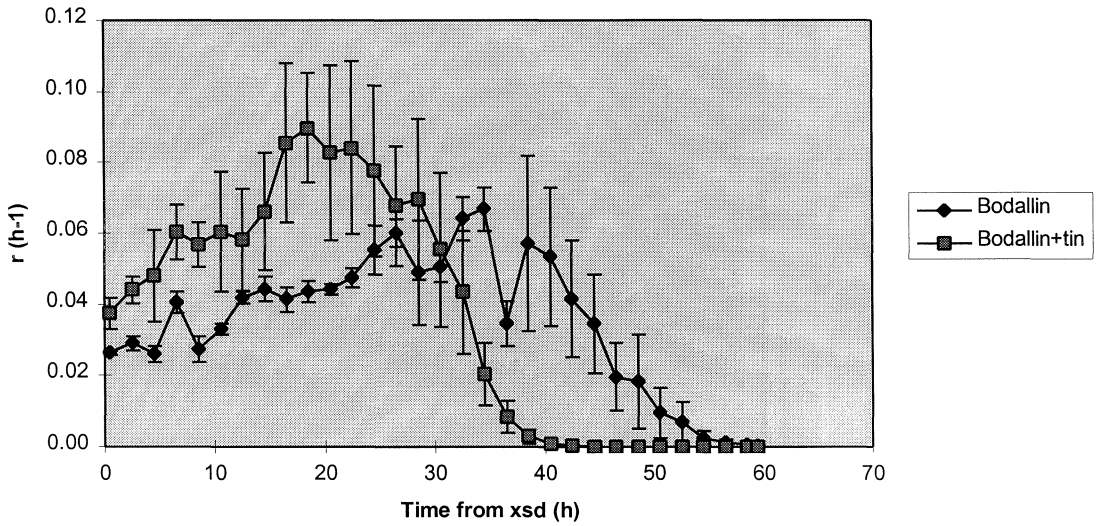


Figure 3.15 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 6 of Morex and Unimorex plotted as a function of distance from x_{sd}

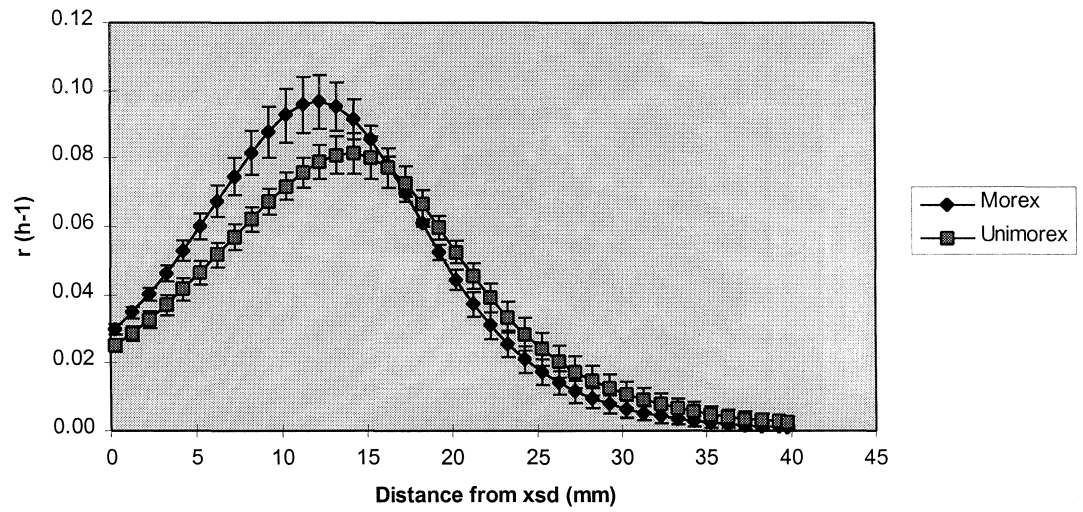


Figure 3.16 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 6 of Morex and Unimorex plotted as a function of cell rank from x_{sd}

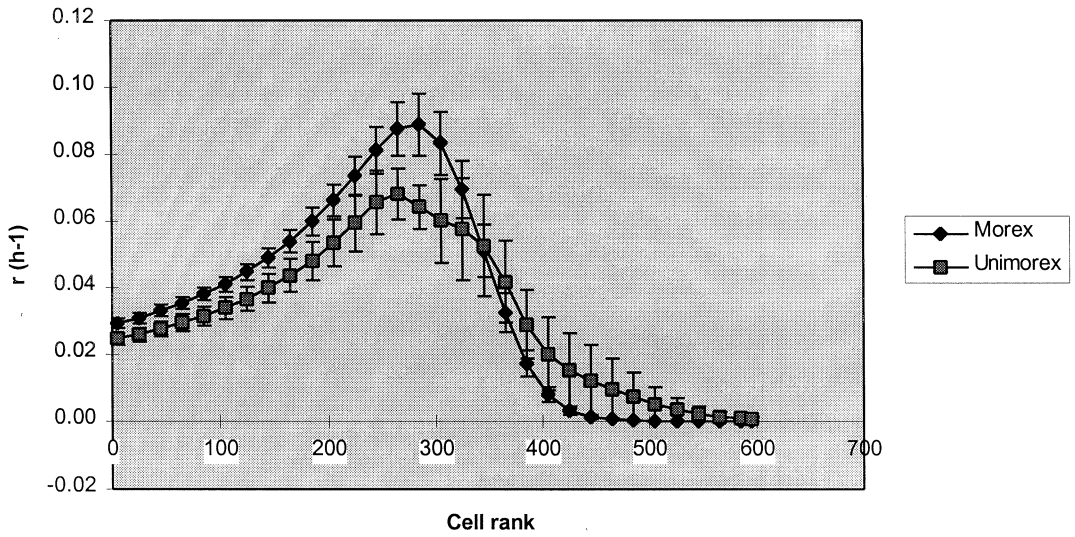


Figure 3.17 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 6 of Morex and Unimorex plotted as a function of time since the cell moved out of the division zone

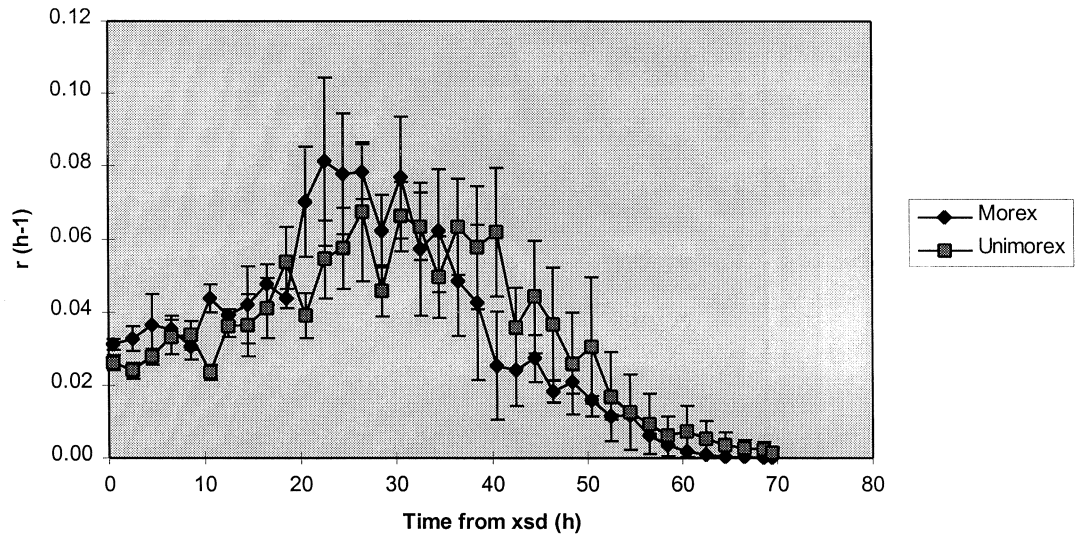


Figure 3.18 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 7 of Morex and Unimorex plotted as a function of distance from x_{sd}

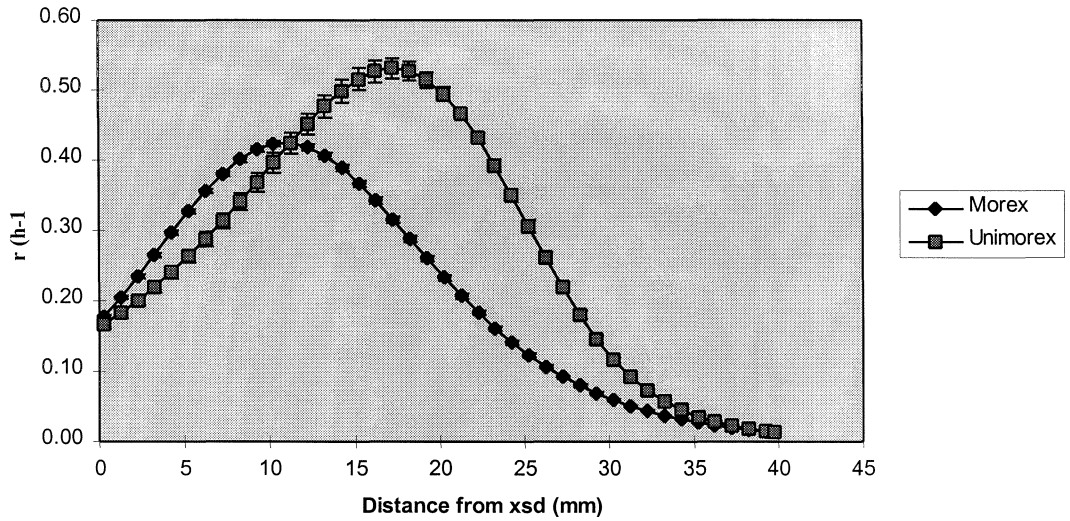


Figure 3.19 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 7 of Morex and Unimorex plotted as a function of cell rank from x_{sd}

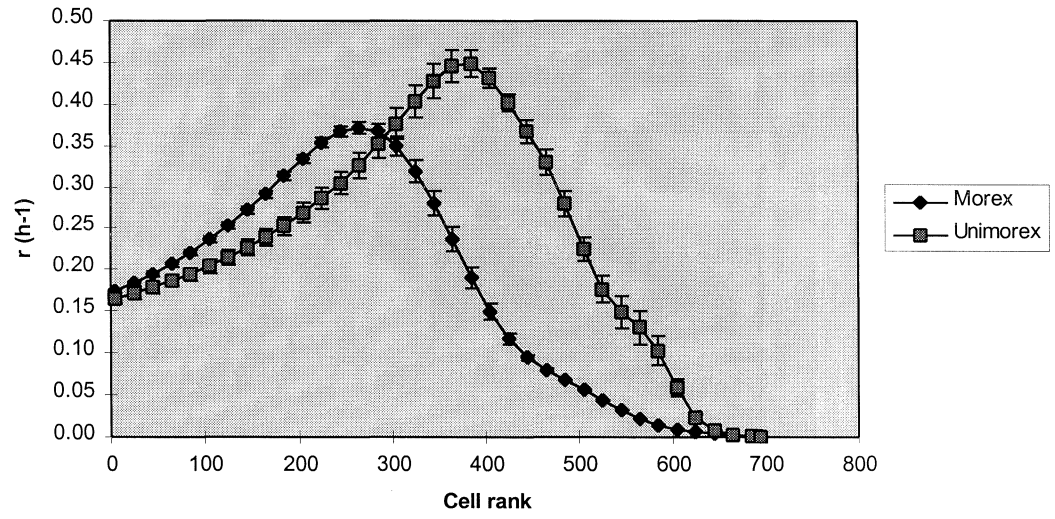
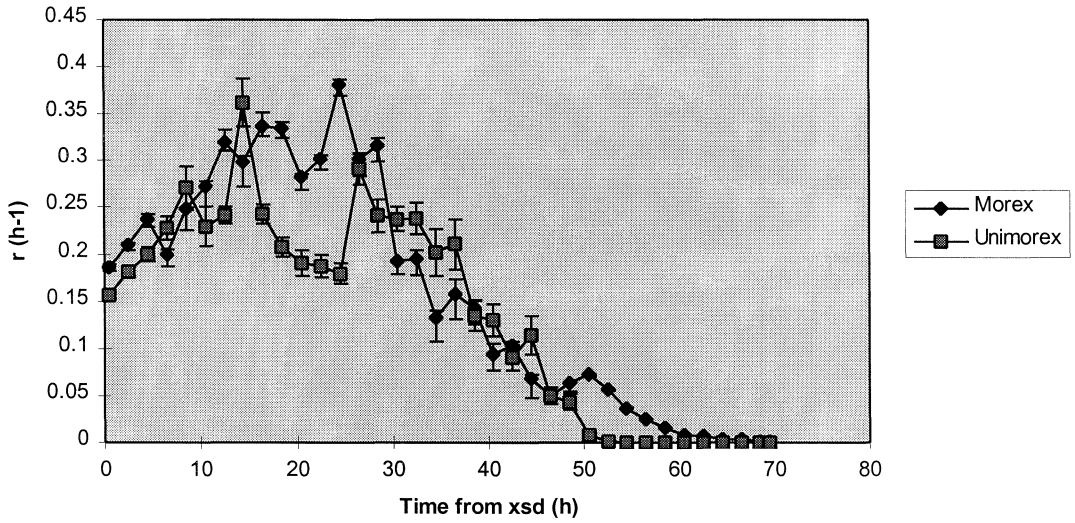


Figure 3.20 Local average relative cell elongation rates of cells (±standard errors) along the elongation zone of leaf 7 of Morex and Unimorex plotted as a function of time since the cell moved out of the division zone



3.5 DISCUSSION

3.5.1 Developmental and fully expanded leaf measurements

Kirby (1973) noted that the rate of elongation of the leaves was the same for lines with and without the *uc2* gene. Similar results were found for leaf 6 of the barley pair in the experiments reported here, although by leaf 7 the rate was significantly higher for Unimorex. Leaves 5 and 6 of Bodallin+*tin* and leaf 7 of Unimorex all elongated faster than their near-isogenic pairs as a result of an increase in the flux, although what causes this increase in flux is unclear. It is tempting to speculate that the inhibition of tillers brought about by the presence of the *tin* gene allows more assimilate to be diverted into the growing leaf. However if it was purely an increase in assimilate supply that caused the increase in cell flux and subsequently leaf elongation, then surely a more dramatic effect would be observed for the barley near-isogenic pair. Therefore the increased rate of elongation of leaves 5 and 6 of Bodallin+*tin* and leaf 7 of Unimorex relative to their near-isogenic pairs must be an intrinsic function of the *tin* gene and not simply be associated

with assimilate supply unless the *uc2* gene in some way counteracts the effect of an increase in the supply of assimilate in a way in which the *tin* gene does not.

By the time leaf 6 was emerging, the number of tillers visible was significantly lower for the *tin* lines, indicating that tiller bud suppression was occurring when leaf 4 or 5 was emerging in the wheat plants while they were suppressed throughout the life of the Unimorex plants. This could account for the significantly lower SLA values from leaf 4 in Bodallin+*tin* and from leaf 1 in Unimorex in addition to an increase in leaf lengths and widths. Whether this is an intrinsic effect of the *tin* and *uc2* genes or it is due to an increase in assimilate availability is unknown. If the mature cell widths are unchanged like cell length in the *tin* and *uc2* lines, the increased leaf widths and lower specific leaf areas also must be the result of an increase in the total cell number per unit area. Alternatively, it may be due to a difference in cell wall thickness.

3.5.2 Growth zone

There were no significant differences in the lengths of the growth zones for the leaves of either the wheat or barley near-isogenic pairs. However the number of cells in the total growth zone was higher for Bodallin+*tin* relative to Bodallin for both leaf 5 and 6. This was reflected in the shorter average cell lengths for much these zones as displayed in Figs 3.5 and 3.6. While the final cell lengths are shorter for Bodallin+*tin* in leaf 6, much of the reduced average cell length could be explained by the fact that the flux was greater and the cells were being pushed along at a faster rate. A cell in the leaf of a Bodallin+*tin* plant at a given position would therefore have had less time to elongate.

The tendency for the *tin* gene to increase leaf elongation rates may be a function of an increase in cellular division in the leaf meristem which in turn could result in a faster rate at which cells entering the growth zone, and/or an increase in carbohydrate concentration. Volenec and Nelson (1984a) found that the leaf elongation rate of two genotypes of tall fescue (*Festuca arundinacea* Schreb.) differed by 50%. The authors claimed that this was due to an increase in the size of the leaf meristem and that the carbohydrate synthesis and transport to the leaf meristem did not limit leaf growth (Volenec and Nelson, 1984b). Working with root meristems, Van't Hof (1968) found that

deprivation of carbohydrate resulted in a "stationary phase" of mitotic division and cells were prevented from undergoing cellular division. An increase in carbohydrate availability may be causing the increase in flux and tendency for an increase in NCW for both leaf 5 and 6 of Bodallin+*tin* relative to Bodallin.

3.5.3 Zone of symmetrical division

There were no differences in the length, number of cells or the rate of partitioning in the zone of symmetrical division in any leaf observed of the wheat or barley near-isogenic pairs. The only differences that existed within the zones of symmetrical division of any of the samples was for leaf 5 of Bodallin and Bodallin+*tin* where the number of NCW was significantly greater for Bodallin+*tin* (13.6 NCW compared to 8.7) and the faster rate of cell elongation for leaf 6 of Bodallin+*tin* (351 μm hr^{-1} compared to 220 μm hr^{-1}). It would appear then that with some exceptions the zones of symmetrical division are largely unaffected by the *tin* and *uc2* genes.

3.5.4 Zone of elongation

The *tin* gene did not appear to affect the length of the elongation zone of leaves 5 and 6 of the wheat near-isogenic pair although the number of cells in this zone was greater for leaf 6 of Bodallin+*tin*. However for Bodallin+*tin*, the rate of extension of cells in the zone of elongation was 84% higher for leaf 5 and 43% higher for leaf 6 relative to Bodallin. The rate of elongation was always greater for Bodallin+*tin* in both leaves when comparing on the basis of distance from x_{sd} or cell rank. This may be a function of the competition for assimilate between cell leaf elongation and stem elongation. Bodallin may have a greater demand from the later due to the greater number of tillers.

The apparent contradiction between the average cell length versus distance from x_{sd} (Fig 3.5) and the rate of cell extension versus distance from x_{sd} (Fig 3.9) for leaf 5 of the wheat near-isogenic pair can be explained when the higher flux of Bodallin+*tin* is taken into account. The increased flux forces cells into the elongation zone away from x_{sd} at a faster rate for Bodallin. As a result, the rate of elongation of cells in the zone of elongation (r_{el}) for Bodallin+*tin* is almost double that of Bodallin. When comparing on

the basis of time from x_{sd}, the cells in the elongation zone of Bodallin+*tin* reached their maximum elongation rate faster after having passed through x_{sd} before the rate quickly declined. The cells of Bodallin however increased their rate of extension much more slowly and reached their maximum rate of extension much later despite being the same size as those of Bodallin+*tin* when they entered the zone of elongation. The cells of leaf 5 and 6 ceased elongating sooner and they reached their maximum length earlier, possibly because more carbohydrates were available due to the significant suppression of tillering which had occurred by this stage.

The process by which the *uc2* gene affects the expansion of cells in the elongation zone of the leaf is markedly different to that of the *tin* gene. The length of the elongation zone was greater for Unimorex than Morex for leaf 6 and 7 and while the length of the cells entering the zone of elongation in leaf 6 were similar for Morex and Unimorex, they expand faster for the former. The acceleration rate was initially higher for Morex on the basis of distance from x_{sd}, cell rank and time. After the maximum rate of elongation was achieved, the rate of deceleration was similar to Unimorex and there were no significant differences in the time, position in the cell file or distance from x_{sd} when the cells ceased elongating.

For leaf 7, the cells of Unimorex achieved higher maximum rates of extension, but did so at a greater distance from x_{sd} and later in the cell rank. Over time however, the maximum rate of extension was achieved at the same time for Morex and Unimorex (23 hours after passing through x_{sd}). The flux was higher and the cells tended to be pushed away from x_{sd} faster for Unimorex than they were for Morex for leaf 7, although there was no significant difference observed for leaf 6.

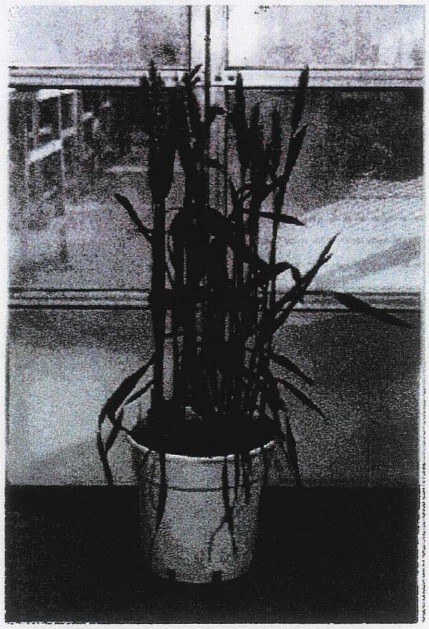
3.6 CONCLUSION

Both the *tin* and *uc2* genes increased leaf length and width and decreased the specific leaf area of mainstem leaves although they appeared to affect the kinematics of leaf cell expansion in different ways. The *tin* gene increased the rate of elongation of the leaf through an increase in the cell flux. Leaf cell final length was either the same or reduced by the presence of the *tin* gene. While it did not affect the length of the growth

zone or its component zones, the *tin* gene increased the maximum rate of cell elongation in leaf 6 and the number of cells in the growth zone through an increase in the number of cellular divisions in the zone of symmetrical division for leaf 5. For leaf 5, the maximum rate of elongation was reached sooner in terms of time after passing through xsd for Bodallin relative to Bodallin+*tin*, although there was little difference in terms of cell rank or distance from x_{sd}. However for leaf 6 the maximum rate was reached earlier for Bodallin+*tin* in terms of time while there was little difference in terms of cell rank or distance from x_{sd}. The uc_2 gene by contrast made no impact on the leaf elongation rate of leaf 6, but increased it in leaf 7 through an increase in cell flux. It also made no significant impact on leaf cell final length. It increased the rate of cellular elongation in the elongation only zone for leaf 7. Maximum rates of elongation were reached sooner and closer to x_{sd} for leaf 6 of Morex relative to Unimorex while they occurred closer to x_{sd} and later in the cell rank for leaf 7.

CHAPTER 4

THE EFFECT OF THE *tin* GENE ON GROWTH AND DEVELOPMENT



Kite+*tin* at anthesis

4.1 INTRODUCTION

Donald (1968) proposed the idea that a cereal crop consisting of uniculm plants would produce more grain than a freely tillering crop. The reasoning behind this concept was that there would be no internal competition between the developing spike and younger, possibly sterile, tillers for assimilate. A reduction in tillering would affect the partitioning of assimilate throughout the plant as the assimilate that would otherwise have been invested in tillers would be directed into other parts of the plant.

The later order leaves of reduced tillering wheat lines thought to contain the *tin* gene were found to be larger than those of freely tillering lines (Marshall and Boyd, 1985). While the apices of these lines were the same size at floral initiation, the rate of initiation of spikelet primordia was greater for the reduced tillering lines while the duration was unchanged. The *uc2* gene in barley, which results in the suppression of all tiller buds, was found to increase the relative growth rates of the mainstem leaves, while the time the leaf spent in the phase of constant relative growth was shorter resulting in an extra leaf being produced (Kirby, 1973). The mature leaves of lines containing the *uc2* gene were also heavier and the shoot apex longer.

Assimilate that would otherwise be used in the growth of tillers could be used in a variety of ways in lines containing the *tin* gene. Possible areas to which assimilate could be directed are the mainstem as well as those tillers that are not suppressed. The thicker stems (Atsmon and Jacobs, 1977) and the larger leaves (Atsmon and Jacobs, 1977; Marshall and Boyd, 1985) of the *tin* lines may be the result of increases in the availability of assimilate to those organs that are produced. To date, there is no known study as to the effect of the *tin* gene on the root system of plants.

It is hypothesised then that lines containing the *tin* gene accumulate biomass at the same rate as their freely tillering near-isogenic pairs but partition it into fertile stems rather than sterile and possibly later formed less productive stems. The aims of the experiments outlined in this chapter were to investigate how the *tin* gene affects the partitioning of assimilate throughout the wheat plant in terms of differences in tillering, leaf area and the weights and changes in weights of various plant organs during the early growth stages in pots and throughout the life of the plant under field conditions. The *tin* gene would be expected to direct assimilate into productive

"sinks", such as fertile tillers and in particular the spikes. The larger heads of the *tin* lines would also be expected to contain more kernels due to their larger size while the thicker stems may not only provide greater lodging resistance but may increase the soluble reserves available for kernel filling.

4.2 MATERIALS AND METHODS

4.2.1 Pot Experiment

Seeds of two wheat cultivars (Banks and Kite) and one barley cultivar (Morex) as well as their near-isogenic lines containing the *tin* or *uc2* gene (Banks+*tin*, Kite+*tin*, Unimorex) were sown outdoors in Canberra, ACT (149°06'E, 35°19'S Alt 600m) in cylindrical PVC pots (500mm high, 87mm diameter) containing a sandy-loam soil on the 18th of August, 1995. Two seeds were sown and then thinned to one plant per pot shortly after emergence. Growth stages of the plants were rated according to the Zadoks (DC) code (Zadoks *et al.* 1974). Plants were harvested at DC 13, 14, 15 and 16 (33, 41, 45 and 51 days after sowing respectively). Average maximum daily temperatures ranged from 16.0°C to 19.3°C, average minimum temperature ranged from 1.8°C to 7.7°C, and daylength increased from 10.7 hours to 12.5 hours over the duration of the experiment. At each harvest, roots were washed to remove the soil and the number of tillers was determined. Plants were separated into roots, mainstem leaves and stem, tiller leaves and stems, then dried at 70°C for two days before being weighed. Leaf area of each plant at each harvest was determined using a planimeter. The root:shoot ratio was calculated as the ratio of root mass to the mass of leaves plus stems. Specific leaf area (SLA) was determined from the quotient of leaf area to leaf mass. Leaf area ratio (LAR) was determined from the quotient of leaf area to mass of leaves plus stems. Leaf weight ratio (LWR) was determined from the quotient of leaf mass to mass of leaves plus stems. There were four replicates of each genotype at each harvest.

4.2.2 Field Experiments

Experiments were conducted over three seasons (1995, 1996 and 1997) at four sites in central and southern New South Wales and sown from late May until early July (Table 4.1). Growth stages of the plants were rated according to the Zadoks (DC)

code (Zadoks *et al.* 1974). Plant densities were determined between DC 12 and DC 14 and are give in Table 4.2.

Table 4.1 Designation, description, sowing date, harvesting date and previous year's history for trials to investigate the interaction between *tin* and density

Site	Designation	Description	Sowing date	Harvesting date	Previous year
Condobolin	C95.1	*tin**density	May 16 1995	Nov 27 1995	Long Fallow
Condobolin	C95.2	*tin**density	June 19 1995	Nov 27 1995	Long Fallow
Condobolin	C96	*tin**density	May 27 1996	Nov 24 1996	Pasture
Wagga Wagga	W96	*tin**density	May 22 1996	Dec 4 1996	Pasture
Moombooldool	M96	*tin**density	July 4 1996	Dec 3 1996	Pasture
Condobolin	C97	*tin**density	May 28 1997	Nov 20 1997	Pasture
Wagga Wagga	W97	*tin**density	May 23 1997	Dec 3 1997	Pasture
Moombooldool	M97	*tin**density	May 30 1997	Nov 25 1997	Long Fallow
Condobolin	C96N	*tin**nitrogen	May 27 1996	Nov 14 1996	Wheat
Ariah Park	AP97	*tin**nitrogen* density	May 20 1997	Nov 18 1997	Canola

Table 4.2 Plant density (plants m^{-2}) at *tin* by density trial sites

Sowing density (kernels m^{-2})		C95.1	C95.2	C96	M96	W96	C97	M97	W97
		----------Plants m^{-2}----------							
Cultivars	150	146	135	149	N.A.	140	103	145	116
Cultivars+*tin*	150	147	141	147	N.A.	130	105	135	130
Cultivars	300	291	286	234	N.A.	212	225	269	212
Cultivars+*tin*	300	258	261	224	N.A.	241	214	281	200

N.A. = not available

4.2.3 Condobolin

Trials were conducted at New South Wales Agriculture's Research and Advisory Station at Condobolin (33° 04'S, 147° 13'E, Alt 195m) in central New South Wales (long term annual rainfall 427mm). The soil is a gradational red earth with a neutral reaction trend, classified as Dr 2.43 (Northcote *et al.* 1971). Four near-isogenic pairs (three spring wheat: Bodallin±*tin*, Banks±*tin*, Kite±*tin*; and one winter wheat: Osprey±*tin*) were sown on May 16 (C95.1) and June 19 (C95.2) in 1995 and on May 27 (C96) in 1996. In 1997, the trial was sown on May 28 (C97) with the same pairs used as in 1995 and 1996 with the exception of Banks±*tin*. All trials were arranged as three replicate, randomised complete block designs. All plots sown with 103kg ha^{-1} of Starter 15 (15.0% N, 13.1% P and 10.0% S). Sowing rates were adjusted according to kernel size to sow 150 and 300 kernels m^{-2}. Plant densities for trials to investigate the interaction between the *tin* gene and sowing rate are shown in Table 4.2. Plots in 1995 measured 10m long by 10 rows wide at 19cm row spacings while in 1996 and 1997 they were 6m long. In 1995, plots were sprayed on June 29 with Legumex ® (active ingredient 2,4-DB) at 3L ha^{-1} to control subterranean clover and with Bayleton ® (active ingredient triadamefon) at 500mL ha^{-1} to prevent fungal diseases. A severe pre-DC 65 drought in 1995 severely reduced the yield potential of all the crops with many stems failing to produce viable grain. Significant rainfall after DC 65 saved the trial from being abandoned. In 1996, plots were sprayed with Folicur ® (active ingredient tebuconazole) at 200mL ha^{-1} on August 16 to prevent fungal diseases. In 1997, plots were sprayed with Roundup ® (active ingredient glyphosate) at 2L ha^{-1} prior to emergence on June 3 to control volunteer grasses and broad-leaf weeds and with Folicur ® at 200mL ha^{-1} on August 19 and September 24 to prevent fungal diseases. Machine harvest took place on November 27 in 1995, November 14 in 1996 and November 20 in 1997.

A trial to investigate the interaction between nitrogen and the *tin* gene (C96N) was sown on May 27, 1996. Plots measured 10m long by 10 rows wide at 19cm row spacings and were arranged as three replicate randomised complete block design with nitrogen and cultivar combinations randomised within a replicate. Four near-isogenic pairs of wheat differing in the presence of the *tin* gene were sown (Bodallin±*tin*, Banks±*tin*, Kite±*tin* and Osprey±*tin*) with Starter 15 at 103kg ha^{-1} and urea at rates of

0 and 50kg ha^{-1} of nitrogen. Sowing rates were adjusted according to kernel size to sow 150 kernels m^{-2}. There were 84 plants m^{-2} established for the cultivars and 89 plants m^{-2} established for the *tin* lines. At DC 65, 2.5m of each plot was irrigated with 50mm of water in an attempt to provide insight into how lines containing the *tin* gene respond when the only variable is the amount of water available during grainfilling. From hereon, plots that were irrigated are know as "irrigated" while those that were not are referred to as "rainfed". As with the *tin**density trial, all plots were sprayed with Folicur ® at 200mL ha^{-1} on September 16 to prevent fungal diseases. Plots were harvested on November 14. Meteorological data for Condobolin in 1995, 1996 and 1997 are given in Table 3 of the Appendix.

4.2.4 Moombooldool

Trials were conducted on Murray and Dianne Long's property, "Coondara" at Moombooldool (34° 11'S, 145° 48'E, Alt 200m) in central New South Wales (long term average annual rainfall at Ardlethan, 21kms south southeast, is 490mm) (Ardlethan is 34° 21'S, 146° 54'E, Alt 270m). The soil is a red brown earth with a neutral reaction trend, Gn 2.12 (Northcote *et al.* 1971). Trials were sown on July 4, 1996 (M96) and May 30, 1997 (M97). Three replicates of four near-isogenic pairs of wheat differing in the presence of the *tin* gene (Bodallin±*tin*, Banks±*tin*, Kite±*tin* and Osprey±*tin*) were sown as a randomised complete block in 1996 while in 1997 the same varieties were used with the exception of Banks±*tin*. Plots measured 5.5m long by 10 rows wide at 19cm row spacings and were sown with Starter 15 at 103kg ha^{-1}. Sowing rates were adjusted according to kernel size to sow 150 and 300 kernels m^{-2}. In 1996, plots were sprayed with Folicur ® at 200mL ha^{-1} on August 15 to prevent fungal diseases and with Bromoxynil ® (active ingredient bromoxynil) at 1L ha^{-1} and Hoegrass ® (active ingredient diclofop methyl) at 1L ha^{-1} on September 4 to control broad-leaf weeds and annual ryegrass respectively. In 1997, Thiodan ® (active ingredient endosulfon) was sprayed on plots at 500mL ha^{-1} on July 13 to control Red legged earth mite, while Bromoxynil ® at 1L ha^{-1} and Hoegrass ® at 1L ha^{-1} were sprayed onto plots on July 22 to control broad-leaf weeds and annual ryegrass respectively. Folicur ® was sprayed onto plots at 250mL ha^{-1} on August 20 and September 23 to control plant rusts. Harvest index cuts were taken shortly before

harvesting which took place on December 3 in 1996 and November 25 in 1997. Meteorological conditions at Moombooldool are assumed to be similar to Yanco Agricultural Institute, 41kms south southwest of Moombooldool. Combined data for Moombooldool and Yanco are given in Table 4 of the Appendix.

4.2.5 Wagga Wagga

Trials were sown at New South Wales Agriculture's Research Institute at Wagga Wagga (35° 03'S, 147° 10'E, Alt 210m) in southern New South Wales (long term average annual rainfall 579mm). The soil was a red brown earth with an acid trend reaction, Gn 4.12 (Northcote *et al.* 1971). Plots were sown on May 22 in 1996 (W96) and May 23 in 1997 (W97) and measured 5m long by eight rows wide at 26cm row spacings with Starter 15 at 81kg ha^{-1}. Sowing rates were adjusted according to kernel size to sow 150 and 300 kernel m^{-2} with three replicates which were arranged in a randomised complete block design. Four near-isogenic pairs of wheat differing in the presence of the *tin* gene were sown in 1996 (Bodallin±*tin*, Banks±*tin*, Kite±*tin* and Osprey±*tin*). Plots were sprayed with Folicur ® at 200mL ha^{-1} on August 14 to prevent fungal diseases. In 1997, the same varieties were used as in 1996 with the exception of Banks±*tin*. Plots were sprayed with Hoegrass ® at 2L ha^{-1} to control annual ryegrass on July 7, Ally ® (active ingredient metsulfon methyl) at 5g ha^{-1} on July 18 and Igran ® (active ingredient terbutryn) at 850mL ha^{-1} on August 4 to control broad-leaf weeds and Folicur ® at 200mL ha^{-1} on August 21 to prevent fungal disease. Harvest index cuts were taken shortly before harvest, with plots were harvested on December 4 in 1996 and December 3 in 1997. Meteorological data for Wagga Wagga are given in Table 5 of the Appendix.

4.2.6 Ariah Park

This experiment was sown at the Davey family's property, "Taradale" at Ariah Park (34° 20'S, 147° 10'E, Alt 260m) in south central New South Wales (long term average annual rainfall 513mm). The soil is a red earth, Dr 2.32 (Northcote *et al.* 1971). There were three replicates of three spring near-isogenic pairs (Bodallin±*tin*, Janz±*tin* and Hartog±*tin*) which were sown as a nine by nine lattice on May 20, 1997 (AP97) with 125kg ha^{-1} of Triphos (20.7% P). Plots were 6.5m long by 10 rows wide

at 19cm row spacings. Sowing rates were adjusted to achieve 170 kernels m^{-2} for lines with and without the *tin* gene as well as at 205 kernels m^{-2} for lines with the *tin* gene. There were 147 plants m^{-2} established for the cultivars and 145 and 158 plants m^{-2} established for *tin* lines. Nitrogen was top-dressed onto the plots as urea 23 days after sowing (DC 13) at 0, 80 and 160kg ha^{-1}. Plots were sprayed with Folicur ® at 250mL ha^{-1} on July 31 and September 6 to prevent fungal diseases. Harvest index cuts were taken shortly before harvest, which took place on October 18, 1997. Meteorological conditions are assumed to be similar to Temora Agricultural Research Station, 31km east-southeast of Ariah Park. Combined meteorological conditions for Ariah Park and Temora are given in Table 6 of the Appendix.

4.2.7 Biomass, leaf area index (LAI), light interception, tillering and root length density measurements

Quadrat cuts on the inner six (1.2m^2 at Condobolin, Moombooldool and Ariah Park) or four (1.0m^2 at Wagga Wagga) rows were taken throughout the growing season at all sites. Plants were partitioned into stems, leaves and at DC 65 (anthesis), spikes. Stem lengths were measured and leaf area index (LAI) values determined using a ΔT area measurement system fitted with a RCA video camera. Samples were dried at 70°C for 48 hours before being weighed. The number of tillers were counted at C96N and AP97 by marking 10 and 12 (or 15 at the higher sowing rate at AP97) plants per 50cm of row respectively and recording the total number of tillers every 7 to 10 days. Soil cores were taken at physiological maturity (DC 92) at C96N (rainfed) to a depth of 1.5m below the soil surface with a 52mm diameter steel coring tube which was pushed into the soil with a hydraulic ram. The core was divided into sections at 0.1, 0.2, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3 and 1.5m and roots washed out manually. The ΔT area measurement system fitted with a RCA video camera was calibrated with white cotton of known length and a subsample of 54 root samples scanned. A relationship between the dry weight of the subsamples and their estimated root length was determined and the root lengths calculated from the dry weights of the remaining root samples.

4.2.8 Meteorological data

Meteorological data were collected on site at the Condobolin Agricultural Research and Advise Station, Ardlethan Post Office, Temora Agricultural Research Station and Wagga Wagga Agricultural Research Station by the Bureau of Meteorology, as well as at Yanco Agricultural Institute. Rain gauges were installed at Moombooldool in 1996 and 1997. Meteorological data are presented in Tables 3 to 6 in the Appendix.

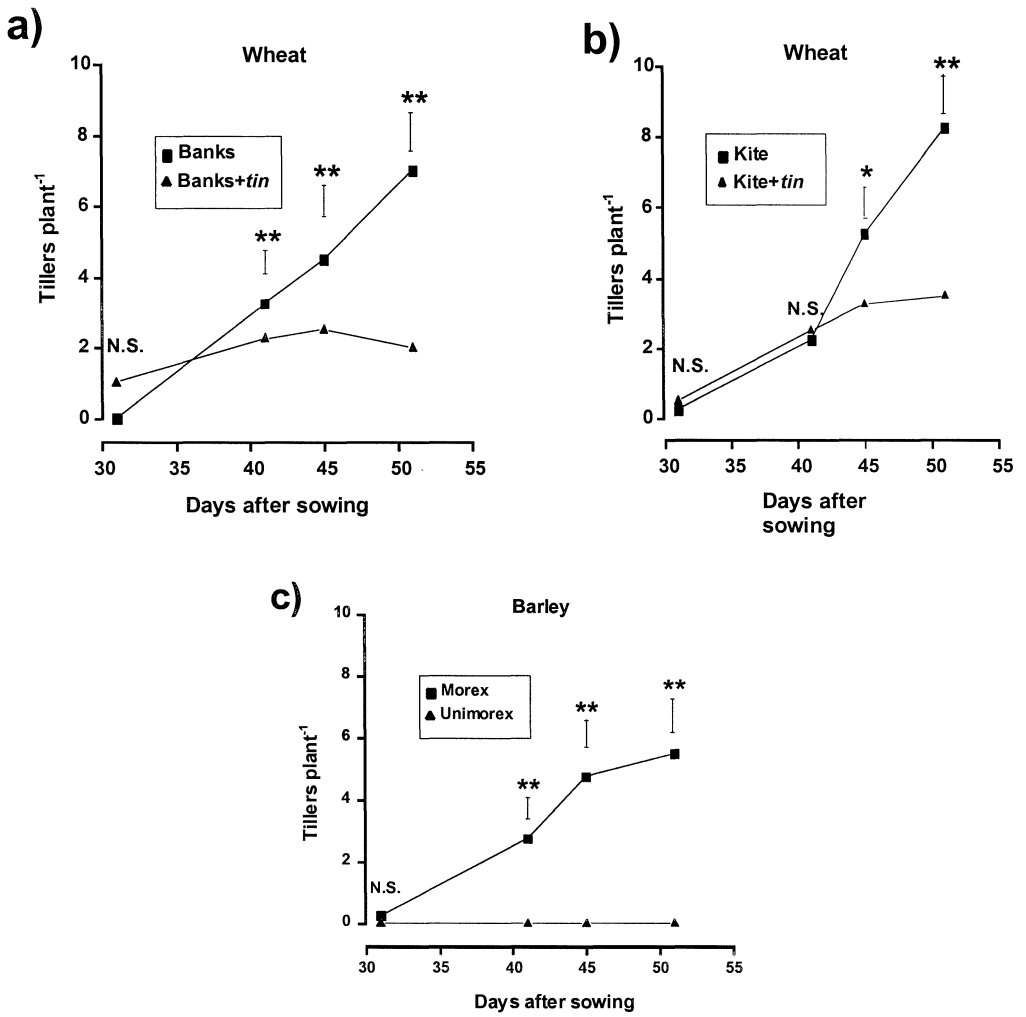
4.3 RESULTS

4.3.1 Pot Experiment

Unimorex failed to produce any tillers throughout the experiment. Near-isogenic pairs of both Banks and Kite developed the earliest tillers at the same time, however the rate of tillering rapidly decreased in both Banks+*tin* and Kite+*tin* soon afterwards compared to their isogenic counterparts (Fig 4.1). Tillering had ceased by DC 14 (41 days after sowing) in Banks+*tin* and by DC 15 (45 days after sowing) in Kite+*tin* whereas tillering was still occurring at DC 16 (51 days after sowing) in Banks and Kite.

Despite the large differences in tiller number plant^{-1} between the near-isogenic pairs, differences in leaf area and total biomass were small (Figs 4.2 and 4.3). No significant differences in leaf area between Banks and Banks+*tin* or between Kite and Kite+*tin* were found, except at the final harvest for the Kite pair. There was no significant difference in total biomass between the near-isogenic pairs at the final harvest. However, the biomass of Banks+*tin* was greater than that of Banks at DC 14 and 15 (41 and 45 days after sowing respectively), while it was at DC 14 for Kite+*tin*. The leaf area of Morex was significantly greater than Unimorex at DC 15 (45 days after sowing) and this difference continued until the final harvest. Differences in biomass between Morex and Unimorex were small with Morex only producing significantly more biomass at DC 15 (45 days after sowing) while there was again no difference by the final harvest (Fig 4.3c). The relative growth rates (g g^{-1} day^{-1}) were not significantly affected by the *tin* or *uc2* genes at most harvests and where the effects were significant, they were inconsistent across varieties and harvests (data not shown).

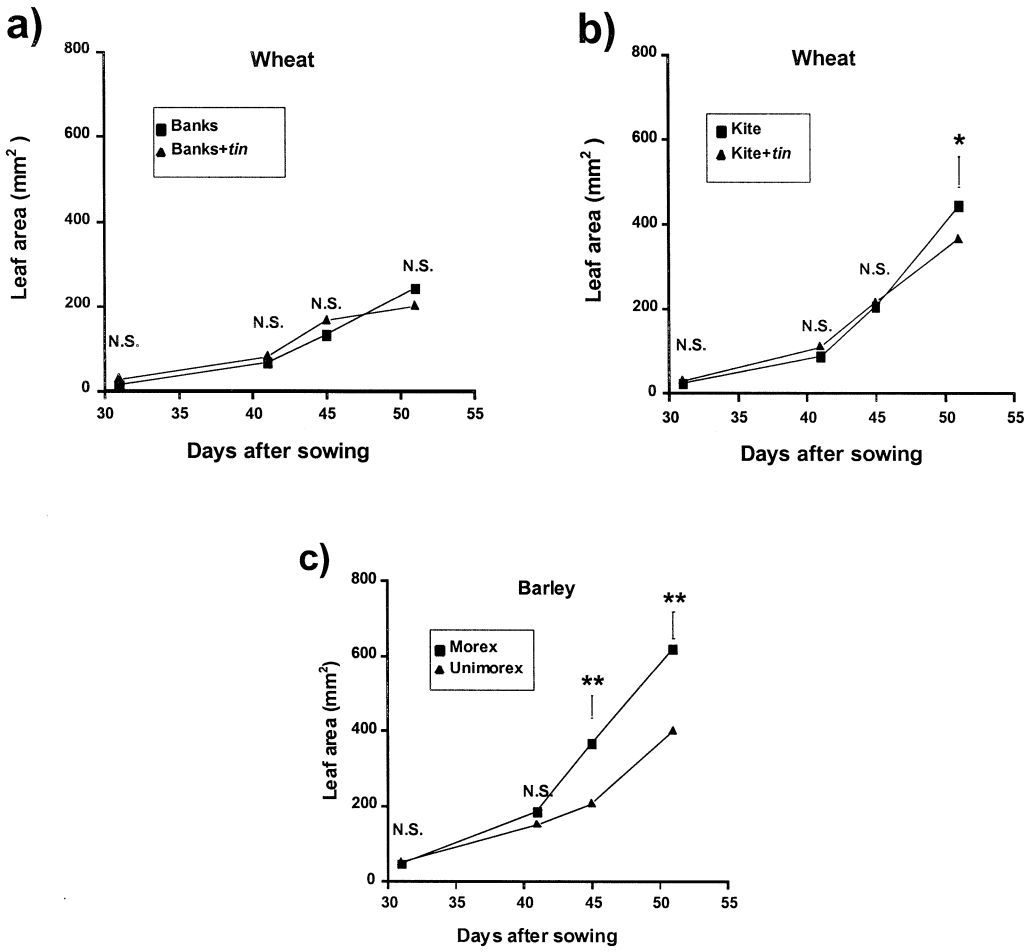
Figure 4.1 Average number of tillers plant^{-1} of a) Banks and Banks+*tin*, b) Kite and Kite+*tin* and c) Morex and Unimorex



LSD significance levels given at P=0.05

N.S. = not significant, * significant at P=0.05, ** significant at P=0.001

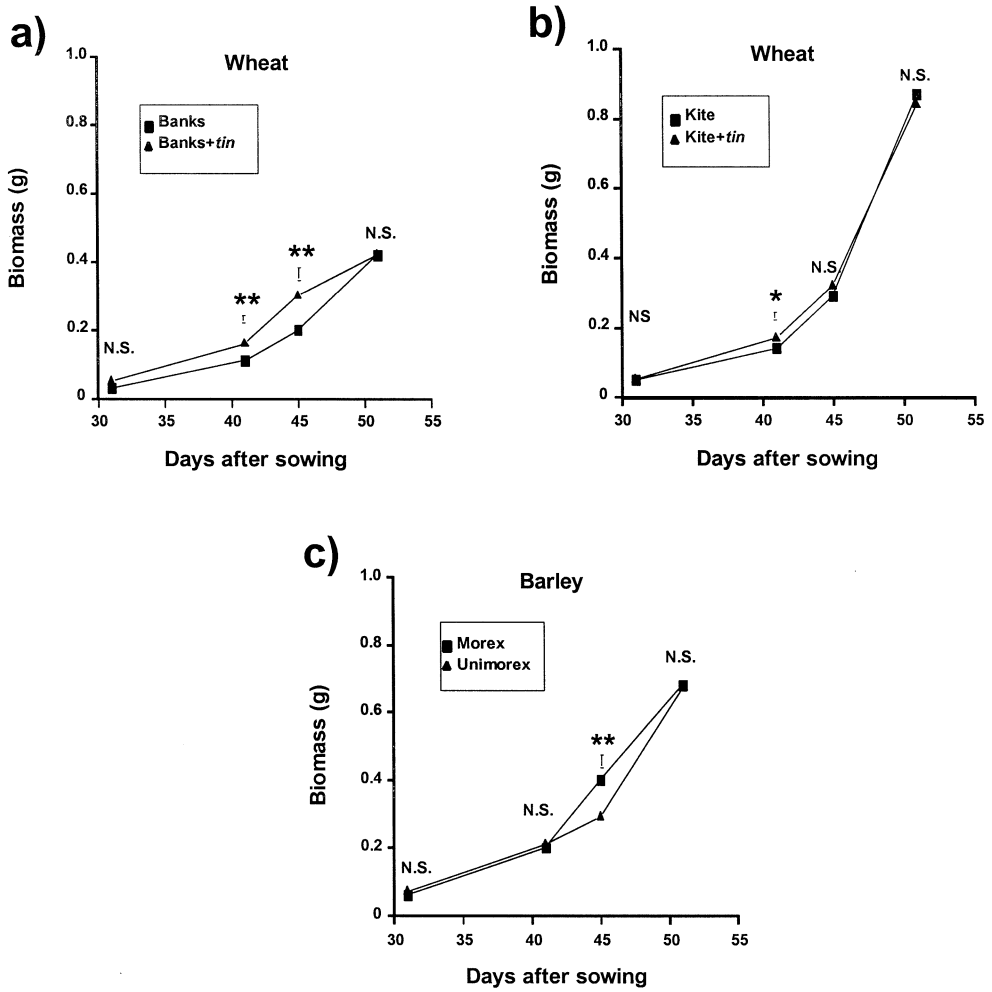
Figure 4.2 Leaf area (mm^2) per plant of a) Banks and Banks+*tin*, b) Kite and Kite+*tin* and c) Morex and Unimorex



LSD significance levels given at P=0.05

N.S. = not significant, * significant at P=0.05, ** significant at P=0.001

Figure 4.3 Above ground biomass (g) per plant for a) Banks and Banks+*tin*, b) Kite and Kite+*tin* and c) Morex and Unimorex

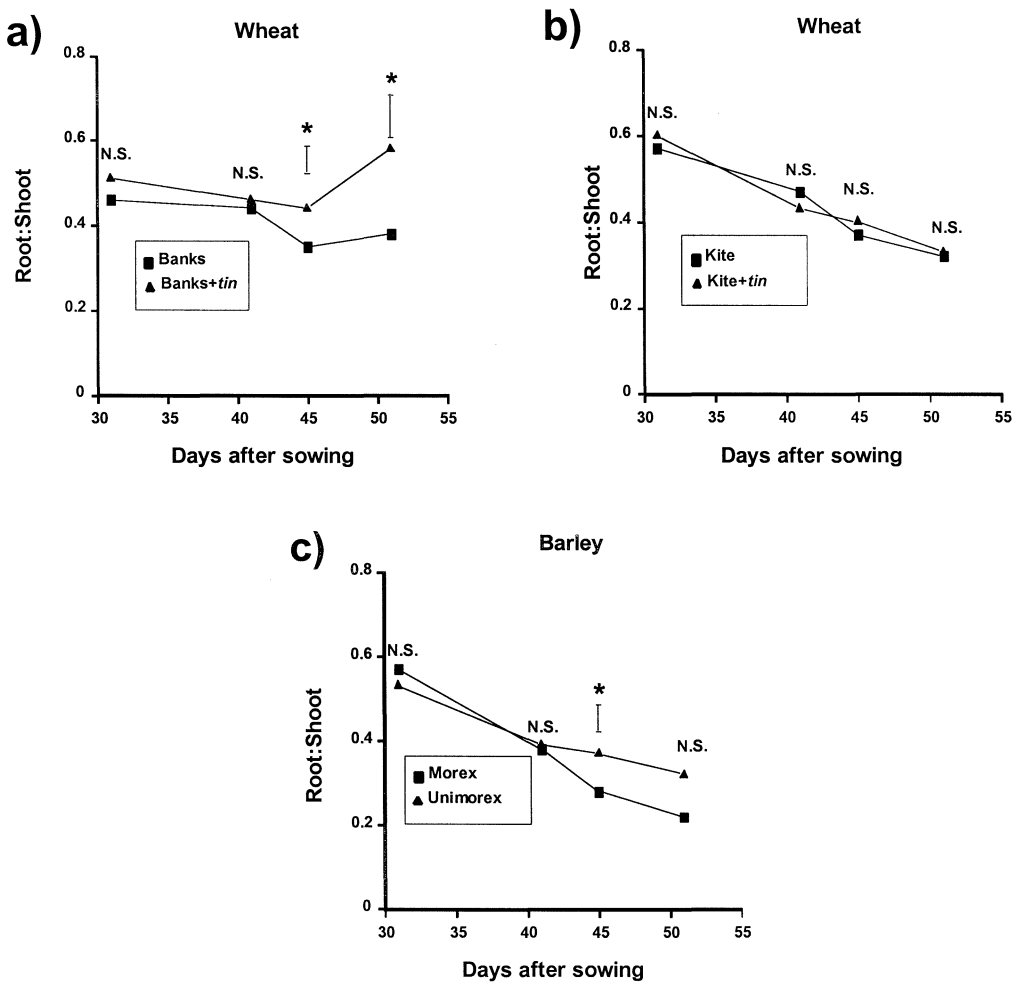


LSD significance levels given at P=0.05

N.S. = not significant, * significant at P=0.05, ** significant at P=0.001

The root:shoot ratio of all lines generally declined with progressive harvests (Fig 4.4), the only exception being Banks+*tin* where the root:shoot ratio was highest at the final harvest (51 days after sowing). At the final two harvests for Banks+*tin* and at the second last harvest for Unimorex, the root:shoot ratio was significantly greater than that of their near-isogenic pair. No differences in root:shoot ratio were found in the Kite near-isogenic pair at any harvest.

Figure 4.4 Root:shoot ratio at each harvest for a) Banks and Banks+*tin*, b) Kite and Kite+*tin* and c) Morex and Unimorex



LSD significance levels given at P=0.05

N.S. = not significant, * significant at P=0.05, ** significant at P=0.001

Some differences in the allocation of biomass between leaves and shoots were observed as well as in the ratio of leaf area to biomass (LAR). While there was no consistent effect of the *tin* gene on the ratio of leaf weight to shoot weight (LWR), Banks+*tin* and Unimorex had significantly lower SLA and LAR values than their commercial parents at harvests 2, 3 and 4. Values at the final harvest are given in Table 4.3.

Table 4.3 Specific leaf area (cm^2g^{-1}), Leaf area ratio (cm^2g^{-1}) and Leaf weight ratio for the final harvest of wheat and barley lines

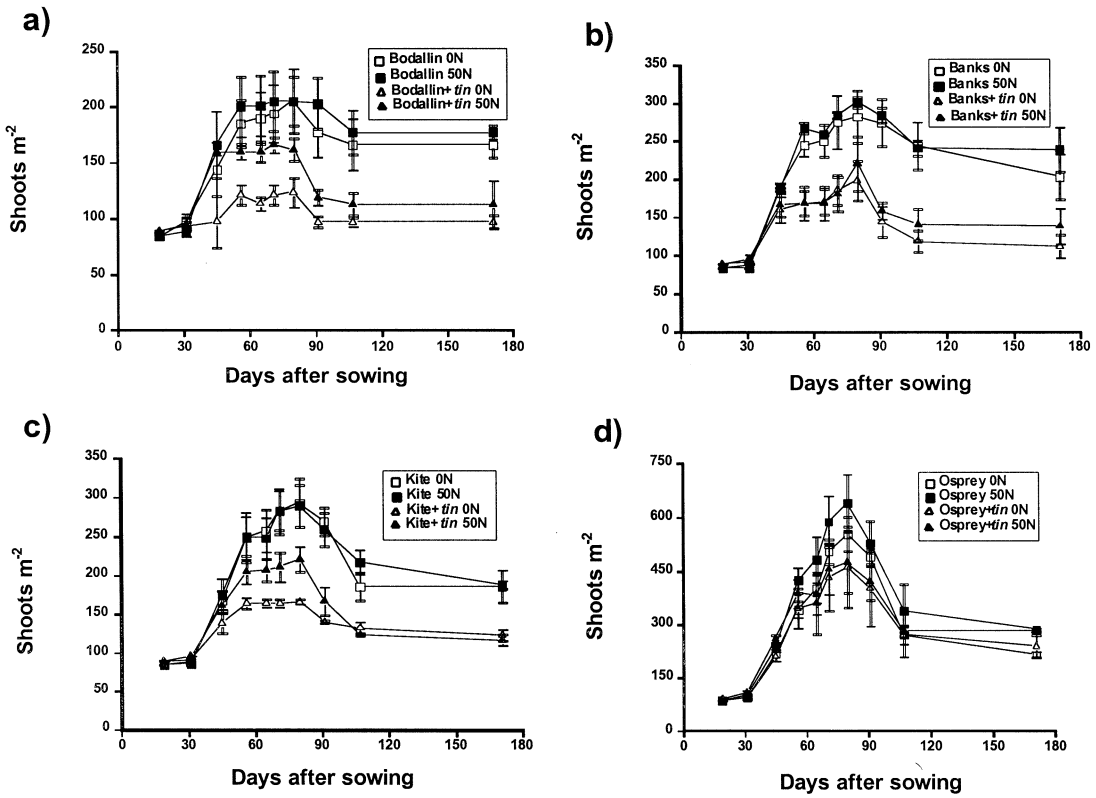
	SLA (cm^2g^{-1})	LAR (cm^2g^{-1})	LWR
Banks	310	203	0.65
Banks+*tin*	222	150	0.68
Kite	275	179	0.65
Kite+*tin*	265	164	0.62
Morex	426	292	0.68
Unimorex	329	214	0.65
LSD (0.05)	*37*	*27*	*0.03*

4.3.2 Field experiments

The *tin* gene resulted in a reduction in the number of tillers in all lines relative to their commercial parents at C96N and AP97 (Figs 4.5 and 4.6 respectively). While the application of nitrogen at 50kg ha^{-1} at C96N and at 160 kg ha^{-1} at AP97 increased tillering in the cultivars as well as the *tin* lines, the increased tillering in the *tin* lines did not usually approach the levels displayed by the cultivars. The reduction in tillering associated with the *tin* gene was due to inhibition of later formed tillers. There was some interaction between the genotype and nitrogen with several of the *tin* lines at both C96N and AP97 not demonstrating an increase in tiller production in response to nitrogen. Final tiller number was reduced on average by 34% in the presence of the *tin* gene at C96N and by 39% at AP97.

At C96N, the LAI of the *tin* lines differed significantly from the cultivars at DC 65 (Fig 4.7). This was almost certainly a function of the poor establishment (see Table 4.2) and the inability of the *tin* lines to produce tillers that would compensate for gaps in the canopy (see Fig 4.5). At AP97 where establishment was good however the LAI values for the *tin* lines at the standard and high sowing densities was the same as for the cultivars from sowing until DC 65.

Figure 4.5 Shoots m^{-2} of a) Bodallin±*tin*, b) Banks±*tin*, c) Kite±*tin* and d) Osprey±*tin* at 0 and 50 kg of nitrogen ha^{-1} at C96N

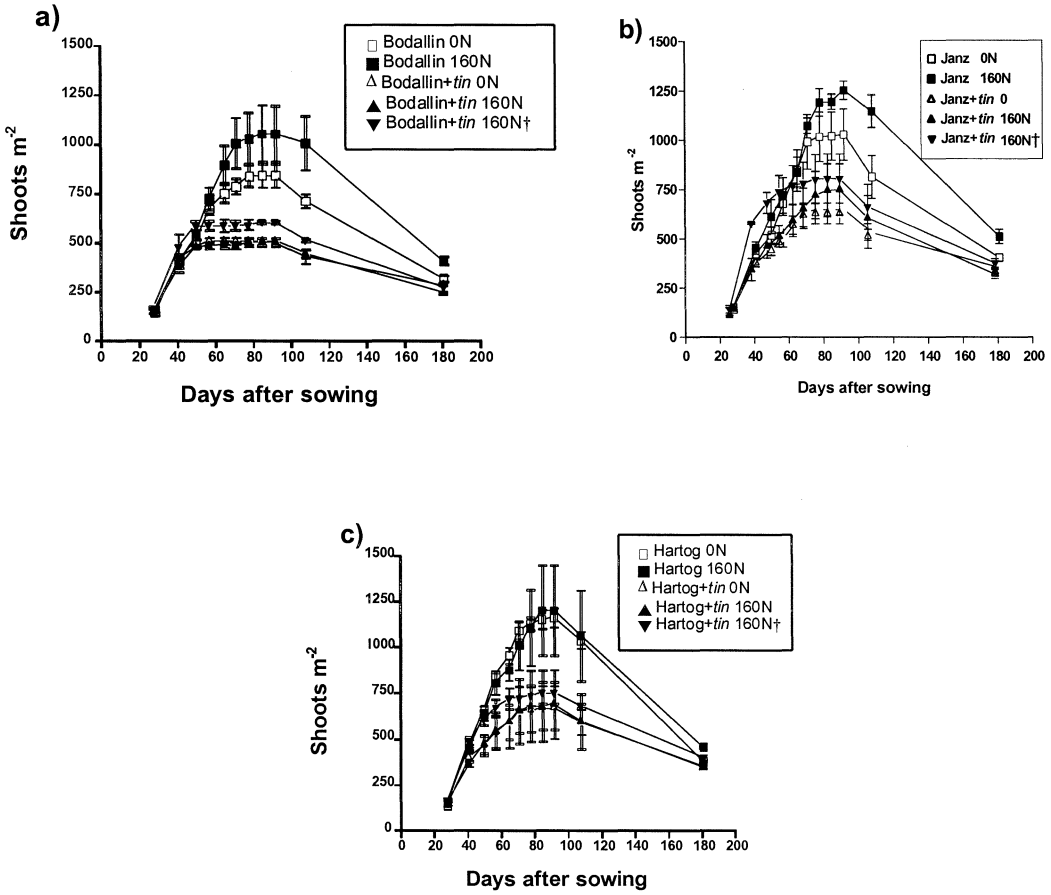


Error bars = ±standard error

There was less light intercepted by the *tin* lines at the lower sowing density at C96 (DC 37 and DC 65) and at C97 (DC 65) while they intercepted less light at the higher sowing density at C96 (DC 37 and DC 65), C97 (DC 65) and M97 (DC 15 and DC 65) (Table 4.4). Increasing the sowing density significantly increased light interception for the cultivars at C97 (DC 30) and W97 (DC 37) while it increased it for the *tin* lines at W96 (DC 65) and W97 (DC 37). While there was no significant difference in light interception between any treatment at AP97 at DC 16, by DC 30 the cultivars were intercepting more light than the *tin* lines at both sowing densities across all nitrogen treatments except at the higher sowing density where 80 kg ha^{-1} of nitrogen was applied (Table 4.5). However by DC 65 the cultivars were only intercepting more light than the *tin* lines at both sowing densities where no nitrogen had been applied and at the same sowing rate where 160 kg ha^{-1} had been applied. At both DC 30 and DC 65, more light was intercepted by all crops which had had 80 kg

ha^{-1} of nitrogen applied compared to those that had had none, although there was not significantly more light intercepted when a further 80 kg ha^{-1} was applied.

Figure 4.6 Shoots m^{-2} of a) Bodallin±*tin*, b) Janz±*tin* and c) Hartog±*tin* at 0 and 160 kg of nitrogen ha^{-1} at Ariah Park in 1997

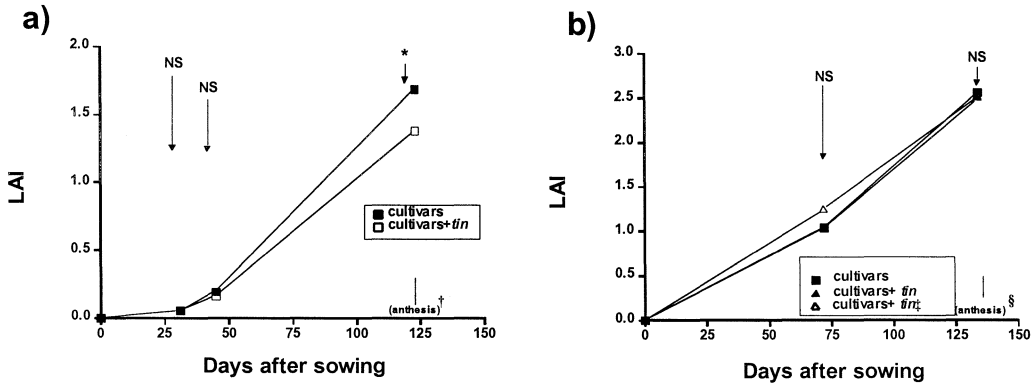


† higher sowing density plots

Error bars = ±standard error

The biomass of the cultivars and the *tin* lines did not differ significantly at any time between sowing and DC 92 at C95.1 or C95.2 (Fig 4.8). Under both rainfed and irrigated conditions at C96N the cultivars produced significantly more biomass by DC 92 than the *tin* lines despite there being no significant difference at DC 65. At AP97, the cultivars had produced significantly more biomass than the *tin* lines at either sowing density but by DC 92 the difference between the cultivars and the *tin* lines at the higher sowing density was no longer significant.

Figure 4.7 Leaf area index (LAI) of the cultivars and *tin* lines from sowing until DC 65 at a) C96N and b) AP97



LSD significance levels given at P=0.05

N.S. = not significant, * = significant at P=0.05, ** = significant at P=0.001

† At C96N, Bodallin±*tin* reached DC 65 115 days after sowing, Kite±*tin* and Banks±*tin* after 120 days and Osprey±*tin* after 133 days

‡ higher sowing density

§ At AP97, Bodallin±*tin* reached DC 65 134 days after sowing, while Janz±*tin* and Hartog±*tin* reached anthesis after 142 days

Table 4.4 Percentage light interception of commercial cultivars and lines containing the *tin* gene at two sowing rates at five environments at various times throughout the growing season

	Sowing density (kernels m^{-2})	C96 DC 37	C96 DC 65	W96 DC 65	C97 DC 30	C97 DC 65	M97 DC 15	M97 DC 65	W97 DC 37
Cultivars	150	92.1	74.4	63.4	38.1	78.8	49.3	74.4	54.4
Cultivars+*tin*	150	80.2	67.3	58.7	44.2	71.8	47.8	73.1	55.9
Cultivars	300	95.3	80.3	77.4	50.9	80.6	60.8	76.6	75.1
Cultivars+*tin*	300	87.0	73.5	72.8	53.3	73.2	56.2	72.5	75.2
LSD (P=0.05)		*3.3*	*4.2*	*6.8*	*9.4*	*5.3*	*4.5*	*3.9*	*5.6*

DC 15 = 5th leaf emerging; DC 30 = start of stem elongation; DC 37 = flag leaf emerging; DC 65 = anthesis

Table 4.5 Percentage light interception of commercial cultivars and lines containing the *tin* gene (at two sowing rates) at three rates of nitrogen (0, 80 and 160 kg ha^{-1}) at Ariah Park, 1997

	N fertiliser (kg ha^{-1})	DC 16	DC 30	DC 65
Cultivars	0	35.1	71.7	78.1
Cultivars+*tin*	0	30.0	63.5	72.2
Cultivars+*tin* (hsd)	0	34.3	66.3	72.4
Cultivars	80	35.5	76.9	85.7
Cultivars+*tin*	80	32.7	70.4	82.2
Cultivars+*tin* (hsd)	80	39.8	72.8	81.5
Cultivars	160	35.3	78.9	88.3
Cultivars+*tin*	160	34.1	69.4	83.7
Cultivars+*tin* (hsd)	160	38.5	71.8	84.1
LSD (P=0.05)		*N.S.*	*5.0*	*4.6*

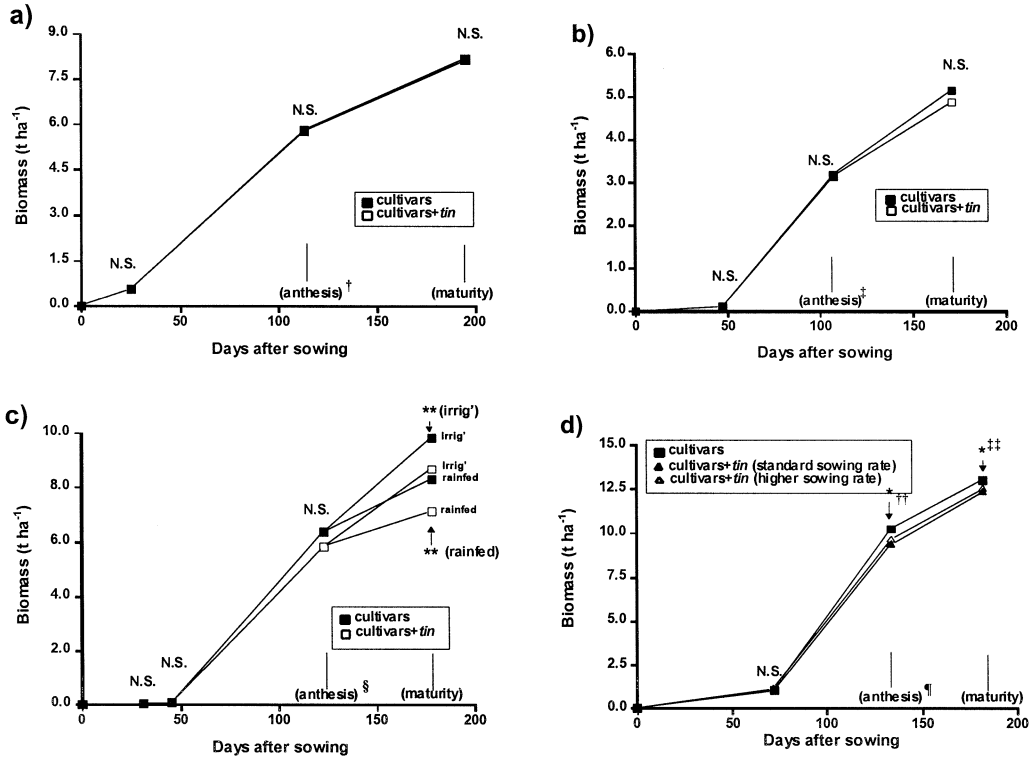
DC 16 = 6th leaf emerging; DC 30 = start of stem elongation; DC 65 = anthesis
hsd = higher sowing density

Although the proportion of the stem weight declined for both the cultivars and the *tin* lines from DC 65 to DC 92 at C95.1, the decrease was much less than at most other sites (Table 4.6). For all components of biomass there was no significant difference between the cultivars and the *tin* lines at DC 65 and DC 92 except for dead leaves of which there was a significantly greater proportion for the *tin* lines compared to the cultivars at DC 65. At C95.2, the proportion of the biomass allocated as stem at DC 65 was significantly greater for the cultivars relative to the *tin* lines. For all other biomass components at DC 65 and all biomass components at DC 92 however there was no difference in allocation between the cultivars and the *tin* lines. A significantly greater proportion of biomass was allocated as spikes for the *tin* lines compared to the cultivars as DC 65 and under both rainfed and irrigated conditions at DC 92. There was proportionally significantly more leaf biomass at DC 92 under both rainfed and irrigated conditions for the cultivars relative to the *tin* lines and although there was no

difference in green leaf biomass at DC 65 there was significantly more dead leaf for the cultivars than the *tin* lines. There was no difference in the proportion of biomass allocated as stems between the cultivars and the *tin* lines at DC 65 although by DC 92 it was much lower for the *tin* lines under both rainfed and irrigated conditions. There was also little difference in the allocation of biomass between the cultivars and the *tin* lines at DC 65 and DC 92 at M97. The only component that differed significantly at this site was the stem biomass, which was greater for the *tin* lines relative to the cultivars at both DC 65 and DC 92. At AP97, significantly more biomass was allocated to the stems of the cultivars than the *tin* lines at either sowing density at both DC 65 and DC 92. There was no difference in the proportion of dead leaf material between the cultivars and the *tin* lines at DC 65, although there was significantly more green leaf material for the *tin* lines at both sowing densities relative to the cultivars. However, by DC 92 the difference in leaf material had disappeared.

The *tin* gene did not significantly affect the rooting pattern when it was examined for the rainfed treatment at C96N, except between 20 and 30 cm where it significantly increased root length density (Fig 4.9). For all samples taken below this however, the root length density tended to be the same or lower for the *tin* lines, resulting in there being no difference when averaged to a depth of 150cm (7.5mm cm^{-3} for both cultivars and *tin* lines).

Figure 4.8 Biomass of the cultivars and *tin* lines from sowing until DC 92 at a) C95.1, b) C95.2, c) C96N and d) AP97



LSD significance levels given at P=0.05

N.S. = not significant,* = significant at P=0.05, ** = significant at P=0.001

† At C95.1, Bodallin±*tin* reached DC 65 113 days after sowing, Kite±*tin* and Banks±*tin* after 127 days and Osprey±*tin* after 141 days

‡ At C95.2, Bodallin±*tin*, Kite±*tin* and Banks±*tin* reached DC 65 after 97 days and Osprey±*tin* after 112 days

§ For DC 65 dates at C96N, see Fig 4.7

¶ For DC 65 dates at AP97, see Fig 4.7

†† Significant (P≤0.05) difference between the cultivars and the *tin* lines at both sowing densities

‡‡ Significant (P≤0.05) difference between the cultivars and the *tin* lines at the standard sowing density

Table 4.6 Proportional biomass allocation for cultivars and *tin* lines at anthesis (DC 65) and maturity (DC 92) at 5 sites over 3 years

	C95.1			C95.2			C96N					
Anthesis												
(DC 65)	*-tin*	*+tin*		*-tin*	*+tin*		*-tin*	*+tin*				
Stem	56.4	57.1	N.S.	57.0	57.9	*	63.0	62.0	N.S.			
Leaf	18.5	16.4	N.S.	20.4	19.4	N.S.	14.5	14.5	N.S.			
Dead leaf	12.9	13.1	**	2.4	2.6	N.S.	4.7	3.4	*			
Spike	12.2	13.4	N.S.	20.1	20.2	N.S.	17.7	20.1	**			
Maturity							Rainfed			Irrigated		
(DC 92)	*-tin*	*+tin*		*-tin*	*+tin*		*-tin*	*+tin*		*-tin*	*+tin*	
Stem	54.7	56.9	N.S.	35.4	37.5	N.S.	32.2	28.7	**	39.5	37.4	**
Leaf	15.2	15.8	N.S.	8.1	18.1	N.S.	10.6	10.2	*	9.6	9.5	*
N.G. spike	11.7	11.0	N.S.	18.9	7.7	N.S.	15.8	17.1	*	13.9	15.5	**
Grain	18.4	16.3	N.S.	37.6	36.8	N.S.	41.4	44.0	**	37.0	37.7	**

	M97			AP97			
Anthesis							
(DC 65)	*-tin*	*+tin*		*-tin*	*+tin*	*+tin* (hsd)	
Stem	71.6	70.6	*	65.8	64.0	64.2	*
Leaf	8.2	8.7	N.S.	10.8	12.3	12.0	*
Dead leaf	4.0	4.0	N.S.	5.0	4.7	5.0	N.S.
Spike	16.2	16.7	N.S.	18.4	19.0	18.8	N.S.
Maturity							
(DC 92)	*-tin*	*+tin*		*-tin*	*+tin*	*+tin* (hsd)	
Stem	39.5	37.4	**	41.3	38.6	38.8	*
Leaf	9.6	9.5	N.S.	9.4	9.6	9.3	N.S.
N.G. spike	13.9	15.5	N.S.	15.9	15.5	15.4	N.S.
Grain	37.0	37.7	N.S.	33.4	36.5	37.0	*

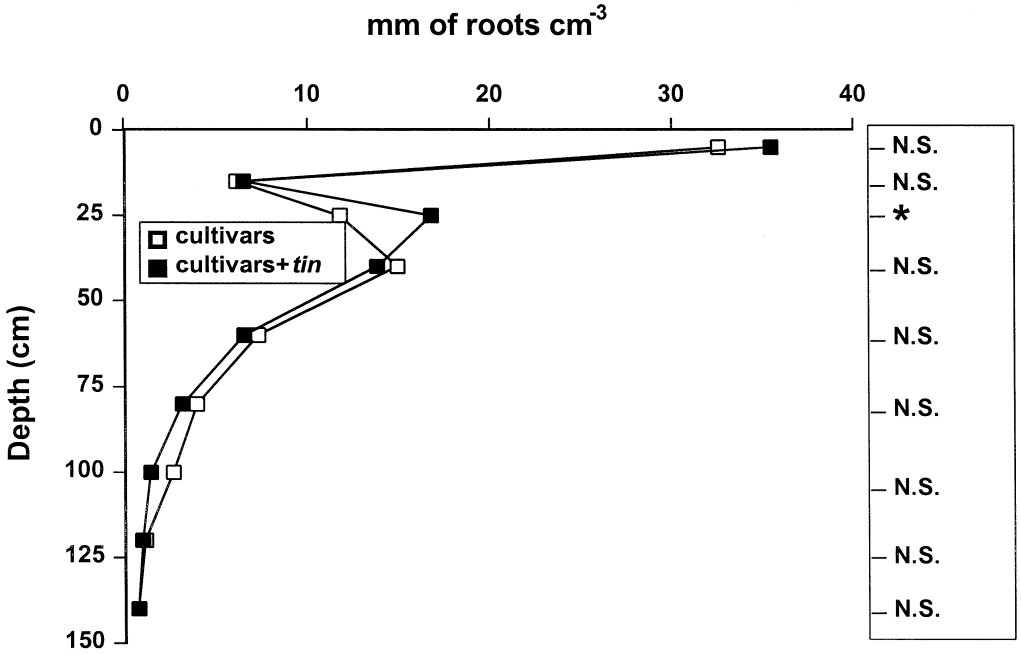
N.G. spike = non-grain spike

LSD significance levels given at P=0.05

N.S. = not significant,* = significant at P=0.05, ** = significant at P=0.001

(hsd) = higher sowing density

Figure 4.9 Root length density (mm of root per cm^3 of soil) throughout the soil profile of cultivars and *tin* lines at C96N (rainfed) at maturity (DC 92)



N.S. = not significant at P=0.05, * = significant at P=0.05

4.4 DISCUSSION

4.4.1 Tillering

The *tin* gene results in a significant reduction in tiller number in spring wheat by the time the plants had produced between 4 and 5 mainstem leaves (DC 15 to 16) in both pot and field conditions. The total number of shoots (tillers+mainstem) produced tended to be lower in the winter line Osprey+*tin* compared to Osprey at C96N, although at no point was the difference significant. The effect of the *tin* gene may occur too late to significantly affect tillering in the winter lines which cease tillering later than spring lines. Tillering slows after double ridge formation (Gomez-MacPherson *et al*, 1998a) and has usually ceased by terminal spikelet (Rawson, 1971; Baker and Gallagher, 1983; Thorne and Wood, 1988; Craufurd and Cartwright, 1989; Gomez-MacPherson *et al*, 1998a) in wheat. Although records of phenological development dates were not taken on the data presented in this paper, results presented Chapter 2 indicate that there is a significant difference in the number of

tillers produced by a wheat plant shortly after double ridge formation. Exactly when this occurs is largely influenced by both genotype and the environment, although by 450 degree days after sowing the *tin* lines have ceased tillering whereas their near-isogenic pairs have not yet reached their maximum number of tillers per plant. The action of the *tin* gene must therefore occur at or slightly prior to double ridge formation. This would explain the reduced expression of the *tin* gene in winter wheat lines as a greater proportion of the total number of tillers in the winter lines would be laid down before they reached double ridge formation compared to the spring lines making a difference in tiller number more difficult to observe.

4.4.2 LAI and light interception

The leaf area of the *tin* lines in the pot experiment remained similar to the cultivars at all times except for the Kite near-isogenic pair where the cultivar produced significantly more leaf area than the *tin* line. This is not surprising, as the mainstem leaves would contribute the majority of the leaf area to this point. Data from Chapter 3 would also appear to indicate that the early mainstem leaves of lines containing the *tin* gene are longer and wider than their freely tillering near isogenic pairs. Whether this is the result of in increased availability of assimilates that would otherwise have been directed into tillers or an intrinsic effect of the *tin* gene is unknown. The leaf area of the *tin* lines under field conditions was not significantly different from that of the cultivars, except at DC 65 at C96N when it was significantly lower. The cultivars appeared to have been better able to compensate for the gaps in the canopy and tillers that would have failed to elongate under higher plant densities did so and contributed significantly to leaf area. The leaf area of the cultivars at AP97 was not significantly different from that of the *tin* lines at either the same or at the 20% higher sowing density, even though shoots m^{-2} was significantly higher. Atsmon and Jacobs (1977), Marshall and Boyd (1985), Richards (1988) and data presented in Chapter 3 have all reported that the later leaves of the *tin* lines are longer and wider than the leaves of lines without the *tin* gene. The leaf area of the crops therefore appears to be maintained, provided plant density is not too low, as some of the assimilate that is normally invested in tillers in the cultivars is invested in the leaves of those tillers that are produced in the *tin* lines.

The *tin* gene resulted in a significant reduction in light interception at both sowing rates at C96 (DC 37 and 65) and C97 (DC 65), as well as at the higher sowing rate at M97 (DC 15 and 65). What is interesting is that the measurements taken at the standard (i.e. lower) sowing rate early in the season at M97 (DC 15) as well as at all the sowing densities and all nitrogen treatments at AP97 (DC 16) indicates that there is no difference in light interception between the cultivars and the *tin* lines, agreeing with the LAI data. This is an important finding as in Mediterranean-like environments shading of the soil is important in that it significantly reduces soil evaporation (Cooper *et al.* 1987) as well as the plant's competitive ability against weeds (Lemerle *et al.* 1996).

The *tin* gene reduced light interception at AP97 at DC 30 at all rates of nitrogen and at 0 and 160kg ha^{-1} of nitrogen at DC 65. Nitrogen fertiliser at 80kg ha^{-1} increased light interception in the cultivars and the *tin* lines at both sowing rates. This is almost certainly due to the increase in tillering and subsequently leaf area. The further application of 80kg ha^{-1} of nitrogen had no significant impact on light interception by the crop and it may be that any extra tillering associated with the additional nitrogen may have produced no significant increase in leaf area.

It should be noted that while transpiration is correlated with LAI up to a value of approximately 3 (Ritchie and Burnett, 1971), light interception by the crop is not necessarily correlated with leaf area. Light interception does not appear to be valid in estimating LAI when comparing cultivars with *tin* lines. The LAI data indicates that there is no significant difference between the cultivars and the *tin* lines at DC 65 at M97, while the data presented here indicates that more light was intercepted by the cultivars. This would appear to indicate that leaves of the *tin* lines are more erect, thus allowing greater light penetration into the canopy. The thicker (greater SLA values – see Table 4.3) leaves of some of the *tin* lines may provide enough support so that a significant percentage of the leaves do not "flop over" and shade the lower canopy.

4.4.3 Biomass accumulation and partitioning

The above ground plant biomass of the *tin* lines was the same and sometimes significantly greater than that of the cultivars early in their development under both pot and field conditions. This indicates that the *tin* lines are able to produce as much

assimilate as the cultivars early in their growth. It is unclear why the *tin* lines had significantly greater biomass at DC 14 in the pot experiment and why this continued for Banks+*tin* at DC 15 but the difference for both *tin* lines was again non-significant by DC 16. It is also difficult to explain the higher root:shoot ratio for Banks+*tin* at the DC 14 and 15, but as this line is extreme in its suppression of tillering (see Chapter 5), it may be that this is the results in the increased partitioning of assimilates to root growth early in the plant's development. The biomass of the *tin* lines also did not differ from the cultivars except under field conditions at C96N (rainfed and irrigated) at DC 92 and at AP97 at DC 65 (when the cultivars produced significantly more biomass than the *tin* lines under both sowing rates) and DC 92 (when the cultivars produced more biomass than the *tin* lines at the standard sowing density). The lower plant density may have allowed greater light penetration and the extra biomass to be produced by the cultivars in both the irrigated and rainfed plots containing the cultivars at C96N due to later tillers which were not produced by the *tin* lines continuing to develop (see Fig 4.8). The small but significant reduction in biomass at AP97 brought about by the *tin* gene may also be due to lack of later formed sterile tillers. This result indicates that sowing rates would not need to be significantly adjusted when sowing lines containing the *tin* gene, except at very low densities when they are unable to produce enough tillers, and subsequently biomass, to compensate and yield is reduced.

There did not appear to be any consistency between sites at anthesis for partitioning of assimilate although at the sites where pre-DC 65 drought did not occur (i.e. C96N, M97 and AP97) there was a tendency for relatively more stem biomass for the cultivars. This difference was exaggerated further by maturity with more biomass being partitioned into non-grain spike biomass at C96N (rainfed and irrigated) and grain at C96N (rainfed and irrigated) and AP97 for the *tin* lines. These results, discussed in further detail in Chapter 5, indicate a more efficient partitioning of biomass into grain for the *tin* lines.

4.4.4 Root length density

The root length density data from rainfed conditions at C96N indicates that the root system of lines containing the *tin* gene do not differ from those without when

grown under field conditions. The significant increase in root length density between 20 and 30cm is difficult to explain as it is not significantly different at any other depth in the profile. If root growth has virtually ceased completely by DC 65 as reported by Gregory *et al* (1978), then the root:shoot ratio must be the same for the cultivars and *tin* lines at DC 65 as the biomass of the cultivars and the *tin* lines is not significantly different despite the tendency for the ratio to be higher for the *tin* lines early in the plant's growth (Fig 4.4).

4.5 CONCLUSION

The *tin* gene reduced tiller number in the spring wheat lines by causing an earlier cessation in tiller production and under field conditions it also tended to reduce the number of tillers produced by the one winter line tested in the experiments listed in this paper. Leaf area for seedlings and the LAI for the crop were not usually significantly affected by the presence of the *tin* gene except under field conditions when plant density was very low and the *tin* lines were unable to compensate for gaps in the canopy. However light interception by crops was lower for the *tin* lines on various points throughout the growing season at several sites. Biomass and root:shoot ratios were as high and sometimes higher for seedlings grown in pots up to DC 16. Under field conditions biomass was typically the same for the lines containing the *tin* gene and the cultivars early in the growing season although it was occasionally lower later in the season at some sites. At sites that experienced a pre-DC 65 drought, there were very few significant differences in the proportional allocation of biomass. However at the other sites the allocation of assimilate to stems was often considerably lower for the *tin* lines compared to the cultivars at both DC 65 and DC 92. How the biomass was redistributed throughout the plant varied between sites, although there was a tendency for a greater proportion of it to exist as grain at DC 92. The rooting pattern of the *tin* lines did not appear to differ significantly from the cultivars indicating that the root:shoot ratio was also unchanged.

CHAPTER 5

THE EFFECT OF THE *tin* GENE ON YIELD, YIELD COMPONENTS, HARVEST INDEX, BIOMASS AND WATER USE

Field trial at Ariah Park, NSW, in 1997

5.1 INTRODUCTION

Genetically manipulating leaf area has been proposed as a means of increasing yield under terminal drought conditions through the conservation of soil moisture (Richards, 1983; Richards and Townley-Smith, 1987). Passioura (1977) found that plants, which used a greater percentage of their water after anthesis (DC 65) gave higher harvest, index values regardless of the level of water that was supplied for a variety of cultivars. Any means by which plants could conserve soil moisture under terminal drought conditions (i.e. when soil moisture becomes limited during grain filling) could therefore potentially increase grain yields.

The transpiration rate of plants in a crop canopy is proportional to the leaf area index (LAI) until it reaches a value of around 3, above which it becomes proportional to potential evaporation (Ritchie and Burnett, 1971). As LAI rarely exceeds 3 in areas where lack of water limits yield, transpiration is related to leaf area for most of the life of the crop (Richards, 1983). Under prolonged drought conditions, Richards and Townley-Smith (1987) found that lines with reduced leaf areas conserved soil moisture that they were able to use after DC 65. These lines had higher harvest index values and produced greater yields. Similar improvements were made in water-use efficiency, harvest index values and grain yield when leaf areas were artificially reduced by cutting off half the lamina of each leaf produced, as well as by removing tillers by cutting them at the base (Richards, 1983).

Under semi-arid Mediterranean environments, Islam and Sedgley (1981) found that crops consisting of plants which were detillered to form biculms gave greater yields than those consisting of plants that averaged four spikes per plant. Detillered plants had a lower LAI prior to DC 65 and were better able to conserve soil moisture for grain filling than the freely tillering lines. Even though there were fewer spikes per plant, the biculm plants produced more kernels, a similar total biomass at physiological maturity (DC 92) and significantly higher harvest index values (Islam and Sedgley, 1981).

Using genetically low tillering lines, Yunusa and Sedgley (1992) found no difference in the yields of reduced tillering lines compared to freely tillering lines under semi-arid Mediterranean environments as leaf area index was not significantly suppressed in the low tillering lines due to an increase in individual leaf size resulting

in there being no difference in water use efficiency. Uniculm barley lines were also shown to produce yields comparable to those of freely tillering lines (McDonald, 1990). However it was claimed that they might be unsuitable for environments were water stress occurs late in the season due to their greater sink capacity which the plants would be unable to accommodate. However, when grown at higher sowing rates where they achieved spike densities similar to those produced by a commercial crop, the uniculm lines yielded more than freely tillering cultivars (Donald, 1979).

The aims of the experiments reported in this chapter were to examine the effect that the *tin* gene has on yield, yield components, harvest index, canopy light interception and water use under field conditions. Sites were chosen in areas of the eastern Australian wheat-belt which commonly experience post-DC 65 moisture stress as it has been hypothesised by Islam and Sedgley (1981) and Richards (1983) that this will be the area where any benefits of reduced tillering will be observed. Experiments designed to evaluate whether lines containing the *tin* gene benefit from being grown at a higher plant density or at higher levels of nitrogen will also be reported.

5.2 MATERIALS AND METHODS

Experiments were conducted over three seasons (1995, 1996 and 1997) at four sites in central and southern New South Wales and sown from late May until early July. Site descriptions, sowing dates, plant establishment counts and agronomic practices are given in Chapter 4. At all sites, crop growth stages were identified using the Zadoks (DC) code system (Zadoks *et al.* 1974)

5.2.1 Biomass, yield and yield components

Quadrant cuts on the inner six ($1.2m^2$ at Condobolin, Moombooldool and Ariah Park) or four ($1.0m^2$ at Wagga Wagga) rows were taken shortly before machine harvest to determine biomass at DC 92. Samples were weighed and the number of fertile spikes determined. A sub-sample was taken and partitioned into stems, leaves and heads. Heads were then threshed in an F Walter and H Wintersteiger LD single head thresher and harvest index values determined. Plot yields were determined by machine harvesting of plots while kernel weights were determined using machine

harvested kernels. Kernels m^{-2} was determined by dividing yield m^{-2} by kernel weight while kernels $spike^{-1}$ was determined by dividing kernel number m^{-2} by the number of fertile spikes m^{-2}.

5.2.2 Soil water

Water use for C95.1, C95.2 and C96N was determined with a neutron moisture meter (Troxler Electronic Laboratories Inc. Model 3222 Gauge) using a locally determined calibration. Aluminium access tubes (52mm diameter by 160cm) were inserted into the soil in the centre of the plots shortly after sowing. Water content of the soil from the cores was used to calibrate the moisture meter and readings were taken soon after sowing, DC 65 and DC 92 to determine the volumetric water content. Readings were taken at 10, 20, 30, 40, 50, 60, 80, 100, 120, 140 and 160cm below the soil surface. Total soil water to 160cm was determined by summing the volumetric water content at each sampling depth to determine soil water content.

5.3 RESULTS

5.3.1 Yield

Although site by *tin* gene interaction was significant (P=0.028), across all sites where it was tested there was no significant effect of the *tin* gene on yield (P=0.360) Grain yields ranged from 1.0t ha^{-1} at C95.1 to 5.2t ha^{-1} at W96 (Tables 5.1, 5.2 and 5.3). The interaction between the *tin* gene and density was significant however (P=0.050) with the *tin* lines on average yielding 0.2t ha^{-1} more at the higher sowing density while it was unaffected for the cultivars. Yield was significantly lower for the *tin* lines at C96N for both levels of nitrogen under rainfed conditions as well at the higher level of nitrogen under irrigated conditions (Table 5.2). This was a direct result of the poor plant establishment achieved at this site. The application of nitrogen fertiliser increased the yield of the *tin* lines under rainfed conditions but made no significant difference under irrigated conditions. The *tin* gene reduced yield at AP97 when sown at the higher density compared to the cultivars sown at the standard density when no nitrogen was applied (Table 5.3). However, when 80kg ha^{-1} of nitrogen was applied the *tin* lines at the higher sowing density yielded significantly more than both the cultivars and the *tin* lines at the standard sowing density.

Table 5.1 Yield (t ha^{-1}) of commercial cultivars and lines containing the *tin* gene at two sowing rates (150 and 300 kernels m^{-2}) at eight sites over three growing seasons

	Sowing rate (kernels m^{-2})	C95.1	C95.2	C96	M96	W96	C97	M97	W97
		Yield (t ha^{-1})							
Cultivars	150	1.05	1.92	2.61	2.44	5.14	2.09	2.64	4.31
Cultivars+*tin*	150	1.04	1.75	2.78	2.32	4.89	2.13	2.57	4.44
Cultivars	300	1.11	1.96	2.58	2.37	5.32	2.25	2.65	4.41
Cultivars+*tin*	300	0.92	1.72	2.96	2.43	5.54	2.34	2.50	4.88
LSD (P=0.05)		*N.S.*	*0.20*	*N.S.*	*N.S.*	*0.27*	*N.S.*	*N.S.*	*0.31*

N.S. = not significant

Table 5.2 Yield and yield components of commercial cultivars and lines containing the *tin* gene (sown at 150 kernels m^{-2}) at two rates of nitrogen at C96N under rainfed and irrigated conditions

	N fertiliser (kg ha^{-1})	Yield (t ha^{-1})	Spikes m^{-2}	Kernels $spike^{-1}$	Kernel wt (mg)	Harvest index	Mature biomass (t ha^{-1})
Rainfed							
Cultivars	0	3.35	193	44	40.1	0.42	8.04
Cultivars+*tin*	0	2.97	146	51	44.5	0.45	6.71
Cultivars	50	3.53	223	42	39.8	0.41	8.55
Cultivars+*tin*	50	3.29	182	49	41.8	0.44	7.51
LSD (P=0.05)		*0.26*	*26*	*6*	*1.7*	*0.01*	*0.70*
Irrigated							
Cultivars	0	3.90	203	47	42.9	0.42	9.29
Cultivars+*tin*	0	3.67	158	55	46.2	0.44	8.34
Cultivars	50	4.27	226	48	41.2	0.42	10.17
Cultivars+*tin*	50	3.81	179	55	43.9	0.44	8.66
LSD (P=0.05)		*0.40*	*25*	*8*	*1.8*	*0.01*	*0.96*

Table 5.3 Yield and yield components of commercial cultivars (sown at 170 kernels m^{-2}) and lines containing the *tin* gene at two sowing rates (170 and 205 kernels m^{-2}) with three rates of nitrogen at AP97

	N fertiliser (kg ha^{-1})	Yield (t ha^{-1})	Spikes m^{-2}	Kernels $spike^{-1}$	Kernel wt (mg)	Harvest index	Mature biomass (t ha^{-1})
Cultivars	0	3.68	368	27	33.5	0.34	12.76
Cultivars+*tin*	0	3.63	317	35	35.4	0.37	12.56
Cultivars+*tin* (hsd)	0	3.49	316	31	36.0	0.36	12.35
Cultivars	80	3.47	421	33	27.6	0.31	13.20
Cultivars+*tin*	80	3.46	327	34	29.7	0.34	12.14
Cultivars+*tin* (hsd)	80	3.94	374	33	31.9	0.36	12.64
Cultivars	160	3.40	459	33	25.8	0.30	13.01
Cultivars+*tin*	160	3.53	323	40	28.6	0.33	12.24
Cultivars+*tin* (hsd)	160	3.57	355	39	28.4	0.32	12.44
LSD (P=0.05)		*0.18*	*22*	*7*	*0.9*	*0.02*	*N.S.*

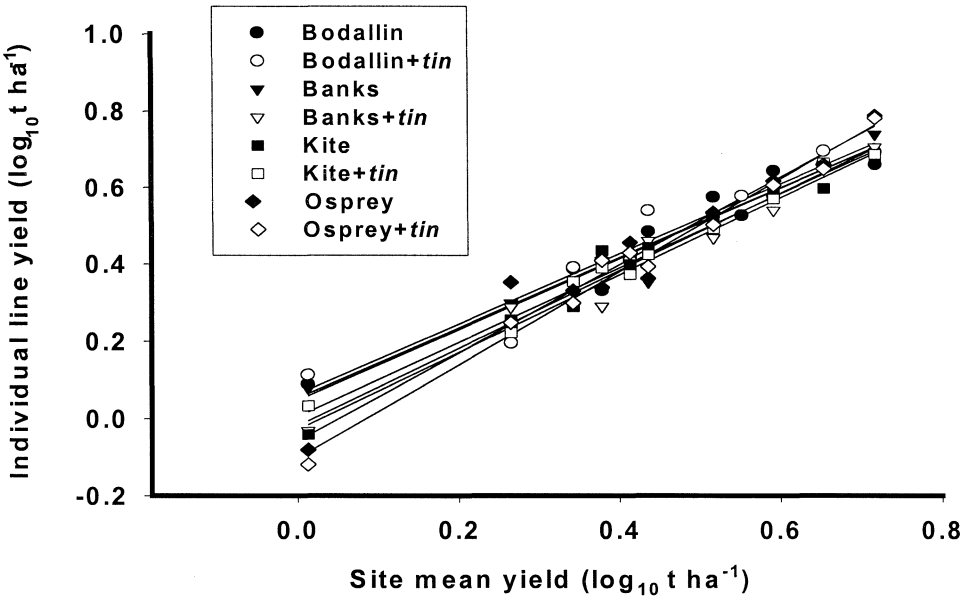
(hsd) = higher sowing density

N.S. = Not significant

Using the Finlay and Wilkinson (1963) method for identifying the adaptability of lines to a range of environments, it was observed that all lines displayed regression coefficients of approximately 1.0 (Fig 5.1). The Osprey near-isogenic pair showed the highest regression coefficients (1.20 and 1.14 for Osprey and Osprey+*tin* respectively), indicating that they responded well to favourable conditions, while the Bodallin near-isogenic pair and Banks all displayed the lowest value of 0.91. There was no apparent effect of the *tin* gene on adaptability with the cultivars averaging regression coefficients of 0.99 while the *tin* lines averaged 1.01. Banks+*tin* was the most poorly adapted line to all environments while Banks displayed above average yield stability and was better adapted to unfavourable environments (Fig 5.2). Bodallin and Bodallin+*tin* also displayed above average yield stability but tended to be better adapted to all environments than the Banks near-isogenic pair. The Osprey

near-isogenic pair displayed lower than average yield stability, while the Kite near-isogenic pair displayed average yield stability and were the most poorly adapted of the lines tested to all environments with the exception of Banks+*tin*.

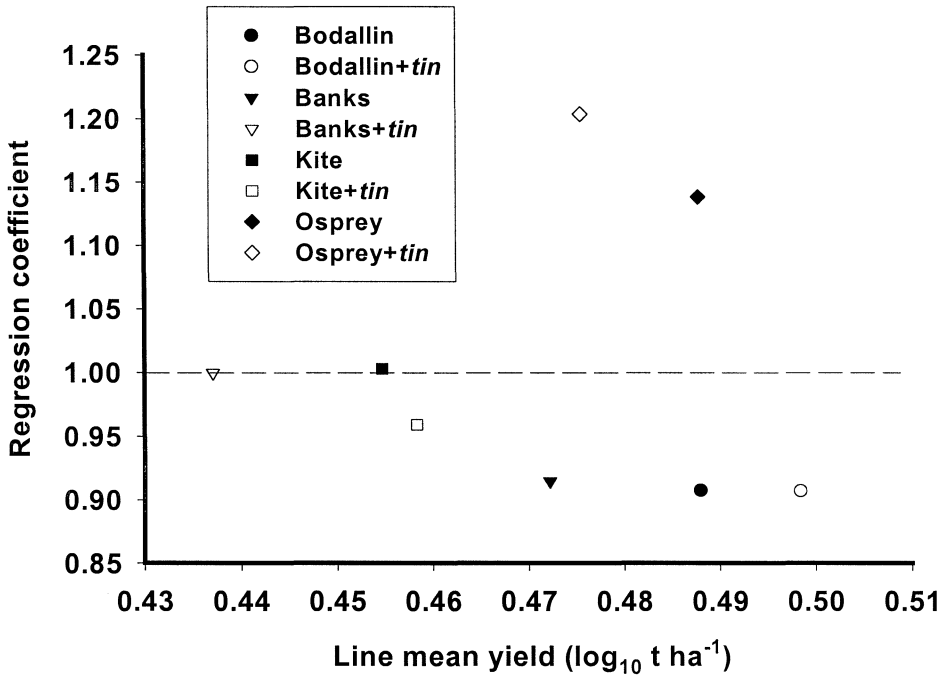
Figure 5.1 Regression lines showing the relationship between the yield of four near-isogenic pairs of wheat differing in the presence of the *tin* gene over 11 environments



5.3.2 Spike density

The *tin* lines produced significantly fewer spikes m^{-2} than the cultivars ($P<0.001$) with an average reduction of 14% averaged across the *tin* gene by density trials. Site by *tin* gene interaction was significant ($P=0.010$) with the *tin* gene causing a reduction in spike density of between 4% at C96 to 21% at C95.1 (Table 5.4). On average, the spike density of Bodallin was reduced by 16% by the presence of the *tin* gene while it was reduced by 27% for Banks, 9% for Kite and 2% for Osprey.

Figure 5.2 Relationship between the line adaptation (regression coefficient) and line mean yield for four near-isogenic pairs of wheat grown over 11 environments



Spike densities were also found to be between 18 and 22% lower for the *tin* lines compared to the cultivars at C96N (Table 5.2). At AP97, the *tin* lines produced significantly lower spike densities at both sowing densities under all levels of nitrogen (Table 5.3). While there was no effect observed in the *tin* lines at the standard sowing density, at the highest sowing density the initial application of nitrogen fertiliser resulted in an increase in spike density. However, the application of a further 80 kg ha^{-1} of nitrogen had no further effect.

Table 5.4 Spike density (spikes m^{-2}), kernels $spike^{-1}$ and kernel weight (mg) of commercial cultivars and lines containing the *tin* gene at two sowing rates (150 and 300 kernels m^{-2}) at eight sites over three growing seasons

	Sowing rate (kernels m^{-2}	C95.1	C95.2	C96	M96	W96	C97	M97	W97
		----------Spikes m^{-2}----------							
Cultivars	150	273	289	177	N.A.	N.A.	359	293	328
Cultivars+*tin*	150	212	230	169	N.A.	N.A.	281	270	310
Cultivars	300	287	293	180	N.A.	N.A.	405	363	457
Cultivars+*tin*	300	230	292	173	N.A.	N.A.	364	339	422
LSD (P=0.05)		*26*	*47*	*N.S.*	N.A.	N.A.	*44*	*22*	*22*
		----------Kernels $spike^{-1}$----------							
Cultivars	150	11	24	43	N.A.	N.A.	25	27	42
Cultivars+*tin*	150	17	28	46	N.A.	N.A.	32	27	45
Cultivars	300	11	22	42	N.A.	N.A.	24	21	34
Cultivars+*tin*	300	11	19	48	N.A.	N.A.	26	21	39
LSD (P=0.05)		*4*	*1*	*6*	N.A.	N.A.	*3*	*1*	*5*
		----------Kernel weight (mg)----------							
Cultivars	150	36.2	29.6	36.2	31.1	38.6	24.2	34.4	32.2
Cultivars+*tin*	150	35.2	29.4	38.0	29.8	41.6	27.6	36.6	33.9
Cultivars	300	40.4	35.4	35.0	31.7	36.6	24.4	35.0	29.8
Cultivars+*tin*	300	39.0	32.2	37.0	29.1	39.8	26.9	35.6	31.4
LSD (P=0.05)		*3.4*	*1.6*	*0.5*	*N.S.*	*1.1*	*2.1*	*1.7*	*1.1*

N.S. = not significant

N.A. = not available

5.3.3 Kernels $spike^{-1}$

Significantly more kernels $spike^{-1}$ were set by the *tin* lines ($P<0.001$) when averaged across the *tin* by density trials with the spikes of the *tin* lines containing an average of 4.6 extra kernels. The Banks near-isogenic pair displayed the greatest response with Banks+*tin* producing 8.0 kernels $spike^{-1}$ more than Banks while there was no difference between the Osprey pair. While increasing the sowing density decreased the number of kernels $spike^{-1}$, there was no significant interaction between

the *tin* gene and the sowing density (P=0.138) with similar proportional decreases in both cultivars and *tin* lines when the sowing density was increased. However at C95.1, C95.2 and C97, kernels spike^{-1} was significantly greater for the *tin* lines at the lower sowing density while at C96 it was significantly greater at the higher sowing density (Table 5.4).

At C96N, the *tin* lines produced more kernels spike^{-1} than the cultivars under both nitrogen treatments under rainfed and irrigated conditions (Table 5.2). At AP97 however, the *tin* lines produced significantly more kernels spike^{-1} than the cultivars at 0 and 160kg ha^{-1} of nitrogen when sown at the standard density (Table 5.3). Unlike C96N, the application of nitrogen fertiliser did not significantly affect kernel set for any treatment with the exception of the *tin* lines at the highest sowing density where it was significantly greater at 160kg ha^{-1}.

5.3.4 Kernel weight

Higher kernel weights for *tin* lines were reported at both sowing densities at several of the *tin* gene by density sites (Table 5.4). Averaged across all sites, kernel weights were 3% heavier (P<0.001) for the *tin* lines. The kernel weights of all three spring wheat lines were increased by 5 to 6% by the *tin* gene, while there was no difference between the kernel weights of Osprey and Osprey+*tin*. Both density (P=0.569) and the interaction between the *tin* gene and density (P=0.598) had no significant impact on kernel weight. The kernel weights were however greater for the *tin* lines at the lower sowing density at C96, W96, C97, M97 and W97 while they were greater at the higher sowing density at C96, W96, C97 and W97.

The kernel weights of the *tin* lines at C96N were greater than those of the cultivars for both nitrogen treatments under rainfed and irrigated conditions (Table 5.2). Increasing the nitrogen reduced the kernel weight of the *tin* lines but had no significant effect on the cultivars under both rainfed and irrigated conditions. At AP97, the *tin* lines at both densities produced greater kernel weights than the cultivars at all levels of nitrogen (Table 5.3). As was the situation at C96N, the application of nitrogen reduced the kernel weights of the *tin* lines, although the cultivars were also reduced as the level of nitrogen applied was increased.

5.3.5 Biomass at maturity

Across the *tin* by density trials, biomass at DC 92 was significantly decreased (P=0.020) by an average of 3% by the presence of the *tin* gene, although at some sites (C95.1, M96 and W97) the effect was either reversed or neutral (Table 5.5). Increasing the sowing density significantly increased biomass (P<0.001), although there was no interaction between the *tin* gene and density with biomass at DC 92 significantly greater for both cultivars and *tin* lines at the higher sowing density.

Table 5.5 Biomass at maturity (DC 92) (t ha^{-1}) and harvest index values of commercial cultivars and lines containing the *tin* gene at two sowing rates (150 and 300 kernels m^{-2}) at eight sites over three growing seasons

	Sowing rate	C95.1	C95.2	C96	M96	W96	C97	M97	W97
	(kernels m^{-2}	----------Biomass (t ha^{-1})----------							
Cultivars	150	4.82	4.76	7.76	6.20	12.85	9.60	10.21	10.08
Cultivars+*tin*	150	5.99	4.62	7.14	5.92	11.94	8.62	10.55	10.74
Cultivars	300	6.88	4.85	8.15	5.87	14.48	10.22	11.27	10.89
Cultivars+*tin*	300	6.02	4.75	7.93	6.18	14.15	9.78	10.74	10.91
LSD (P=0.05)		*N.S.*	*0.61*	*N.S.*	*N.S.*	*0.86*	*0.93*	*N.S.*	*0.77*
		----------Harvest index----------							
Cultivars	150	0.20	0.37	0.33	0.41	0.40	0.22	0.38	0.34
Cultivars+*tin*	150	0.20	0.39	0.39	0.40	0.41	0.27	0.37	0.36
Cultivars	300	0.19	0.33	0.31	0.41	0.37	0.23	0.36	0.27
Cultivars+*tin*	300	0.17	0.37	0.37	0.40	0.40	0.29	0.38	0.32
LSD (P=0.05)		*0.02*	*0.02*	*0.04*	*N.S.*	*0.02*	*0.06*	*N.S.*	*0.03*

N.S. = not significant

At C96N the *tin* lines produced less biomass at maturity than the cultivars under both levels of nitrogen under rainfed conditions and the higher level of nitrogen under irrigated conditions (Table 5.2). There was a trend for biomass to be greater at DC 92 at the higher level of nitrogen although this was only significantly so for the *tin* lines under rainfed conditions. The biomass of the *tin* lines at either sowing density

across all levels of nitrogen did not differ significantly from that of the cultivars at DC 92 at AP97 (Table 5.3).

5.3.6 Harvest index

Harvest index values were significantly increased (P=0.008) by an average of 0.01 by the presence of the *tin* gene at the *tin* by density sites and this was reported at most sites with the exception of C95.1, M96 and M97 which displayed no significant effects (Table 5.5). While increasing the sowing density decreased harvest index values (P<0.001), there was no significant interaction between the *tin* gene and sowing density (P=0.487) with increasing the density resulting in a significant decrease in the harvest index values of both the cultivars and the *tin* lines.

At C96N the *tin* lines produced significantly higher harvest index values at both levels of nitrogen under both rainfed and irrigated conditions (Table 5.2). Applying nitrogen at 50kg ha^{-1} significantly reduced the harvest index values of both the cultivars and the *tin* lines in the rainfed treatment, but failed to make an impact for the irrigation treatment. The harvest index values of the *tin* lines at both sowing densities were higher than those of the cultivars at the standard sowing rate across all nitrogen treatments at AP97 (Table 5.3). The only difference between the two sowing densities of the *tin* lines was for the treatment where 80kg ha^{-1} of nitrogen was applied and those at the higher sowing density produced a significantly greater harvest index value.

5.3.7 Soil Water

There were no significant differences in the amount of water extracted at any depth or for the whole soil profile for Bodallin and Bodallin+*tin* at DC 65 at C95.1 or C95.2 (Figs 5.3 and 5.5). There were however significant differences at DC 92 at 30, 40 and 120 cm below the surface at C95.1 (Fig 5.4) with Bodallin+*tin* extracting 15mm more water than Bodallin to a depth of 160cm (Total water use from sowing to DC 92 was 244mm for Bodallin and 258mm for Bodallin+*tin*). However at C95.2 there was no significant difference at any depth for soil water extraction at DC 65 (Fig 5.5) or DC 92 (Fig 5.6) and no significant differences in total soil water extraction at DC 92 (Total water use from sowing to DC 92 was 215mm for Bodallin and 214mm

for Bodallin+*tin*). There was no significant difference of sowing density on the amount of soil water extracted for either the cultivars or the *tin* lines for either C95.1 or C95.2 at DC 65 and 90 (data not shown).

Figure 5.3 Difference in water content (mm) throughout the soil profile at C95.1 between sowing and anthesis (DC 65) for Bodallin±*tin* (N.S. = P>0.05)

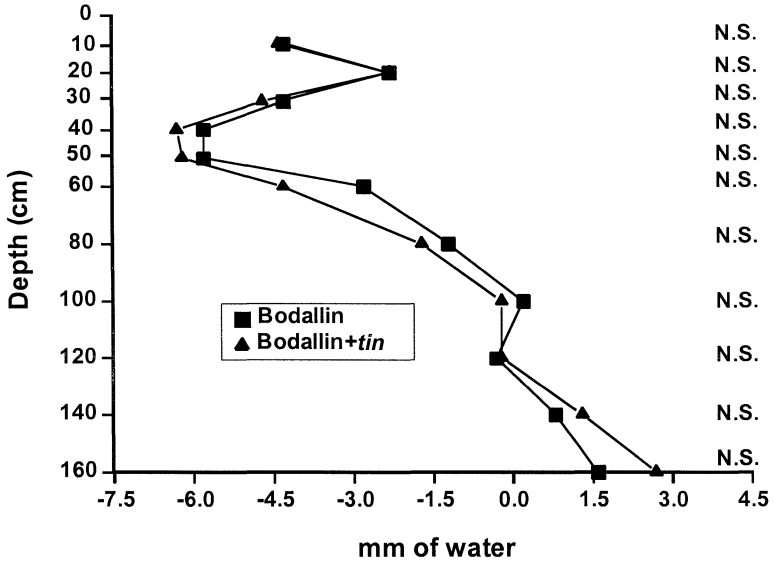


Figure 5.4 Difference in water content (mm) throughout the soil profile at C95.1 between sowing and maturity (DC 92) for Bodallin±*tin* (N.S. = P>0.05)

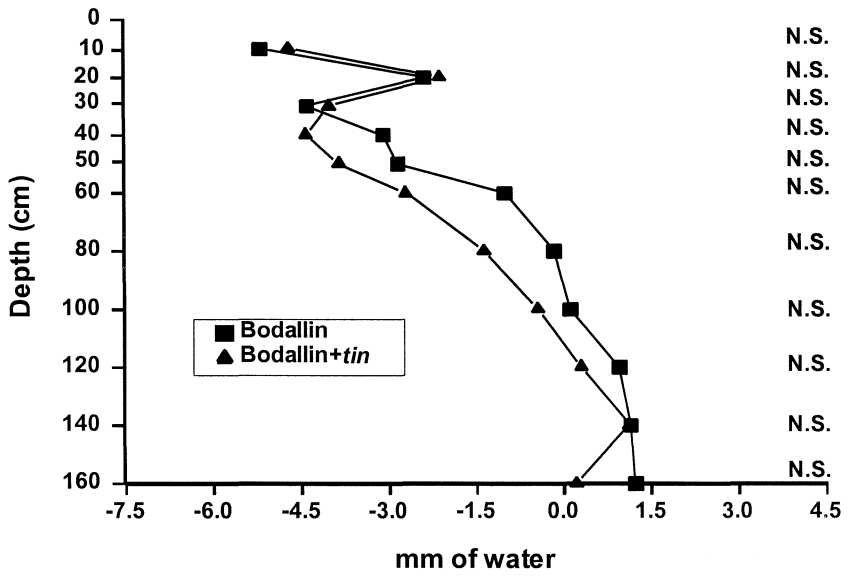


Figure 5.5 Difference in water content (mm) throughout the soil profile at C95.2 between sowing and anthesis (DC 65) for Bodallin±*tin* (N.S. = P>0.05, *=P≤0.05))

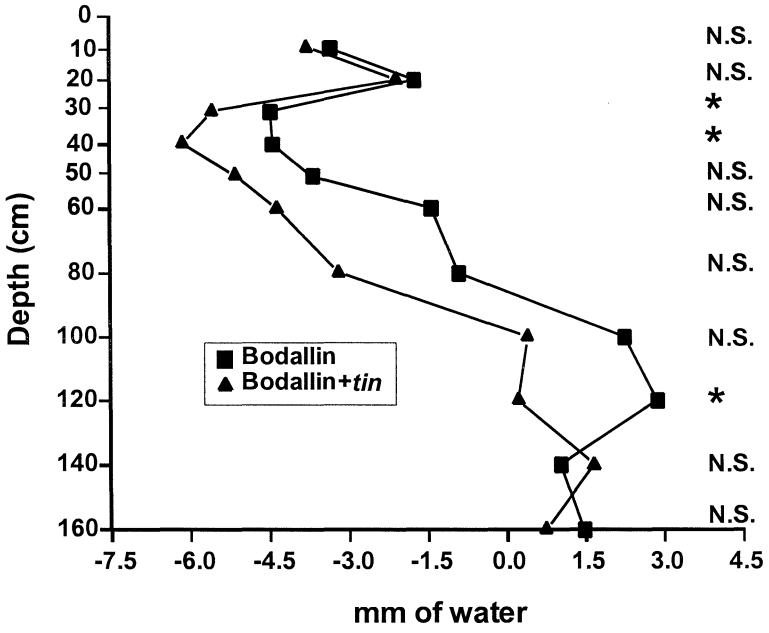
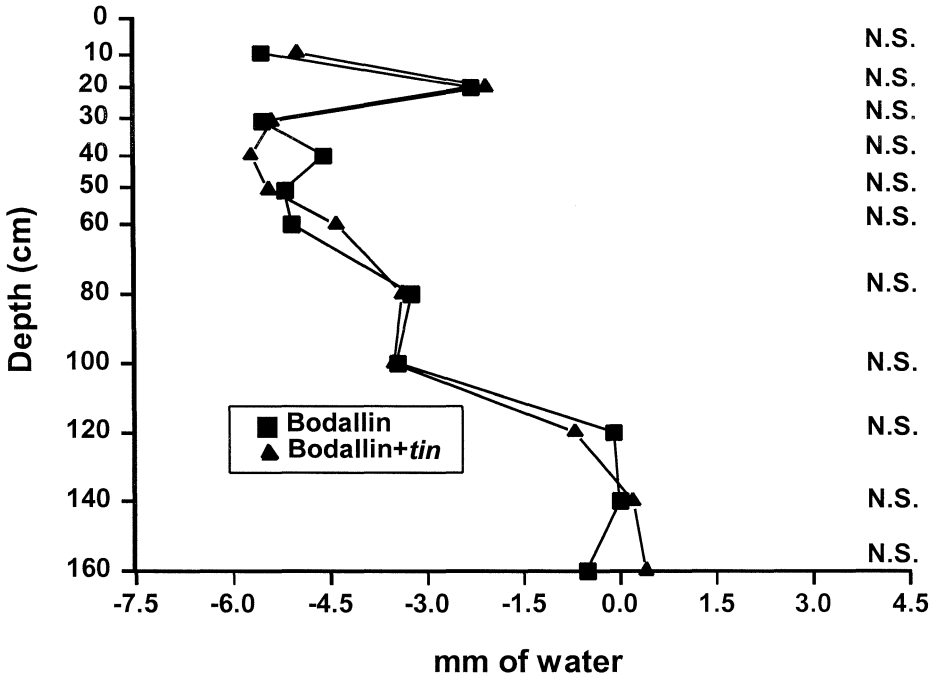


Figure 5.6 Difference in water content (mm) throughout the soil profile at C95.2 between sowing and maturity (DC 92) for Bodallin±*tin* (N.S. = P>0.05)



At C96N there was no difference in the volumetric soil water extracted from the whole soil profile, nor at any depth, between the cultivars or the *tin* lines from sowing to DC 65 or DC 92 (Figures 5.7 and 5.8). By DC 65, the cultivars had extracted 12mm of water from the soil profile and the *tin* lines had extracted 11mm while by DC 92 the cultivars had extracted 25mm and the *tin* lines had extracted 22mm (Total water use from sowing to DC 92 was 243mm for the cultivars and 240mm for the *tin* lines). The application of nitrogen did not significantly affect the amount of water extracted from the soil profile at DC 65 or DC 92 (data not shown).

Figure 5.7 Difference in water content (mm) throughout the soil profile at C96N between sowing and anthesis (DC 65) for cultivars±*tin* (N.S. = P>0.05)

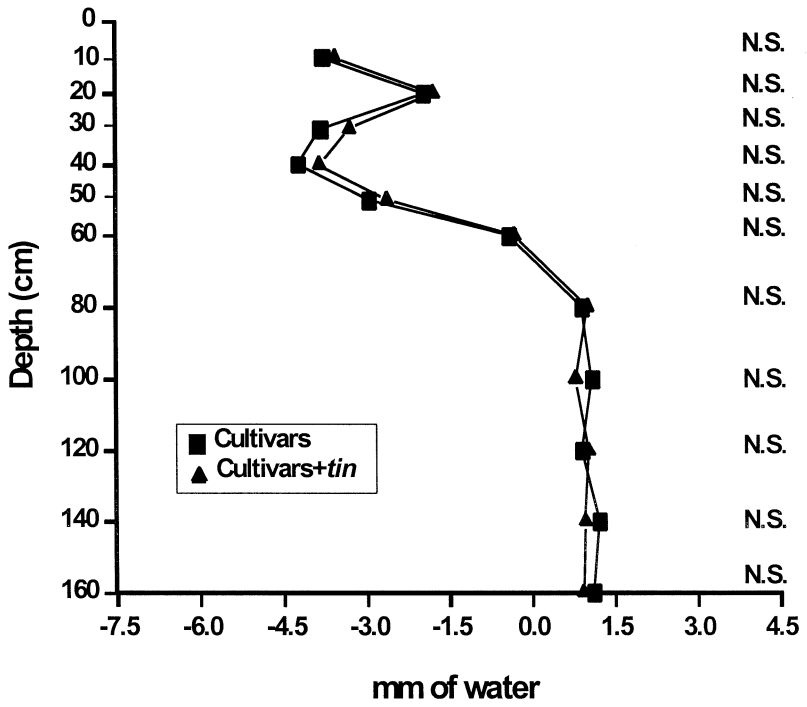
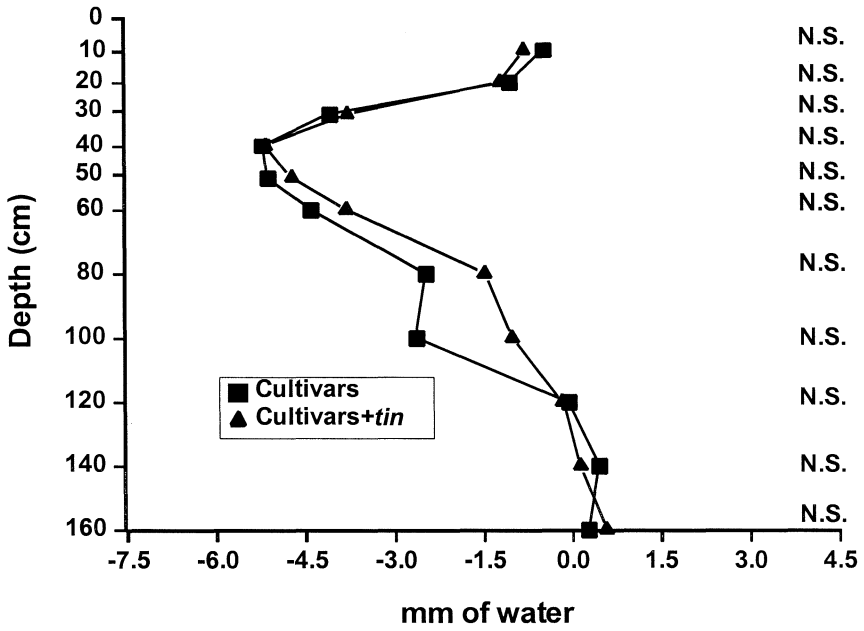


Figure 5.8 Difference in water content (mm) throughout the soil profile at C96N between sowing and maturity (DC 92) for cultivars±*tin* (N.S. = P>0.05)



5.4 DISCUSSION

5.4.1 Yield

At most sites the *tin* lines produced the same yield as the commercial lines when sown at both sowing densities. However at C95.2, where yields were lower for the *tin* lines at the higher sowing density, spike density was almost identical for the cultivars and the *tin* lines. This may have been due to a combination of the lateness of sowing (Rawson, 1988), the high plant density (Kasperbauer and Karlen, 1986) and the pre-DC 65 drought (Smith, 1925; Gardner, 1942; Schönfeld *et al.* 1989) which may have reduced tillering in the cultivars. The only other site where the *tin* gene had a significant impact on yield was at W97 where it significantly increased yield at the higher sowing density. At this site all lines, but particularly the cultivars, appeared to suffer from herbicide damage when plots were sprayed to control annual ryegrass. For unknown reasons, *tin* lines appeared better able to recover and while spike density still tended to be lower, it was more than compensated for by the significant increase in kernel size.

Figures 5.1 and 5.2, based on the work of Finlay and Wilkinson (1963), emphasized that the *tin* gene had a mixed effect on yield stability and adaptability. Averaged across four genetic backgrounds, the *tin* gene did not alter the adaptability of lines to more or less favourable environments. However, it is interesting to note that the Osprey near-isogenic pair tended to be below average in terms of yield stability and were better adapted to favourable environments. This could be partly due to their ability to tiller more profusely and take advantage of favourable conditions and/or their tendency to mature later than the other lines tested may have adversely affected them under terminal drought conditions. By maturing earlier and avoiding terminal drought, the Bodallin near-isogenic pair were better adapted to all environments and displayed greater yield stability than the other lines tested.

5.4.2 Spike density

With the exception of C96 and W97, spike densities for the *tin* lines were always significantly lower than the cultivars at the lower sowing rate. At the higher sowing rate, spike density was only significantly lower for the *tin* lines at C95.1 and M97. The only sites where the *tin* gene did not have a significant impact on spike density was at C96 and W97. The result at W97 may be explained as being due to the apparent herbicide damage to the cultivars. At C96, ample soil moisture at sowing and the mild conditions early in the crop's development provided excellent conditions for plant growth. However, this may have depleted soil water and rainfall in September and October appeared to be insufficient to meet demands by the crop. The subsequent water deficit caused sterility in many stems which otherwise may have produced grain. The water deficit appears to be so severe that many plants at the higher sowing rate failed to produce a head before senescing.

Nitrogen increased spike density in both the cultivars as well as the *tin* lines at C96N under non-irrigated conditions, although there was no effect on either under irrigated conditions. At AP97, nitrogen increased spike density in the cultivars as well as in the *tin* lines at the higher sowing rate. It is interesting to note that this did not occur for the *tin* lines sown at the lower rate. Even at the higher sowing rate, the *tin* lines were not capable of achieving the same spike density as the cultivars. However, their yields were the same when they were sown at the same rate and were in some

situations higher when sown at the increased rate at some of the higher rates of nitrogen. Therefore it seems as though sowing rate may not need to be increased greatly compared to the sowing rates of current cultivars to achieve a yield benefit from the lines containing the *tin* gene.

5.4.3 Kernels spike^{-1}

The *tin* gene increased the number of kernels spike^{-1} at several sites, particularly at the lower sowing rate. At C95.1 and C97, the *tin* lines produced significantly more kernels spike^{-1} at the lower sowing density. However at C96 and W97, this was reversed with no difference in the number of kernels spike^{-1} at the lower sowing density. The trend for the number of kernels spike^{-1} to either remain the same or increase significantly for lines containing the *tin* gene was only reversed at C95.2 when at the higher sowing rate the *tin* lines produced significantly fewer kernels spike^{-1}. A strong positive correlation between spike density and yield at C96N indicates that poor plant establishment resulted in sub-optimal spike densities for both the cultivars and the *tin* lines (data not shown).

5.4.4 Kernel weight

With the exception of C95.2, the number of kernels weight for the *tin* lines was greater than that of the cultivars, a result supported by the findings of Atsmon and Jacobs (1977), Richards (1988) and Whan *et al.* (1988). The only sites where the *tin* gene did not significantly impact on kernel weight was when there was a pre-DC 65 drought (C95.1 and C95.2) or a late sowing (M96). Atsmon and Jacobs (1977) and Marshall and Boyd (1983) also reported increased kernel weights in low tillering wheat lines. The *tin* gene significantly increased kernel weights under rainfed and irrigated conditions at C96N, as well as at AP97. The application of nitrogen fertiliser significantly decreased kernel weights for the *tin* lines at the rainfed and irrigated trials at C96N, but surprisingly did not do so for the cultivars. At AP97 however, the kernel weights of both the cultivars and the *tin* lines were reduced as a consequence of the nitrogen fertiliser. Nitrogen fertiliser application usually results in a reduction in kernel weight and in its most severe manifestation in association with a water deficit results in haying-off (Barley and Naidu, 1964; Fischer and Kohn, 1966b; van

Herwaarden *et al*, 1998a). In Australia, haying-off can lead to grain being down-graded in value due to a high proportion of screenings (i.e. kernels passing through a 2mm slotted sieve) at a significant cost to the cereal industry every year. The increase in seed sizes due to the *tin* gene regardless of the soil nitrogen status therefore may help wheat to tolerate haying-off conditions.

5.4.5 Biomass at maturity

The *tin* gene tended to reduce maturity biomass at DC 92 at all sites except W97 (where herbicide damage early in the season affected cultivars more than the *tin* lines), but only significantly reduced it at both sowing densities at C95.2 and at the lower sowing rate at W96. A large biomass at DC 92 is advantageous for grain yield, but only if harvest index can be maintained. As biomass increases, the soil moisture level begin to fall (Fischer and Kohn, 1966a) as transpiration increases (extracted from van Herwaarden *et al.* 1998a). Passioura (1977) found that harvest index was positively correlated with the percentage of water used after DC 65, so in situations of a terminal drought, harvest index and subsequently grain yield can be reduced due to excessive total biomass. As there was no significant difference in biomass at most sites, the increased harvest index values of the *tin* lines therefore can not be a function of a reduction in biomass resulting in more water being available for grain filling.

5.4.6 Harvest Index

The trend for the *tin* gene to maintain or increase harvest index would indicate that it results in a more efficient partitioning of assimilates into grain through translocation of pre-DC 65 assimilation or greater water use efficiency. The fact that the *tin* lines had higher harvest index values at the relatively drier sites of C95.2 and C96, as well as at higher sowing densities at other sites where it may be expected that moisture becomes more limiting compared to the lower sowing density plots (Pelton, 1969), indicates that although harvest index values may not be high, they are still higher for the *tin* lines than the cultivars. This means that the ability of the *tin* lines to partition assimilates is greater across a wide range of degrees of post-DC 65 water deficits. Nitrogen also tended to reduce the harvest index with a significant reduction for both the cultivars and the *tin* under rainfed conditions at C96N and at AP97. This

finding is supported by those of van Herwaarden *et al.* (1998a) who found that harvest index values fell as rates of nitrogen fertiliser were increased, particularly at drier sites where haying-off occurred.

5.4.7 Soil water

Contrary to what was hypothesised, measurements reported in this chapter did not conclude that the *tin* lines do not conserve soil water from sowing to DC 65. The finding that the *tin* lines extracted significantly more water at 30, 40 and 120cm as well as from the whole profile (15mm) compared to the cultivars at DC 92 may indicate that they partition more assimilate into roots and are better able to extract soil moisture. At C95.2 and C96N however, there were no significant differences in volumetric soil moisture extraction at any depth or for the whole profile. Root length density measurements were made from soil cores taken at DC 92 at C96N but failed to find any significant difference between cultivars or the *tin* lines at any depth (see Chapter 4, Fig 4.9). The sowing of C95.2 was relatively late in the season and data from spike density indicated that the effect of the *tin* gene is less pronounced when lines containing it are sown late while it is difficult to say what effect the poor plant establishment at C96N may have had on water extraction.

5.5 CONCLUSION

There was no significant yield advantage or disadvantage of the *tin* lines over the cultivars at most sites mentioned in this chapter. The *tin* gene tended to cause major shift in the balance of yield components. There was a tendency for the spike density to be reduced by the *tin* gene, except when sowing was late, at high sowing densities or when there was a severe pre-DC 65 drought. Kernel m^{-2} was affected to a lesser extent by the *tin* gene as it tended to increase the number of kernels $spike^{-1}$. The *tin* gene also tended to increase kernel weight, except where there was a severe drought or sowing was late. Harvest index values were increased by the *tin* gene indicating that lines containing it are more efficient at partitioning assimilate towards grain, while final biomass tended to be lower for the *tin* lines. If plant establishment is poor, the *tin* gene can be detrimental and significantly reduce yield due to its inability to compensate for low spike density. When sown at a slightly higher sowing density,

or with higher rates of nitrogen however, the *tin* gene increased yield and may provide a genetic resource for tolerance to haying-off. There was no significant difference in the amount of water extracted by lines containing the *tin* gene compared to cultivars at DC 65, although there was more water extracted by the *tin* lines at one site by DC 92.

CHAPTER 6

THE INTRINSIC BENEFITS OF THE *tin* GENE AND ITS ABILITY TO STORE ADDITIONAL WATER SOLUBLE CARBOHYDRATES AND NITROGEN IN STEMS

Kite and Kite+*tin* at heading

6.1 INTRODUCTION

The morphology of plants containing the *tin* gene results in an increase in allocation of resources towards productive stems (see Chapter 4). While several authors have reported that sterile tillers contribute to grain yield through their ability to translocate assimilate and nutrients to fertile tillers (Tincker and Jones, 1931; Palfi and Daszi, 1960; Bremner, 1969; Lauer and Simmons, 1988; Palta *et al.* 1994), others have claimed that any benefit is small and insignificant and that the tillers may be a burden through their wastage of soil water (Rawson and Donald, 1969) and nutrients (Sharma, 1995). Grain yields have been found to be higher for de-tillered crops in terminal drought situations where yields were 2 t ha^{-1} or less and spike densities were less than 300 m^{-2} (Islam and Sedgley, 1981).

Palta *et al.* (1994) claimed that that there were benefits for fertile stems from the remobilisation of carbon and nitrogen from unproductive tillers. While Palta and Fillery (1995) reported an increase in the remobilisation when dry matter and/or spike density was increased, data extracted from van Herwaarden *et al.* (1998a,b) demonstrates that there is a greater potential retranslocation of stem reserves in terms of % of stem biomass when spike densities are low. This would appear to be the result of a more open canopy allowing greater penetration of light and allowing photosynthesis to continue in the lower leaves in the period up to anthesis (DC 65) as stem water soluble carbohydrate (WSC) levels are higher at DC 65 for crops at lower spike densities (van Herwaarden *et al.* 1998b). Crops consisting of plants containing the *tin* gene may therefore be able to accumulate more WSCs in their stems as they typically produce canopies with lower spike densities (see Chapter 5). The thicker stems produced by *tin* lines may also act as greater storage capacity for pre-DC 65 assimilated carbon and nitrogen. This could be of particular importance in areas where terminal drought (water-limiting conditions post-DC 65) can limit yield. In these areas, plants rely more on pre-DC 65 stored assimilate for grain filling than on current assimilation (Gallagher *et al.* 1975; Austin *et al.* 1977; Bidinger *et al.* 1977; Austin *et al.* 1980; Pheloung and Siddique, 1991; van Herwaarden *et al.* 1998a, Villegas *et al.* 1998). However calculating the change in non-grain biomass between DC 65 and physiological maturity (DC 92) can prove an inaccurate way of

determining pre- and post-DC 65 contributions to grain filling due to effects such as loss of leaf material (Barley and Naidu, 1964; Austin *et al.* 1980) and saprophytic leaf decay (Bidinger *et al.* 1977) which can overestimate the pre-DC 65 contribution, and cell wall thickening and lignification (Stoy, 1965; Pearce *et al.* 1988) and stem elongation post-DC 65 (Borrell *et al.* 1989 and 1993; Bonnett and Incoll, 1992) which can underestimate the contribution. van Herwaarden *et al.* (1998b) proposed the analysis and determination of changes in the stem WSC and nitrogen levels between DC 65 as the best way of estimating retranslocation to avoid these errors.

The *tin* gene may have 'intrinsic' benefits other than those directly arising from a reduction in tillering. Plants containing the *tin* gene have significantly more spikelets spike^{-1} and heavier spikes at DC 65 than their near-isogenic freely tillering pairs (Atsmon and Jacobs, 1977 and Chapter 3). Data from Fischer *et al.* (1976) however indicate that the number of spikelets spike^{-1} in cultivars without the *tin* gene decreases as spike density increases, while data from Chapter 3 indicates that the same relationship exists for *tin* lines up to at least 450 spikes m^{-2} under field conditions.

Lines containing the *tin* gene have been shown to have desirable features such as larger kernel weights than their freely tillering near-isogenic pairs under field conditions and may have increased WSC levels (van Herwaarden *et al.* 1998b). What is not known however is whether these are intrinsic traits associated with the *tin* gene and are displayed under all conditions, or whether they are simply a responses associated with lower spike densities. The aims of the experiments listed in this chapter are to investigate the effect of the *tin* gene on WSC and nitrogen levels when plants are grown in field plots (where spike densities are typically lower for the *tin* lines) as well as under controlled conditions where they achieve the same tiller and spike densities by either growing them under conditions of high plant density, or by detillering plants to achieve similar spike densities as the *tin* lines. The 'gigas' characteristics of the *tin* lines will also be investigated at the same tiller and spike density to see whether they truly are intrinsic traits of the *tin* gene or merely a response to the reduction in plant competition.

6.2 MATERIALS AND METHODS

6.2.1 Experiment 1

Six microplots (0.4m by 0.5m) consisting of two cultivars (Bodallin and Kite), their near-isogenic pair containing the *tin* gene (Bodallin+*tin* and Kite+*tin*) and the two cultivars detillered to the same tiller density as the *tin* lines (Bodallin detillered and Kite detillered) were grown on a sandy-clay soil in Canberra, ACT (149°06'E, 35°19'S, Alt 600m) as one larger plot (1.2m by 1.0m). There were four repetitions per treatment and plots were arranged in a randomised block design. The growth stage of the plants was determined using the Zadoks (DC) code (Zadoks *et al.* 1974). Kernels were sown on August 20, 1996 using a planting board at a uniform sowing density of 696 kernels m^{-2} at a depth of 15mm and were thinned to a uniform density of 348 seedlings m^{-2} shortly after emergence. Detillering comprised of removing tillers throughout the tillering phase with a surgical blade so that plants had achieved the same number of tillers as their near-isogenic pair containing the *tin* gene by the commencement of stem elongation (DC 30). Clear plastic rain covers (which reduced the photosynthetically active incident radiation by 15%) were placed over one third of the plots (from here on referred to as 'droughted'), while one third of the plots were irrigated to field capacity every two or four days (from here on referred to as 'irrigated') on October 28 (9 days prior to DC 65 for the Bodallin lines (DC 39) and 15 days for the Kite lines (DC 37)). The remaining one third of the plots only received water as rainfall (from here on referred to as 'rainfed'). The only water received by the droughted plants was on November 4 and 17 when rain covers were removed and 11mm and 20mm of rain fell respectively. All plants were harvested at physiological maturity (DC 92) at ground level on December 28, stem lengths measured, samples dried at 70°C for 48 hours and yield, yield components, biomass and harvest index values determined. Maximum and minimum temperature and rainfall data for August until December 1996 are given in Figure 2 in the appendix.

6.2.2 Experiment 2

Stem samples taken at anthesis (DC 65) at C96N, M97 and AP97 and at physiological maturity (DC 92) at M97 and AP97 were ground in a Wileymill grinder with a 1mm screen (N.B. site abbreviations are given in Chapter 4). These samples were dried at 70°C before being scanned by near infra-red reflectance spectroscopy using a NIRS systems model 500 scanning monochromator with an IBM compatible Osborne computer loaded with Infrasoft International software as described in van Herwaarden *et al.* 1998b). The near infra-red spectrum (1100-2500nm) was used to scan the samples at 2nm intervals. Carbohydrates were extracted from a subset of samples as described by van Herwaarden *et al.* (1998b) and the concentrations determined using the anthrone method as described in Yemm and Willis (1954). The nitrogen concentrations were determined using the semi-micro Kjeldahl method for foliage digestion described in Heffernan (1985). Calibration curves were developed for WSC and nitrate levels using the NIR values against the anthrone and Kjeldahl methods of the sample subset respectively and the WSC and nitrogen concentrations of the remaining subset samples estimated.

6.2.3 Experiment 3

Kernels of spring wheat lines Bodallin and Kite as well as their near-isogenic pairs containing the *tin* gene (Bodallin+*tin* and Kite+*tin*) were imbibed for 24 hours at 4°C and then sown three kernels per cylindrical PVC pot (86mm diameter, 500mm high) containing a low nitrogen potting mix on June 29, 1998 in Canberra, ACT (149°06'E, 35°19'S, Alt 600m). Seedlings were thinned to two seedlings per pot when the second leaf was fully emerged to achieve a plant density of 270 plants m^{-2}. At the commencement of stem elongation (DC 30), stem (i.e. those tillers that appeared vigorous and thought to produce a fertile spike) numbers were counted with both Bodallin and Bodallin+*tin* averaging 295 stems m^{-2}, while Kite and Kite+*tin* averaged 589 stems m^{-2}. Throughout stem elongation, stem density levels were maintained at these levels with any latter tillers removed with a scalpel blade.

On October 2 (flag leaf collar visible for the Bodallin pair (DC 39) and flag leaf partly emerged for the Kite pair (DC 37)), one third of the plants were placed under a

frame with shade cloth on the top and sides which reduced the incident radiation by 45% (from here-on referred to as 'shaded'), another third were spaced at half the plant density (i.e. 135 plants m^{-2}) (from here-on referred to 'spaced') while the remaining third were maintained at the same density (from here-on referred to as 'normal'). At anthesis (DC 65) (October 23 for the Bodallin pair and October 27 for the Kite pair) all plants were returned to their original plant density. Four plants (two pots) per treatment were harvested at DC 65, stem heights and spikelet numbers recorded and leaf areas determined using a ΔT area measurement system fitted with a RCA video camera. Samples were then dried at 70°C for 48 hours and weighed. A further four plants were harvested at both 14 days (kernels watery ripe, clear liquid - DC 71) and 30 days (very late milk, half solid/half liquid - DC 79) after DC 65 and the same measurements taken as at DC 65. At physiological maturity (DC 92) (48 and 45 days after DC 65 for the Bodallin and Kite near-isogenic pair respectively) the remaining plants were harvested and the yield determinates measured. Stem samples from all sampling dates were ground in a Cyclotec sample mill, model 1093 with a 1.0mm sieve and analysed for WSC as described for Experiment 2. Maximum and minimum temperature and rainfall data for June until December 1998 are given in the appendix.

6.3 RESULTS

6.3.1 Experiment 1

Detillering either significantly reduced or at least tended to reduce the number of spikes m^{-2} produced relative to the cultivars (Table 6.1). However detillered plants tended to produce more spikes m^{-2} than the lines containing the *tin* gene under droughted and irrigated conditions and in the case of the rainfed conditions this was significant. This may be due to the detillered plants producing tillers after stem elongation had commenced which still produced a fertile spike. The smallest tillers were removed leaving all remaining tillers to produce a head for the detillered treatment while some of the stems of the *tin* lines may have been small and sterile. It seems unlikely that the *tin* lines may preferentially direct assimilate towards the mainstem and early tillers which resulted in some sterile tillers being produced as sterile tillers were not observed in the

crops of *tin* lines in Experiment 1. Increasing the availability of water also tended to increase the number of spikes m^{-2} regardless of whether they were cultivars, the detillered cultivars or the *tin* lines.

Table 6.1 Spikes m^{-2} under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	503	531	574	N.S.
Cultivars D.T.	435	522	518	N.S.
Cultivars+*tin*	409	396	455	N.S.
LSD (0.05)	55	78	89	

N.S. = not significant; D.T. = detillered

Yield did not differ significantly between the droughted, rainfed or irrigated treatments, and only differed significantly between the tiller manipulation treatments under irrigation when the *tin* lines significantly out-yielded the detillered cultivars (Table 6.2). Both water availability and tiller manipulation treatment failed to significantly impact upon total biomass at DC 92 (Table 6.3).

Table 6.2 Yield (g m^{-2}) under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	810	737	844	N.S.
Cultivars D.T.	707	756	773	N.S.
Cultivars+*tin*	802	768	964	N.S.
LSD (0.05)	N.S.	N.S.	149	

N.S. = not significant; D.T. = detillered

Table 6.3 Maturity biomass (g m^{-2}) under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	1651	1563	1808	N.S.
Cultivars D.T.	1461	1579	1633	N.S.
Cultivars+*tin*	1613	1574	1936	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	

D.T. = detillered

The *tin* gene resulted in higher harvest index values compared to the cultivars under rainfed and irrigated treatments while detillering resulted in significantly higher harvest index values than the cultivars under rainfed conditions (Table 6.4). The cultivars produced the highest harvest index values under droughted conditions while the harvest index values of the other tiller manipulation treatments were unaffected by the water availability. Independent of the availability of water, harvest index values of the *tin* lines were consistently higher than the cultivars and the detillered treatments ($P<0.001$). The *tin* lines also had consistently shorter stems than the cultivars while detillering resulted in the stems being on average 20mm longer at DC 92 ($P<0.001$) (Table 6.5). The reduction in stem length brought about by the *tin* gene is most obvious in the rainfed and the irrigated treatments where it reduced the length by 50mm. The *tin* lines were significantly shorter than the detillered cultivars across all treatments.

Kernel weight was significantly greater for the *tin* lines compared to both the cultivars and the detillered cultivars across all treatments (Table 6.6). Averaged across all treatments, the kernel weights of the *tin* lines were 12% greater than those from the cultivars and 10% greater than those from the detillered cultivars. It is interesting to note that the water availability treatments had no impact on kernel weight except when comparing between the values for the cultivars under droughted and rainfed conditions when the average weight of the kernels from droughted plants was higher.

Table 6.4 Harvest index values under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	0.49	0.47	0.47	0.02
Cultivars D.T.	0.50	0.49	0.49	N.S.
Cultivars+*tin*	0.50	0.50	0.51	N.S.
LSD (0.05)	N.S.	0.02	0.03	

N.S. = not significant; D.T. = detillered

Table 6.5 Stem length (mm) under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	714	698	749	40
Cultivars D.T.	736	743	748	50
Cultivars+*tin*	675	650	698	51
LSD (0.05)	35	32	39	

D.T. = detillered

Table 6.6 Average kernel weight (mg) under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	51.6	48.6	49.9	1.9
Cultivars D.T.	52.3	51.0	50.6	2.0
Cultivars+*tin*	55.7	55.7	57.4	2.5
LSD (0.05)	2.3	2.0	2.1	

D.T. = detillered

Kernels spike^{-1} was significantly higher for the *tin* lines for both the rainfed and the irrigated treatments and tended to be so for the droughted treatment (Table 6.7). There was no effect of detillering on kernels spike^{-1} relative to the cultivars. As detillering took place after terminal spikelet formation when the spikelet number had been determined (for the mainstem) or almost determined (for the tillers), any increase in kernels spike^{-1} would almost certainly have been the result of an increase in the number of kernels spikelet^{-1}. Data from Chapter 4 also indicates that the *tin* gene does not affect kernels spikelet^{-1}.

Table 6.7 Kernels spike^{-1} under droughted, rainfed and irrigated conditions for cultivars, detillered cultivars and their near-isogenic pairs differing in the presence of the *tin* gene

	Droughted	Rainfed	Irrigated	LSD (0.05)
Cultivars	31.2	29.2	29.2	3.7
Cultivars D.T.	31.6	28.8	29.5	3.6
Cultivars+*tin*	35.2	34.6	37.4	6.5
LSD (0.05)	5.8	4.0	3.9	

D.T. = detillered

6.3.2 Experiment 2

Stem WSC levels for the *tin* lines at DC 65 were higher than those of the cultivars at M97, tended to be higher at AP97 while there was no significant difference at C96N (Table 6.8). By DC 92, significantly more WSC had been mobilised from the stems of the *tin* lines relative to the cultivars at M97. There was no difference in the levels mobilised at AP97 however and resulted in there being no difference in the levels at DC 92 either.

Stem nitrogen levels tended to be lower for the *tin* lines at all sites at DC 65 but not significantly different at P=0.05 (Table 6.9). By DC 92 however more nitrogen had been mobilised out of the stems of the cultivars than the *tin* lines at M97 while there was no difference at AP97.

Table 6.8 WSC levels (g m^{-2}) in the stems at C96N at anthesis (DC 65) and M97 and AP97 at DC 65 and maturity (DC 92) as well as the difference in levels of remobilisation

Stem WSC (g m^{-2}) at anthesis				
	C96N	M97	AP97	
Cultivars	136	368	199	
			s.s.r.	h.s.r.
Cultivars+*tin*	131	402	208	215
LSD (0.05)	N.S.	20	N.S.	

Stem WSC (g m^{-2}) at maturity			
	M97	AP97	
Cultivars	65	36	
		s.s.r.	h.s.r.
Cultivars+*tin*	64	41	46
LSD (0.05)	N.S.	N.S.	

Difference in stem WSC (g m^{-2}) anthesis - maturity			
	M97	AP97	
Cultivars	303	163	
		s.s.r.	h.s.r.
Cultivars+*tin*	338	167	169
LSD (0.05)	35	N.S.	

N.S. = not significant; s.s.r. = standard sowing rate; h.s.r. = higher sowing rate

The *tin* gene had a varied effect on grain protein, as it significantly increased at some sites (W96 and C96N irrigated and rainfed), while it decreased at others (C96 and W97) (Table 6.10). At the majority of sites however, grain protein was not significantly altered by the presence of the *tin* gene. Grain protein tended to be higher for the sites where yield was low although there was no significant difference in the relationship between the cultivars and *tin* lines (Fig 6.1).

Table 6.9 Nitrogen levels (g m^{-2}) in the stems at C96N at anthesis (DC 65) and M97 and AP97 at DC 65 and maturity (DC 92) as well as the difference in levels of remobilisation

Stem nitrogen (g m^{-2}) at anthesis				
	C96N	M97	AP97	
Cultivars	3.19	3.61	4.41	
			s.s.r.	h.s.r.
Cultivars+*tin*	2.87	3.20	4.31	4.26
LSD (0.05)	N.S.	N.S.	N.S.	

Stem nitrogen (g m^{-2}) at maturity			
	M97	AP97	
Cultivars	0.72	1.40	
		s.s.r.	h.s.r.
Cultivars+*tin*	0.84	1.31	1.28
LSD (0.05)	N.S.	N.S.	

Difference in stem nitrogen (g m^{-2}) anthesis - maturity			
	M97	AP97	
Cultivars	2.89	3.02	
		s.s.r.	h.s.r.
Cultivars+*tin*	2.36	3.00	2.98
LSD (0.05)	0.39	N.S.	

N.S. = not significant; s.s.r. = standard sowing rate; h.s.r. = higher sowing rate

6.3.3 Experiment 3

The *tin* gene only significantly altered the yield of the Bodallin near-isogenic pair under normal conditions where Bodallin+*tin* yielded 19% more than Bodallin (Table 6.11) while it made no impact under any conditions for the Kite near-isogenic pair. Spacing plants increased the yield for both cultivars and *tin* lines while shading reduced the yield for both Bodallin and Bodallin+*tin*, while it only tended to have this effect on Kite and Kite+*tin*.

Table 6.10 Grain protein % from 11 field sites over three years (site descriptions given in Chapter 5)

	C95.1	C95.2	C96	M96	W96	C97
Cultivars	18.35	16.86	15.45	14.52	11.94	17.18
Cultivars+*tin*	18.65	17.01	14.62	14.78	12.47	16.99
LSD (0.05)	N.S.	N.S.	0.47	N.S.	0.20	N.S.

	M97	W97	C96 (irrig)	C96 (rainfed)	AP97	
Cultivars	10.97	16.20	10.97	11.29	13.99	
					s.s.r.	h.s.r.
Cultivars+*tin*	10.95	15.87	11.64	11.91	14.38	13.96
LSD (0.05)	N.S.	0.31	0.44	0.55	N.S.	

N.S. = not significant, irrig' = irrigated

Figure 6.1 Relationship between grain protein and yield for spring cultivars and *tin* lines at C95.1, C95.2, C96, C96N rainfed and irrigated, M96, W96, C97, M97, W97 and AP97

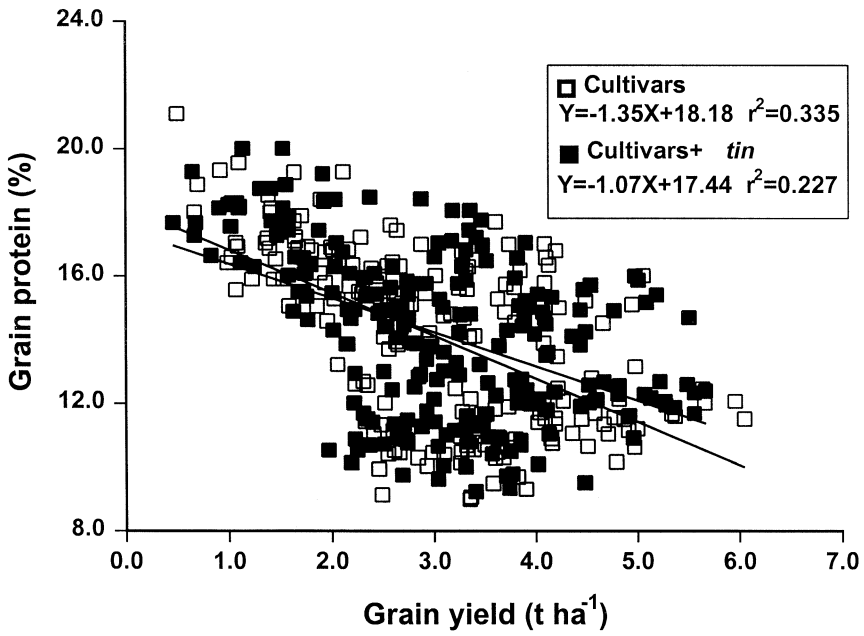


Table 6.11 Grain yield (g m^{-2}) of Bodallin±*tin* and Kite±*tin* exposed to shaded, normal and spaced conditions prior to anthesis (DC 65)

	Shaded	Normal	Spaced	LSD (0.05)
Bodallin	748	900	1042	179
Bodallin+*tin*	710	1072	1074	181
LSD (0.05)	N.S.	N.S.	N.S.	
Kite	396	483	491	N.S.
Kite+*tin*	378	430	545	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	

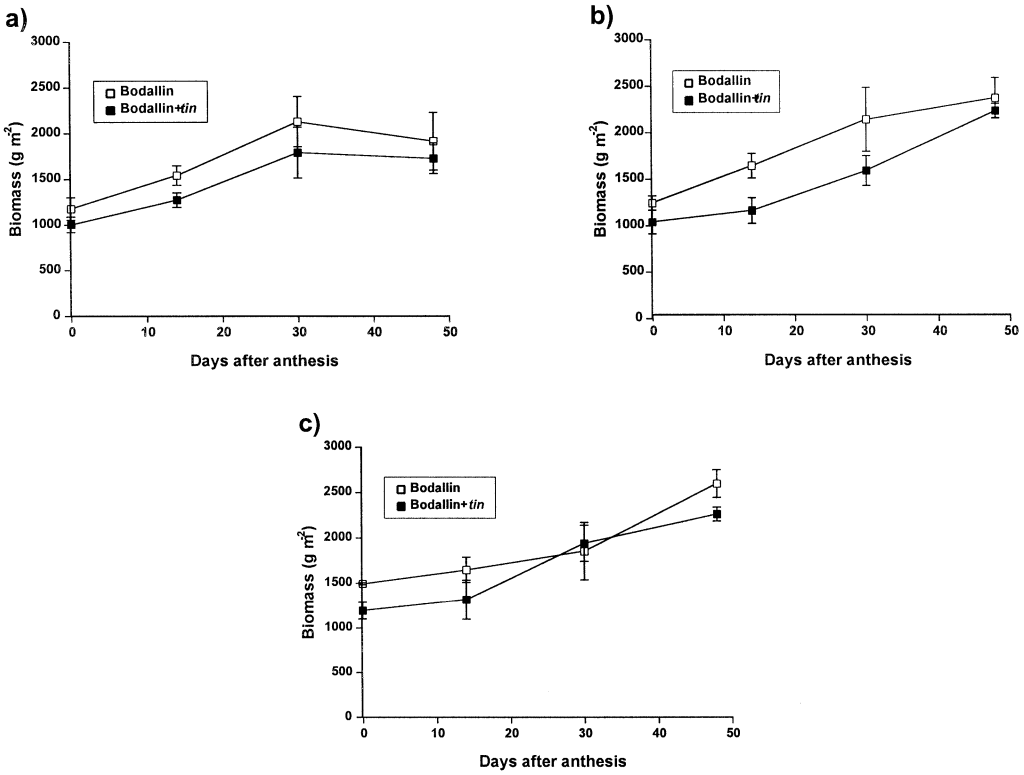
N.S. = not significant

During grain filling, biomass produced by Bodallin tended to be, and in several situations was significantly greater than that produced by Bodallin+*tin* (Fig 6.2). By DC 92 however there was no significant difference in biomass between the lines under shaded, normal or spaced conditions. There was no significant difference in biomass between Kite and Kite+*tin* at any stage between DC 65 and DC 92 except under normal and spaced conditions at DC 65 when the Kite+*tin* plants were heavier (Fig 6.3).

Harvest index values were the same or significantly higher for the *tin* lines compared to the commercial cultivars, particularly as the amount of light they received prior to DC 65 increased (Table 6.12). For all the lines except Bodallin, harvest index values increased between shaded and spaced treatments as the amount of light received prior to DC 65 increased.

Increasing the amount of light that plants received prior to DC 65 tended todid not increase head weights at DC 65 for either the Bodallin or Kite near-isogenic pairs (Table 6.13). The *tin* gene also failed to significantly alter the head weights or the number of spikelets $spike^{-1}$ for either of the near-isogenic pairs under any tested.

Figure 6.2 Above ground biomass (g m^{-2}) (±standard error) of Bodallin±*tin* between anthesis (DC 65) and maturity (DC 92); a = shaded; b = normal; c = spaced



Increasing the amount of light the plants were exposed to prior to DC 65 increased the number of kernels spikelet^{-1} at DC 92 of both Bodallin and Bodallin+*tin*, but did not significantly affect Kite or Kite+*tin* (Table 6.14). The presence of the *tin* gene did not affect the number of kernels spikelet^{-1}, except under spaced conditions where it was significantly greater for Kite+*tin*.

Figure 6.3 Above ground biomass (g m^{-2}) (±standard error) of Kite±*tin* between anthesis (DC 65) and maturity (DC 92); a = shaded; b = normal; c = spaced

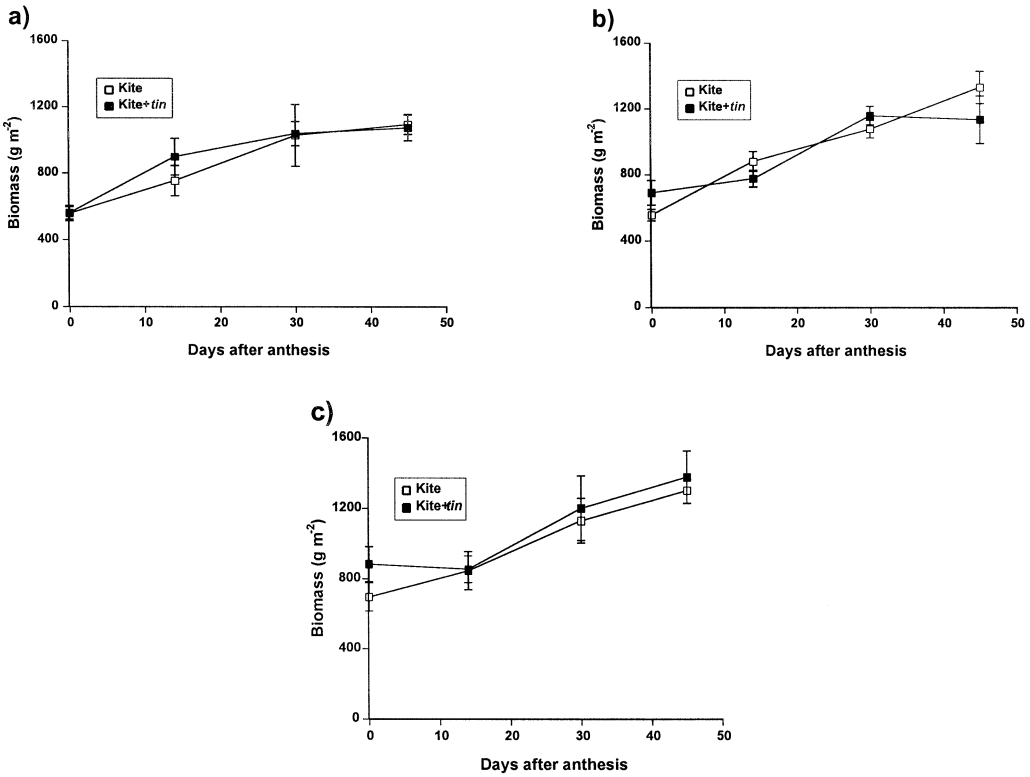


Table 6.12 Harvest Index values of Bodallin±*tin* and Kite±*tin* exposed to shaded, normal and spaced conditions prior to anthesis (DC 65)

	Shaded	Normal	Spaced	LSD (0.05)
Bodallin	0.40	0.37	0.40	N.S.
Bodallin+*tin*	0.41	0.48	0.48	0.05
LSD (0.05)	N.S.	0.06	0.05	
Kite	0.36	0.36	0.38	N.S.
Kite+*tin*	0.35	0.37	0.40	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	

N.S. = not significant

Table 6.13 Average spike weights (g) at anthesis (DC 65) of Bodallin±*tin* and Kite±*tin* exposed to shaded, normal and spaced conditions prior to DC 65

	Shaded	Normal	Spaced	LSD (0.05)
Bodallin	0.84	0.88	1.00	N.S.
Bodallin+*tin*	0.92	0.90	1.06	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	
Kite	0.40	0.38	0.42	N.S.
Kite+*tin*	0.41	0.47	0.59	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	

N.S. = not significant

Table 6.14 Average number of kernels spikelet^{-1} at maturity (DC 92) of Bodallin±*tin* and Kite±*tin* exposed to shaded, normal and spaced conditions prior to anthesis (DC 65)

	Shaded	Normal	Spaced	LSD (0.05)
Bodallin	3.16	3.83	4.22	0.67
Bodallin+*tin*	2.64	3.76	4.03	0.45
LSD (0.05)	N.S.	N.S.	N.S.	
Kite	2.26	2.27	2.29	N.S.
Kite+*tin*	2.32	2.11	2.56	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	

N.S. = not significant

The number of spikelets spike^{-1} was not significantly altered by the presence of the *tin* gene when grown at the same spike density (Table 6.15). The conditions under which plants were grown prior to DC 65 also made no impact on the number of spikelets spike^{-1}.

Table 6.15 Average number of spikelets spike^{-1} at anthesis (DC 65) of Bodallin±*tin* and Kite±*tin* exposed to shaded, normal and spaced conditions prior to DC 65

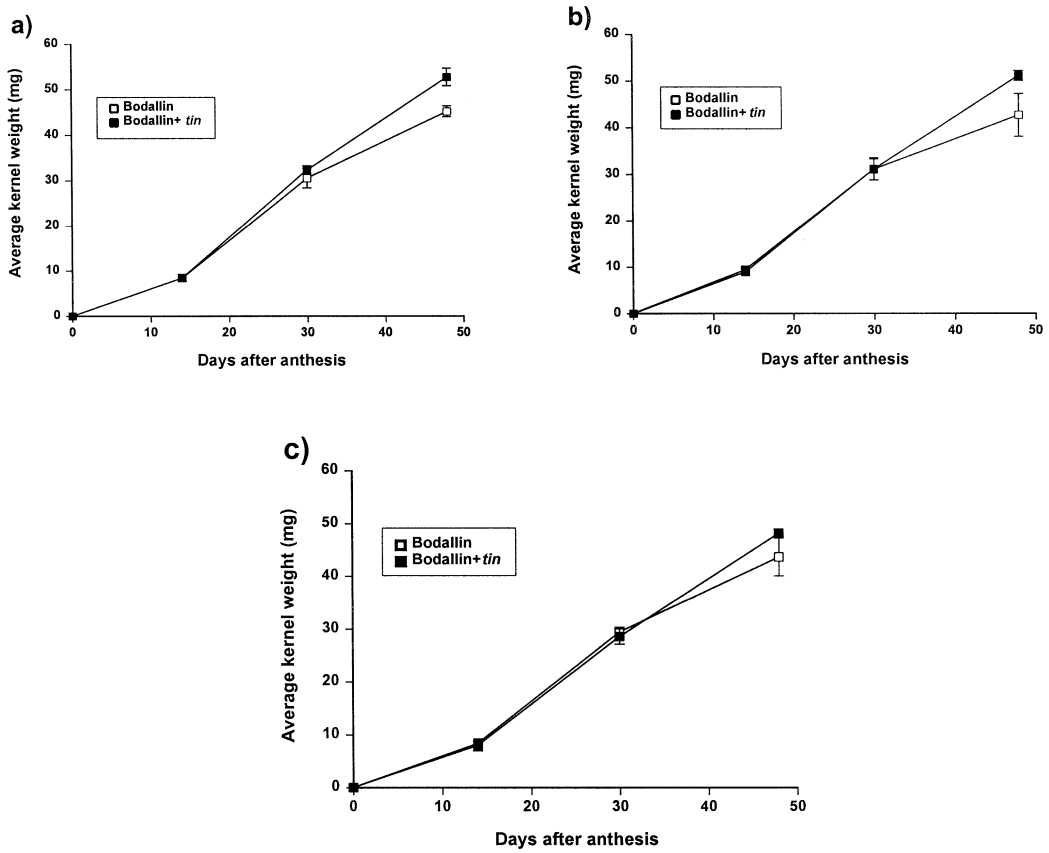
	Shaded	Normal	Spaced	LSD (0.05)
Bodallin	20.3	20.0	20.3	N.S.
Bodallin+*tin*	20.8	20.3	20.8	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	
Kite	17.8	17.9	18.2	N.S.
Kite+*tin*	17.9	17.3	19.3	N.S.
LSD (0.05)	N.S.	N.S.	N.S.	

N.S. = not significant

As with the results from the field trials (see Chapter 5, Tables 5.2, 5.3, 5.4) and Experiment 1, the kernel weights of Bodallin+*tin* were greater than those of Bodallin under shaded, normal and spaced conditions (Fig 6.4). Under normal conditions, the kernel weights of Kite+*tin* were also greater than those of Kite, although under shaded and spaced conditions there were no significant differences (Fig 6.5). Where the final kernel weights of the *tin* lines were greater than those of the cultivars, it was the result of an increased rate of grain filling between DC 79 (30 days after DC 65) and DC 92 rather than higher rates throughout grain filling.

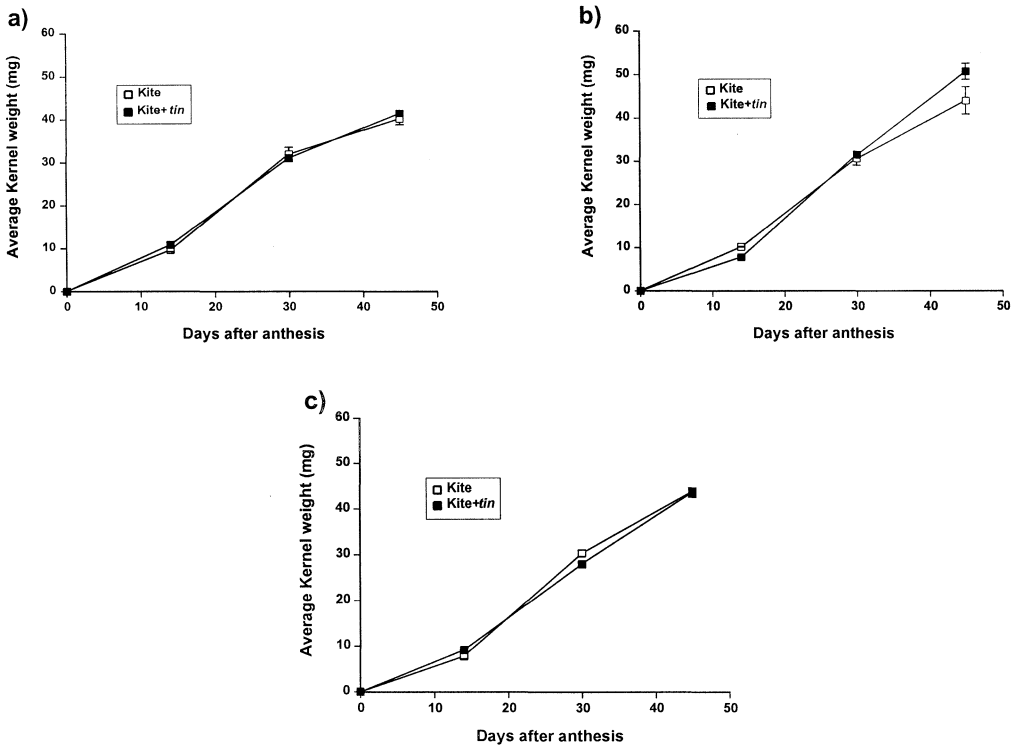
Leaf area index was the same or significantly greater for both the *tin* lines compared to the cultivars at DC 65 (Figs 6.6 and 6.7). By DC 79 (30 days after DC 65), Bodallin+*tin* under normal and spaced conditions and Kite under all conditions had greater green leaf areas than their near-isogenic pair under the same conditions.

Figure 6.4 Average kernel weight (mg) (±standard error) of Bodallin±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced



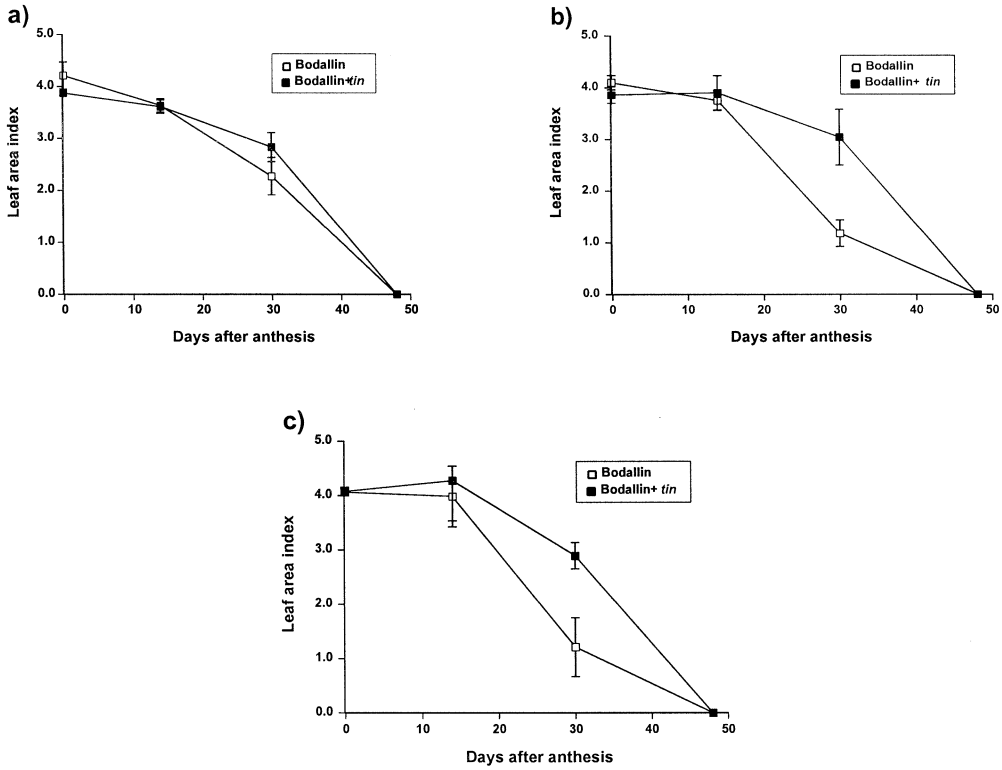
The stem length densities (SLD) of the *tin* lines were either the same or lower than that of the cultivars under all conditions for the duration of grain filling with the exception of Kite+*tin* at DC 65 under spaced and normal conditions (Figs 6.8 and 6.9). Spacing the plants and increasing the amount of light the plants were exposed to before DC 65 increased the SLD for both near-isogenic pairs, although the difference was not significant when comparing between normal and shaded treatments.

Figure 6.5 Average kernel weight (mg) (±standard error) of Kite±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced



Stem lengths were reduced by the presence of the *tin* gene for both near-isogenic pairs under all conditions, except for the Kite pair under shaded conditions where there was no difference until DC 92 by which time the stems of Kite were significantly longer than those of Kite+*tin* (Figs 6.10 and 6.11).

Figure 6.6 Leaf area index values (±standard error) of Bodallin±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced



Contrary to the findings reported earlier in Experiment 2, the water soluble carbohydrate (WSC) level in the stems of the *tin* lines were often the same or significantly lower than those of the cultivars (Figs 6.12 and 6.13). Under both shaded and normal conditions, there was no difference in the level of stem WSC at DC 65 between Bodallin and Bodallin+*tin*. After DC 65 however, the rate at which Bodallin accumulated WSC in the stems was greater than that of Bodallin+*tin* and a significant difference between the levels was maintained until DC 92. Under spaced conditions, Bodallin had a significantly higher level of stem WSC compared to Bodallin+*tin* except at DC 79 when there was no difference in the levels. The level of stem WSC was higher for Kite+*tin* relative to Kite under spaced conditions at DC 65, but for all other conditions at all other sampling times the level was the same or significantly lower than that of Kite.

Figure 6.7 Leaf area index values (±standard error) of Kite±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced

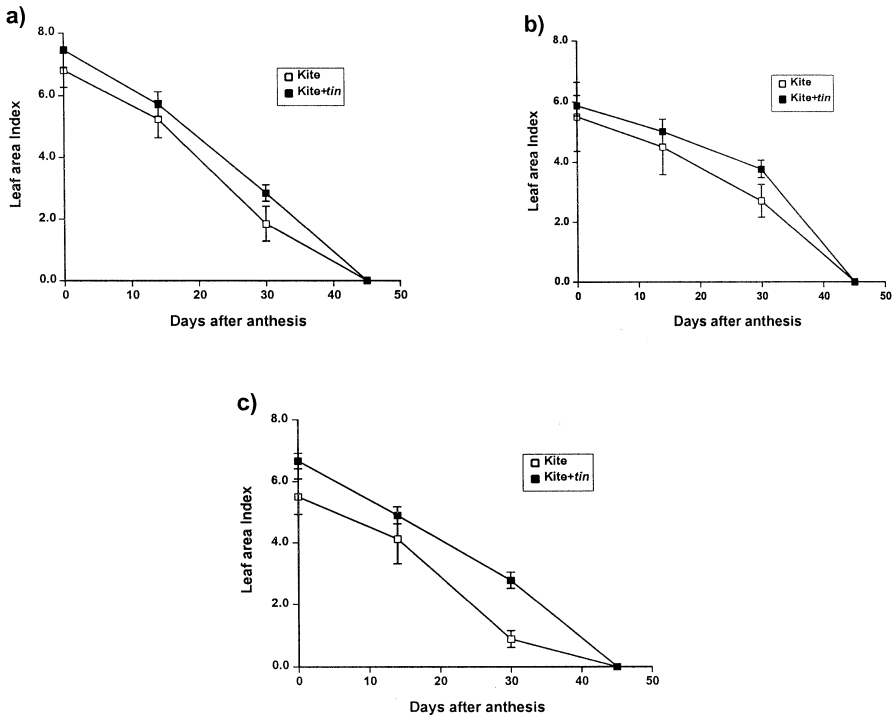


Figure 6.8 Stem length densities (g m^{-1}) (±standard error) of Bodallin±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced

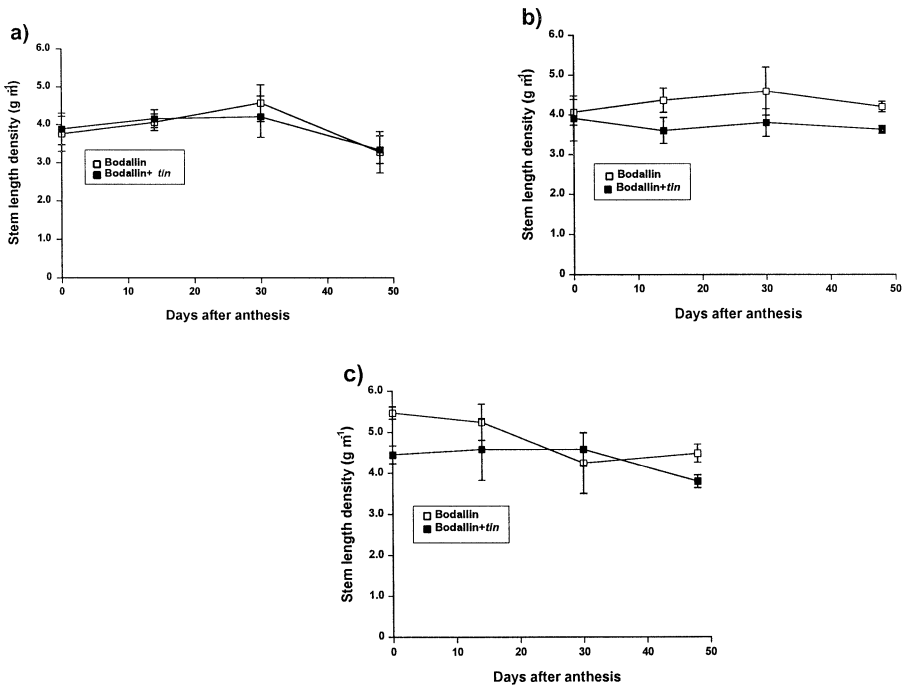


Figure 6.9 Stem length densities (g m^{-1}) (±standard error) of Kite±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced

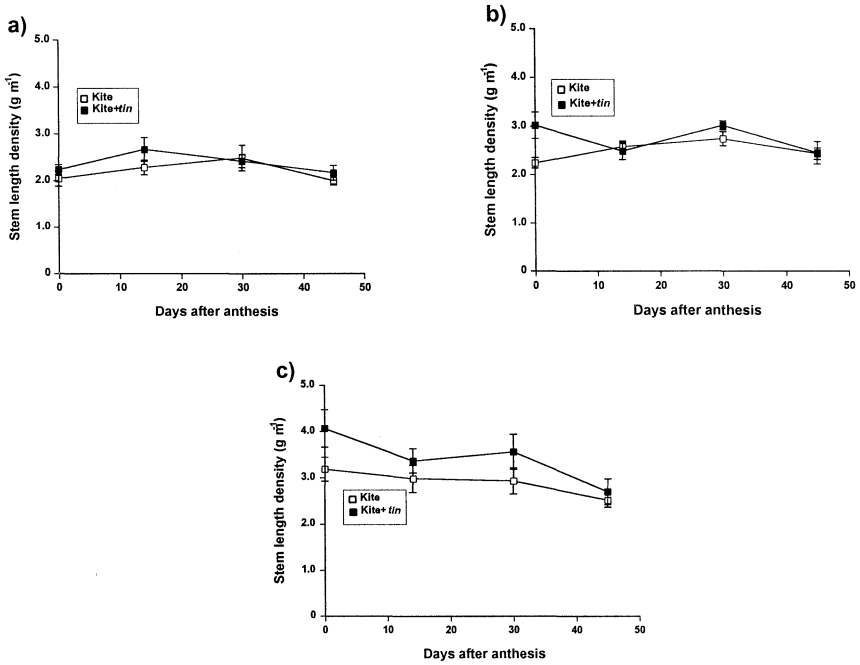


Figure 6.10 Stem lengths (mm) (±standard error) of Bodallin±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced

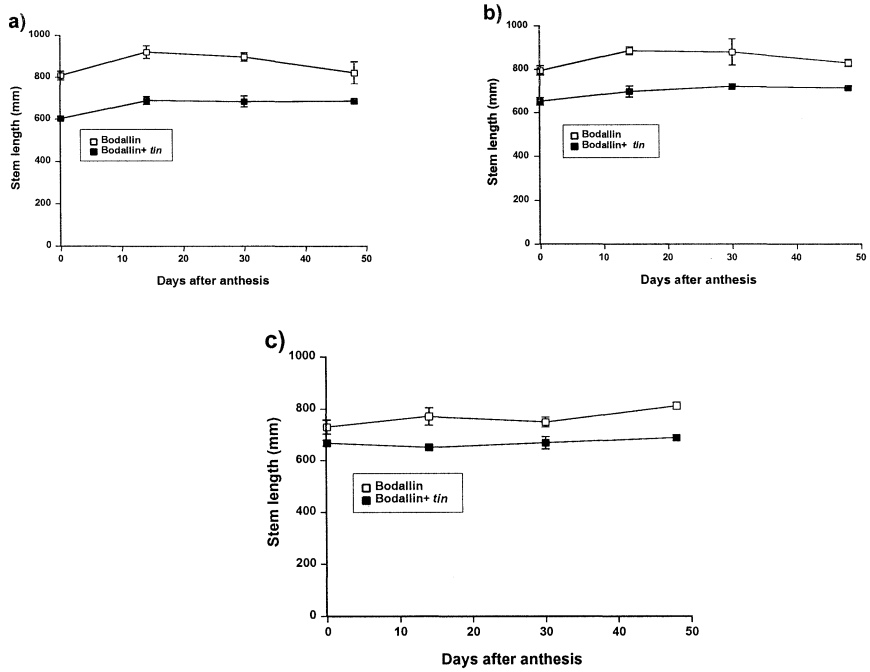


Figure 6.11 Stem lengths (mm) (±standard error) of Kite±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced

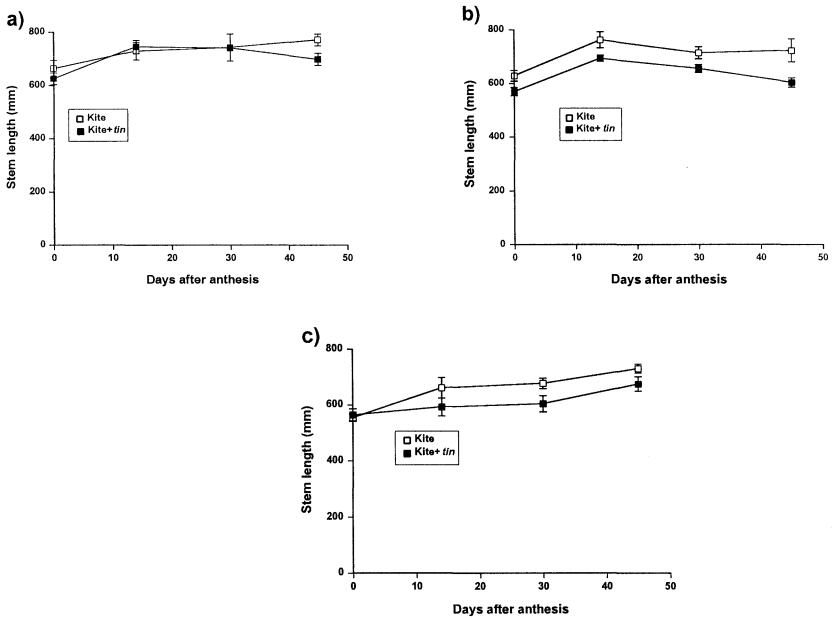


Figure 6.12 Stem water soluble carbohydrate levels (g m^{-2}) (±standard error) of Bodallin±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced

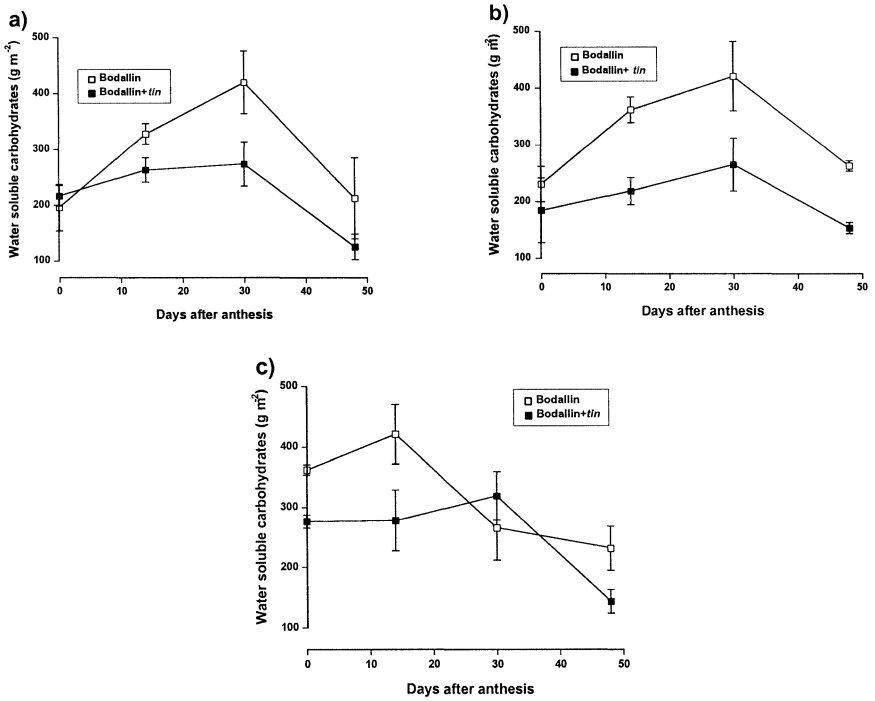
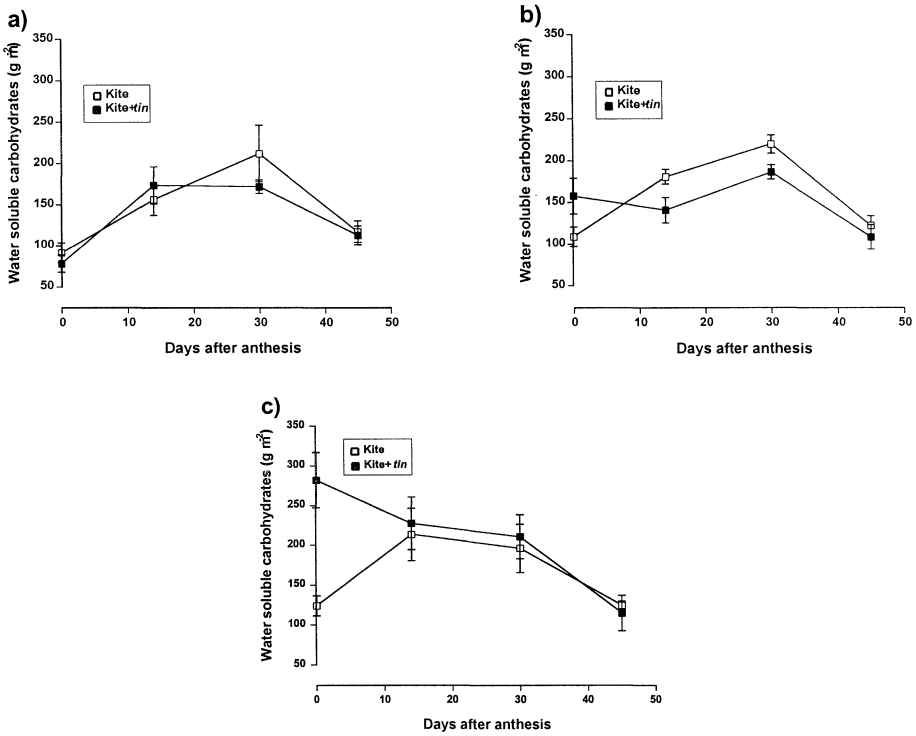


Figure 6.13 Stem water soluble carbohydrate levels (g m^{-2}) (±standard error) of Kite±*tin* between anthesis (DC 65) and maturity (DC 92); a) shaded; b) normal; c) spaced



6.4 DISCUSSION

6.4.1 Intrinsic effects - yield and yield components

While the *tin* lines produced significantly fewer spikes m^{-2} compared to their freely tillering near-isogenic pairs in Experiment 1, detillering the cultivars did not successfully reduce the number of spikes m^{-2} to the same levels produced by the *tin* lines. Under all conditions the spike density of the detillered crops tended to be higher than that produced by the *tin* lines. The cultivars (both detillered and freely tillering treatments) appeared to be able to respond to water and produce tillers that developed into fertile spikes after detillering occurred.

Contrary to the findings of Islam and Sedgley (1981), detillering did not significantly increase yield under terminal drought nor under rainfed or irrigated conditions. The *tin* lines did however maintain yield levels or tended to increase them relative to the cultivars. As spike densities of the *tin* lines were comparable with those of

the detillering treatment and therefore may have an intrinsic advantage over the cultivars other than a reduction in tiller number. Under irrigated conditions, while detillering tended to lower the spike density of the cultivars to levels comparable to those produced by the *tin* lines, the *tin* lines produced a significantly higher yield than the detillered cultivars and tended to produce higher yields than the cultivars. Why the droughted crops tended to yield more than the rainfed ones is puzzling. One possible explanation is that the plastic used to construct the rain out shelters and induce the drought diffused the light, thus allowing the lower leaves to photosynthesise more than the lower leaves of the plants under rainfed conditions that were subject to direct radiation.

In many ways it may be unfair to compare the findings presented here with those of Islam and Sedgley (1981) as the plant density in the experiments presented here were higher and there was less apparent moisture stress resulting in greater biomass and harvest index values. Like the finding of Islam and Sedgley however, final biomass was not affected by detillering. The yield advantage reported by Islam and Sedgley was due to an increase in harvest index values with detillering and this was observed under rainfed and irrigated conditions in this experiment while there was a tendency for it to also occur under drought. These findings support the hypotheses of Rawson and Donald (1969), Kirby and Jones (1977) and Sharma (1995) that sterile tillers only act as storage organs for nutrients such as nitrogen and that if anything they are parasitic on the plant for nutrients and water. Proportionally more biomass was partitioned into grain for the *tin* lines as demonstrated by the significantly higher harvest index values under rainfed and irrigated conditions and the tendency for it to be higher under drought. Sterile tillers of cocksfoot were found to not produce roots (Lambert, 1967) and therefore rely on the roots of fertile tillers for their uptake of nutrients. As a result, sterile tillers are incapable of providing a source of uptake of nutrients for the plant.

Shading wheat prior to DC 65 has been shown to decrease yield, primarily through a reduction in the number of kernels spikelet^{-1} rather than spike density or kernel weight (Fischer, 1975; Fischer and Stockman, 1980; Fischer, 1985). Yield reductions were observed in both the cultivars and the *tin* lines as the amount of light received by the plants prior to DC 65 fell from the spaced to shaded conditions and in the Bodallin near-

isogenic pair was due primarily to a reduction in the number of kernels spikelet^{-1}. Fischer (1975) observed that kernel weights increased as the intensity of shading was increased as a compensatory measure to the reduction in kernels set. However this phenomenon was not observed in Experiment 3 with DC 92 average kernel weights unaffected by the intensity of the shading except Kite+*tin* where the average kernel weights of the shaded treatment were significantly lower than those of the normal and spaced crops (see Fig 6.5). Fischer (1975) also observed a trend for a reduction in spike density as shading increased from the control to 70% shading and some of the increased assimilate which contributed to the increase in kernel weight may have come from the remobilisation of assimilate from some of these sterile stems. As the spike density was the same for all treatments in Experiment 3, there was no extra assimilate from sterile tillers for the shaded plants to draw on for grain filling.

Across almost all conditions, kernel weights at DC 92 were higher for the *tin* lines relative to those of their near-isogenic pairs in Experiment 3. Although they were similar up until DC 79 under most conditions, they increased significantly for the *tin* lines thereafter. Although only one set of data is reported here, as spike densities were the same it appears as though the increased kernel size must be an intrinsic effect associated with the *tin* gene rather than simply a result of lower spike densities

One trait that appears to not be intrinsically associated with the *tin* gene is the gigas spikes described by Atsmon and Jacobs (1977). The number of kernels spike^{-1} was greater for the *tin* lines compared to the cultivars for Experiment 1 and results from the field trials reported in Chapter 4 indicate that where spike densities were lower for the *tin* lines their spike weights and the number of spikelets spike^{-1} were greater. The cultivars do not have the ability to produce spikes with as many spikelets or as heavy at DC 65 as those produced by the *tin* lines at low densities (see Fig 4.17 and 4.18). The large gigas spikes of the *tin* lines therefore appear to be a function of the *tin* gene and the spike density with spikelets spike^{-1}, kernels spikelet^{-1} and spike weight at DC 65 the same as the cultivars when grown at the same, high spike densities in Experiment 3. Data from Chapter 4 indicates that as spike density increases up to 450 spikes m^{-2}, spikelets spike^{-1}, kernels spikelet^{-1} and spike weight at DC 65 all decrease for the *tin* lines. What happens

beyond this spike density remains unknown although both Kite and Kite+*tin* achieved spike densities of 589 spike m^{-2} in Experiment 3 and spikelets $spike^{-1}$ was unaffected by the presence of the *tin* gene. At DC 65 spike weight and kernels $spikelet^{-1}$ were only significantly higher for Kite+*tin* relative to Kite for the plants that were spaced for 25 days prior to DC 65 when spike density was halved. This would indicate that beyond approximately 450 spike m^{-2} the spike traits of the *tin* lines are the same as their near-isogenic pairs. Fischer (1985) found that the period 30 days prior to DC 65 was where environmental influences had the largest impact on the number of spikelets $spike^{-1}$ and kernels $spikelet^{-1}$. It is not surprising to find then that the water availability treatments, which were imposed shortly before DC 65 and remained in place for the duration of grain filling in Experiment 1, also did not impact significantly on the number of kernels $spike^{-1}$. The *tin* gene did however significantly increase the number of kernels $spike^{-1}$ in Experiment 1 while detillering had no effect relative to freely tillering cultivars. This was due to the fact that detillering would have occurred after the terminal spikelet stage and maximum spikelet number would have already been determined.

6.4.2 Biomass, harvest index, stem length and stem length density

The biomass of Bodallin+*tin* throughout grain filling tended to be lower than Bodallin during Experiment 3, particularly for the shaded and normal treatment although there was no trend for a difference between the Kite or the Bodallin near-isogenic pairs under spaced conditions. By DC 92 however there was no significant difference in biomass between either of the near-isogenic pairs under any conditions, the only exception being Bodallin which had produced more biomass than Bodallin+*tin* under spaced conditions. As with the field trials, harvest index tended to be higher in the *tin* lines in both Experiments 1 and 3. As concluded in Chapters 4 and 5, *tin* lines have an increased ability to partition assimilate towards grain and Experiment 3 indicates that it not a function of spike density but rather an intrinsic effect associated with the *tin* gene.

Stem lengths were reduced by the *tin* gene relative to the cultivars in both Experiments 1 and 3 in agreement with the findings reported in Chapter 4. Detillering however did not have the same effect with stem length either the same (droughted and

irrigated) or significantly longer (rainfed) than the freely tillering cultivars. In Experiment 3, neither the stems of the cultivars nor the *tin* lines elongated significantly after DC 65. The stems of the *tin* lines were usually significantly shorter than the cultivars throughout grain filling and by DC 92 they were significantly shorter under all conditions. While these findings do not indicate that the *tin* lines can reduce stem elongation during grain filling, thus remove the assimilate competition process between stem elongation and grain filling suggested by Wardlaw (1970), it may provide a way of reducing the height of wheat plants.

At the same spike density, the SLD values of the *tin* lines were not significantly different from those of the cultivars and at several occasions were significantly lower. The only exception was the Kite pair under normal and spaced conditions at DC 65 where Kite+*tin* had significantly higher values than the cultivars. These results were surprising as SLD values for *tin* lines reported in Chapter 4 were typically higher than those for cultivars. This would indicate then that the *tin* gene does not significantly alter the SLD but rather it is the lower spike density resulting from the *tin* gene that causes higher SLD values.

6.4.3 Water soluble carbohydrates and leaf area index values

The higher DC 65 water soluble carbohydrate levels in the stems of the *tin* lines at M97 and the trend for it to be higher at AP97 indicates that under field conditions the stems of the *tin* lines are better able to store WSC for grain filling than the cultivars. The *tin* lines also seemed more adept at mobilising these reserves with significantly more WSC being mobilised out of the stems between DC 65 and DC 92 at M97 (see Table 6.8), although there was no difference recorded at AP97. In Experiment 3 when spike densities of the cultivars and *tin* lines were the same however, the WSC levels of the *tin* lines were the same or significantly lower than those of the cultivars with the exception of the Kite near-isogenic pair under normal and spaced conditions at DC 65. As the *tin* lines produced as much or more WSCs than the cultivars under field conditions it can only be deduced that the *tin* gene does not significantly affect the level of WSC in the stems

under field conditions *per se*, but it is a function of the increased light penetration into the canopy or few sinks resulting from a lower spike density.

As the *tin* lines do not appear to rely on stored WSC to the same extent as the cultivars when grown at the same spike density but have either the same or greater rates of grain filling after DC 79, they must rely on assimilate for grain filling from other sources. Under all conditions used in Experiment 3, the LAI of the *tin* lines was as high or significantly higher than the cultivars at DC 65. However by DC 79 the leaf area of the *tin* lines was significantly greater than that of the cultivars, the only exception being the Bodallin near-isogenic pair under shaded conditions. Whether this is a response to the lower WSC levels or a truly intrinsic benefit of the *tin* gene is unclear, although it would appear to account for the increased rate of grain filling before DC 79 and DC 92 in Experiment 3. Certainly a greater persistence in leaf area or delay in maturity was not observed for the *tin* lines compared to their near-isogenic pairs for any of the field experiments reported in this thesis.

6.4.4 Stem nitrogen and grain protein

The ability to uptake and store nitrogen in the stem was not enhanced by the *tin* gene under field conditions with stem nitrogen levels not differing significantly between the cultivars and the *tin* lines. There was a significant reduction in the amount of nitrogen mobilised out of the stems between DC 65 and DC 92 for the *tin* lines relative to the cultivars at M97, although no difference was reported at AP97. This difference was not reflected in grain protein which, like at many other sites, was unaffected by the presence of the *tin* gene. As kernel weights are typically higher for *tin* lines, the possibility existed that the increase in kernel weight may come solely from carbohydrates that would dilute the protein levels in the grain. Results from Experiment 2 indicate that this was not the case. Total kernel nitrogen increases linearly over time from approximately 15 days after DC 65 in a similar manner to kernel weight (Simpson *et al.* 1983). Fischer *et al.* (1993) demonstrated that grain protein levels fall as yields rise up to about 6 t ha^{-1} and a similar trend exists with the data presented in this chapter (see Fig 6.1). Much of the variability in this graph would be due to the differences in the ability of the various cultivars to

accumulate protein as well as the agronomic and environmental differences between sites. However despite the increase in kernel weights associated with the *tin* gene, the protein contents were not consistently diluted, or enhanced, by its presence. At the two lowest yielding sites, C95.1 and C95.2, where kernels weights were low and grain protein contents were high, there was no difference in protein contents between the cultivars and the *tin* lines, while at the highest yielding sites, W96 and W97, grain protein contents were significantly higher for the *tin* lines at the former, but significantly lower for the latter.

6.5 CONCLUSION

As with field trials reported in Chapter 5, the *tin* gene did not result in yield or biomass penalties. The intrinsic effects of the *tin* gene appear to be higher harvest index values, shorter stems and greater kernel weights. Increased kernel weights of the *tin* lines appears to be due to their ability to fill kernels for a longer duration or at a faster rate during the final phases of grainfilling than the cultivars when spike densities were the same. Until late milk development (DC 79), rates of grain filling of the *tin* lines mirrored those of the cultivars. Those traits which appear to be a function of the lower spike density rather than the *tin* gene *per se* are the greater head weights at DC 65, increased number of spikelets spike^{-1}, increased number of kernels spike^{-1} and increased stem length density which are normally observed under field conditions when the *tin* lines produced crops with lower spike densities.

Stem water soluble carbohydrate levels at DC 65 were the same or higher for the *tin* lines under field conditions, although when grown at the same spike density the values were the same or lower, indicating that it is more a function of the lower spike density than the *tin* gene. Stem nitrogen levels were the same or lower for the *tin* lines under field conditions, although there was no consistent effect on grain protein levels.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

Stunted and normal phenotypes of the *tin* breeding line J/BKU #2 at Ginninderra Experimental Station, Canberra (photograph courtesy of Dr Hiroshi Tsuyuzaki)

7.1 INTRODUCTION

There were several objectives outlined at the commencement of this project. An understanding was required to determine what conditions brought about the stunting phenomenon. In addition to this, an understanding as to how stunting can be avoided or prevented as lines that had a tendency to stunt under regular growing conditions could never be released commercially. How the *tin* gene affects cellular dynamics was also deemed important to provide an insight into the kinematic processes that result in the gigas features such as large leaves. With the morphology of wheat plants radically altered by the presence of the *tin* gene an understanding of how it affects plant development and partitioning of assimilate was also required. Finally, assessment of the potential for the *tin* gene to increase yield was required as was an investigation as to whether the beneficial traits associated with the *tin* gene are purely a function of the gene, or were merely a function of the lower spike densities.

7.2 STUNTING AND TILLERING

The presence of the *tin* gene resulted in a reduction in the number of tillers produced regardless of the conditions under which lines containing it were sown. In some more susceptible lines, and only under certain conditions of low minimum temperature and long days/high light intensity, it resulted in stunting. Data from Experiment 5 of Chapter 2 indicates that the amount of assimilate availability plays an important role in determining whether a plant containing the *tin* gene stunts. Results from this chapter also demonstrate that it is the suppression of the apex that causes those plants containing the *tin* gene to stunt. However if the apex can develop beyond terminal spikelet, then it will avoid the severe and fatal forms of stunting.

Apical dominance and nutritional conditions control tiller bud elongation in grasses. When the mainstem apex of barley and teosinte (Leopold, 1949) and oats (Harrison and Kaufman, 1980) were destroyed, tillering was found to be promoted, indicating a role for the mainstem apex in the control of tiller bud elongation. The hormone most commonly associated with apical dominance is auxin that is produced in the apex (White *et al.* 1975), which migrates to tiller buds where it suppresses their growth. As the plant develops it produces increasing amounts of auxin (Leopold, 1949) which suppresses later formed, more juvenile tiller buds. In addition to being produced

in the mainstem, auxin is also produced in the leaves with production increasing as daylength increases (Leopold, 1949). Cytokinins, which are produced in the roots, are known to increase tillering (Langer *et al.* 1973; Johnston and Jeffcoat, 1977), and Jewiss (1972) suggested that it was the cytokinin:auxin ratio that regulated tillering in cereals rather than the individual auxin or cytokinin concentration. Plants containing the *tin* gene may simply produce more auxin in their leaves and/or less cytokinins in their roots compared to plants without the *tin* gene when exposed to long days and low temperatures which in turn results in the suppression of the tiller buds. Alternatively, the apex may become more sensitive to one of these hormones. While it is tempting to speculate as to which hormone(s) are involved in tiller suppression resulting from the presence of the *tin* gene, results presented in this thesis do not definitively implicate specific hormone(s).

The fact that increased light intensity, increased photoperiod and increased CO_2 all increase the incidence and severity of stunting indicates that assimilation or levels of assimilate regulate the expression of the *tin* gene. Increasing assimilate or the rate of assimilation along with low minimum temperatures promotes the expression of the *tin* gene. The reduction in tillering and stunting are linked and the latter is simply a more extreme manifestation of the former. Fig 2.12 demonstrated that when minimum temperatures were low and light intensities were high and stunting was induced, plants containing the *tin* gene produced fewer tillers than those grown under the same conditions except at lower light intensities. However when the minimum temperature was raised (but average temperature maintained), plants at the high light intensity not only failed to stunt but produced more tillers than those plants grown at the low light intensities. The severity of the expression of the *tin* gene almost certainly varies between lines due to the presence of minor genes that may be more prevalent in lines such as line 492 and Banks+*tin* than line 380. These genes however do not normally impact on the rate of phenological development as there was no trend between time from sowing to double ridge formation or flowering with stunting or tiller inhibition.

The possibility exists that stunting could be a problem if lines containing the *tin* gene were grown in the Australian wheatbelt if appropriate action is not taken. Fig 2.5 displayed the percentage stunting in line 492 plants when it was sown at various times throughout the year in Canberra. The highest incidence of stunting occurred in late winter and spring, although a high incidence of stunting was also reported when seeds

were sown in mid-autumn. However, sowing in late autumn sowing resulting in now stunting. Most of the Australian wheat crop is sown in the late autumn (i.e. May), although if seasonal rains are early or late then sowing could take place at any time between early April and late July. Line 492 is the most susceptible to stunting of the lines tested reported in this project and any cultivars that may be developed that contain the *tin* gene would need to be much less susceptible to this phenomenon. The other lines containing the *tin* gene that were sown throughout the year in this experiment failed to stunt so it is certainly possible to develop lines that display a lower incidence of stunting than line 492. However, if cultivars containing the *tin* gene are to be produced in the future, farmers who purchase this seed should be warned of the conditions that cause stunting and be advised not to sow their seed too early or too late in the season.

7.3 CELLULAR DYNAMICS

The presence of the *tin* gene resulted in longer and wider leaves, provided that plants are not grown under conditions that induce or partly induce stunting. Elongation rates of the leaves of *tin* lines were also promoted by the presence of the *tin* gene by increasing the maximum rate of cell expansion and number of cells entering the growth zone per unit of time. One possible reason why the *tin* gene did not have the expected yield advantage under terminal drought conditions hypothesised by Innes *et al.* (1981) and Islam and Sedgley (1981) may be due to the larger leaves produced by the *tin* lines. The larger individual leaf area may have compensated for the reduction in total number of leaves through the reduction in tiller number brought about by the presence of the *tin* gene and the crops would not have been able to conserve soil water for grain filling as had been hypothesised.

Why Bodallin+*tin* produced more cells and those cells elongated at a faster rate than those produced by Bodallin is unclear, although it may be more an intrinsic effect of the *tin* gene rather an increase in assimilate availability. As stated in Chapter 3, if the increase in cell flux and leaf elongation rates were due to the increase in assimilate availability associated with the suppression of tiller buds then it would be expected that there would be an even greater increase seen in the uniculm barley line compared to its near-isogenic pair. Leaf width also contributed significantly to the greater leaf area of Bodallin+*tin* relative to Bodallin and further investigation is required to determine

whether it is due to an increase in the number of cell files or whether the individual cell with is increased.

7.4 DEVELOPMENT AND PARTITIONING

Under both pot and field conditions, all lines containing the *tin* gene initially produced tillers at the same rate as their near-isogenic pairs, but ceased shortly after double-ridge formation whereas the cultivars continued well beyond this stage. As it takes a few days before tillers begin to emerge from the axil of the leaf, then the *tin* gene must start to affect tiller bud development at or shortly prior to double ridge formation. The application of nitrogen promoted tillering in all the *tin* lines, but not to the same extent as in the cultivars. This was due to an increase in the initial rate rather than the duration of the tillering. The *tin* lines produced similar leaf area index values and levels of biomass as the cultivars under most conditions and the partitioning of assimilate was largely unaffected by the presence of the *tin* gene except for the consistent increase in harvest index. Light interception was however lower for the *tin* lines indicating that the leaves may be more erect. It was hypothesised that the lower specific leaf area (SLA) values associated with the *tin* gene may have resulted in the leaves being less likely to flop, thus allowing more light to penetrate into the lower canopy. Stem water soluble carbohydrates tended to be the same or slightly higher for the *tin* lines at DC 65 under field conditions where spike densities were lower for the *tin* lines, while stem nitrogen levels were typically the same or lower compared to the cultivars. It was hypothesised that the lower spike density of the *tin* lines allowed more light into the lower canopy, which in turn allowed the stems of the *tin* lines to accumulate more water soluble carbohydrates. This was shown to be the case as when near-isogenic pairs differing in the presence of the *tin* gene were sown to achieve the same spike density then the level of stem water soluble carbohydrates was the same or even slightly lower for the *tin* lines. Root growth appeared to be unaffected by the presence of the *tin* gene with root-length density values the same for the *tin* lines and the cultivars throughout most of the profile.

7.5 YIELD AND YIELD COMPONENTS

Although the site by *tin* interaction was significant (P=0.028), across sites reported in this thesis the *tin* gene did not significantly alter yield, except when plant density was low and cultivars were able to compensate for gaps in the canopy. While spike density was lower for *tin* lines relative to the cultivars, they were able to compensate with more kernels spike^{-1} and an increase in kernel weight. These characteristics were typical across most field sites. When growing at the same plant density, lines containing the *tin* gene also seemed more efficient at partitioning assimilate towards grain as demonstrated by the increase in harvest index values. This finding supports the view that sterile tillers do not provide an effective store of nutrients for the plant. Results presented here indicated that sterile tillers are largely a net waste of biomass that may otherwise have been directed into fertile stems and subsequently grain yield.

The finding that some lines (e.g. Bodallin+*tin*) consistently yield more than their commercial near-isogenic pair demonstrates that there are benefits associated with the *tin* gene and that it can be useful in commercial wheat breeding programs. Other lines, such as Banks+*tin*, may still contain genes that have a negative impact on yield and may out-yield their commercial near-isogenic pair with further back-crossing. Why Bodallin+*tin* consistently yielded well compared to Bodallin is unknown. It may simply be that there are other advantageous genes have been retained in the development of this line and/or there are deleterious genes have been retained in the development of lines such as Banks+*tin*. The absence of any difference in yield or yield components between Osprey and Osprey+*tin* may mean that the *tin* gene has no effect in winter wheat lines. As winter wheat lines produce most of their tillers prior to double ridge formation then the *tin* gene would not be expressed in these lines until most of the tillering had taken place. As a result the effects of the *tin* gene, both intrinsic and associated, are more difficult to observe in winter wheat grown under Australian wheatbelt conditions.

7.6 INTRINSIC VERSUS ASSOCIATED EFFECTS

The *tin* gene appears to have some characteristic responses that will be produced regardless of the prevailing conditions and other associated effects that are secondary and are only the result from the lower spike density. The reduction in height is clearly

an intrinsic trait as both Kite+*tin* and Bodallin+*tin* are shorter than their near-isogenic pairs regardless of the spike density. The same is true of increased kernel weights and harvest index values. However many of the gigas traits (i.e. more spikelets spike^{-1}, more kernels spike^{-1} and thicker stems) seem to be a function of the lower spike density rather than the *tin* gene *per se*. These gigas traits must therefore be a result of increased assimilate availability as a result of the reduction in tiller number. Increased stem water soluble carbohydrate levels also appear to be a function of lower spike densities/more open canopies and the *tin* gene may in fact have a negative effect on water soluble carbohydrate storage in the stems. The fact that leaf area index was conserved for longer when spike densities were the same for both the Kite+*tin* and Bodallin+*tin* lines may be a result of the lower stem water soluble carbohydrate levels. As the larger grains would place a greater demand on the plant for reserves towards the end of grain filling, plants containing the *tin* gene may need to conserve leaf area in an attempt to continue the supply of assimilate.

7.7 CONCLUSION

The *tin* gene results in the suppression of tillers and can result in the extreme manifestation where plants are stunted under conditions of long photoperiod, high light intensity and low minimum temperature. There appears to be good evidence from the light intensity and CO_2 experiments that the phenomenon is driven by assimilate supply and an auxin or auxin-like hormone may be the mode of action. The lower tiller and spike numbers are the result of an early cessation rather than a lower rate of tillering. Yield is usually unaffected by the *tin* gene, although yield components are altered with fewer kernel produced per unit area while kernel weights are increased. Partitioning is more efficient in the *tin* lines with harvest index values consistently higher than those of the commercial cultivars. Many of the gigas traits associated with the *tin* gene such as large spikes and thick stems are not conserved when lines containing the *tin* gene are sown to achieve the same spike density as cultivars. However some traits such as increased kernel weight, increased harvest index and reduced height are still exhibited and must therefore be considered intrinsic effects.

BIBLIOGRAPHY

Wagga Wagga, August 1997

Allan, R.E. (1989). Agronomic comparisons between *Rht1* and *Rht2* semidwarf genes in winter wheat. *Crop Science*. **29**, 1103-1108.

Aspinall, D. (1961). The control of tillering in the barley plant I. The pattern of tillering and its relation to nutrient supply. *Australian Journal of Biological Science*. **14,** 493-505.

Aspinall, D. and Paleg, L.G. (1963). Effects of daylength and light intensity on growth of barley. I Growth and development of apex with a fluorescent light source. *Botanical Gazette*. **124,** 429-437.

Atsmon, D. and Jacobs, E. (1977). A newly bred 'Gigas' form of bread wheat (*Triticum aestivum* L.): Morphological features and thermophotoperiodic responses. *Crop Science*. **17,** 31-35.

Atsmon, D., Bush, M.G. and Evans, L.T. (1986a). Effects of environmental conditions on expression of the 'Gigas' characters in wheat. *Australian Journal of Plant Physiology*. **13,** 365-379.

Atsmon, D., Bush, M.G. and Evans, L.T. (1986b). Stunting in 'Gigas' wheats as influenced by temperature and daylength. *Australian Journal of Plant Physiology*. **13,** 381-389.

Austin, R.B., Edrich, J.A. and Ford, M.A. (1974). Vernalisation requirements of winter wheats. *Plant Breeding Institute, Cambridge: UK, Plant Breeding Institute, Cambridge: Annual report 1973.. 1974,* pg 168-169

Austin, R.B., Morgan, C.L., Ford, M.A. and Blackwell, R.D. (1980). Contributions to grain yield from pre-anthesis assimilate in in tall and dwarf barley phenotypes in two contrasting seasons. *Annals of Botany*. **45,** 309-319.

Austin, R.B., Edrich, J.A., Ford, M.A. and Blackwell, R.D. (1977). The fate of the dry matter, carbohydrates and ^{14}C lost from the leaves and stems of wheat during grain filling. *Annals of Botany.* **41,** 1309-1321.

Baker, C.K. and Gallagher, J.N. (1983). The development of winter wheat in the field I. Relation between apical development and plant morphology within and between seasons. *Journal of Agricultural Science., Cambridge.* **101**, 327-335.

Barley, K.P. and Naidu, N.A. (1964). The performance of three Australian wheat varieties at high levels of N supply. *Australian Journal of Experimental Agriculture and Animal Husbandry.* **4,** 39-48.

Beemster, G.T.S., Masle, J., Williamson, R.E. and Farquhar, G.D. (1996). Effects of soil resistance to root penetration on leaf expansion in wheat (*Triticum aestivum* L.): kinematic analysis of leaf elongation. *Journal of Experimental Botany.* **47:** 1663-1678.

Begg, J.E. and Wright, M.J. (1962). Growth and development of leaves from intercalary meristems in *Phalaris arundinacea* L. *Nature.* **194:** 1097-1098.

Bell, G.D.H. and Kirby, E.J.M. (1966). Utilisation of growth responses in breeding new varieties of cereals. *In* 'The growth of cereals and grasses'. *Proceedings of the 12th Easter school in agricultural science., University of Nottingham.* (Eds Milthorp and Ivins). Butterworths., London. pg 308-319.

Benbelkacem, A., Mekni, M.S. and Rasmusson, D.C. (1984). Breeding for high tiller number and yield in barley. *Crop Science.* **24**, 968-972.

Bhagsari, A.S. and Brown, R.H. (1986). Leaf photosynthesis and its correlation with leaf area. *Crop Science.* **26,** 127-132.

Bidinger, F., Musgrave, R.B. and Fischer, R.A. (1977). Contributions of stored pre-anthesis assimilation to grain yields in wheat and barley. *Nature.* **270**, 431-433.

Black, A.L. (1970). Adventitious roots, tillers and grain yields of spring wheat as influenced by N-P fertilization. *Agronomy Journal.* **62,** 32-36.

Boffey, S.A., Sellden, G. and Leech, R.M. (1980). Influence of cell age on chlorophyll formation in light-grown and etiolated wheat seedlings. *Plant Physiology.* **65:** 680-684.

Bonnett, G.D. and Incoll, L.D. (1992). The potential pre-anthesis and post-anthesis contributions of stem internodes to grain yield in crops of winter barley. *Annals of Botany*. **69**, 219-225.

Borrell, A.K., Incoll, L.D. and Dalling, M.J. (1993). The influence of Rht_1 and Rht_2 alleles on the growth of wheat stems and ears. *Annals of Botany*. **67**, 103-110.

Borrell, A.K., Incoll, L.D., Simpson, R.J. and Dalling, M.J. (1989). Partitioning of dry matter and the deposition and use of stem reserves in a semi-dwarf wheat crop. *Annals of Botany*. **63**, 527-539.

Bremner, P.M. (1969). Growth and yield of three varieties of wheat, with particular reference to the influence of unproductive tillers. *Journal of Agricultural Science., Cambridge.* **72,** 281-287.

Cannell, R.Q. (1969). The tillering pattern in barley varieties I. Production, survival and contribution to yield by competent tillers. *Journal of Agricultural Science., Cambridge.* **72,** 405-422.

Casal, J.J., Deregibus, V.A. and Sanches, R.A. (1985). Variations in tiller dynamics and morphology in *Lolium multiflorum* Lam. Vegetative and reproductive plants as affected by differences in red/far red irradiation. *Annals of Botany.* **56,** 553-559.

Christopher, D.A., Atsmon, D. and Feldman, M. (1985). Mode of inheritance and chromosomal allocation of stunting genes in common wheat. *Crop Science.* **25,** 147-151.

Cline, M.G. (1991). Apical Dominance. *The Botanical review.* **57,** 318-358.

Cooper, P.J.M., Gregory, P.J., Keatinge, J.D.H. and Brown, S.C. (1987). Effects of fertilizer, variety and locations on barley production under rainfed conditions in northern Syria 2. Soil water dynamics and crop water use. *Field Crops Research.* **16,** 67-84.

Craufurd, P.Q. and Cartwright, P.M. (1989). Effect of photoperiod and chlomequat on apical development and growth in a spring wheat (*Triticum aestivum*) cultivar. *Annals of Botany.* **63**, 515-525

Crofts, H.J. (1989). On defining a winter wheat. *Euphytica.* **44,** 225-234.

Davidson, D.J. and Chevalier, P.M. (1990). Preanthesis tiller mortality in spring wheat. *Crop Science.* **30**, 832-836.

Davis, M.H. and Simmons, S.R. (1994). Far-red light reflected from neighbouring vegetation promotes shoot elongation and accelerates flowering in spring barley plants. *Plant, Cell and Environment.* **17**, 829-836.

Donald, C.M. (1968). The breeding of crop ideotypes. *Euphytica.* **17,** 385-403.

Donald, C.M. (1979). A barley programme based on an ideotype. *Journal of Agricultural Science., Cambridge.* **93**, 261-269.

El Alaoui, A.C., Simmons, S.R. and Crookston, R.K. (1992). Allocation of photoassimilate by main shoots and nonsurviving tillers in barley. *Crop Science.* **32**, 1233-1237.

Engledow, F.L. and Wadham, S.M. (1924). Investigations on yield in cereals I. *Journal of Agricultural Science., Cambridge.* **14,** 66-98.

Erickson, R.O. and Sax, K.B. (1956). Elemental growth rate of the primary root of *Zea mays. Proceedings of the American Philosophical Society.* **100:** 487-514.

Evans, L.T., Wardlaw, I.F. and Williams, C.N. (1964). Environmental control of growth. *In* 'Grasses and Grasslands'. (Ed Barnard, C.). McMillian and Co. London, Melbourne and New York. pg 102-125.

Farrer, W. (1898). The making and improvement of wheats for Australian conditions. *The Agricultural Gazette of New South Wales.* **9,** 131-168.

Finlay, K.W. and Wilkinson, G.N. (1963). The analysis of adaptation in a plant-breeding programme. *Australian Journal of Agricultural Research.* **14,** 742-754.

Fischer, R.A. (1975) Yield potential of dwarf spring wheat and the effect of shading. *Crop Science.* **15**, 607-613.

Fischer, R.A. (1985). Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science., Cambridge.* **105**, 447-461.

Fischer, R.A., Aguilar, I.M., Maurer, R.O. and Rivas, S.A. (1976). Density and row spacing effects on irrigated short wheats at low latitude. *Journal of Agricultural Science., Cambridge.* **87**, 137-147.

Fischer, R.A., Howe, G.N. and Ibrahim, Z. (1993). Irrigated spring wheat and timing and amount of nitrogen fertilizers I. Grain yield and protein content. *Field Crops Research.* **33**, 37-56.

Fischer, R.A. and Kohn, G.D. (1966a). The relationship between evapotranspiration and growth in the wheat crop. *Australian Journal of Agricultural Research.* **17,** 255-267.

Fischer, R.A. and Kohn, G.D. (1966b). The relationship of grain yield to vegetative growth and post-flowering leaf area in the wheat crop under conditions of limited soil moisture. *Australian Journal of Agricultural Research.* **17,** 281-295.

Fischer, R.A. and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I Grain yield responses. *Australian Journal of Agricultural Research.* **29**, 897-912.

Fischer, R.A. and Stockman, Y.M. (1980). Kernel number-per spike in wheat (*Triticum aestivum* L.). Responses to preanthesis shading. *Australian Journal of Plant Physiology*. **7**, 169-180.

Fraser, J., Dougherty, C.T. and Langer, R.H.M. (1982). Dynamics of tiller populations of standard height and semi-dwarf wheats. *New Zealand Journal of Agricultural Research.* **25**, 321-328.

Friend, D.J.C. (1965). Tillering and leaf production in wheat as affected by temperature and light intensity. *Canadian Journal of Botany.* **43,** 1063-1076.

Gallagher, J.N., Biscoe, P.V. and Scott, R.K. (1975). Barley and its environment V. Stability of grain weight. *Journal of Applied Ecology.* **12**, 319-336.

Gandar, P.W. and Hall, A.J. (1988). Estimating position-time relationships in steady-state, one-dimensional growth zones. *Planta.* **175:** 121-129.

Gandar, P.W. and Rasmussen, H. (1991). Growth patterns and movement of epidermal cells within the leaves of *Asphodelus tenuifolius* Cav. *Annals of Botany.* **68**, 307-315.

Garcia del Moral, L.F., Ramos, J.M. and Recalde, L. (1984). Tillering dynamics of winter barley as influenced by cultivar and N fertilizer: A field study. *Crop Science.* **24,** 179-181.

Gardner, J.L. (1942). Studies in tillering. *Ecology.* **23(2),** 162-174.

Gomez-MacPherson, H., Richards, R.A. and Masle, J. (1998a) Growth of near-isogenic wheat lines differing in development I. Spaced plants. *Annals of Botany.* **82**, 315-322.

Gomez-MacPherson, H., Richards, R.A. and Masle, J. (1998b) Growth of near-isogenic wheat lines differing in development II. Plants in a simulated canopy. *Annals of Botany.* **82,** 323-330.

Green, P.B. (1976). Growth and cell pattern formation on an axis: critique of concepts, terminology, and modes of study. *Botanical Gazette.* **137(3):** 187-202.

Gregory, P.J., McGowan, P., Biscoe, P.V. and Hunter, B. (1978). Water relations of winter wheat I. Growth of the root system. *Journal of Agricultural Science., Cambridge.* **91**, 91-102.

Gu, J. and Marshall, C. (1988). The effect of tiller removal and tiller defoliation on competition between the main shoot and tiller of spring barley. *Annals of Applied Biology.* **112,** 597-608.

Harrison, M.A. and Kaufman, P.B. (1980). Hormonal regulation of lateral bud (tiller) release in oats (*Avena sativa* L.). *Plant Physiology.* **66**, 1123-1127.

Haus, T.E., Ramage, R.T. and Tsuchuja, T. (eds) (1971). Barley Genetics Newsletter. Dept of Agronomy, Colorado State University, Fort Collins, U.S.A. pp 156.

Heffernan, B. (1985). 'A handbook of methods of inorganic chemical analysis for forest soils, foliage and water'. (CSIRO Division of Forest Research, Canberra).

Holliday, R. and Willey, R.W. (1969). Variety potential in cereals and its improvement. *Agricultural progress.* **44**, 56-77.

Holmes, M.G. and Smith, H. (1975). The function of phytochrome in plants growing in the natural environment. *Nature.* **254**, 512-514.

Hotsonyame, G.K. and Hunt, L.A. (1998). Effects of sowing date, photoperiod and nitrogen on variation in main culm leaf dimensions in field-grown wheat. *Canadian Journal of Plant Science.* **78**, 35-49.

Inbal, E. and Atsmon, D. (1983). Morphological, physiological and genetic aspects of stunting expression in the uniculm 'Gigas' wheat. *Proceedings of the 6th International Wheat Genetics Symposium, Kyoto, Japan.* 439-445.

Innes, P., Blackwell, R.D., Austin, R.B. and Ford, M.A. (1981). The effects of selection for number of ears on the yield and water economy of winter wheat. *Journal of Agricultural Science., Cambridge.* **97,** 523-532.

Ishag, H.M. and Taha, M.B. (1974). Production and survival of tillers of wheat and their contribution to yield. *Journal of Agricultural Science., Cambridge.* **83,** 117-124.

Islam, T.M.T. and Sedgely, R.H. (1981). Evidence for a 'uniculm effect' in a spring wheat (*Triticum aestivum* L.) in a Mediterranean environment. *Euphytica.* **30,** 277-282.

Jewiss, O.R. (1972). Tillering in grasses - its significance and control. *Journal of the British Grasslands Society.* **27,** 65-82.

Johnston, G.F.S. and Jeffcoat, B. (1977). Effects of some growth regulators on tiller bud elongation in cereals. *New Phytologist.* **79,** 239-245.

Kasperbauer, M.J. (1971). Spectral distribution of light in a tobacco canopy and effects of end-of-day light quality on growth and development. *Plant Physiology*. **47**, 775-778.

Kasperbauer, M.J. and Karlen, D.L. (1986). Light-mediated bioregulation of tillering and photosynthate partitioning in wheat. *Physiologia Plantarum*. **66**, 159-166.

Khalil, M.S.H. (1956). The interrelationship between growth and development of wheat as influenced by temperature, light and nitrogen. *Mededelingen van de Landbouwhogeschool te Wageningen/Nederland.* **56(7),** 1-73.

Kirby, E.J.M. (1970). Evapotranspiration from barley grown at different plant densities. *Journal of Agricultural Science., Cambridge*. **75**, 445-450.

Kirby, E.J.M. (1973). The control of leaf and ear size in barley. *Journal of Experimental Botany*. **24**, 567-578.

Kirby, E.J.M. and Faris, D.G. (1972). Effect of plant density on tiller growth and morphology in barley. *Journal of Agricultural Science., Cambridge.* **78,** 281-288.

Kirby, E.J.M. and Jones, H.G. (1977). The relations between main shoot and tillers in barley plants. *Journal of Agricultural Science., Cambridge.* **88,** 381-389.

Lambert, D.A. (1967). Cocksfoot (*Dactylis glomerata*) grown for production of seed. *Annals of Botany.* **31**, 371-383.

Langer, R.H.M., Prasad, P.C. and Laude, H.M. (1973). Effects of kinetin on tiller bud elongation in wheat (*Triticum aestivum* L.). *Annals of Botany.* **37,** 565-571.

Lauer, J.G. and Simmons, S.R. (1988). Photoassimilate partitioning by tillers and individual tiller leaves in field-grown spring barley. *Crop Science.* **28,** 279-282.

Leeky, R.R.B and Longman, K.A. (1986). Physiological, environmental and genetic variation in apical dominance as determined by decapitation in *Triplochiton scleroxylon*. *Tree Physiology.* **1**, 193-207.

Lemerle, D., Verbeek, B., Cousens, R.D. and Coombes, N.E. (1996). The potential for selecting wheat varieties strongly competitive against weeds. *Weeds Research.* **36**, 505-513.

Leopold, A.C. (1949). The control of tillering in grasses by auxin. *American Journal of Botany.* **36,** 437-440.

Lim, R. and Tamas, I.A. (1989). The transport of radiolabeled indoleacetic acid and its conjugates in nodal stem segments of *Phaseolus vulgaris* L. *Plant growth regulators.* **8**, 151-164.

Marshall, C. and Boyd, W.J.R. (1985). A comparison of the growth and development of biculm wheat lines with freely tillering cultivars. *Journal of Agricultural Science., Cambridge.* **104,** 163-171.

McDonald, G.K. (1990). The growth and yield of uniculm and tillered barley over a range of sowing rates. *Australian Journal of Agricultural Research.* **41**, 449-461.

Miller, C.O., Skoog, F., von Saltza, M.H. and Strong.F.M. (1955). Kinetin, a cell division factor from deoxyribonucleic acid. *Journal of the American Chemical Society.* **77**, 1392.

Mitchell, K.J. (1953a). Influence of light and temperature on the growth of ryegrass (*Lolium* spp.). I Pattern of vegetative development. *Physiologia Plantarum.* **6,** 21-46.

Mitchell, K.J. (1953b). Influence of light and temperature on the growth of ryegrass (*Lolium* spp.). II The control of lateral bud development. *Physiologia Plantarum.* **6,** 425-443.

Mohamed, G.E.S. and Marshall, C. (1979). Physiological aspects of tiller removal in spring wheat. *Journal of Agricultural Science., Cambridge.* **93,** 457-463.

Morris, A.K. and Silk, W.K. (1992). Use of flexible logistic function to describe axial growth of plants. *Bulletin of Mathematical Biology*. **54**, 1069-1081.

Nix, H.A. (1976). Climate and crop productivity in Australia. *In* International Rice Research Institute. 1976. Climate and Rice. Los Baños, Philippines. pg 495-508.

Nix, H.A. (1987). The Australian climate and its effects on grain yield and quality. In *Australian field crops. Volume 1: Wheat and other temperate cereals. Second edition.* (Eds Lazenby, A. and Matheson, E.M.) (Angus and Robertson Publishers) pg 183-226.

Northcote, K.H. (1971). A factual key for the regonition of Australian soils. (Rellim: Glenside. South Australia).

Palfi, G and Daszi, L (1960). The translocation of nutrients between fertile and sterile shoots of wheat. *Acta Botanica Hungarica.* **6,** 65-74.

Palta, J.A. and Fillery, I.R. (1995). N application enhances remobilization and reduces losses of pre-anthesis N in wheat grown on a duplex soil. *Australian Journal of Agricultural Research.* **46**, 519-531.

Palta, J.A., Kobata, T., Turner, N.C. and Fillery, I.R. (1994). Remobilization of carbon and nitrogen in wheat as influenced by post-anthesis water deficits. *Crop Science.* **34**, 118-126.

Paolillo Jr, D.J., Sorrells, M.E. and Keyes, G.J. (1991). Gibberellic acid sensitivity determines the length of the extension zone in wheat leaves. *Annals of Botany.* **67:** 479-485.

Passioura, J.B. (1977). Grain yield, harvest index, and water use of wheat. *The Journal of the Australian Institute of Agricultural Science.* **43**, 117-120.

Pearce, G.R., Lee, J.A., Simpson, R.J. and Doyle, P.T. (1988). Sources of variation in the nutritive value of wheat and rice straws. *In* 'Plant breeding and the nutritive value of crop residues'. (Eds J.D. Reed, B.S. Capper, and P.J.H. Neate.) pp 195-229. (International Livestock Centre for Africa: Ethiopia).

Pelton, W.L. (1969). Influence of low seeding rates on wheat yield in southwestern Saskatchewan. *Canadian Journal of Plant Science*. **49**, 607-614.

Pheloung, P.C. and Siddique, K.H.M. (1991). Contribution of stem dry matter to grain yield in wheat cultivars. *Australian Journal of Plant Physiology*. **18**, 53-64.

Pinthus, M.J. (1967). Evaluation of winter wheat as a source of high yield potential for the breeding of spring wheat. *Euphytica*. **16**, 231-251.

Power, J.F. and Alessi, J. (1978). Tiller development and yield of standard and semidwarf spring wheat varieties as affected by nitrogen fertilizer. *Journal of Agricultural Science., Cambridge*. **90,** 97-108.

Puckridge, D.W. and Donald, C.M. (1967). Competition among wheat plants sown at a wide range of densities. *Australian Journal of Agricultural Research*. **18**, 193-211.

Rasmusson, D.C. (1987). An evaluation of ideotype breeding. *Crop Science*. **27,** 1140-1146.

Rawson, H.M. (1971). Tillering patterns in wheat with special reference to the shoot at the coleoptile node. *Australian Journal of Biological Science*. **24**, 829-841.

Rawson, H.M. (1987). Effects of high temperatures on the development and yield of wheat and practices to reduce deleterious effects. *In* Wheat production constraints in tropical environments. *Proceedings of the International conference, January 19-23, 1987, Chang Mai, Thailand*. (ed Klatt, A.R.) pg 44-61.

Rawson, H.M. (1988). High temperature is not a stress. *Proceedings of the International conference of Plant Physiology, February 15-20, 1988, New Delhi, India*. pg 923-928.

Rawson, H.M. and Donald, C.M. (1969). The absorption and distribution of nitrogen after floral initiation in wheat. *Australian Journal of Agricultural Research.* **20,** 799-808.

Rawson, H.M., Gardner, P.A. and Long, M.J. (1987). Sources of variation in specific leaf area in wheat grown at high temperature. *Australian Journal of Plant Physiology.* **14,** 287-298.

Reid, D.A. and Wiebe, G.A. (1968). Taxonomy, botany, classification and world collection. *In* Barley: Origin, botany, culture, winterhardiness, genetics, utilization, pests. Volume 38, U.S. Department of Agriculture, Washington D.C. pp 61-84.

Richards, R.A. (1983). Manipulation of leaf area and its effect on grain yield in droughted wheat. *Australian Journal of Agricultural Science.* **34**, 23-31.

Richards, R.A. (1988). A tiller inhibition gene in wheat and its effect on plant growth. *Australian Journal of Agricultural Research.* **39,** 749-757.

Richards, R.A. and Townley-Smith, T.F. (1987). Variation in leaf area development and its effect on water use, yield and harvest index of droughted wheat. *Australian Journal of Agricultural Research.* **38,** 983-992.

Ritchie, J.T. and Burnett, E. (1971). Dryland evaporative flux in a subhumid climate. II Plant influences. *Agronomy Journal.* **63**, 56-62.

Saini, A.D. and Nanda, R. (1979). The effect of culm number on yield in wheat (*Triticum aestivum* L). *Indian Journal of Plant Physiology.* **22**, 97-101.

Scarth, R., Kirby, E.J.M. and Law, C.N. (1985). Effects of the photoperiod genes *Ppd1* and *Ppd2* on growth and development of the shoot apex in wheat. *Annals of Botany.* **55**, 351-359.

Schnyder, H. and Nelson, C.J. (1987). Growth rates and carbohydrate fluxes within the elongation zone of tall fescue leaf blades. *Plant Physiology*. **85:** 548-553.

Schnyder, H. and Nelson, C.J. (1988).Diurnal growth of tall fescue leaf blades I. Spatial distribution of growth, deposition of water, and assimilate import in the elongation zone. *Plant Physiology*. **86:** 1070-1076.

Schnyder, H. and Nelson, C.J. (1989). Growth rates and assimilate partitioning in the elongation zone of tall fescue leaf blades at high and low irradiance. *Plant Physiology*. **87:** 1201-1206.

Schnyder, H., Nelson, C.J., and Spollen, W.G. (1988). Diurnal growth of tall fescue leaf blades II. Dry matter partitioning and carbohydrate metabolism in the elongation zone and adjacent expanded tissue. *Plant Physiology*. **86:** 1077-1083.

Schnyder, H. Seo, S., Rademacher, I.F. and Khübauch, W. (1990). Spatial distribution of growth rates and of epidermal cell lengths in the elongation zone during leaf development in *Lolium perenne* L. *Planta.* **181:** 423-431.

Schönfeld, M., Johnson, R.C. and Ferris, D.M. (1989). Development of winter wheat under increased atmospheric CO_2 and water limitation at tillering. *Crop Science.* **29,** 1083-1086.

Sharma, R. (1995). Tiller mortality and its relationship to grain yield in spring wheat. *Field Crops Research*. **41**, 55-60.

Sharman, B.C. (1942). Developmental anatomy of the shoot of *Zea mays* L. *Annals of Botany, N.S.* **6 no 2:** 245-282.

Silk, W.K., Lord, E.M. and Eckard, K.J. (1989). Growth patterns inferred from anatomical records. Empirical tests using longisections of roots of *Zea mays* L. *Plant Physiology*. **90:** 708-713.

Simpson, R.J., Lambers. H. and Dalling, M.J. (1983). Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.) IV. Development of a quantitative model of the translocation of nitrogen to the grain. *Plant Physiology*. **71**, 7-14.

Sims, H. (1963). Changes in the hay production and the harvest index of Australian oat varieties. *Australian Journal of Experimental Agriculture and Animal Husbandry.* **3,** 198-202.

Smith, R.W. (1925). The tillering of grain as related to yield and rainfall. *Journal of the American Society of Agronomy*. **17**, 717-725.

Smith, H.F. (1933). The physiological relations between tillers of a wheat plant. *Journal of the Council for Scientific and Industrial Research, Australia.* **6,** 32-42.

Smith, H.H. and Webster, J.A. (1974). Leaf pubescence and cereal beetle resistance in *Triticum* and *Avena* species. *Crop Science.* **14**, 241-243.

Stoy, V. (1965). Photosynthesis, respiration and carbohydrate accumulation in spring wheat in relation to yield. *Physiologia Plantarum Supplementum*. **IV**. 92-104.

Thorne, G.N. and Wood, D.W. (1988) Contributions of shoot categories to growth and yield of winter wheat. *Journal of Agricultural Science., Cambridge*. **111**, 285-294.

Tincker, M. and Jones. M. (1931). Yield studies in oats. III The inter-relationship of the parts of the oat plant during development. *Annals of Applied Biology.* **18,** 37-53.

Tomar, Y.S. (1983). Reflections on apical dominance in relation to mineral nutrients in *Nicotiana tobacum* L. cv Calcutti. *Proceedings of the National Academy of Science, India*. **53(B)2**, 109-114.

Trione, E.J. and Metzger,R.J. (1970). Wheat and barley vernalization in a precise temperature gradient. *Crop Science*. **10**, 390-392.

van Dillewijn, C. (1952). Tillering. *In* Botany of sugarcane. The Chronica Botanica Co. Waltham, Massachusetts, U.S.A. pg 77-97

van Herwaarden, A.F., Farquhar, G.D., Angus, J.F., Richards, R.A. and Howe, G.N. (1998a). 'Haying-off', the negative grain yield response of dryland wheat to nitrogen fertiliser I. Biomass, grain yield, and water use. *Australian Journal of Agricultural Research*. **49**, 1067-1081.

van Herwaarden, G.D., Angus, J.F., Richards, R.A. and A.F., Farquhar. (1998b). 'Haying-off', the negative grain yield response of dryland wheat to nitrogen fertiliser II.Carbohydrate and protein dynamics. *Australian Journal of Agricultural Research*. **49**, 1083-1093.

van Herwaarden, A.F., Richards, R.A., Farquhar, G.D. and Angus, J.F. (1998c). 'Haying-off', the negative grain yield response of dryland wheat to nitrogen fertiliser III. The influence of water deficit and heat shock. *Australian Journal of Agricultural Research*. **49**, 1095-1110.

van Sanford, D.A. and Utomo, H. (1995). Inheritance of tillering in a winter wheat population. *Crop Science*. **35**, 1566-1569.

Van't Hof, J. (1968). Control of cell progression through the mitotic cycle by carbohydrate provision I. Regulation of cell division in excised plant tissue. *The Journal of Cell Biology.* **37:** 773-780.

Villegas, D., Aparicio, N. and Royo, C. (1998). Drought effect on the pattern of biomass accumulation of durum wheat genotypes selected for contrasting environments. *In* 'Proceedings of the 9th International Wheat Genetics Symposium, Saskatoon, Canada' (Compiled by A.E. Slinkard). Vol 4, pp 104-107.

Volenec, J.J. and Nelson, C.J. (1983) Response of tall fescue leaf meristems to N fertilization and harvest frequency. *Crop Science.* **23:** 720-724.

Volenec, J.J. and Nelson, C.J. (1984A). Carbohydrate metabolism in leaf meristems of tall fescue I. Relationship to genetically altered leaf elongation rates. **74:** 590-594.

Volenec, J.J. and Nelson, C.J. (1984B). Carbohydrate metabolism in leaf meristems of tall fescue II. Relationship to leaf elongation rates modified by nitrogen fertilization. **74:** 595-600.

Wardlaw, I.F. (1970). The early stages of grain development in wheat: Response to light and temperature in a single variety. *Australian Journal of Biological Science.* **23**, 765-774.

Warington, K. (1933). The influence of length of day on the response of plants to boron. *Annals of Botany.* **47,** 430-458.

Watson, D.J. (1936). The effect of applying a nitrogenous fertiliser to wheat at different stages of growth. *Journal of Agricultural Science., Cambridge.* **26,** 391-414.

Whan, B.R., Delane, R. and Gilmour, R. (1988). The potential of reduced tillering wheats in dry environments. *Proceedings of the 7th International Wheat Genetics Symposium, Cambridge, Great Britain.* pg 907-911.

White, J.C., Medlow, G.C., Hillman, J.R. and Wilkins, M.B. (1975). Correlative inhibition of lateral bud growth in *Phaseolus vulgaris* L. Isolation of Indolacetic acid from the inhibitory region. *Journal of Experimental Botany.* **26,** 419-424.

Wiggans, S.C. (1959). Responses of oat plants to various percentages of continuous shade. *Botanical Gazette.* **121(1),** 55-60.

Williams, R.F. and Langer, R.H.M. (1975). Growth and development of the wheat tiller II. The dynamics of tiller growth. *Australian Journal of Botany.* **23**, 745-759.

Williams, R.F. and Metcalf, R.A. (1975). Physical constraint and tiller growth in wheat. *Australian Journal of Botany.* **23**, 213-223.

Williams, R.F. Sharman, B.C. and Langer, R.H.M. (1975). Growth and development of the wheat tiller I.Growth and form of the tiller bud. *Australian Journal of Botany.* **23**, 715-743.

Woolley, J.T. (1971). Reflectance and transmittance of light by leaves. *Plant Physiology.* **47**, 656-662.

Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *The Biochemical Journal.* **57**, 508-514.

Yunusa, I.A.M. and Sedgley, R.H. (1992). Reduced tillering spring wheats for heavy textured soils in semi-arid Mediterranean environment. *Journal of Agronomy and Crop Science.* **168,** 159-168.

Zadoks, J.C., Chang, T.T. and Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weeds Research*. **14,** 415-421.

APPENDIX

Lines 492 (left) and 380 grown under conditions of 21/16°C, 16hour daylength and high (700ppm) CO_2

Table 1. Composition of Hoagland's Solution

Macro nutrients

Compound	mg L^{-1}	mM
$Ca(NO_3)_2.4H_20$	950	4.0
$(NH_4).H_2PO_4$	120	1.0
KNO_3	610	6.0
$MgSO_4.7H_20$	490	2.0

Micro nutrients

Compound	mg L^{-1}	μM
H_3BO_3	0.6	9.7
$MnCl_2.4H_2O$	0.4	2.0
$ZnSO_4.7H_2O$	0.09	0.3
$CuSO_4.5H_2O$	0.05	0.2
H_2MoO_4	0.02	0.1
$Co(NO_3)_2.6H_2O$	0.025	0.1
$FeSO_4.7H_2O$	24.8	89.2
NaOH	6.6	165.0
$[CH_2.N(CH_2.COOH).CH_2.COONa]_2.2H_2O$	33.2	89.2

Table 2. Composition of Hewitt's Solution (nitrite type)

Macro nutrients

Compound	mg L^{-1}	mM
KNO_3	404	4.0
$Ca(NO_3)_2.4H_20$	945	4.0
$MgSO_4.7H_20$	368	1.5
$NaH_2PO_4.2H_20$	208	1.5
EDTA (FeNa)	20	0.05

Micro nutrients

Compound	mg L^{-1}	μM
$MnSO_4.H_2O$	0.223	1.3
$ZnSO_4.7H_2O$	0.290	1.0
$CuSO_4.5H_2O$	0.250	1.0
H_3BO_3	3.100	50.0
$Na_2MoO_4.H_2O$	0.120	0.5
NaCl	5.800	100.0
$Co(NO_3)_2.6H_2O$	0.056	0.2

Table 3. Rainfall, temperature (maximum and minimum) and pan evaporation for Condobolin

	J	F	M	A	M	J	J	A	S	O	N	D
Monthly rainfall (mm)												
Mean[a]	55	40	40	35	38	27	38	35	29	53	38	41
1995[c]	181	22	0	2	89	33	29	1	4	36	79	26
1996 [c]	3	7	13	6	84	26	63	41	26	48	40	1
1997 [c]	22	6	4	1	45	16	16	27	122	24	28	7
Average Daily Maximum temperature (°C)												
Mean[a]	33.3	32.5	29.4	24.4	19.4	15.6	14.8	16.6	20.0	24.7	28.2	31.6
1995 [c]	30.0	31.5	29.0	22.1	17.9	15.0	12.8	20.1	20.7	26.0	27.7	30.0
1996 [c]	33.1	30.7	29.4	23.8	N.A.	16.8	13.8	15.9	19.9	25.6	26.9	N.A.
1997 [c]	32.3	35.8	29.8	26.2	19.6	15.9	14.9	16.0	18.7	25.0	32.1	32.3
Average Daily Minimum temperature (°C)												
Mean[a]	17.6	17.8	14.6	9.7	6.7	3.6	2.5	3.4	5.4	9.3	12.5	15.4
1995 [c]	18.7	16.8	13.1	7.4	9.1	5.4	3.0	3.0	5.4	9.9	14.7	14.9
1996 [c]	18.2	15.0	14.6	8.0	N.A.	4.6	4.8	4.3	5.8	9.6	11.6	N.A.
1997 [c]	18.2	21.1	14.5	8.1	8.6	3.0	0.3	3.0	6.8	9.5	18.7	16.5
Monthly Pan Evaporation (mm)												
Mean[b]	310	251	217	129	74	48	50	78	111	174	231	304
1995 [c]	214	224	248	141	56	36	47	87	105	192	195	285
1996 [c]	283	274	218	148	60	50	36	63	108	165	227	304
1997 [c]	278	274	234	158	75	43	60	72	84	168	265	343

[a] 42 year mean from Condobolin AR and AS

[b] 23 year mean from Condobolin AR and AS

[c] Condobolin AR and AS

N.A. =not available

Table 4. Rainfall, temperature (maximum and minimum) and pan evaporation for Moombooldool

	J	F	M	A	M	J	J	A	S	O	N	D
Monthly rainfall (mm)												
Mean[a]	44	38	38	38	42	41	43	44	38	46	40	37
1996[b]	67	33	50	7	25	34	92	35	52	55	30	32
1997 [b]	58	18	37	2	33	46	15	35	59	30	14	10
Average Daily Maximum temperature (°C)												
Mean[c]	31.6	31.1	27.8	23.0	17.9	14.4	13.5	15.2	18.1	22.3	26.7	29.5
1996 [d]	31.4	29.0	27.6	21.5	19.9	16.6	13.6	15.6	19.2	23.7	25.2	29.7
1997 [d]	32.5	35.5	27.6	24.6	19.5	15.6	14.5	11.6	19.1	25.4	29.9	32.4
Average Daily Minimum temperature (°C)												
Mean[c]	16.9	17.5	14.4	10.2	7.3	4.1	3.2	4.0	5.9	9.2	12.0	14.9
1996 [d]	17.1	14.5	14.2	8.1	7.2	6.3	4.7	5.3	6.4	9.5	11.7	14.8
1997 [d]	18.0	21.3	13.1	9.1	8.7	4.8	1.6	0.9	6.7	9.0	14.6	16.4
Monthly Pan Evaporation (mm)												
Mean[c]	319	271	242	132	74	45	50	78	111	177	243	298
1996 [d]	295	248	219	120	82	56	40	76	131	178	235	323
1997 [d]	369	288	116	143	85	53	58	81	96	216	267	337

[a] 88 year average for Ardlethan Post Office

[b] "Coondara", Moombooldool

[c] 55 year average from Yanco Agricultural Research Institute

[d] Yanco Agricultural Research Institute

Table 5. Rainfall, temperature (maximum and minimum) and pan evaporation for Wagga Wagga

	J	F	M	A	M	J	J	A	S	O	N	D
						Monthly rainfall (mm)						
Mean[a]	42	38	45	43	56	49	58	53	51	61	47	46
1996[b]	83	46	80	13	40	76	81	55	49	43	59	28
1997 [b]	55	5	64	4	50	40	28	47	87	25	24	17
						Average Daily Maximum temperature (°C)						
Mean[a]	31.2	30.7	27.4	22.2	17.1	13.7	12.5	14.3	17.3	21.1	25.2	29.1
1996 [b]	30.1	27.9	26.7	20.2	18.4	14.8	12.2	14.1	17.2	22.2	23.6	28.9
1997 [b]	31.7	35.0	29.5	24.3	18.0	15.0	13.7	14.1	18.1	23.0	29.0	32.3
						Average Daily Minimum temperature (°C)						
Mean[a]	15.9	16.3	13.4	9.1	6.0	3.6	2.7	3.6	5.1	7.8	10.4	13.7
1996 [b]	15.7	13.0	12.8	7.0	5.9	4.3	4.4	5.1	5.9	8.6	9.0	12.0
1997 [b]	15.8	19.1	13.6	7.9	9.4	4.0	1.8	3.7	5.1	7.8	13.0	14.9
						Monthly Pan Evaporation (mm)						
Mean[a]	310	257	214	120	62	36	37	59	87	146	210	295
1996 [b]	245	220	186	90	56	39	31	53	96	140	195	279
1997 [b]	330	301	230	143	74	44	49	64	80	170	280	392

[a] 55 year mean for Wagga Wagga Agricultural Research Institute

[b] Wagga Wagga Agricultural Research Institute

Table 6. Rainfall, temperature (maximum and minimum) and pan evaporation for Ariah Park

	J	F	M	A	M	J	J	A	S	O	N	D
Monthly rainfall (mm)												
Mean[a]	49	37	41	44	46	41	47	46	42	54	45	41
1997[b]	30	4	33	2	46	62	19	48	52	40	8	5
Average Daily Maximum temperature (°C)												
Mean[c]	31.3	30.6	27.6	22.3	17.7	13.8	12.9	14.7	18.0	22.0	25.8	29.5
1997 [b]	31.6	35.2	28.1	25.2	18.9	14.7	13.8	14.3	18.0	24.1	31.3	32.3
Average Daily Minimum temperature (°C)												
Mean[c]	15.9	16.1	13.2	8.9	5.6	3.1	2.0	3.0	4.6	7.7	10.5	13.7
1997 [b]	18.1	19.4	12.3	7.3	7.7	2.5	0.6	3.0	4.8	7.5	14.1	17.4
Monthly Pan Evaporation (mm)												
Mean[d]	267	220	180	105	59	33	37	56	78	130	189	242
1997 [b]	285	241	189	135	62	42	43	65	84	233	393	344

[a] 64 year mean from Temora Agricultural Research station

[b] Temora Agricultural Research station

[c] 54 year mean from Temora Agricultural Research station

[d] 19 year mean from Temora Agricultural Research station

Figure 1. Daily maximum and minimum temperatures for Canberra betweenAugust 1995 and September 1996

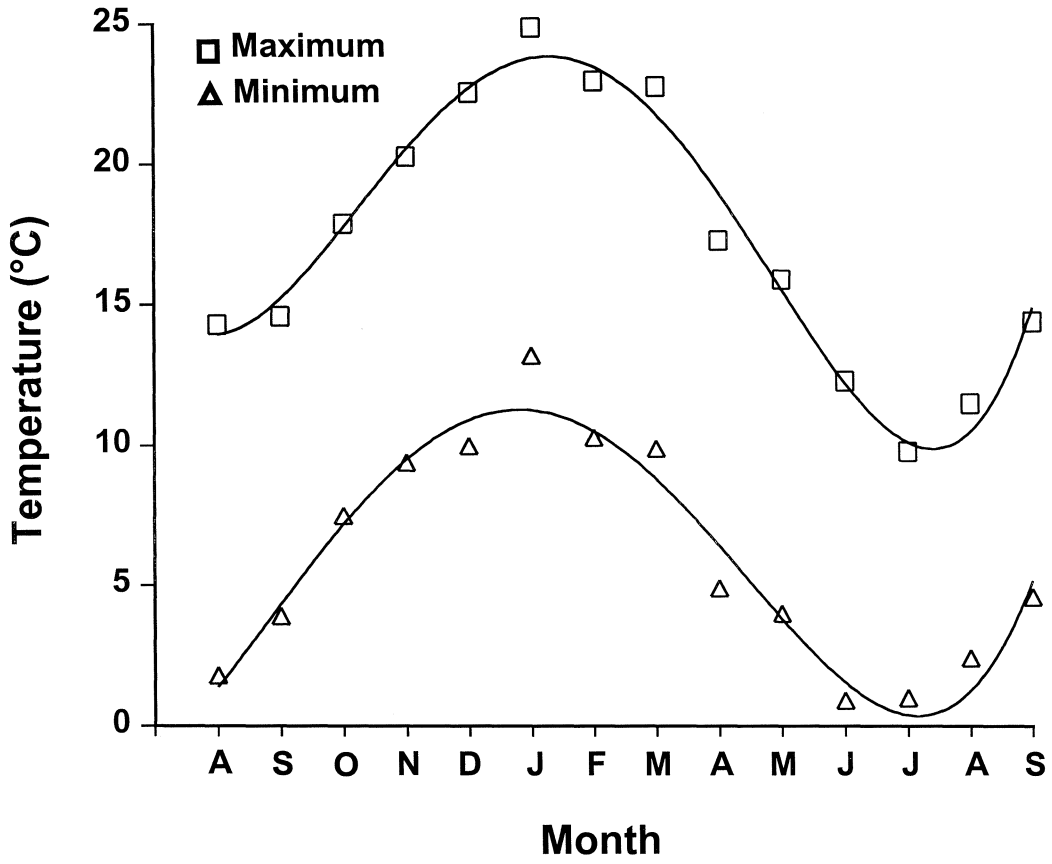


Figure 2. Mean maximum and minimum temperature for Canberra, August to December, 1996

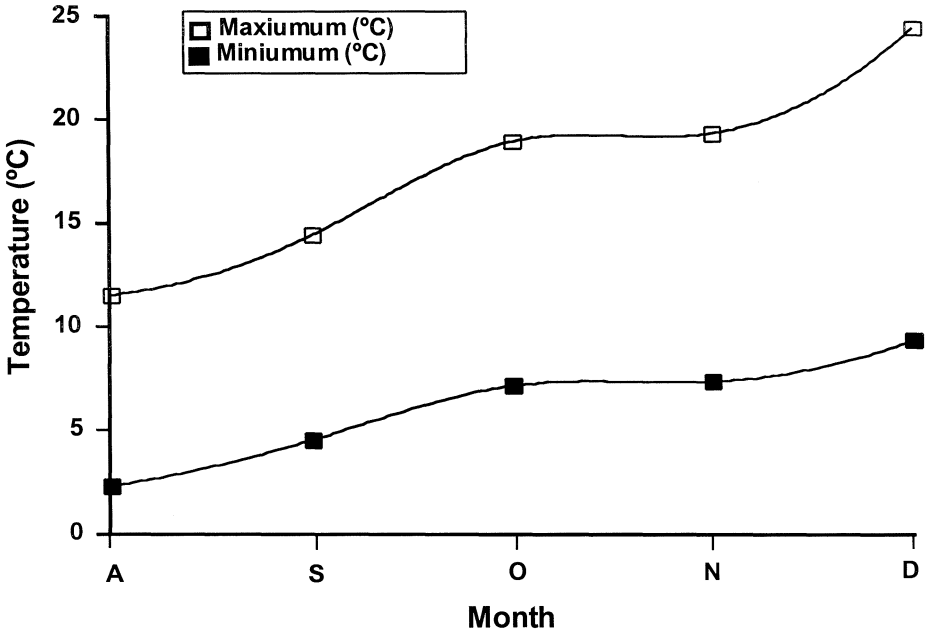


Figure 3. Monthly rainfall (mm) for Canberra, August to December, 1996

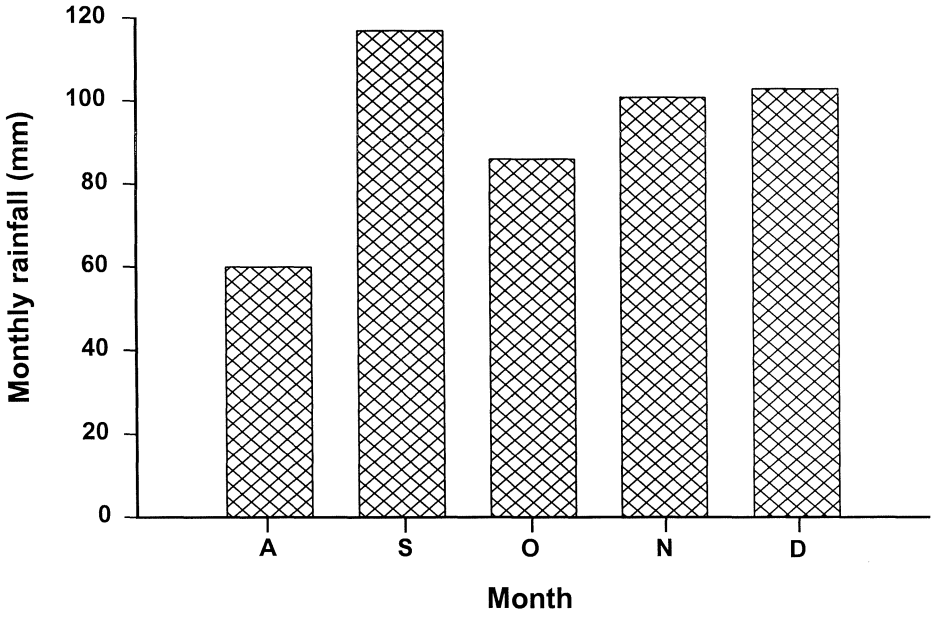
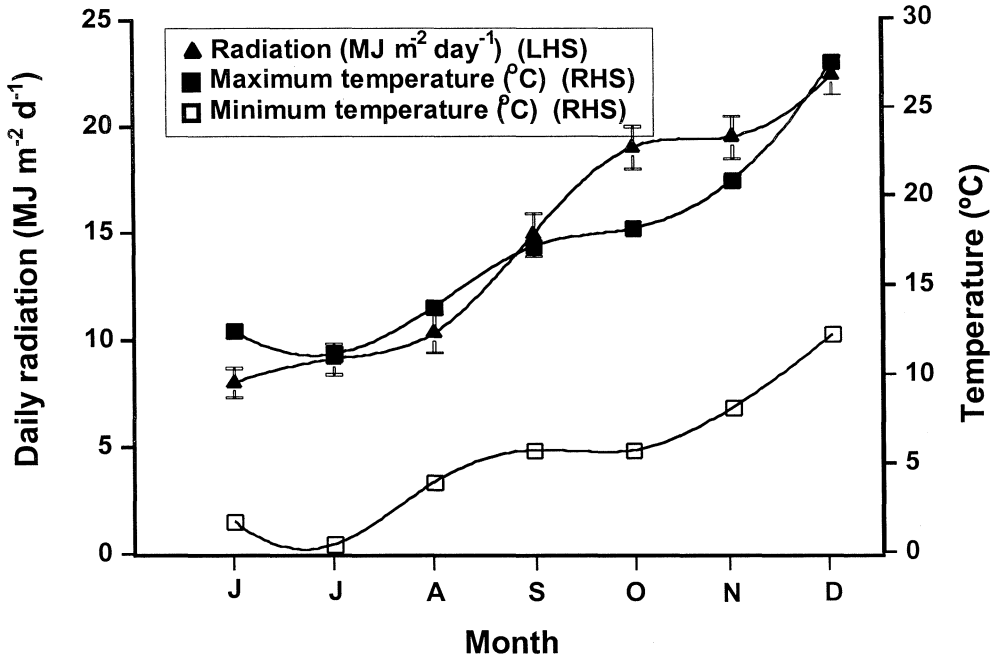


Figure 4. Daily radiation (LHS) and maximum and minimum temperatures (RHS) for Canberra between June and December, 1998



Error bars for radiation indicate ± standard error