CARBON, NITROGEN AND WATER DYNAMICS IN DRYLAND WHEAT, WITH PARTICULAR REFERENCE TO HAYING-OFF

by

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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.

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ABSTRACT

Haying-off is a colloquial term that is used to describe the phenomenon of the premature cessation of grain filling in cereals due to water stress. It typically results in low yields and small or shrivelled grains. Haying-off is moderately common in well fertilised cereal crops in south-eastern Australia and it represents an increasing risk as farmers seek higher yield and protein content by increasing the nitrogen status of their soils. Haying-off typically occurs in well-fertilised crops during a post-anthesis drought at a time when temperatures are increasing. Curiously, the exact causes of haying-off have never been understood but there are thought to be several requirements. There must be vigorous vegetative growth stimulated by high soil nitrogen levels and accompanied by high water use prior to anthesis and lastly dry conditions after anthesis with some periods of extreme evaporative demand.

The major symptom of haying-off is small or pinched kernels. It is proposed in this thesis that, in the absence of other severe biological constraints, when kernel weight for small-seeded wheat varieties is above 30 mg, that haying-off has not occurred. If kernel weight falls somewhere between 30 - 25 mg then haying-off is likely to have occurred and to have reduced grain yield, whereas if kernel weight falls below 25 mg then haying-off has almost certainly occurred.

The principal aim of the work presented in this thesis was to determine the physiological basis of haying-off by monitoring the carbon and nitrogen dynamics and the water use of wheat crops and their responses to applied nitrogen. Six field experiments are described which were established to provide differences in soil water availability. Increasing amounts of nitrogen fertiliser were applied in all experiments and at different times of development. A controlled environment study is also described. This was established to more precisely separate the effects of drought from high temperatures on haying-off.

The field experiments varied substantially in their responses to nitrogen. At all sites the application of nitrogen resulted in a higher biomass at anthesis and increased spike densities and kernel numbers. Grain yields at the most responsive site varied from 132 g m^{-2} for the low nitrogen control to 570 g m^{-2} for the high nitrogen treatment. For the site where haying-off was most severe, yields declined from 374 g m^{-2} in the control to 283 g m^{-2} for the high nitrogen treatment.

Carbon, nitrogen and water budgets were developed for different nitrogen treatments in each experiment. The carbon budget showed that the loss in leaf weight between anthesis and maturity could not be accounted for by the loss in water soluble carbohydrates (WSC) and protein, nor by saprophytic decay or leaf fall. It was shown

that hemicellulose in the cell walls of leaves was an important source of pre-anthesis assimilate that is available for retranslocation to the grain. Hemicellulose contributed about 30% of the dry matter exported from the leaves to the grain between anthesis and maturity. Unexpectedly, the water budget for crops that hayed-off established that about 10 mm more water was left behind in the soil than crops that did not hay-off.

Nitrogen application increased kernel number in all crops irrespective of drought. The nitrogen content of the spike at anthesis was highly correlated with kernel number at each site and this strong relationship between spike nitrogen content and kernel number was maintained when data were combined over all sites. The WSC content of the spike was also positively correlated with kernel number in each experiment. However, when data for WSC from all sites was related to kernel number there was no relationship which suggests that nitrogen content rather than carbon supply is the principal determinant of kernel number in these environments.

In line with current beliefs haying-off was only found where there was water stress. Surprisingly, it was found that brief periods of high temperatures were not necessary to induce haying-off. Continued increases in kernel number in response to nitrogen fertiliser, despite the onset of drought stress prior to anthesis, renders the wheat crop vulnerable to haying-off. It was found that haying-off was due to a combination of high water use prior to anthesis and to a low level of WSC reserves in well-fertilised, high biomass crops. This latter finding was contrary to expectations as it is often assumed that high nitrogen leads to high anthesis biomass and proportionately more pre-anthesis assimilate reserves stored as WSC. Thus, it is concluded that haying-off occurs when there is a terminal drought which reduces photosynthesis during grain filling and to a lack of WSC reserves. A low nitrogen crop is unlikely to hay-off because it uses less water before anthesis and so suffers less post-anthesis water stress. It also has greater reserves of pre-anthesis WSC. These findings lead to the suggestion of a plant type which is expected to have more stable yields in fertile environments which experience large seasonal fluctuations in water availability. The findings also lead to a better definition of where haying-off is most likely to occur.

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T_{SA}	Transpiration from sowing to anthesis
VPD	Vapour pressure deficit
W_{ET}	Water use efficiency
W_{ET}^{B}	Water use efficiency for above ground biomass
W_{ET}^{G}	Water use efficiency for grain yield
W_T	Transpiration efficiency
W_T^B	Transpiration efficiency for above ground biomass
$W_T^B{}_{A\text{-}M}$	Transpiration efficiency of above ground biomass from anthesis to maturity
$W_T^B{}_{S\text{-}A}$	Transpiration efficiency of above ground biomass from sowing to anthesis
W_T^G	Transpiration efficiency for grain yield
WSC	Water soluble carbohydrate
Δ	Carbon isotope discrimination
θ_v	Volumetric soil water content
$\mu E\ m^{-2}$	Micro Einsteins per square metre
40LT, 80LT.....	Rates of nitrogen applied at late tillering
40MT, 80MT.....	Rates of nitrogen applied at mid tillering
200ST	Split application of 200kg N ha^{-1} applied from sowing to late tillering
40SW, 80SW.....	Rates of nitrogen applied at sowing

CHAPTER 1

INTRODUCTION AND OBJECTIVES

"Like all young men I set out to be a genius,

but mercifully laughter intervened."

Lawrence Durrell

1.1 INTRODUCTION

1.1.1 A brief history of nitrogen fertiliser trials in Australia

Several series of extensive field trials have been conducted throughout the history of wheat production in Australia, attempting to determine the nitrogen status of cropping soils and the scope for nitrogen fertilisation to increase grain yield and protein. Work in the early 1900's indicated that phosphorus was the major limiting nutrient for wheat production and that there was little or no positive response in yield to nitrogenous fertiliser in the presence or absence of superphosphate (Guthrie and Helms 1902; Howell 1904). In the ensuing years the soil-exploitative practice of fallow led to a decline in soil nitrogen (Cornish 1949) such that in the 1930's positive yield responses to nitrogen fertiliser were observed and cautious recommendations for its use were advocated (Callaghan and Breakwell 1937). As soil nitrogen levels continued to decline and wheat yields suffered, the clover ley was introduced into the farming landscape to restore soil nitrogen levels between cropping phases (Penman 1949). Extensive field trials in the 1950s and 1960s (Colwell 1963a,1963b; Russell 1967; Mason 1968; Taylor *et al.* 1978) identified the widespread occurrence of nitrogen deficiency but could only provide broad regional recommendations for the use of nitrogen fertilisers due to the variability of seasonal rainfall and supply of mineral nitrogen from the soil. With the introduction of the nitrogen responsive semi-dwarf wheat varieties (Syme *et al.* 1976) in the 1970s and the decline in the protein concentration of Australian Standard White (ASW) category of wheat (McDonald 1989), presumably due to increased cropping intensity (Angus and Fischer 1991), extensive field trials (Angus *et al.* 1989; McDonald 1992) reassessed the responsiveness of commercial wheat crops to nitrogen fertiliser. Tests to determine the nitrogen status of wheat crops were assessed (van Herwaarden *et al.* 1989; Handson and Amenta 1989) with the aim of topdressing nitrogen to crops several months after sowing when more is known about the availability of soil water than at the time of sowing.

Each of these research programs, from the turn of the century to recent times, has looked at the scope for increased production and/or maintaining grain quality and all but the work in Western Australia was confronted with the phenomenon of "haying-off". The effects of the phenomenon known as haying-off in Australia are well known in regions around the world where the cultivation of cereals occur and is referred to as hay curing in parts of the United States of America, hot spell damage in Italy, zachvat or zapal in Russia (Fischer 1980).

1.1.2 What is haying-off ?

Haying-off has had almost as many different definitions as there have been reports of its occurrence. Taylor (1965b) provided a general description stating that "haying-off is regarded as the phenomenon in which cereal crops fail to yield grain in accordance with their vegetative potential." This definition is, however, too broad to be of any practical use since any wheat crop which matures under terminal drought is likely to yield less than its vegetative potential. Colwell (1963a,1963b) similarly described it as maturation of crops before grain development has been completed, apparently due to exhaustion of soil moisture reserves. While acknowledging the association between increasing nitrogen supply and declining water supply, several workers contend that wheat crops hay-off due to premature senescence (Storrier 1962, 1965a, 1975; Simpson 1992), premature ripening (Fischer and Kohn 1966c) or "burning" (Hunter *et al.* 1958). The most complete definition of haying-off comes from Dann (1969) who states that haying off is the premature cessation of grain filling due to exhaustion of soil moisture by the vigorous vegetative growth of crops stimulated by high soil nitrogen levels. However, this definition also fails to describe the point at which a water-stressed crop can be considered to hay-off. Still other works refer to haying-off without defining it (Johnston and Fowler 1991; McDonald 1992), report the classical symptoms without discussing it (Kirkegaard *et al.* 1994) or were at a loss to account for it (Stanford and Hunter 1973).

Experimental treatments which increase early biomass production make it possible to identify haying-off as the absence of a positive yield response or a yield decline. However haying-off is difficult to define or identify in the absence of different levels of soil nitrogen and because of the unique combination of factors which contribute to yield. Most commonly, the interaction of the three factors of soil water, phosphorus and nitrogen determine grain yield at any given site assuming the lack of significant interaction with other factors such as disease (Taylor 1965b) or sowing density (Fischer and Kohn 1966c). When phosphorus is not limiting to growth and nitrogen supply is in excess of that needed to balance water supply during grain filling, then haying-off occurs. Haying-off will remain a subjective description of the phenomenon which results in reduced grain yields due to the absence of any measurable criteria which would provide a yes or no answer. This is due to the fact that the yield response to nitrogen is a curve of diminishing returns which ultimately becomes negative (Fischer 1979) and not an on or off response.

Obvious features of haying-off at maturity are a tall, dark-coloured biomass, small and usually pinched grain of a high protein content, low harvest index and low yields (Angus *et al.* 1989). If the drought stress occurs early, grain yield is reduced due to poor tiller survival (Barley and Naidu 1964; Fischer and Kohn 1966c) and low grain set per spike

(Barley and Naidu 1964) but kernel weight may not be affected (Barley and Naidu 1964). The term haying-off is not usually used in this situation since a reduction in hectolitre weight or kernel weight caused by inadequate water supply during grain filling is the most common expression of haying-off (Taylor 1965b; Dann 1969). Indeed, Russell (1967) found that haying-off did not occur in seasons which were dry throughout but was associated with adequate winter rainfall followed by dry, hot spring conditions. This late stress may not reduce tiller survival or grain set per spike (Fischer and Kohn 1966c) but acts through dramatically reducing kernel weight and hence harvest index (McDonald 1991; Frederick and Marshall 1985). It is proposed in this thesis that, in the absence of other severe biological constraints, when kernel weight for small-seeded wheat varieties is above 30mg, that haying-off has not occurred. If kernel weight falls somewhere between 30 - 25 mg then haying-off is likely to have had an effect on grain yield, while if kernel weight falls below 25 mg haying-off has almost certainly occurred.

1.1.3 Confirmation of the role of water stress in haying-off

There have been many field experiments in Australia reported in the scientific literature that have been affected by the phenomenon known as haying-off but very few have specifically set out to investigate the problem. In most studies water stress is usually inferred from the appearance of experimental crops and not by direct measurement of the soil water status.

However, Barley and Naidu (1964) and Fischer and Kohn (1966a) measured greater water use due to increased biomass production in response to nitrogen application. Fischer and Kohn (1966a, 1966c) reported that at anthesis wheat crops which had received 112 kg N ha^{-1} had produced an additional 263 gm^{-2}, used 31 mm more water and went on to produce 38 g m^{-2} more grain yield than control crops. In the work of Barley and Naidu (1964), however, crops receiving 134 kg N ha^{-1} produced an additional 450 g m^{-2} by ear emergence, used an extra 25 mm of water (assuming a mean bulk density of 1.6 g m^{-3}) but yielded the same as the control crops. In the latter study the high nitrogen treatment used 13 mm less water than the control between ear emergence and maturity. van Herwaarden *et al.* (1993) reported greater water use for positive yield responses to nitrogen under terminal drought conditions. Their data show that water use was greater when flowering was delayed and that water use increased in response to nitrogen to a greater extent in a later maturing variety despite lower grain yield. These data support the inference that haying-off is due to water stress during grain filling (eg. Collwell 1963a), that rainfall distribution is critical in the development of haying-off (Russell 1967) and that harvest index (and hence grain yield) decreases as the proportion of total water use transpired after anthesis decreases (Passioura 1977).

1.1.4 Do factors other than water supply contribute to haying off ?

There is evidence that nitrogen fertilisation has special, unknown effects on a wheat crop (Fischer and Kohn 1966c; Dann 1969; Lipsett and Simpson 1973) and that there is more to the phenomenon of haying-off than excessive biomass production to anthesis (Fischer 1979). There is evidence that nitrogen induced nutrient deficiency (Freney and Lipsett 1965) or the interaction with nitrogen of root disease (Butler 1961) and foliar disease Roth *et al,* 1984) may also exacerbate haying-off. Storrier (1965b) found that there was a linear decline in wheat yield with nitrogen applications and that 32mm of post anthesis irrigation, while increasing yield at a given level of nitrogen, failed to alter the magnitude of the yield decline due to nitrogen. The application of nitrogen also reduced kernel weight even when there was no response in the biomass measured in October (prior to anthesis) and little evidence of water stress (Dann 1969; Lipsett and Simpson 1973). Indeed, for wheat grown in Australia, kernel weight rarely increases and usually decreases in response to nitrogen fertiliser under both dryland (eg. Fischer and Kohn 1966b; McDonald 1991) and irrigated conditions (eg. Strong 1986; Fischer 1993). This decrease in kernel weight, even in the absence of water stress, may be explained by an increased tiller production in response to nitrogen which results in denser crops and greater competition for limiting resources (Fischer and Kohn 1966c), especially under water stress (Storrier 1965b).

Fischer and Kohn (1966c) contend that there was not likely to be any increase in water use and hence water stress in response to nitrogen in their 1961 experiment due to only a small increase in leaf area. Barley and Naidu (1964), however, hypothesise 'that there may have been changes in foliar characteristics that reduced resistance to water loss from the leaf to air at given water deficits'. Such a response could be possible if stomates opened wider than necessary to balance the increase in photosynthetic capacity induced by nitrogen fertilisation (Wong *et al.* 1979; Evans 1983).

Another possible factor affecting haying-off is high-temperature stress. Fischer (1980) showed that a single 6 hr high temperature (46 °C) stress on well-watered wheat plants 12 or 20 days after anthesis reduced leaf relative water content at the end of the stress cycle and reduced final kernel weight to a greater extent at high nitrogen than at low nitrogen. These data indicate that wheat plants of high nitrogen status are less able to control water loss during periods of high vapour pressure deficit (VPD) than low nitrogen plants and that high nitrogen status may predispose wheat crops to haying-off. Hot dry weather during grain filling has been implicated in haying-off by Russell (1967) More specifically, Azzi (1956) described the effects of high temperature and associated 'Sirocco' winds on a wheat crop during grain filling, and credits it with the cause of haying-off known as 'hot-spell damage' in Italy. Indeed, as already mentioned, high

VPD events associated with high temperatures and dry winds are common during grain filling in various cereal growing regions of the world. Damage is claimed to occur after only brief periods of atmospheric stress and even under wet soil conditions (Fischer 1980). Sudden severe drought leading to the death of tissues may result in greater reductions in kernel weight at high nitrogen due to the possibility of incomplete translocation of all available assimilate to the kernels (Fischer 1979).

1.1.5 Clear and present danger ?

Haying-off is a term that originated with the introduction of superphosphate fertiliser for wheat in the 1890's (Colwell 1963a). Descriptions of the symptoms of haying-off were common in the scientific literature of the time (Guthrie and Helms 1902; Callaghan and Breakwell 1937) but gradually disappeared as haying-off became less common, probably due to the depletion of soil nitrogen levels (Cornish 1949). With the adoption of clover ley into the farming system in the 1950's wheat growers again began reporting yield losses in wheat from haying-off, prompting Colwell (1963a) to investigate the problem with a series of experiments in the late 1950's. Pugsley (1963) felt that there was a danger that fertility levels could be allowed to build up under the clover ley to a level too high for safe wheat growing. More recently, increased cropping intensity, the introduction of reputedly nitrogen-responsive semi-dwarf wheats, and better control of soil-borne pathogens due to breakcrops has led many researchers to believe that the threat of haying-off is no longer a problem (J.F. Angus, pers. comm.).

However, a study of responses to nitrogen fertiliser across a range of semidwarf wheat varieties in commercial crops (Angus *et al.* 1989) has shown that haying off is at least as prevalent now as it was with tall wheats in the 1950's and 60's (Russell 1967, Taylor *et al.* 1978). With the initiative started in 1994 by the Australian Wheat Board to achieve a national average grain yield of 2 tonnes ha^{-1} at 10 percent protein by the year 2000 for the ASW wheat category (Anon. 1994), increased nitrogen fertiliser usage and efforts to improve the nitrogen status of cropping soils through better pastures and/or increased usage of grain legumes is likely to lead to an increased incidence of haying-off. Unfortunately it will be growers who bear the increased risk of haying-off while payment schemes continue to favour yield rather than protein concentration.

1.2 OBJECTIVES

This study was conducted because haying-off is a continuing problem for the Australian wheat industry and as yet there is no satisfactory explanation of its cause. At the onset of the experimental work the hypothesis was that haying-off is initiated when a high nitrogen wheat crop experiences both drought and a high-temperature event after

anthesis, that is, once the kernel number has been set. Haying-off subsequently develops due to desiccation and death of photosynthetic tissues thereby reducing the supply of current assimilate, and the incomplete retranslocation of pre-anthesis stored reserves due to the rapid senescence of the crop. Consequently, there is a shortfall in assimilate supply for the kernel number set by the crop, resulting in reduced kernel weight and lower yield and harvest index.

The overall objective of this research was to gain sufficient understanding of the physiological responses of wheat crops to nitrogen fertiliser to explain the mechanisms of haying-off. Field experiments were established in environments which were expected to provide contrasting water availability during grain filling. In each experiment increasing rates of nitrogen fertiliser were applied at different stages of crop development. The mechanism of haying-off was investigated by monitoring the carbon and nitrogen dynamics through measurements of biomass production, nitrogen uptake and water use during crop development.

In addition to the field studies, a controlled environment study was conducted to separate the effects of drought and high temperature on haying-off. Wheat plants were grown as a simulated crop (microcanopy) at low and high nitrogen status, under post-anthesis drought and well-watered conditions, so as to assess the effect of a heat shock event on haying-off. The heat shock event was designed to be as realistic as possible so as to simulate that which can occur through spring and summer in the southeastern Australian wheatbelt. The simulated crops were labelled with ^{14}C several times prior to anthesis to examine respiratory losses during grain filling.

A secondary objective, but no less important to the graingrowers who funded this project, was to identify possible strategies to reduce the risk of haying-off thereby increasing yield and protein while maintaining or improving sustainable production. Analysis of these strategies expands our crop physiological knowledge and offers speculation to stimulate further thought and discussion and so encourage future research.

CHAPTER 2

THE EFFECT OF RATE AND TIME OF NITROGEN APPLICATION ON WHEAT BIOMASS PRODUCTION, WATER USE, NITROGEN UPTAKE AND YIELD

And days went by on dancing feet,
With harvest-hopes immense,
And laughing eyes beheld the wheat
Nid-nodding o'er the fence.

Said Hanrahan
P.J. Hartigan
(pen name- John O'Brien)

2.1 INTRODUCTION

There has been a gradual decline in the protein concentration of Australian Standard White (ASW) wheat since 1968 (McDonald 1989; Hamblin and Kyneur 1993). Extensive field experiments (Mason 1968;Taylor *et al.* 1974; Angus *et al.* 1989) and farm surveys (Martin *et al.* 1988; Wegener *et al.* 1988) have shown that nitrogen deficiency of commercial wheat crops is widespread in the wheat belt. Yield response to applied nitrogen fertiliser is highly variable, ranging from strongly positive (Russell 1967; Angus *et al.* 1989; McDonald 1991) to negative (Colwell 1963a; Storrier 1965a; Angus *et al.* 1989; McDonald 1991). The major factor limiting response of grain-yield to nitrogen was found to be seasonal rainfall and, to a lesser extent, soil mineral nitrogen (Taylor *et al.* 1974). While it could be intuitively expected that paddock history and particularly the number of years of previous pasture should also correlate well with grain yield, in reality variable pasture composition in the ley phase negates its predictive ability (van Herwaarden *et al.* 1989). In addition, soil-borne disease adds to the variability of response to nitrogen fertiliser (Angus *et al.* 1989). Such variability in response to nitrogen fertiliser and the high cost of nitrogen relative to returns for wheat has led to low nitrogen fertiliser usage in Australia (2-3 kg ha^{-1} national average, McDonald 1991). Unless clear guidelines on nitrogen fertiliser application can be determined for distinct cropping regions and/or a computer-based decision support system established for dryland wheat, the risks associated with nitrogen fertiliser usage will continue to be too great for many farmers. Much of the literature on nitrogen usage in wheat deals with strategic application of nitrogen fertiliser whereas a tactical approach mid season based on crop tissue tests, soil water availability and selected managerial criteria is one obvious way of reducing risks. Work of Angus and Fischer (1991) showed only a small penalty for delaying nitrogen fertiliser application until the terminal spikelet stage in a wet environment (640 mm average annual rainfall [a.a.r.]).

The aim of experiments detailed in this chapter was to examine the effects of the addition of nitrogen fertiliser at various stages during crop growth up to the beginning of stem elongation on the dynamics of biomass production and conversion of biomass to grain yield in relation to the rate and time periods of water use in a dry (425 mm a.a.r.) and wet (706 mm a.a.r.) environment. In addition, examination of the physiological responses of wheat crops to nitrogen fertiliser was expected to indicate responses which may predispose crops to haying-off.

2.2 MATERIALS AND METHODS

2.2.1 Cultural Conditions

The sites were at Barellan on "Gipson's Lease", the property of Neville and Pamela Semmler and at Ginninderra on the CSIRO Experiment Station. A map is presented in Appendix 1. Experimental sites were chosen to follow a long fallow or a breakcrop (Angus *et al.* 1991; Kirkegaard *et al.* 1994) to minimise the detrimental effects of soil-borne disease on nitrogen response (McDonald 1991).

2.2.1.1 *Barellan*

The Barellan site is 320 km west of Canberra (146°39' long, 34°12' lat, 170 m above sea level [a.s.l.]) (Appendix 1) in the western half of the New South Wales wheat belt (425 mm a.a.r.). Experiments were conducted in a flat 50 hectare paddock. The soil is a red brown earth, Dr 2.33 (Northcote *et al.* 1971) with a texture contrast at 30-40 cm.

The paddock was in pasture for two years prior to the first experiment. In late June 1990 the pasture was winter cleaned (grasses killed with selective herbicide) and mechanically fallowed in early September. Fallow weeds were controlled using non-selective herbicides. The paddock was scarified in April 1991. Two hectares were sown to wheat (*Triticum aestivum* L. cv. Janz) using a 16 row International 511 drill at a rate of 57 kg ha^{-1} with triadamefon double superphosphate supplying 26 kg P ha^{-1}. Two hectares set aside for experimentation in 1992 were sown to canola (*Brassica napus* cv Barossa) at a rate of 4 kg ha^{-1} on 24 May with Starter 12 supplying 16 kg P ha^{-1} and 9 kg N ha^{-1}.

The timing of nitrogen fertiliser application and crop sampling was related to the decimal code (DC) of Zadoks *et al.* (1974). Nitrogen treatments in 1991 were control, 40, 80 and 120 kg N ha^{-1} applied at sowing (DC10), 5 leaf stage (DC15) and the start of stem elongation (DC30) plus treatments of 160 and 200 kg N ha^{-1} with the nitrogen applied at DC10, DC15, DC16, DC30, and DC31 in 4 or 5 stages respectively in an effort to make crops hay-off.

Wheat was sprayed with diclofop-methyl and canola with haloxyfop on June 26 to control monocotyledonous weeds. Wheat was also sprayed with a tank mix of dicamba, MCPA and terbutryn on 12 July for the control of dicotyledonous weeds.

In 1992 the canola stubble was incorporated with an offset disc plough on the first sufficient rain following harvest. The wheat stubble was burned after grazing in March 1992 and the whole paddock scarified after rain in April. The four hectares were sprayed

with the pre-emergent herbicide trifluralin and sown to wheat (cv. Janz) on May 7 using a 16 row International 511 drill. Seed was sown at a rate of 65 kg ha^{-1} with single superphosphate supplying 19 kg P ha^{-1}. The new experiment was marked out over the canola stubble and the previous experiment located to assess the residual effects of nitrogen applied in the previous season. In 1992 treatments were control, 80, 160 and 240 kg N ha^{-1} applied at the same growth stages as in 1991. Rates were high in 1992 because the site was anticipated to be low in nitrogen after the canola crop and the rates of nitrogen applied in 1991 were insufficient to induce haying-off. The site was sprayed on July 17 with a tank mix of dicamba, MCPA and terbutryn to control a range of dicotyledonous weeds.

2.2.1.2 *Ginninderra*

Wheat cv Janz was sown at CSIRO Ginninderra Experiment Station, Australian Capital Territory, using a 16 row International 511 drill in 1992. The site was 17 km north-west of Canberra (149° 06' long, 35° 12' lat, 600 m a.s.l.) (Appendix 1) in the southern tablelands of NSW (706 mm a.a.r.). The experiments were conducted in a two hectare trial site with a 1 percent slope. The area to be used for experimentation in 1992 was sown on May 31, 1991 to canola cv Barossa at a rate of 5.5 kg ha^{-1} with Starter 12 compound fertiliser supplying 20 kg P ha^{-1} and 9 kg N ha^{-1}. The soil is a yellow podzolic soil Gn 3.85 (Northcote *et al.* 1971). The paddock was cultivated following rain in February 1992 with offset discs to incorporate canola stubble. In early May the paddock was scarified, sprayed with trifluralin pre-emergent herbicide and harrowed. The whole paddock was sown to wheat cv. Janz at a rate of 82 kg ha^{-1} on 15 May with single superphosphate supplying 20 kg P ha^{-1}. Rates of nitrogen application in 1992 were 0, 80, 160, 240 and 320 kg N ha^{-1} in the form of urea applied soon after DC 10 or at DC 30. Rates were split 7-10 days apart depending on rainfall events to reduce the possible losses by ammonia volatilisation at high rates of nitrogen. The paddock was sprayed with diclofop-methyl and phenoxaprop-p-ethyl to control monocotyledonous weeds on July 30 and with bromoxynil and MCPA to control dicotyledonous weeds on August 20.

2.2.2 Experimental design

All experiments were 4-replicate, randomised block designs. Plots were superimposed over the bulk area of wheat at 90° to the direction of sowing with dimensions of 3 m x 20 m or 2.5 m x 24 m. Plots were marked by spraying out narrow pathways at DC15 using the non-selective herbicide glyphosate. Nitrogen fertiliser was applied as urea topdressed on the designated crops at the appropriate time. Topdressing of urea was timed to occur just before the anticipated arrival of rain and on all but one occasion rain commenced within 12 hours. Experiments at Barellan will be referred to as BAR91 and

BAR92 and the experiment at Ginninderra as GES92. Nitrogen treatments applied at DC10 will be referred to as 40SW, 80SW, 120SW, 160SW, 240SW, 320SW (sowing) while nitrogen treatments imposed at DC15 and DC30 will have the sufficies MT (mid tillering) and LT (late tillering) respectively. The split application of 200 kg N ha^{-1} at BAR91 will be known as 200ST (sowing to tillering).

2.2.3 Meteorological data

All sites had rain gauges installed at the time of sowing. After reading the gauge at each visit a small amount of liquid paraffin was placed into the gauge to prevent evaporation of subsequent rainfall until the gauge was next read. There were weather stations 300 m from the site at Ginninderra and at Yanco Agricultural Research Institute 35 km south of

Table 2.1. Monthly observed and derived weather data at the Yanco Agricultural Institute and at BAR91.

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
				Maximum temperature (°C)					
Mean[a]	23.0	17.8	14.3	13.6	15.1	18.0	22.1	26.7	29.5
1991[b]	23.3	19.6	16.4	13.9	15.0	17.8	25.8	27.1	28.6
				Minimum temperature (°C)					
Mean[a]	10.4	7.2	4.0	3.2	4.0	5.9	9.1	11.9	14.9
1991[b]	8.5	6.8	8.7	3.8	3.8	5.6	10.0	11.3	14.7
				Rainfall (mm)					
Mean[c]	35	40	36	37	40	32	41	36	33
Mean[d]	36	44	35	38	39	39	46	32	31
1991[e]	8	23	75	35	17	67	15	6	15
				Class A Pan Evaporation (mm)					
Mean[a]	132	74	45	50	78	111	174	243	295
1991[b]	123	75	47	39	70	108	215	273	280
				Vapour pressure deficit (k Pa)[f]					
1991[b]	1.7	1.2	0.6	0.7	0.8	1.0	2.2	2.6	2.9
Range	0.7-2.7	0.4-2.3	0.1-1.1	0.4-1.3	0.3-1.3	0.6-1.7	1.1-4.3	1.3-4.9	1.0-5.5

[a] Long-term mean (53 years) Yanco Agricultural Institute.

[b] Yanco Agricultural Institute.

[c] Long-term mean (81 Years) "Euronga", Moombooldool.

[d] Long-term mean (53 years) Yanco Agricultural Institute.

[e] Values from experimental site BAR91.

[f] Mean daily maximum vapour pressure deficit

the site at Barellan. Measurements taken at each station included maximum and minimum temperatures, 9am and 3pm wet and dry bulb temperatures, solar radiation, precipitation, windrun and Class A pan evaporation. Daily maximum vapour pressure deficit was calculated for Barellan using 9am wet and dry bulb temperatures and daily maximum temperature. At Ginninderra 3pm wet and dry bulb temperatures were used due to the possible influence of sea breezes on the 9am measurements. Data for each site are summarised in Tables 2.1 and 2.2 (Barellan) and 2.3 (Ginninderra). Justification for the use of Yanco weather data for Barellan is by way of the similar long-term mean monthly rainfall data presented in Figure 2.1.

Table 2.2. Monthly observed and derived weather data at Yanco Agricultural Institute and at BAR92.

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
				Maximum temperature (°C)					
Mean[a]	23.0	17.8	14.3	13.6	15.1	18.0	22.1	26.7	29.5
1992[b]	24.3	18.1	14.0	14.0	13.8	16.2	20.5	22.6	27.0
				Minimum temperature (°C)					
Mean[a]	10.4	7.2	4.0	3.2	4.0	5.9	9.1	11.9	14.9
1992[b]	11.9	7.4	5.1	3.4	3.4	5.5	9.5	10.3	15.5
				Rainfall (mm)					
Mean[c]	35	40	36	37	40	32	41	36	33
1992[d]	46	33	31	34	70	69	85	77	109
				Class A Pan Evaporation (mm)					
Mean[a]	132	74	45	50	78	111	174	243	295
1992[b]	160	59	34	44	63	93	150	171	201
				Vapour pressure deficit (k Pa)[e]					
1992[b]	1.9	0.9	0.6	0.7	0.7	0.9	1.2	1.5	2.0
Range	0.6-3.3	0.4-1.6	0.1-1.0	0.4-1.2	0.1-1.2	0.4-1.6	0.5-2.2	0.9-2.4	0.8-3.8

[a] Long-term mean (53 years) Yanco Agricultural Institute.

[b] Yanco Agricultural Institute.

[c] Long-term mean (81 Years) "Euronga", Moombooldool.

[d] Values from experimental site BAR91.

[e] Mean daily maximum vapour pressure deficit

Table 2.3. Monthly observed and derived weather data at CSIRO Ginninderra Experiment Station

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
					Maximum temperature (°C)				
Mean[a]	19.3	14.7	11.3	10.3	11.8	14.5	18.0	21.5	25.1
1992	19.7	16.0	12.0	11.9	12.0	13.9	18.3	19.4	22.8
					Minimum temperature (°C)				
Mean[a]	6.5	3.5	1.2	0.2	1.5	3.5	6.1	8.4	11.0
1992	7.0	3.5	0.2	-0.2	0.2	3.1	6.9	7.6	12.0
					Rainfall (mm)				
Mean[a]	56	55	39	59	63	69	73	65	58
1992[b]	38	30	23	15	104	84	93	109	77
					Class A Pan Evaporation (mm)				
Mean[a]	87	53	38	40	58	86	124	162	204
1992	97	53	41	58	69	95	107	132	143
					Vapour pressure deficit (k Pa)[d]				
1992[c]	1.3	0.88	0.6	0.7	0.7	0.8	1.0	1.2	1.4
Range	0.6-2.0	0.3-1.9	0.3-0.8	0.1-1.2	0.3-1.0	0.5-1.4	0.4-1.8	0.5-2.0	0.4-2.8

[a] Long-term mean (32 years) Ginninderra Experiment Station.

[b] Values from experimental site Ginninderra Experiment Station.

[c] Canberra airport 1992

[d] Mean daily maximum vapour pressure deficit

2.2.4 Crop growth and soil measurements

2.2.4.1 *All sites*

The soil profile was sampled using a tractor mounted hydraulic soil corer and thin walled, 50 mm diameter sampling tubes as soon as possible after sowing for determinations of bulk density, soil water content, mineral nitrogen and pH. Bulk density was calculated by accurately cutting lengths of soil core to determine soil volume, placing them into large soil tins and oven drying at 104°C for 72 hours to determine the dry soil weight. Soil water and mineral nitrogen samples were immediately packed into thick plastic bags to prevent water loss and placed into large ice boxes until they could be put into a freezer at -14°C. Gravimetric soil water content was determined by oven drying at 105°C for 72 hours. Volumetric water content was calculated as the product of gravimetric soil water content and bulk density. Water storage, in mm of water, was

derived for each depth by the product of volumetric water content and the appropriate sampling interval and then summing over the profile to 1.7m. Mineral nitrogen content of soil samples was determined by extracting with a solution of 2 molar KCl and was analysed for NH_4^+ (AOAC 1984) and NO_3^- (Markus *et al.* 1985).

Soil water suction was measured using the filter paper method reviewed by Greacen *et al.* (1987). Soil samples were put into a constant temperature room at 21°C and allowed to equilibrate for 2 days. Each sample was then crumbled and mixed well. A soil tin was half filled with soil, a Whatman No. 42, 5.5 cm filter paper placed on top of the soil and the tin almost filled with more soil. The lid was then replaced and the tin sealed with masking tape. The tin was then tapped on the bench twice to consolidate the soil and placed in a wooden carry box. Filter papers were allowed to equilibrate with the soil for 7 days, removed and immediately weighed on a balance accurate to ±0.1 mg. Filter papers were then placed into paper seed envelopes in lots of 9-11, oven dried at 104°C for 24 hours, put into a small freshly recharged desiccator of small air volume, allowed to cool for 5 minutes and weighed. Filter papers were calibrated when it became clear that the calibration of Fawcett and Collis-George (1967), which had been verified by McQueen and Miller (1968) and Hamblin (1981) did not appear to apply. Calibration was performed by determining soil water suction of samples in the range 0.01-8 MPa using an isopiestic thermocouple psychrometer (Boyer, 1995) with sucrose as vapour pressure standard solutions and then equilibrating filter papers with the soil as described above.

Plant density was measured at the three to four leaf stage (DC13 to DC14) by counting the number of plants in a 50 cm length of two adjacent rows 20 times per block if establishment appeared uniform, or 6 times per plot if the stand appeared patchy.

At DC30 harvests were taken by randomly pulling 20-40 plants from within crops. Soil was washed from the roots of each sample, the number of plants and the total number of shoots counted, the crown and roots cut off and discarded, the shoots placed in a paper bag and placed in a dehydrator at 70°C until dry. Estimates of crop performance on a per unit area basis were calculated by multiplying plant density with measurements on a per plant basis. Dried samples were ground with a Wiley mill to pass a 1 mm sieve, sub sampled and ground with a Cyclotec mill to pass a 0.5 mm sieve. Nitrogen content was determined using an elemental analyser (Carlo Erba 1108).

At anthesis (DC65) quadrats were harvested at the soil surface from selected crops to estimate above-ground dry matter production (hereafter called biomass). One quadrat of 0.30 m x 6 rows was harvested from each end of the plot, bulked, weighed and subsampled. Fertile shoots were counted in each subsample, the spikes cut off and

placed in a separate bag and both samples placed in a dehydrator at 70°C until dry. Crop performance was estimated on a per unit area basis. Nitrogen and WSC concentration in the above-ground biomass was analysed by near infrared reflectance spectroscopy using locally determined calibrations. An NIRS Systems model 5000 scanning monochromator with an IBM compatible Osborne computer loaded with Infrasoft International software was used. Samples were scanned throughout the near infrared region (1100-2500nm) with reflectances measured at 2 nm intervals. Stepwise multiple linear regression was used to correlate laboratory determination of nitrogen and WSC concentration for a subset of samples with measured reflectances. This calibration was then used to estimate nitrogen and WSC concentration in all samples. Apparent fertiliser recovery (AFR) is defined by the equation;

$$AFR\ (\%) = (N_F - N_C) / F \times 100 \qquad (2.1)$$

where N_F and N_C are the nitrogen contained in the biomass of the fertilised and control crops respectively, and F is the amount of fertiliser nitrogen applied (Craswell and Godwin 1984). Nitrogen harvest index (NHI) was calculated as the proportion of grain nitrogen in the above-ground nitrogen at maturity. Nitrogen allocation per kernel (NAK) was calculated as the grain nitrogen divided by the kernel number.

At physiological maturity (DC86), or soon after, ten random grab samples were taken from each crop to estimate harvest index and quadrats, as at DC 65, were harvested to calculate biomass at maturity. Harvest index samples were separated into spikes and straw and oven dried at 70°C and weighed. Spikes were threshed and the glumes, awns and rachis (non-grain spikes) put with the straw (collectively the non-grain sample), the grain redried at 70°C and weighed. Kernel weight was calculated from the average of 3 lots of 100 kernels. Grain was ground using a Cyclotec mill with an 0.5 mm sieve and the non-grain sample ground in a Wiley mill to pass a 1 mm sieve. Nitrogen and WSC concentrations in the grain and non-grain sample were analysed by near infrared reflectance spectroscopy using locally determined calibrations.

When crops were harvest ripe (DC92), the plot-ends were trimmed, the length measured and a width of 1.32 m harvested down the middle of each crop to avoid edge effects. The components of yield not measured directly but calculated from the spike number and grain and kernel weights in the grab sample, and from the machine harvest yield were spike density and kernels per spike. Samples harvested from DC86 to DC92 will be collectively referred to as maturity samples.

As soon as possible after harvest soil cores were collected and analysed to determine soil water content, mineral nitrogen and soil water suction (as described for the soil sampling at sowing).

2.2.4.2 *Barellan*

Additional harvests were taken at DC43 prior to anthesis and at DC78 during grainfilling at BAR91 and at DC40, DC 75 and DC 83 at BAR92. In 1991 leaf areas were estimated using a Delta-T-series planimeter on harvested crops but in 1992 only spikes per square metre, biomass and nitrogen uptake were calculated.

2.2.5 Soil water measurements

At Barellan aluminium access tubes were pushed into the holes left at the time of soil sampling for later measurement of soil water using a neutron moisture meter (Troxler Electronic Laboratories Inc. Model 3222.) The total water use and patterns of water use were measured for the control treatment and the treatments in which nitrogen was applied at DC10 and at DC 30. In 1991 the treatments measured were control, 80SW, 80LT and 200ST. In 1992 the treatments measured were control, 80SW 240SW and 80LT and 240LT.

In both years two access tubes were installed in each plot to be measured, one tube for readings to 1.8 m and the other to 1.6 m. The neutron moisture meters were field calibrated at the start and end of each season. Volumetric soil water content, θ_V was determined for each depth and calibration curves fitted. Soil water content to 1.7 m was calculated by summing θ_V for each sampling interval to give soil moisture content in mm of water over the profile.

2.2.6 Radiation interception

In conjunction with sampling dates and moisture meter readings, interception of radiant energy by the crop canopies was estimated for each crop by placing a 1 m line quantum sensor (Decagon, Sunfleck ceptometer) horizontally on the ground beneath the canopy at 45° to the row direction. The mean of eight measurements was taken from each plot. Estimates of incident radiation were obtained at regular intervals during the measurement of canopy interception by holding the sensor horizontal above the crop. All measurements were taken in bright sunshine within one and a half hours either side of solar noon. From the incident and crop canopy measurements the proportion of incident radiation intercepted by the canopy was determined for selected crops. Measurements

were made in conjunction with biomass sampling dates and moisture meter readings, weather permitting.

2.2.7 Crop water use

Crop evapotranspiration, ET, was calculated using the moisture budget equation:

$$ET = P - \Delta S - R - D \qquad (2.2)$$

where P is the precipitation during the given period, ΔS is the change in total moisture in the soil profile, R is the surface runoff and D is the drainage beyond the depth of measurement. There was no evidence of runoff or drainage at either site and these were assumed to be zero in both years. Thus seasonal evapotranspiration could be calculated at Ginninderra and Barellan using rainfall data and start and finish soil water storage. At Barellan evapotranspiration could be calculated between readings of the access tubes with the neutron moisture meter.

At Barellan, evapotranspiration was partitioned into components of soil evaporation, E_S, and transpiration, T, using a model based on radiation penetration to the soil surface under crop canopies (Section 2.2.6) and evaporation from bare soil (Cooper *et al.* 1983). Four access tubes were located to a depth of 1.9 m on bare ground immediately adjacent to the crops at each end of the experiment. These tubes were to measure evaporation from uncropped bare soil, E_S. Crop water-use efficiency, W_{ET} and transpiration efficiency, W_T for above-ground biomass (W_{ET}^B, W_T^B) and grain yield (W_{ET}^G, W_T^G) was calculated based on evapotranspiration and T. Water-use efficiency and transpiration efficiency were calculated as the ratio of dry matter produced to water used and water transpired, respectively, over the periods from DC10 to maturity, DC10 to DC65 and DC65 to maturity.

2.2.8 Carbon isotope discrimination (Δ)

The basis of discrimination against ^{13}C by C_3 plants lies predominantly in two processes: the physical process of diffusion of CO_2 to the sites of carboxylation; and the biochemical process of CO_2 fixation (O'Leary 1981). Farquhar *et al.* (1982) developed the theory that negatively related the discrimination against ^{13}C measured in C_3 plant tissues with the ratio of CO_2 assimilation, to the conductance to diffusion, or equivalently related Δ positively to the ratio of p_i/p_a , where p_a and p_i are the atmospheric and intercellular partial pressures of CO_2 respectively. Subsequent research has found that at the whole plant level transpiration efficiency is negatively correlated with carbon isotope discrimination for a range of crop species including wheat (Farquhar and Richards 1984),

peanuts (Hubick *et al.* 1986) and barley (Hubick and Farquhar 1987). Hence carbon isotope discrimination was used in the present study to corroborate the estimate of transpiration efficiency at different nitrogen levels.

To measure carbon isotope discrimination (Δ) dried plant material was ground to pass a 0.5 mm sieve using a Cyclotec sample mill, model 1093. Subsamples of 2-4mg were combusted in an organic combustion preparation system (Carlo Erba 1108). The CO_2 in the effluent gas was trapped in a liquid-nitrogen cooled glass tube and passed into a ratio mass spectrometer (VG Isomass) for measurement of the molar abundance ratio (R) of ^{13}C to ^{12}C. An internal standard prepared from sucrose was periodically combusted to estimate variation in isotopic ratio determination. Carbon isotope discrimination, (Δ), of the dry matter was calculated assuming an isotopic composition for the air of -8 ‰ relative to Pee Dee Belemnite, the international standard according to the equation:

$$\Delta = \frac{Rair}{Rsample} - 1 = \frac{(-8 \times 10^{-3}) - (\delta sample)}{1 + (\delta sample)} \qquad (2.3)$$

where Rair and Rsample are the abundance ratios, $^{13}C/^{12}C$ of the air and the sample respectively and δsample is the ^{13}C isotopic composition in the sample.

2.3 RESULTS

2.3.1 Weather conditions

A composite summary of long-term mean weather conditions and the years 1991 and 1992 for Yanco and Barellan and 1992 for Ginninderra are given in Tables 2.1, 2.2 and 2.3 respectively. At BAR91 monthly mean maximum and minimum temperatures were similar to the long-term mean except for June and October which were several degrees warmer than the long-term mean. In 1992 monthly mean maximum and minimum temperatures were similar to the long-term mean except for the months August to November when mean maximum temperatures were cooler than the long-term mean. April to December rainfall was below the mean at BAR91 especially during the grain-filling period of October and November. In contrast, the rainfall at BAR92 from August to November was almost double the long-term mean resulting in no visible water stress in any crops during grain filling. August to December rainfall at GES92 was 42% higher than the long-term mean. The onset of terminal drought at BAR91 corresponded to the grain-filling period and coincided with a sharp rise in Class A pan evaporation and VPD in October. In contrast, Class A pan evaporation and VPD showed no such dramatic increase at BAR92 and GES92, but increased gradually as the season progressed.

2.3.2 Growth, shoot density and grain yield

Established seedling density was 115 plants m^{-2} at BAR91 which was within recommended limits for the region (Gammie 1994). This represented an establishment of 82% of seeds sown. In 1992 established seedling density was at the lower end of that recommended for the region at BAR92 (81 plants m^{-2}) and GES92 (117 plants m^{-2}) due to damage to the emerging seedlings by the pre-emergent herbicide trifluralin. Establishment percentages were 51% and 59%, respectively.

The application of nitrogen fertiliser stimulated biomass production at each experiment, though the magnitude of the response and the effect of time of application varied between sites (Tables 2.4, 2.5, 2.6). Nitrogen applied at DC10 increased early biomass production (increased early vigour) at all sites, though increasing rates of nitrogen did not increase biomass production at BAR91. As time of application was progressively delayed from DC10 to DC30 so the response in biomass production diminished at BAR91 and BAR92 while at GES92 compensatory growth between DC30 and DC65 in response to the late applications of nitrogen resulted in no significant difference in DC65 biomass between the two times of nitrogen application. The contrast in growth response between sites was due to differences in shoot survival following nitrogen application (Tables 2.6, 2.7, 2.8). No extra tillers were initiated if nitrogen was applied after DC10 at BAR91 but application of nitrogen at DC15 improved tiller survival relative to the

Table 2.4. Effect of nitrogen fertiliser on the growth and grain yield of wheat at BAR91.

Treatment (kg N ha^{-1})	Dry wt (g m^{-2})						Grain yield (g m^{-2})
	DC15	DC30	DC43	DC69	DC78	DC87	
Control	34.0	94.1	395	634	747	814	327
40SW	49.7	-	-	-	-	967	390
80SW	53.1	170.3	547	910	953	1038	408
120SW	-	173.1	-	916	-	1042	419
40MT	C	-	-	-	-	916	376
80MT	C	101.2	451	811	847	959	393
120MT	C	-	-	817	-	945	388
40LT	C	C	-	-	-	875	362
80LT	C	C	373	728	778	881	369
120LT	C	C		725		872	368
200ST	49.7	154.8	518	828	899	993	399
(l.s.d. P=0.05)	7.4	17.4	51	67	66	38	14

C, values are the same as for the control crop; - not measured.

Table 2.5. Effect of nitrogen fertiliser on the growth and grain yield of wheat at BAR92.

Treatment (kg N ha^{-1})	Dry wt (g m^{-2})							Grain yield (g m^{-2})
	DC15	DC30	DC40	DC65	DC75	DC83	DC87	
Control	25.3	55.7	149	248	311	355	352	132
80SW	30.7	77.2	326	723	888	1044	978	367
160SW	-	-	-	-	-	-	1356	535
240SW	40.1	102.3	471	1029	1315	1474	1416	570
80MT	C	63.1	292	691	868	998	900	356
160MT	C	-	-	-	-	-	1207	511
240MT	C	64.7	308	875	1063	1274	1310	568
80LT	C	C	242	575	714	814	836	349
160LT	C	C	-	-	-	-	1136	491
240LT	C	C	268	749	1030	1233	1212	543
(l.s.d. P=0.05)	3.1	6.5	41	64	74	69	44	16

C, values are the same as for the control crop; - not measured.

Table 2.6. Effect of nitrogen fertiliser on tillering and the growth and grain yield of wheat at GES92.

Treatment (kg N ha^{-1})	Shoot/Spike density (shts m^{-2})			Biomass (g m^{-2})			Grain yield (g m^{-2})
	DC30	DC65	DC87	DC30	DC65	DC87	
Control	650	401	423	89.6	879	1270	530
80SW	691	523	543	116.3	1165	1772	745
160SW	858	627	608	120.1	1302	2003	855
240SW	-	697	707	-	1341	2177	931
320SW	908	767	726	126.0	1442	2276	974
80LT	C	544	538	C	1185	1934	836
160LT	C	617	631	C	1261	2166	948
240LT	C	650	619	C	1243	2155	957
320LT	C	680	643	C	1310	2231	973
(l.s.d. P=0.05)	117	79	62	14.6	119	121	51

C, values are the same as for the control crop; - not measured.

Table 2.7. Effect of nitrogen fertiliser on shoot and spike density of wheat at BAR91.

Treatment (kgN ha^{-1})	Shoot density (shts m^{-2})			Spike density (spks m^{-2})		
	DC15	DC30	DC43	DC69	DC78	DC87
Control	398	386	347	295	308	287
40SW	467	-	-	-	-	353
80SW	517	545	480	405	404	418
120SW	-	550	-	431	-	386
40MT	C	-	-	-	-	337
80MT	C	403	356	333	327	323
120MT	C	-	-	386	-	351
40LT	C	C	-	-	-	304
80LT	C	C	341	322	308	314
120LT	C	C	-	326	-	297
200ST	467	466	396	373	377	357
(l.s.d. P=0.05)	75	35	55	53	39	28

C, values are the same as for the control crop; - not measured.

Table 2.8. Effect of nitrogen fertiliser on shoot and spike density of wheat at BAR92.

Treatment (kgN ha^{-1})	Shoot density (shts m^{-2})			Spike density (spks m^{-2})			
	DC15	DC30	DC40	DC65	DC75	DC83	DC87
Control	229	244	151	147	162	156	150
80SW	283	305	260	273	261	250	250
160SW	-	-	-	-	-	-	305
240SW	349	380	337	352	322	373	350
80MT	C	264	230	275	265	234	244
160MT	C	-	-	-	-	-	286
240MT	C	260	245	320	341	330	350
80LT	C	C	221	239	249	230	250
160LT	C	C	-	-	-	-	330
240LT	C	C	240	334	323	304	349
(l.s.d. P=0.05)	22	25	41	28	49	34	19

C, values are the same as for the control crop; - not measured.

control and nitrogen applied at DC30 crops. At BAR92, application of nitrogen after DC10 also resulted in improved tiller survival, but in addition, new tillers were initiated as evidenced by the increase in shoots/spikes between DC40 and DC65. At GES92 application of nitrogen, and increasing rates of nitrogen, improved tiller survival such that at the highest rate of nitrogen very few if any shoots/spikes were lost between DC30 and DC87. The 200ST crop at BAR91 had a similar biomass to the 80SW crop throughout the season though the shoot density was significantly lower.

The highest grain yield was recorded at GES92 where 320 kg N ha^{-1} whether applied at DC10 or DC30 resulted in 974 g m^{-2}. The lowest yield of 132 g m^{-2} was recorded for the control crop at BAR92. There were positive grain yield responses with diminishing returns to increases in the rate of nitrogen fertiliser at each site. Grain yield response decreased the later the fertiliser was applied at BAR91 and BAR92 while at GES92 the lower rates of nitrogen applied at DC30 outyielded DC10 applications.

2.3.3 Yield components

There was a significant increase in the number of kernels per square metre with applied nitrogen at all sites, the largest increase occurring at GES92 and the lowest at BAR91 (Table 2.9). The number of kernels per spike increased significantly with higher rates of nitrogen at all sites. Kernel weight decreased with increasing rates of nitrogen at BAR91 and at GES92. Kernel weight had a range of 5 mg at BAR91 and GES92. At BAR92 increasing nitrogen application at DC10 increased kernel weight. The low rate of nitrogen at later applications resulted in an initial increase in kernel weight but higher

Table 2.9 Effect of nitrogen fertiliser on grain yield components of wheat at three sites in southern New South Wales and Australian Capital Territory.

Treatment	Kernels m^{-2} x10^3	Kernels $spike^{-1}$	Kernel wt (mg)	Harvest index
		BAR91		
Control	10.53	36.9	31.1	0.402
40SW	12.97	36.9	30.1	0.403
80SW	14.81	35.4	27.6	0.393
120SW	15.19	39.4	27.7	0.403
40MT	13.36	39.8	28.2	0.411
80MT	14.30	44.4	27.5	0.410
120MT	14.80	42.1	26.3	0.411
40LT	12.66	41.8	28.6	0.414
80LT	13.60	43.5	27.2	0.419
120LT	13.49	45.7	27.3	0.423
200ST	15.70	44.2	25.4	0.402
(l.s.d. P=0.05)	0.73	3.1	1.2	0.010
		BAR92		
Control	3.98	26.7	33.2	0.379
80SW	10.42	41.8	35.2	0.376
160SW	14.84	48.6	36.1	0.394
240SW	15.42	44.0	37.0	0.403
80MT	9.94	40.9	35.8	0.395
160MT	14.33	50.8	35.7	0.423
240MT	17.38	49.6	32.7	0.434
80LT	9.82	39.3	35.5	0.417
160LT	13.74	41.8	35.7	0.432
240LT	16.08	46.1	33.8	0.448
(l.s.d. P=0.05)	0.56	3.3	1.2	0.011
		GES92		
Control	13.81	32.7	38.3	0.417
80SW	19.98	36.9	37.3	0.421
160SW	24.73	41.0	34.6	0.427
240SW	27.18	38.5	34.3	0.428
320SW	28.28	39.1	34.4	0.428
80LT	22.46	41.9	37.3	0.433
160LT	26.37	41.9	36.0	0.438
240LT	27.97	45.6	34.3	0.444
320LT	28.98	45.3	33.6	0.436
(l.s.d. P=0.05)	1.38	3.5	1.3	0.016

rates of nitrogen resulted in no significant difference to the control. Harvest index increased with higher rates and later applications of nitrogen at GES92 and BAR92. At BAR91 only the DC30 applications of nitrogen increased harvest index above the control and resulted in a non-significant upward trend in harvest index with increasing rates of nitrogen.

2.3.4 Water Soluble Carbohydrates.

Water Soluble Carbohydrates (WSC) present at DC65 and maturity are presented in Table 2.10. Delayed time of application of nitrogen reduced WSC reserves present in the DC65 biomass at each site. Increasing rates of nitrogen application significantly reduced, or did not alter WSC stem reserves at DC65 at BAR91 except for the application of 80SW crop which significantly increased WSC stem reserves. Similarly, increasing rates of nitrogen applied at DC10 at GES92 initially increased but then decreased WSC reserves at DC65. Application of increasing rates of nitrogen at DC30 decreased WSC reserves at DC65. In contrast, increasing rates of nitrogen application increased WSC reserves at DC65 at BAR92, though the reserves diminished with delayed nitrogen application. At maturity, WSC remaining in the non-grain biomass tended to be lower at higher rates of nitrogen at BAR91 and BAR92 while at GES92 WSC remaining in the non-grain biomass was significantly higher with increasing rates of nitrogen. Delaying the time of nitrogen application significantly reduced the WSC remaining in the non-grain biomass at BAR91 and BAR92 but had no effect at GES92. The multiple application of nitrogen at BAR91 had similar levels of WSC to the rates of nitrogen applied at DC30.

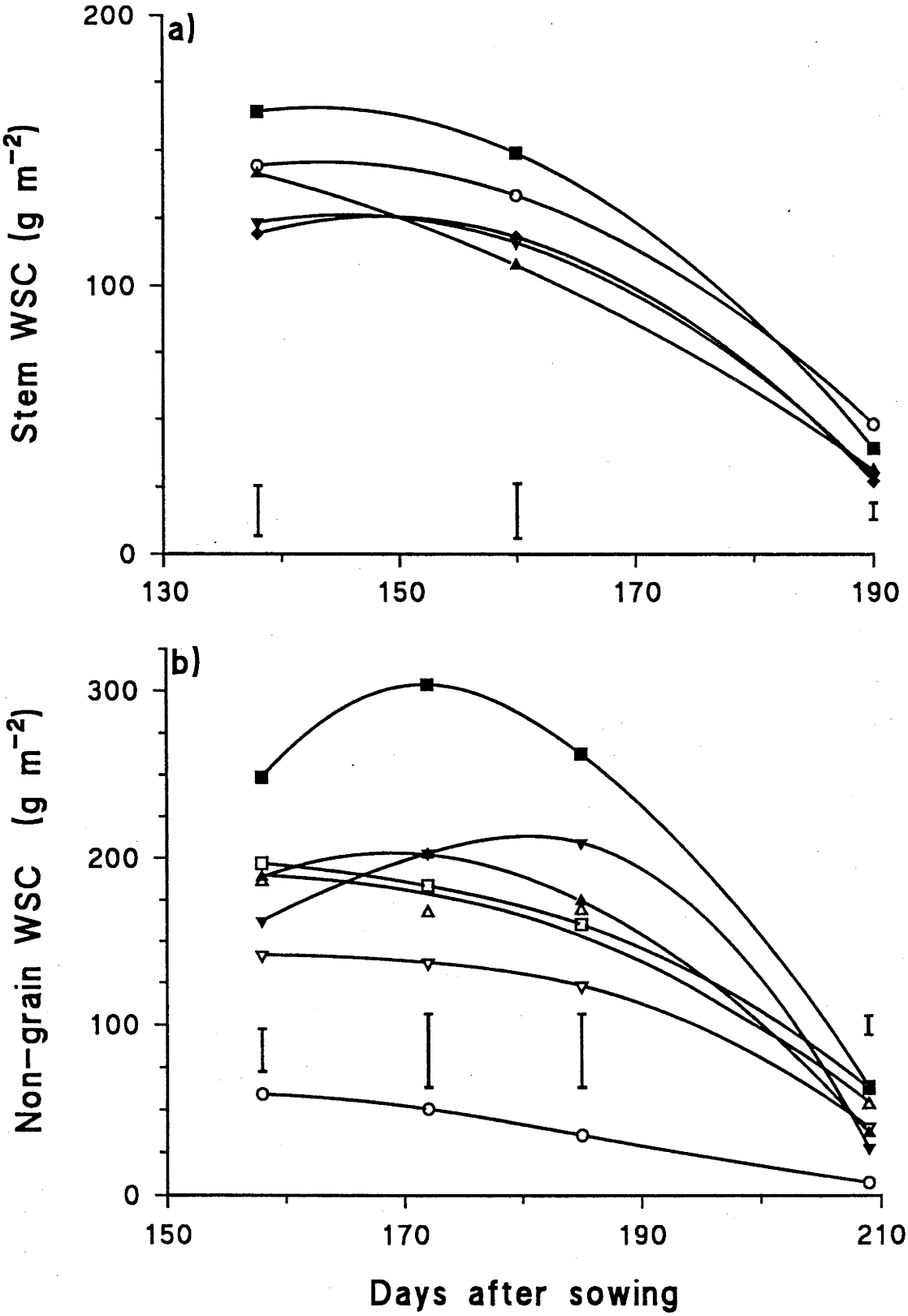
The dynamics of WSC content of the stems at BAR91 and non-grain biomass at BAR92 during grain filling are presented in Figure 2.1a and 2.1b respectively. In the dry year at BAR91, WSC content decreased following DC65 for all crops. Only the crop receiving nitrogen at DC10 contained higher levels of WSC than the control crop during early to mid grain filling. By maturity the control crop had utilised less of its reserves than for the crops that received nitrogen at DC10 which in turn had utilised less of its reserves than other nitrogen fertilised crops. In the wet spring at BAR92 crops receiving the high rate of nitrogen continued to lay down WSC reserves after DC65 while for the crop at the lowest rate of nitrogen and in the control crop WSC reserves decreased following DC65 as at BAR91. Late application of the highest rate of nitrogen resulted in peak storage of WSC measured at the DC83 harvest while DC10 application of nitrogen resulted in peak WSC levels being measured at DC75.

Table 2.10. Effect of nitrogen fertiliser on water soluble carbohydrate (WSC) present in stems of wheat at anthesis (DC65) at BAR91, present in the biomass at anthesis at BAR92 and GES92 and in the non-grain biomass at maturity (DC87) at each site.

Treatment (kg N ha^{-1})	Water soluble carbohydrates (g m^{-2})	
	DC65	DC87
	BAR91	
Control	144 (170)[a]	48
80SW	164 (203)	39
120SW	149 (187)	34
80MT	141 (175)	31
120MT	114 (142)	25
80 LT	123 (152)	29
120LT	119 (148)	27
200ST	119 (157)	27
(l.s.d. P=0.05)	19 (23)	6
	BAR92	
Control	59	8
80SW	197	63
160SW	-	109
240SW	248	64
80MT	186	53
160MT	-	59
240MT	189	37
80LT	142	40
160LT	-	50
240LT	162	28
(l.s.d. P=0.05)	25	10
	GES 92	
Control	269	39
80SW	306	49
160SW	266	50
240SW	232	52
320SW	223	63
80LT	261	55
160LT	221	61
240LT	194	59
320LT	166	69
(l.s.d. P=0.05)	36	15

[a] Numbers in parenthesis are estimates of total WSC calculated using the proportions contained in plant parts at anthesis from GES91 and PUC91 in Chapter 4; -, not measured.

Figure 2.1 WSC changes during grain filling for a) stems of control (O), 80SW (■), 80MT (▲), 80LT (▼) and 200ST (◆) at BAR91 and b) non-grain biomass of control (O), 80SW, 240SW (□,■), 80MT, 240MT (Δ,▲), 80LT, 240LT (∇,▼) at BAR92. Curves were fitted by eye, bars indicate the l.s.d., (P = 0.05).



2.3.5 Nitrogen uptake, apparent nitrogen efficiency and grain protein.

The application of nitrogen fertiliser stimulated nitrogen uptake at each experiment, though the magnitude of the response and the effect of time of application varied between sites (Tables 2.11, 2.12, 2.13). Nitrogen applied at DC10 increased nitrogen uptake at all sites, though increasing rates did not significantly increase uptake at BAR91. As time of application was progressively delayed from DC10 to DC30 so the final nitrogen uptake decreased at BAR91, showed no significant effect of time of fertiliser application at BAR92 but showed a significant increase in uptake with the DC30 application of nitrogen at GES92.

At BAR91 only the control crop took up nitrogen between DC69 and DC87 while other crops lost nitrogen from the biomass over the same period. In contrast at BAR92 nitrogen was taken up for all rates and times of application of nitrogen between DC65 and DC83. There tended to be losses of nitrogen between DC83 and DC87 for earlier applications of nitrogen but continued nitrogen uptake for later applications. At GES92 almost half the nitrogen uptake occurred between DC65 and DC87 for control crops while other crops also took up a substantial proportion of final nitrogen uptake. The application of nitrogen at DC30 resulted in greater net uptake of nitrogen after DC65 than for DC10 applications.

Table 2.11. Effect of nitrogen fertiliser on the nitrogen uptake of wheat at BAR91.

Treatment (kg N ha^{-1})	Nitrogen uptake (g m^{-2})					
	DC15	DC30	DC43	DC69	DC78	DC87
Control	1.90	3.83	4.91	5.31	6.00	6.10
40SW	2.77	-	-	-	-	8.25
80SW	3.09	7.36	9.81	10.73	10.12	9.92
120SW	-	8.09	-	12.35	-	11.22
40MT	C	-	-	-	-	7.74
80MT	C	4.82	8.34	10.47	9.32	9.13
120MT	C	-	-	12.12	-	10.06
40LT	C	C	-	-	-	7.27
80LT	C	C	6.79	8.92	8.41	8.12
120LT	C	C	-	9.65	-	8.80
200ST	2.77	6.78	11.25	11.60	11.31	11.14
(l.s.d. P=0.05)	0.38	0.92	1.07	1.19	1.19	0.60

C, values are the same as for the control crop; - not measured.

Table 2.12. Effect of nitrogen fertiliser on the nitrogen uptake of wheat at BAR92.

Treatment (kg N ha^{-1})	Nitrogen uptake (g m^{-2})						
	DC15	DC30	DC40	DC65	DC75	DC83	DC87
Control	1.08	1.44	1.58	1.67	2.03	2.34	2.66
80SW	1.53	3.03	4.75	5.34	6.07	6.69	6.58
160SW	-	-	-	-	-	-	10.43
240SW	2.27	4.57	9.64	11.33	12.48	14.05	14.33
80MT	C	2.71	4.76	5.48	7.16	7.69	6.81
160MT	C	-	-	-	-	-	11.11
240MT	C	3.02	7.22	11.95	13.07	14.91	15.21
80LT	C	C	3.84	5.00	6.04	6.04	7.37
160LT	C	C	-	-	-	-	11.64
240LT	C	C	5.65	10.08	11.83	13.60	14.29
(l.s.d. P=0.05)	0.18	0.30	0.85	0.91	1.14	0.75	0.59

C, values are the same as for the control crop; - not measured.

Table 2.13. Nitrogen uptake during the season, apparent fertiliser recovery (AFR), grain protein, nitrogen harvest index (NHI) and nitrogen allocation per kernel (NAK) of wheat grown at GES92.

Treatment (kg N ha^{-1})	Nitrogen uptake (gm^{-2})			AFR	Grain	N HI	NAK
	DC30	DC65	DC87	%	Protein[a]		(mg $kernel^{-1}$)
Control	3.23	5.49	10.04	-	8.6	0.798	0.578
80SW	4.74	9.91	13.90	48	8.6	0.806	0.561
160SW	5.49	13.33	16.39	40	8.8	0.803	0.533
240SW	-	16.09	19.92	41	9.6	0.783	0.575
320SW	6.30	19.14	22.68	40	10.3	0.772	0.618
80LT	C	12.06	17.05	88	9.3	0.803	0.611
160LT	C	16.10	21.84	74	10.6	0.809	0.670
240LT	C	20.76	25.64	65	11.9	0.778	0.714
320LT	C	22.84	28.81	59	12.6	0.748	0.743
(l.s.d. P=0.05)	0.61	1.74	1.42	n.a.	0.5	0.024	0.038

n.a. - not applicable; C values are the same as for the control crop; - not measured; [a] Grain protein presented at 12% moisture

At BAR91 AFR decreased with increasing rates of nitrogen application and delayed time of nitrogen application (Table 2.14). At BAR92, however, there was little difference in AFR between rates of nitrogen application or time of nitrogen application (Table 2.15) except for 80LT which continued to take up nitrogen late in the season (Table 2.12). Higher rates of nitrogen application were also associated with lower AFR at GES92 but in contrast to the other sites applying the nitrogen fertiliser at DC30 consistently maximised uptake (Table 2.13).

Grain protein increased with increasing rates of nitrogen application but decreased with delayed time of nitrogen application at BAR91 (Table 2.14). In contrast, grain protein increased with delayed application of nitrogen at BAR92 and GES92 (Tables 2.15 and 2.13). Application of 80SW resulted in no change in grain protein compared to control crops at GES92 but decreased grain protein at BAR92 while the application of 80LT resulted in increased grain protein at both sites. Rate of nitrogen application tended to decrease nitrogen harvest index (NHI) at BAR91 and BAR92 but significantly reduced NHI at GES92. Despite a decrease in grain protein with delayed application of nitrogen at BAR91, NHI increased as time of nitrogen application was delayed. Increased NHI in response to delayed time of nitrogen application was also observed at BAR92 but no significant difference was detected at GES92.

Nitrogen allocation per kernel (NAK) increased with the addition of nitrogen at BAR91 but decreased with delayed time of application of nitrogen (Table 2.14). At BAR92 nitrogen application strongly increased NAK at the higher rates of nitrogen while time of application did not have a consistent effect on NAK (Table 2.15). Application of nitrogen at DC10 at GES92 resulted in lower NAK than when nitrogen was applied at DC30 (Table 2.13). In addition, lower rates of nitrogen applied at DC10 resulted in a decrease in NAK which only exceeded that of the control at the highest rate of nitrogen.

Table 2.14 Apparent fertiliser recovery (AFR), grain protein, nitrogen harvest index (NHI) and nitrogen allocation per kernel (NAK) of wheat grown at BAR91.

Treatment (kg N ha^{-1})	AFR %	Grain protein[a] %	N HI	NAK (mg $kernel^{-1}$)
Control	-	8.9	0.840	0.487
40SW	54	10.0	0.832	0.529
80SW	48	11.3	0.816	0.547
120SW	43	12.4	0.813	0.600
40MT	41	9.9	0.840	0.487
80MT	38	11.1	0.835	0.533
120MT	33	12.2	0.826	0.562
40LT	29	9.7	0.843	0.485
80LT	25	10.5	0.838	0.500
120LT	22	11.6	0.848	0.553
200ST	25	13.1	0.823	0.584
(l.s.d. P=0.05)	n.a.	0.5	0.014	0.017

n.a. - not applicable, [a] Grain protein presented at 12% moisture

Table 2.15 Apparent fertiliser recovery (AFR), grain protein, nitrogen harvest index (NHI) and nitrogen allocation per kernel (NAK) of wheat grown at BAR92.

Treatment (kg N ha^{-1})	AFR %	Grain protein[a] %	N HI	NAK (mg $kernel^{-1}$)
Control	-	9.3	0.810	0.544
80SW	49	8.4	0.824	0.520
160SW	49	9.1	0.823	0.580
240SW	49	11.7	0.817	0.761
80MT	52	9.0	0.820	0.562
160MT	53	10.4	0.841	0.653
240MT	52	12.5	0.819	0.716
80LT	59	10.1	0.835	0.627
160LT	56	11.4	0.842	0.714
240LT	48	12.5	0.835	0.742
(l.s.d. P=0.05)	n.a.	0.5	0.014	0.038

n.a. - not applicable, [a] Grain protein presented at 12% moisture

2.3.6 Water use, water use efficiency and transpiration efficiency.

The cumulative evapotranspiration and soil evaporation curves from DC10 to DC87 for selected crops in experiments BAR91 and BAR92 are presented in Figures 2.2 and 2.3 respectively. During the early phase of crop growth evapotranspiration was higher in 1991 than 1992 due to a warm start to the season and frequent showers of rain. Less frequent rainfall in the early period of crop growth in 1992 and poor crop establishment reduced evapotranspiration. In 1991 the dry spring resulted in terminal drought conditions which limited evapotranspiration and reduced soil evaporation due to a dry soil surface. No water stress was observed in the wet spring in 1992, which resulted in a threefold increase in evapotranspiration and up to a five fold increase in soil evaporation during grain filling relative to the same period at BAR91.

At BAR91 the 80SW and 200ST crops used the same amount of water over the season, and more than the 80LT crop or the control. Losses of water due to soil evaporation were also similar for 80SW and 200ST crops but less than for the 80LT crop or the control. Seasonal transpiration, therefore, was similar for 80SW and 200ST crops, but greater than for the 80LT crop which was in turn greater than the control.

At BAR92 crops receiving nitrogen had greater seasonal evapotranspiration than control crops (Figure 2.3a, 2.3b). Crops receiving 240SW had the highest evapotranspiration while the control crops had the lowest. Crops receiving 80SW used less water than 240SW but those with 80LT and 240LT used similar amounts of water which was midway between the use of the crops receiving nitrogen at DC10. Soil evaporation was highest for control crops and lowest for the highest rate of nitrogen. DC10 applications of nitrogen reduced soil evaporation to a greater extent than DC30 applications.

The differences in soil water suction at DC10 and at maturity for BAR91 is presented in Figure 2.4. Under the terminal drought conditions the control crops dried the soil profile less than the 80LT crops which in turn dried the soil less than the 80SW crops. At depths of 0.6 to 0.8m, below which soil evaporation could not influence water extraction, the fertilised crops were able to dry the soil to greater than 6 MPa suction and control crops dried the soil to more than 4 MPa.

Components of evapotranspiration, and the water use efficiency and transpiration efficiency for biomass production at BAR91 are presented in Table 2.16. Between DC10 and DC69 soil evaporation formed a larger component of evapotranspiration than during grain filling due to frequent rain events and more open crop canopies. Differences in evapotranspiration between crops were established by DC69 and post-anthesis evapotranspiration was similar between crops resulting in greatest final

Figure 2.2 Cumulative evapotranspiration (solid symbol, solid line) and soil evaporation (open symbol, dashed line) curves at BAR91 from DC10 to DC87 for a) control (O,●), 80SW (□,■) and, b) control (O,●), 80LT (□,■) and 200ST (Δ,▲). Curves were fitted by eye, arrow indicates mean anthesis date.

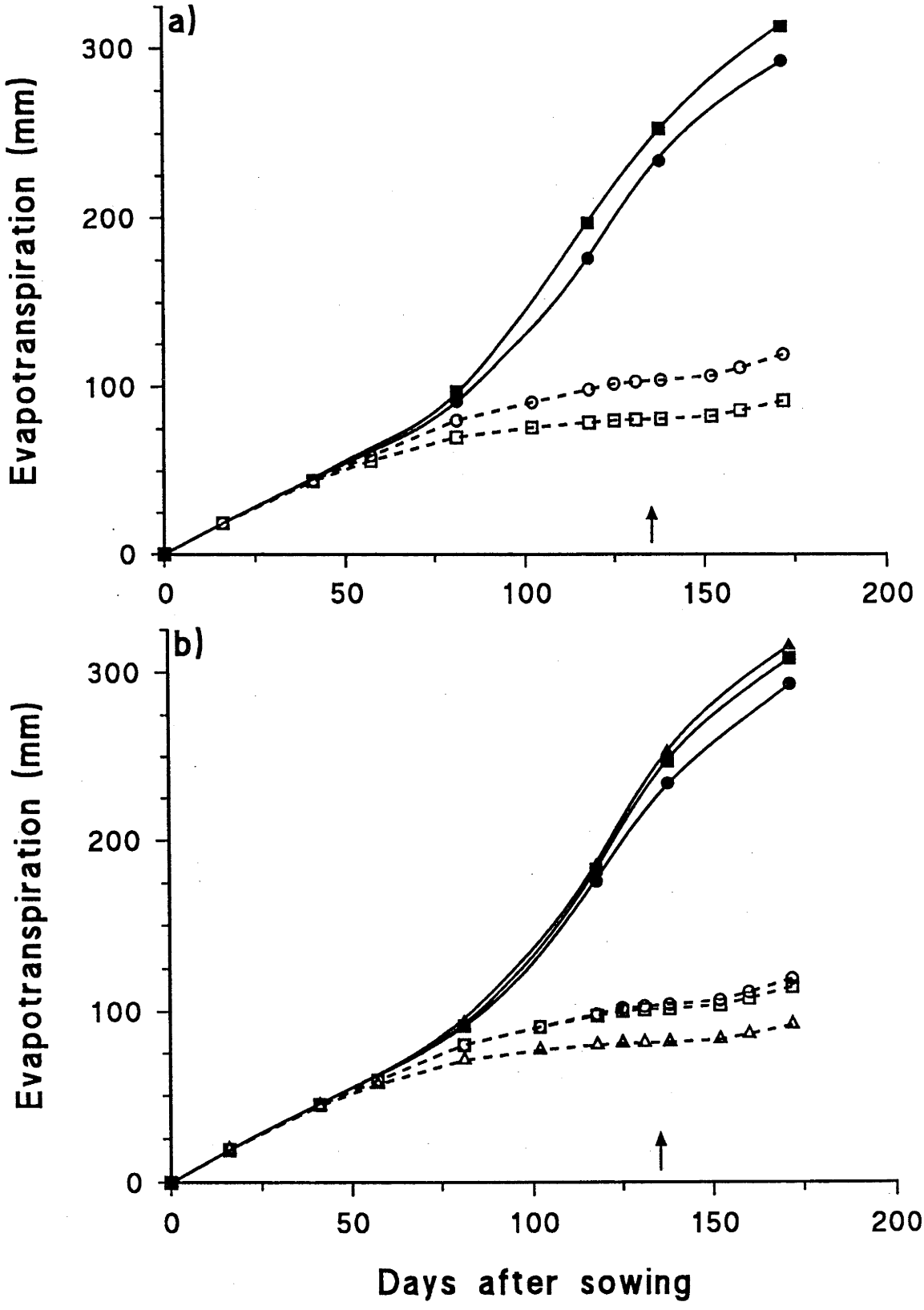
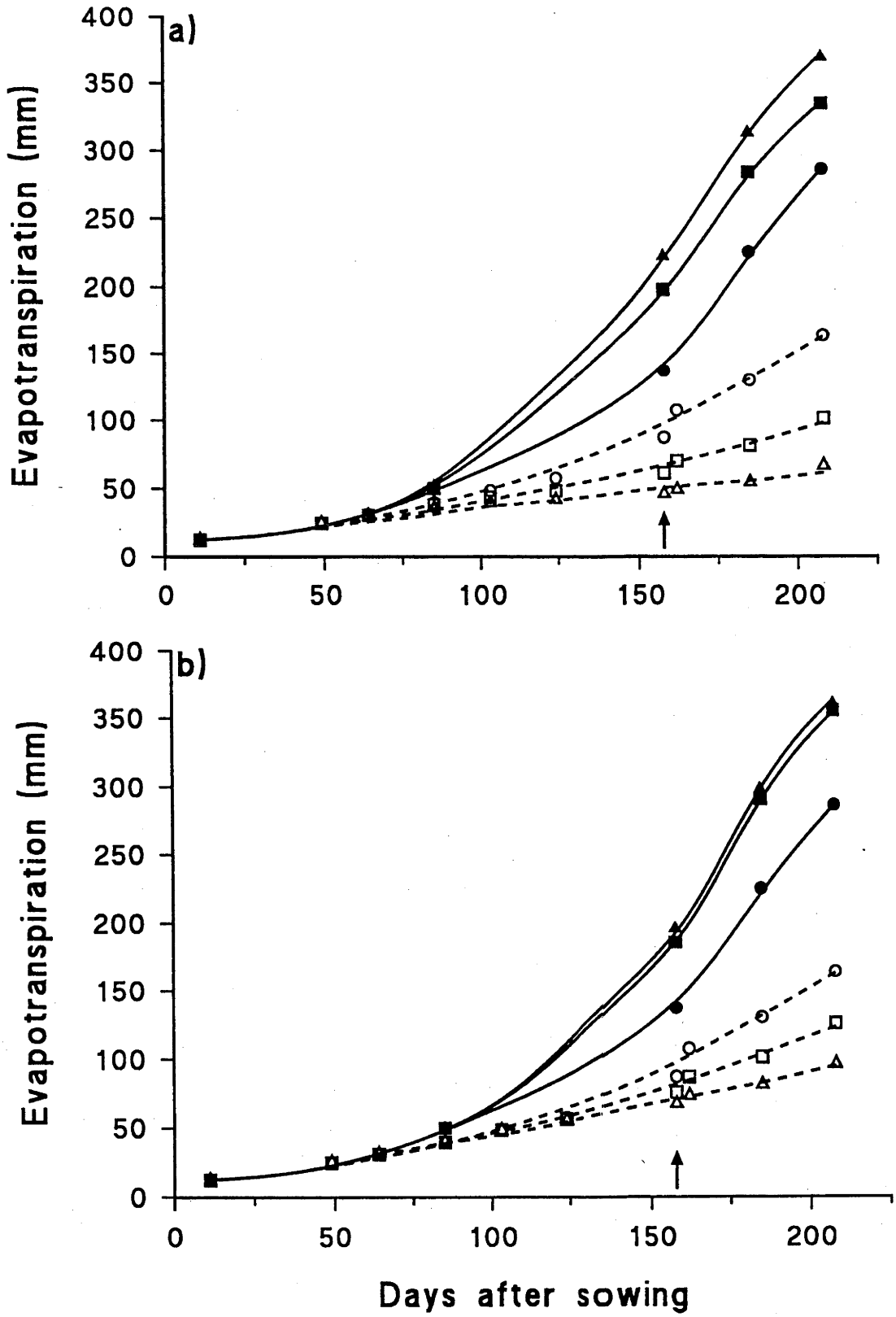


Figure 2.3 Cumulative evapotranspiration (solid symbol, solid line) and soil evaporation (open symbol, dashed line) curves at BAR92 from DC10 to DC87 for a) control (O,●), 80SW (□,■) and 240SW (Δ,▲) and, b) control (O,●), 80LT (□,■) and 240 LT (Δ,▲). Curves were fitted by eye, arrow indicates mean anthesis date.



evapotranspiration and hence soil water extraction for 80SW and 200ST crops. Values for water use efficiency and transpiration efficiency were greater before DC65 than during grain filling. There were no significant differences in transpiration efficiency, though the 80SW crop had the highest value of transpiration efficiency between DC10 and DC69 and the 200ST and control crops the lowest. The application of nitrogen at DC10 resulted in the lowest estimate of transpiration efficiency during the post-anthesis period while control crops had the highest. Over the whole season there was little difference in transpiration efficiency between crops (Table 2.16).

Components of evapotranspiration, water use efficiency for biomass and transpiration efficiency for biomass at BAR92 are presented in Table 2.17. The application of nitrogen increased evapotranspiration and reduced soil evaporation in each of the periods presented. Soil evaporation from control crops constituted more than half the evapotranspiration before and after DC65. Application of nitrogen at DC10 reduced soil evaporation before DC65 to 27% of evapotranspiration while application of nitrogen at DC30 resulted in a pre-anthesis loss of 38% of evapotranspiration. Soil evaporation formed a similar proportion of evapotranspiration during grain filling (21 and 23%) for early or late applied nitrogen respectively. Early application of nitrogen resulted in a higher evapotranspiration and T prior to DC65 than for DC30 applications but post-anthesis the ranking was reversed. Values for water use efficiency and transpiration efficiency were greater for the pre-anthesis period than during grain filling. There were no clear differences in pre-anthesis transpiration efficiency between nitrogen fertilised crops. In contrast to BAR91 there was a trend for higher transpiration efficiency of fertilised crops to be maintained during grain filling. All but the 80LT crops had significantly higher whole season transpiration efficiency than the control crops and the efficiency tended to increase with increasing rates of nitrogen.

The range in water use efficiency was similar between BAR91 and BAR92 both before and after DC65. Pre-anthesis transpiration efficiency tended to be greater at BAR92 than BAR91 but similar for the grain filling and whole season periods.

There was no significant difference in evapotranspiration between nitrogen fertilised crops at GES92 (Table 2.18). Control crops had the lowest water use efficiency and the 320LT crops the highest while the 160LT crops tended to have a higher water use efficiency than the 160SW crops. The control crops had the lowest light interception at both DC30 and DC65. Despite having the same light interception at DC30 as the control crops the 320LT crops had the highest light interception and hence the lowest soil evaporation at DC65.

Figure 2.4 Soil water suction versus soil depth at DC10 and maturity for BAR91 at DC10 (▽), and maturity control (●), 80SW (■) and 80LT (▲). The bar indicates the l.s.d., (P = 0.05) for the main effect of nitrogen at maturity.

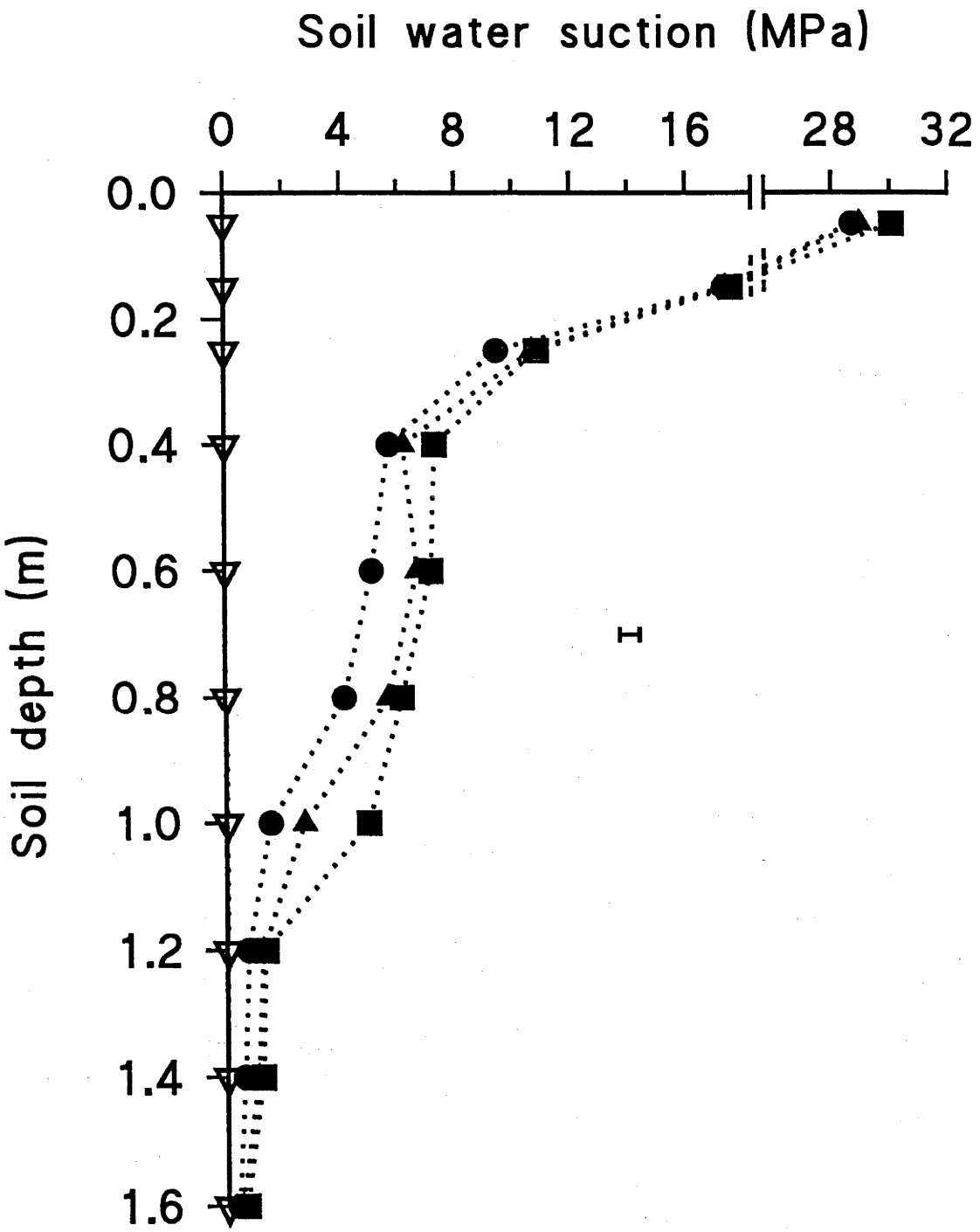


Table 2.16 Evapotranspiration (ET), soil evaporation (E_S), Transpiration (T) and water use efficiency for biomass (W_{ET}^B) and transpiration efficiency for biomass (W_T^B) for periods during the season and for the whole season at BAR91.

Treatment	Components of evapotranspiration (mm)			Efficiency (g m^{-2} mm^{-1})	
(kg N m^{-2})	ET	E_S	T	W_{ET}^B	W_T^B
			DC10 to DC69		
control	234	104	130	2.7	4.9
80SW	253	81	172	3.6	5.3
80LT	247	102	145	3.0	5.0
200ST	253	82	171	3.3	4.9
(l.s.d. P=0.05)	14	n.a.	14	0.2	n.s.
			DC69 to DC87		
control	59	15	44	3.0	4.1
80SW	61	11	50	2.2	2.7
80LT	61	13	49	2.6	3.3
200ST	62	10	52	2.6	3.1
(l.s.d. P=0.05)	n.s.	n.a.	n.s.	n.s.	n.s.
			DC10 to DC87		
control	293	119	174	2.8	4.7
80SW	314	92	223	3.3	4.7
80LT	308	114	194	2.9	4.5
200ST	315	92	223	3.2	4.5
(l.s.d. P=0.05)	16	n.a.	16	0.2	n.s.

n.a. - not applicable, n.s. not significant.

Table 2.17 Evapotranspiration (ET), soil evaporation (E_S), Transpiration (T) and water use efficiency for biomass (W_{ET}^B) and transpiration efficiency for biomass (W_T^B) for periods during the season and for the whole season at BAR92.

Treatment	Components of evapotranspiration (mm)			Efficiency (g m^{-2} mm^{-1})	
(kgN m^{-2})	ET	E_S	T	W_{ET}^B	W_T^B
			DC10 to DC65		
control	134	87	46	1.9	5.4
80SW	191	61	130	3.8	5.6
240SW	219	46	173	4.7	6.0
80LT	183	76	107	3.2	5.5
240LT	196	68	129	3.8	5.9
(l.s.d. P=0.05)	19	n.a.	19	0.4	n.s.
			DC65 to DC87		
control	151	77	73	0.7	1.4
80SW	138	40	97	2.0	3.0
240SW	148	21	127	2.6	3.1
80LT	169	49	120	1.6	2.2
240LT	164	29	135	2.9	3.5
(l.s.d. P=0.05)	20	n.a.	20	1.1	n.s.
			DC10 to DC87		
control	284	164	120	1.2	2.9
80SW	329	102	227	3.0	4.4
240SW	366	67	299	3.9	4.7
80LT	353	125	227	2.4	3.7
240LT	360	96	264	3.4	4.6
(l.s.d. P=0.05)	31	n.a.	31	0.5	0.9

n.a. not applicable; n.s. not significant.

Table 2.18 Percentage of photosynthetically active radiation (PAR) intercepted at DC30 and DC65, and evapotranspiration (ET) and water use efficiency for biomass (W_{ET}^{B}) during crop growth at GES92.

	Treatment (kg N ha^{-1})				l.s.d.
	control	160SW	160LT	320LT	($P = 0.05$)
DC30 PAR (%)	30.9	37.4	30.9	30.9	n.s.
DC65 PAR (%)	70.8	92.6	96.1	98.1	2.8
ET (mm)	562	568	584	580	n.s.
W_{ET}^{B} ($gm^{-2}mm^{-1}$)	2.3	3.5	3.7	3.9	0.4

n.s. not significant

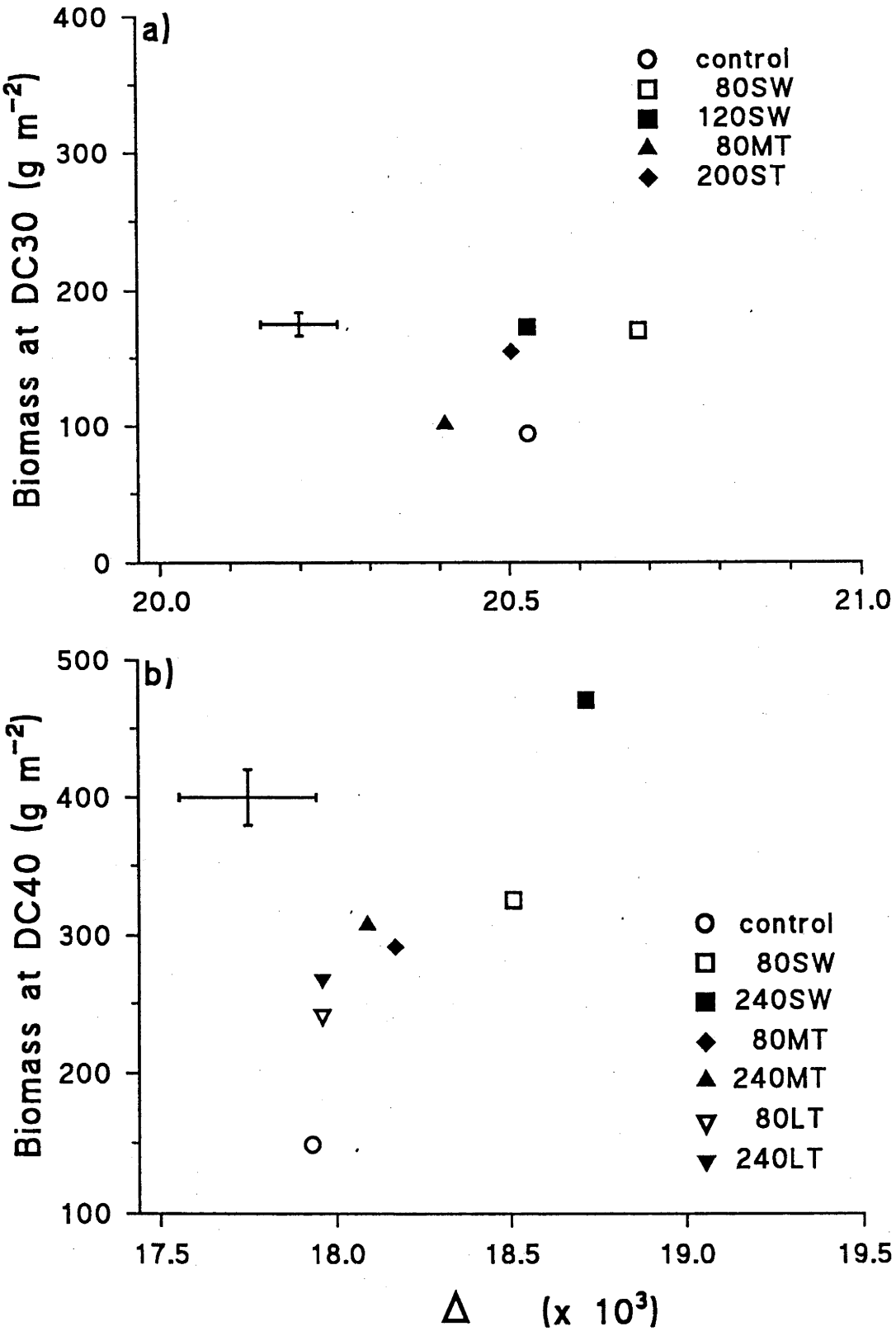
2.3.7 Carbon isotope discrimination

Carbon isotope discrimination (Δ) was measured on selected crops at DC30 from BAR91 and at DC40 from BAR92 (Figure 2.5). At BAR91 the application of 80SW resulted in an increase in Δ and biomass while for the 120SW crops Δ was at the level of the control without a significant increase in biomass. For the 200ST, Δ crop was also not significantly different to the control while the application of 80MT decreased Δ without increasing biomass.

At BAR92 there was a positive relationship between biomass at DC40 and Δ measured at the same time. Application of nitrogen at DC10 resulted in the largest increase in Δ while the application of nitrogen at DC30 (41 days prior to sampling) did not result in a significant increase in Δ. Δ continued to increase with increasing rates of nitrogen applied at DC10 while increasing rates at later applications did not increase Δ.

The site mean Δ at BAR91 was 20.5×10^{-3} while at BAR92 it was 18.2×10^{-3}.

Figure 2.5 The relationship between carbon isotope discrimination (Δ), and biomass as influenced by nitrogen fertiliser application at a) BAR91 and b) BAR92. Bars indicate the l.s.d., (P = 0.05).



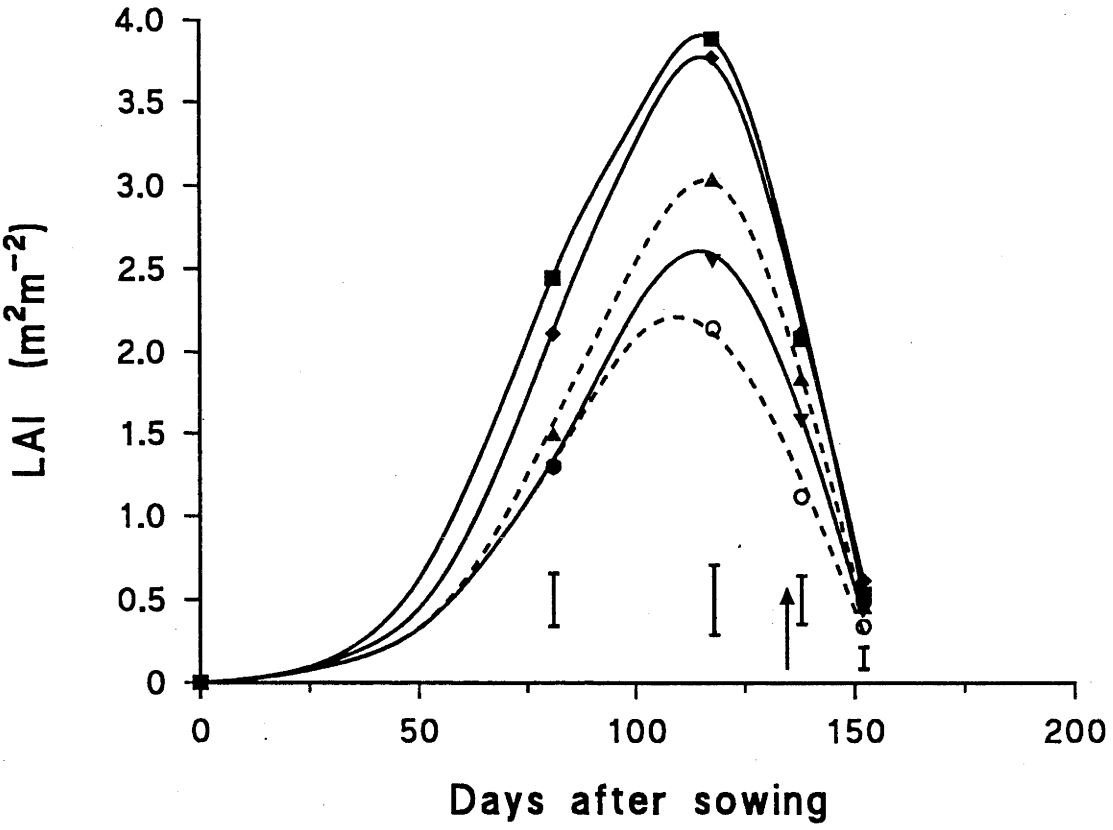
2.4 DISCUSSION

2.4.1 Biomass production and grain yield

Nitrogen fertiliser applied at DC10 stimulated tiller production while applications of nitrogen at DC15 or DC30 encouraged survival of tillers present at the time of fertiliser application (Tables 2.6, 2.7, 2.8). At BAR92 where the crops had a low plant population, later applications of nitrogen encouraged the production of late tillers such that at DC65 there were no differences in shoot density in response to times of nitrogen application. Nitrogen application resulted in significant increases in crop biomass by the anthesis harvest at each site (Tables 2.4, 2.5 2.6). Lower biomass production at DC69 for late applications of nitrogen relative to DC10 applications were associated with reduced shoot density at BAR91 since there was no significant difference in individual spike weight between crops. In contrast, both individual spike weight and shoot density increased in response to nitrogen fertiliser application at BAR92 except for nitrogen applied at DC30 which led to a proliferation of late tillers and reduced the mean spike weight. At GES92 only the crop receiving the highest rate of nitrogen applied at DC30 had significantly less biomass at DC65 than the corresponding rate of nitrogen applied at DC10. The negative trend in DC65 biomass when nitrogen was applied at DC30 was associated with a decrease in shoot density and spike weight. The crop at GES92 was able to compensate for its poor establishment so that by the time the fertiliser was applied at DC30 there were 5.6 shoots per plant compared to BAR92 where plants had only 3.0 shoots each. The greater number of shoots per plant for controls (also crops to receive fertiliser application at DC30) at DC30 at GES92 compared to BAR92 is no doubt due to the higher nitrogen status of GES92 since nitrogen uptake has been shown to limit growth at low levels of soil nitrogen (van Keulen 1981) (3.2 g m^{-2} nitrogen uptake relative to 1.8 g m^{-2}) The greater number of shoots per plant for controls at GES92 may also be due to the positive effects of a higher photothermal quotient (Rawson 1988) on tillering resulting in greater shoot densities at GES92 (Table 2.6) than at BAR92 (Table 2.8).

Water stress was negligible at all growth stages at BAR92 and GES92. At lower rates of nitrogen the GES92 crops reached their nitrogen-limited final biomass as evidenced by significant increases in biomass. At higher rates of nitrogen, crops reached their radiation-limited final biomass as evidenced by the non significant decreasing returns to nitrogen fertiliser (Table 2.6). The BAR92 crops reached a final biomass limited by plant population. At BAR91 nitrogen fertilised crops showed the leaf-rolling symptoms of water stress at DC69 while the low nitrogen crops had no symptoms. It is likely that stress occurred prior to this stage as shown by a decline in Leaf Area Index (LAI) which commenced prior to DC69 (Figure 2.6). Leaf rolling at DC69 was greatest at higher

Figure 2.6 The development of leaf area index (LAI) with time at BAR91 for control (O), 80SW (■), 80MT (▲), 80LT (▼) and 200ST (◆). Curves were fitted by eye, arrow indicates mean anthesis date and bars indicate the l.s.d. (P = 0.05).



rates of nitrogen and for nitrogen applied at DC10. These observations of greater water stress in fertilised crops were corroborated by the 19mm extra water use to DC69 of 80SW crops relative to controls (Table 2.16).

Net biomass production between DC65 and maturity ranged from 126 - 180 g m^{-2} at BAR91, 100 - 463 g m^{-2} at BAR92 and 392 - 921 g m^{-2} at GES92 (Figure 2.7). Regardless of whether a site experienced post-anthesis water stress or not, the greatest increases in biomass between DC65 and maturity at each site occurred when the nitrogen fertiliser was applied later than DC10. Post-anthesis biomass production at BAR91 increased the later the fertiliser was applied and was greatest for the control crop. In contrast, control crops at the wet sites of BAR92 and GES92 produced less additional biomass after DC65 than fertilised crops. Grain yield was negatively correlated with post-anthesis biomass production at the dry site BAR91 and between times of nitrogen application for 240 kg N ha^{-1} at BAR92. This negative correlation with grain yield is associated with decreasing pre-anthesis WSC reserves. At GES92 and within times of application at BAR92 grain yield was positively correlated with post-anthesis biomass production. It is proposed that this positive correlation is due to a greater leaf area duration and greater WSC reserves for higher rates of nitrogen within a time of application at BAR92 and to greater leaf area duration as a result of increased nitrogen uptake at GES92.

Positive grain yield responses to nitrogen fertiliser at all sites were associated with greater spike density, more kernels per spike and hence more kernels per square metre, but also generally associated with reduced kernel weight. Harvest index did not differ significantly with increased rate of nitrogen application but it increased as time of nitrogen application was delayed, corroborating the greater post-anthesis biomass production of late nitrogen application which was predominantly grain.

2.4.2 Retranslocation of pre-anthesis assimilates

The method of Gallagher *et al.* (1976) was used to estimate retranslocation from the non-grain above ground biomass to the grain by assuming that weight loss between DC65 and maturity equates to net retranslocation and in this study is termed 'apparent retranslocation'. This analysis was carried out on the data from the three sites and is presented in Table 2.19. Retranslocation was also estimated from changes in WSC and protein in the biomass between DC65 and maturity. Here it is termed 'estimated retranslocation' and it is also presented in Table 2.19. At the dry site BAR91 apparent retranslocation was greater than estimated retranslocation for all crops except the control. At the wet sites of BAR92 and GES92, however, estimated retranslocation was greater than apparent retranslocation.

Figure 2.7 Relationship between the change in total biomass from anthesis to maturity and grain yield at GES92 (●), BAR91 (□) and BAR92 (■). Time from anthesis to maturity was 50, 34 and 50 days respectively. Curves are fitted regressions.

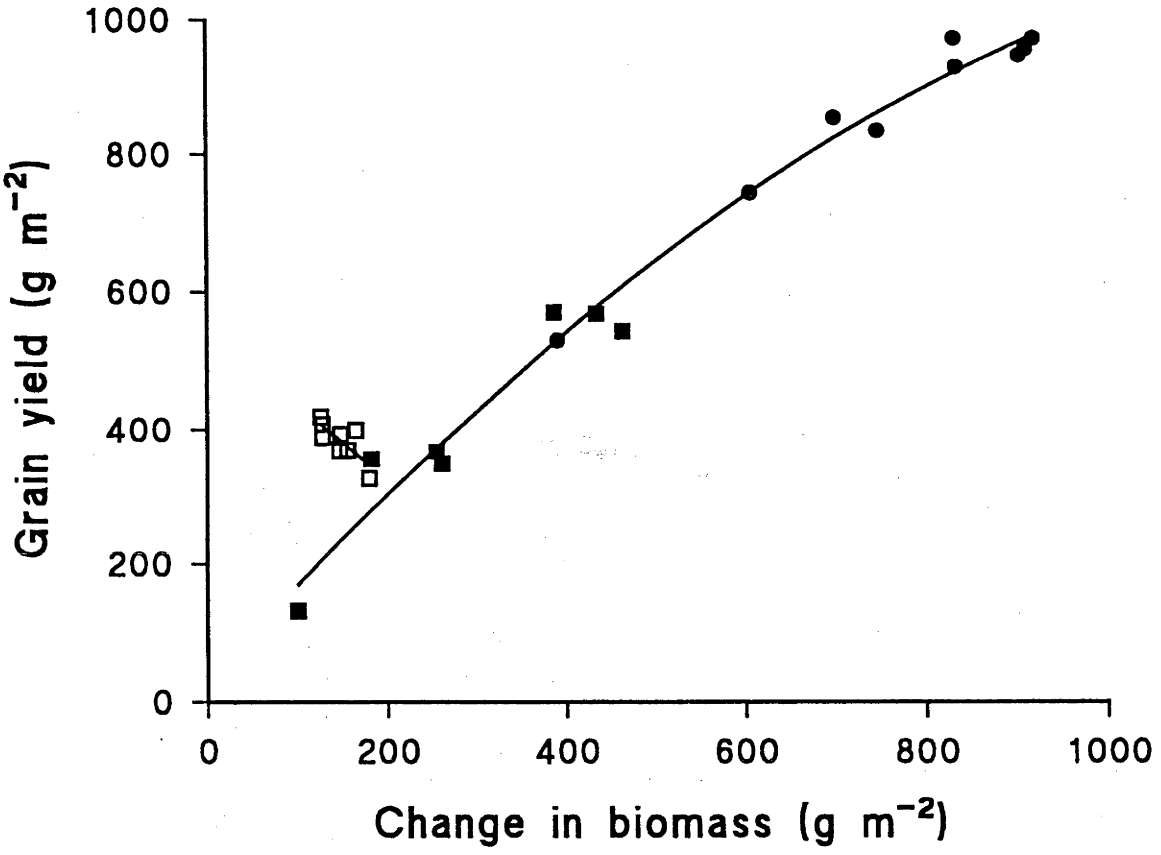


Table 2.19. Comparison of apparent retranslocation (based on weight loss from the non-grain biomass) and estimated retranslocation (based on a WSC and protein budget for the non-grain biomass) between anthesis and maturity as affected by the application of nitrogen fertiliser at each site.

Treatment	Apparent retranslocation (gm^{-2})	Estimated retranslocation ($g\ m^{-2}$)		
(kg N ha^{-1})		WSC[a]	Protein	Total
	BAR91			
Control	148	122	27	149
80SW	280	163	51	214
120SW	294	153	57	209
80MT	245	143	48	191
120MT	261	117	52	169
80LT	216	123	43	166
120LT	222	121	47	168
200ST	234	130	57	187
(l.s.d. P=0.05)	84	25	5	24
	BAR92			
Control	32	52	7	59
80SW	112	134	26	160
240SW	183	184	54	239
80MT	146	133	27	159
240MT	134	152	57	210
80LT	88	102	24	125
240LT	80	134	48	183
(l.s.d. P=0.05)	89	29	6	32
	GES 92			
Control	139	230	22	252
80SW	138	258	45	303
160SW	154	215	63	279
240SW	95	180	74	253
320SW	139	160	87	247
80LT	88	206	54	261
160LT	43	161	75	235
240LT	45	135	94	229
320LT	53	97	97	194
(l.s.d. P=0.05)	n.s.	42	12	46

[a] Estimated WSC retranslocation uses the estimate of total anthesis WSC presented in Table 2.10; n.s., not significant

There are several sources of error which can lead apparent retranslocation to either overestimate or underestimate actual retranslocation. The problem with this approach is that it does not take into account tissue formed before DC65 that is lost in the post-anthesis period, such as respiration, leaf fall (Barley and Naidu 1964, Austin *et al.* 1980b) or saprophytic decay of lower leaves (Bidinger *et al.*1977). This error is likely to overestimate the pre-anthesis contribution to grain filling. Conversely, it does not take into account non-grain tissues formed after DC65 that are measured at maturity but not at DC65, thereby decreasing the magnitude of apparent retranslocation. This error is likely to be greatest in the absence of water stress due to the continued growth of the stem after DC65 (Bonnett and Incoll 1992a; Borrell *et al.*1989, 1993) and cell-wall thickening and lignification (Stoy 1965; Pearce *et al.* 1988) which lead to an increase in structural biomass thereby reducing apparent retranslocation.

Estimated retranslocation takes into account this post-anthesis growth of structural material in the stems. Errors associated with leaf loss are reduced since leaves contain a minor proportion of the total WSC and most of the protein has already been retranslocated from the leaves when they become brittle and tend to fall off the plant. In addition loss of leaf biomass translates into a smaller loss of total WSC and protein because total WSC and protein made up less than 40% of the biomass at DC65 and less than 10% of the non-grain biomass at DC86. Estimated retranslocation is therefore a more complete estimate of actual retranslocation. This calculation assumes that once laid down WSC is not turned over (Winzeler *et al.* 1990) and that post-anthesis respiration is supplied by current rather than stored assimilates (Stoy 1963a, Austin *et al.* 1977, Bell and Incoll 1990, Palta *et al.* 1994, Chapters 3 and 5).

At BAR91 there is good agreement between apparent and estimated retranslocation for the control crop but for nitrogen fertilised crops apparent retranslocation was greater than estimated retranslocation. Due to the terminal drought conditions at BAR91 dead leaves would have became dry and brittle resulting in leaf fall in the field and leaf loss during subsampling of biomass harvests. Austin *et al.*(1977) concluded that leaves export dry matter during grain filling but that it was difficult to quantify because dead leaves are fragile and tend to drop off the plant. Leaf loss may therefore explain a significant proportion of the discrepancy between the two methods but does not explain why agreement between the methods was better for the control and lower rates of nitrogen than for the higher rates of nitrogen. Stirzaker (1983), and data presented in Chapter 4, show that leaves become a higher proportion of the biomass as the rate of nitrogen fertilisation is increased. The greater proportion of leaves in high nitrogen crops at BAR91 was likely to result in greater losses of leaf material, thus increasing the apparent retranslocation. For the control crop reduced water use to DC65 meant that the crop was less water stressed after anthesis and that post-anthesis stem elongation

may cancel the effects of leaf fall. Indeed between DC65 and DC78 at BAR91 stem biomass of control crops fell slightly while the combined WSC and protein decreased to a greater extent indicating the possibility of net stem growth (Table 2.20). In contrast, stem biomass of the nitrogen fertilised crops fell more than for control crops and the sum of WSC and protein also decreased in the same period. However, stem biomass decreased to a greater extent than the decrease in WSC and protein. Stems are not fragile like leaves and losses of stem biomass are not probable. Stem growth was unlikely due to water stress and it is possible that another stem component not considered in this analysis was being remobilised. This possibility is discussed further in Chapter 4.

Table 2.20 Comparison between the change in stem biomass (stem plus leaf sheath) and change in stem biomass accounted for by WSC and protein between DC65 and DC78 at BAR91.

Treatment	Change in stem biomass (g m^{-2})	Change in stem WSC and protein (g m^{-2})		
(kg N ha^{-1})		WSC	Protein	Total
Control	-5	-11	-1	-12
80SW	-61	-15	-5	-20
80MT	-55	-34	-5	-39
80LT	-42	-7	-5	-12
200ST	-38	-11	-5	-16
(l.s.d. P=0.05)	32	12	2	13

In the wet season at BAR92 and GES92 estimated retranslocation exceeded apparent retranslocation and the discrepancy increased as time of nitrogen application was delayed (Table 2.19). In the wet conditions at both BAR92 and GES92, continued stem growth after DC65 probably accounted for the disagreement between the methods, although there may also have been less leaf fall than in a dry year because the leaves were less brittle and thus less subject to losses in the standing crop and during sub-sampling. An analysis of the change in the non-grain biomass between DC65 and DC75 at BAR92 (Table 2.21) showed that there was growth in all crops during this period and the largest increases were at the highest rate of nitrogen. Those crops continued to lay down WSC reserves while the crops at the lower rate of nitrogen and the controls were utilising WSC reserves in the same period (Figure 2.1). Subtracting the increase in WSC from the increase in non-grain biomass gives an estimate of the 'structural growth' between DC65 and DC75 and this was greatest for 240LT which produced many late tillers in response to the nitrogen application.

Table 2.21 Comparison between the change in non-grain biomass and change in non-grain biomass accounted for by WSC and protein for the period between DC65 and DC75 at BAR92.

Treatment	Change in biomass ($g\ m^{-2}$)	Change in WSC and protein ($g\ m^{-2}$)		
(kg N ha^{-1})		WSC	Protein	Total
Control	+18	-9	-3	-12
80SW	+57	-13	-11	-24
240SW	+146	+56	-15	+41
80MT	+65	-19	-6	-25
240MT	+77	+14	-12	+2
80LT	+48	-5	-7	-12
240LT	+168	+41	-8	+33
(l.s.d. P=0.05)	85	42	7	46

In the absence of post-anthesis water stress pre-anthesis reserves contributed between 20% and 48% to the grain yield. The proportion decreasing with fertiliser application (Table 2.22). The magnitude of the contribution also decreased as time of fertiliser application was delayed. For BAR92, the estimated retranslocation decreased by 23% when fertiliser application was delayed from DC10 to DC30 while yield only decreased by 5%. At GES92, however, estimated retranslocation decreased by 15% when fertiliser application was delayed from DC10 to DC30 while yield increased by 6%. Therefore post-anthesis assimilation was greatest with late applications of nitrogen in conditions of adequate water supply. For these conditions, crops were able to compensate for lower soluble reserves by maintaining assimilation for longer after DC65.

Terminal drought at BAR91 resulted in a downward trend in the proportion of grain yield accounted for by estimated retranslocation (49 - 46%) as fertiliser application was increased from 80 to 120 kg N ha^{-1} (Table 2.22). The magnitude of the contribution also decreased as time of fertiliser application was delayed (51 - 45%). Estimated retranslocation decreased by 21% at BAR91 when fertiliser application was delayed from DC10 to DC30 and yield also decreased by 21%. Surprisingly, therefore, the estimates of post-anthesis assimilation did not vary with time of fertiliser application despite the trend for greater water use before DC65 with early nitrogen application. A possible explanation is that the post-anthesis assimilation of the 80LT crops was less than for crops supplied with nitrogen at DC10 due to the reduced AFR of the later application. Lower nitrogen uptake with the later application would have resulted in more competition for nitrogen between the photosynthetic apparatus and the developing grain.

Table 2.22. The effect of nitrogen fertilisation and time of application on the grain yield, estimated retranslocation, estimated net post-anthesis assimilation and the proportion of the grain yield contributed by retranslocation of pre-anthesis reserves at each site.

Treatment (kgN ha^{-1})	Grain yield (gm^{-2})	Estimated retranslocation[a] (gm^{-2})	Estimated net assimilation[b] (gm^{-2})	Pre-anthesis contribution (%)
		BAR91		
Control	327	149	179	45
80SW	408	214	194	52
120SW	419	209	210	50
80MT	393	191	202	49
120MT	388	169	219	43
80LT	369	166	204	45
120LT	368	168	111	45
200ST	399	187	206	47
(l.s.d. P=0.05)	14	24	n.s.	n.s.
		BAR92		
Control	133	59	73	44
80SW	367	160	207	44
240SW	570	239	332	42
80MT	356	159	196	47
240MT	568	210	359	37
80LT	349	125	223	36
240LT	543	183	361	34
(l.s.d. P=0.05)	16	32	44	n.s.
		GES 92		
Control	530	252	278	48
80SW	745	303	442	41
160SW	855	279	576	33
240SW	931	253	678	27
320SW	974	247	726	25
80LT	836	261	576	31
160LT	948	235	713	25
240LT	957	229	728	24
320LT	973	194	778	20
(l.s.d. P=0.05)	51	46	59	6

[a] From Table 2.19

[b] Grain yield - estimated retranslocation; n.s., not significant

Nitrogen uptake at DC65 was 17% less for the late application of nitrogen than the DC10 application while kernel number was reduced by only 8%.

The additional loss of stem weight not accounted for by WSC and protein in Table 2.20 may also have been retranslocated to the grain since loss of stem material was unlikely because stems are not fragile like leaves. If this additional loss of stem weight is also subtracted from the grain yield then the estimates of post-anthesis net assimilation were similar for all crops (188 gm^{-2} for control, 173 gm^{-2} 80SW, 186 gm^{-2} 80LT and 189 gm^{-2} 200ST).

2.4.3 Water use, water use efficiency and transpiration efficiency

2.4.3.1 *BAR91*

At BAR91, evapotranspiration before DC69 was greater for the fertilised crops than for the control. However, evapotranspiration was not significantly different between time of application or the higher rate of nitrogen (Table 2.16). Transpiration efficiency before DC65 tended to be greatest when nitrogen was applied at DC10 while for late or split applications transpiration efficiency was not different from the control. However, after DC65 transpiration efficiency tended to be lower for crops which used more water before DC65. The higher post-anthesis transpiration efficiency for control crops was also probably due to the more efficient photosynthesis of spikes than leaves (Araus *et al.* 1993), in light of the lower leaf area of control crops relative to nitrogen fertilised crops. Transpiration efficiency for the whole season was, therefore, similar for all crops.

Interestingly, the 200ST crop had the same transpiration efficiency as the control up to DC69. The biomass production of the 200ST crop was also less than the biomass produced by 80SW and 120SW crops. A lower transpiration efficiency in response to nitrogen was also observed in one of the experiments discussed in Chapter 4. For the 200ST crop, spike density and biomass at DC65 were lower than the 80SW crop but LAI was similar despite having more than twice the rate of nitrogen applied.

One of the reasons for the poor response of the 200ST crop may have been the delay in applying most of the nitrogen. The first application of 40 kg N ha^{-1} was at DC10 but the next application was not until DC15 when shoot density was already less than for the 80SW crop. The remaining three applications of 40 kg N ha^{-1} were at DC16, DC30 and DC31. Multiple application of nitrogen led to 8% more nitrogen uptake than 80SW and to the same water use by DC69.

Another possible reason for the poor response may have been the biological cost of nitrogen reduction. Assuming that the nitrogen was taken up as nitrate and thus a glucose requirement of 2.48 g glucose g protein^{-1} for the synthesis of protein (Loomis and Connor, 1992), the additional 5 g m^{-2} protein in the 200ST at DC65 would only account for a reduction in biomass of 8 g m^{-2} whereas the biomass was 82 g m^{-2} less than for the 80SW crop. Synthesis of extra protein due to the additional nitrogen uptake cannot account for the lower biomass relative to the 80SW crop.

A greater proportion of the nitrogen uptake for 200ST was, however, contained in a similar leaf area to the 80SW crop. Work by Winzeler *et al.* (1989) found a positive correlation (r^2 = 0.86) between dark respiration rate and nitrogen concentration in the leaves of rye, triticale and wheat. Respiration rates of leaves of field grown wheat supplied with 117 kg N ha^{-1} were shown to be greater throughout the season, and up to double the rate of crops supplied with 39 kg N ha^{-1} (Stoy 1965). In the current experiment leaf nitrogen concentration at the maximum LAI was 35 g kg^{-1} for the 80SW crop and 40 g kg^{-1} for the 200ST crop. A similar increase in leaf nitrogen concentration increased respiration rate approximately 2.5 fold when averaged over all measurement temperatures (Winzeler *et al.* 1989). This large increase in respiration rate for a much smaller increase in nitrogen concentration was possibly due to maintenance of active enzymic protein which is likely to incur a greater maintenance cost than an increase in compartmentalised storage protein in the stem. Higher respiration rate of the leaves and shading of the lower leaves of a higher nitrogen concentration in particular would lead to a less efficient canopy for the multi application crop and may explain the reduction in pre-anthesis transpiration efficiency.

Similar evidence of reduced transpiration efficiency can be derived from Figure 2.5a. The 200ST and 120SW crops have similar Δ values at DC30 which are less than the value for the 80SW crop. This ranking reflects the leaf nitrogen concentrations at DC65 of 24 g kg^{-1} (for 200ST) and 23 g kg^{-1} (for 120SW) compared to 20 g kg^{-1} (for 80SW). The similar, lower Δ values for 120SW and 200ST suggest that they should have had a higher transpiration efficiency than the 80SW crop. However, Figure 2.5a shows that the 200ST and 120SW crops did not produce significantly more biomass than the 80SW crop. These data suggest that the lack of increase in biomass for 120SW and 200ST crops above the 80SW at DC30 or any other growth stage, despite maintaining higher leaf nitrogen concentrations (greater photosynthetic capacity), were due to greater maintenance respiration of the higher protein content (Amthor 1989).

2.4.3.1 *BAR92*

Evapotranspiration to DC65 increased with nitrogen fertiliser application at BAR92 and in contrast to BAR91 differences existed between nitrogen fertilised crops (Table 2.17). The proportion of evapotranspiration lost as soil evaporation was greatly reduced by the application of nitrogen fertiliser, in particular the application at DC10. The crop growth response and resultant small increases in transpiration efficiency of the 80SW or 80LT crops relative to the control were similar to the responses at BAR91. High rates of nitrogen, whether applied at DC10 or DC30 tended to increase transpiration efficiency.

Transpiration efficiency decreased after DC65 for all crops especially the nitrogen deficient control crop. The especially low estimate of transpiration efficiency for the control crop could be due to the loss of stomatal function leading to continued transpiration as the crop senesced under wet conditions. The 80LT crops also showed a substantial decrease in transpiration efficiency and may be related to the high nitrogen uptake of this treatment compared to the 80SW crops. It has been shown that increased nitrogen uptake during grain filling may reduce assimilates available for grain formation (Bänziger *et al.* 1994), presumably due to the costs associated with uptake and synthesis of protein (Loomis and Connor, 1992). It is unlikely that possible errors associated with the determination of the soil evaporation component of evapotranspiration (Cooper *et al.* 1983) increase at low biomass since the method was determined over a large range of biomass and has appeared to work satisfactorily on all treatments in the present study up to DC65. Transpiration efficiency was greatest for the 240LT crops presumably due to the greater leaf area duration (Fischer and Kohn, 1966c, Blacklow and Incoll 1981, Siddique *et al.*1989) than other crops. This greater leaf area duration was inferred from a 29% higher nitrogen concentration of the non-grain biomass for the 240LT crops relative to 240SW for the DC83 biomass harvest (Chapter3, Figure3.9).

The seasonal transpiration efficiency was greater with the application of nitrogen fertiliser. Transpiration efficiency was greatest at the high rate of nitrogen applied at either DC10 or DC30. The 80LT crop had a lower transpiration efficiency than if the same amount of nitrogen was applied at DC10, possibly due to the inefficient use of water prior to the application of nitrogen at DC30, less growth at low VPD when growth is more efficient than for early applied nitrogen (Condon *et al.* 1992b) and/or the reason described above.

2.4.4 Soil water extraction

Under the terminal drought conditions at BAR91 the crops supplied with additional nitrogen dried the soil more than the control crop (Figure 2.4). The 80SW crop tended

to dry the soil to a greater suction than the nitrogen applied at DC30. At depths below which soil evaporation could not influence water extraction, nitrogen fertilised crops were able to dry the soil to almost 8 MPa suction and control crops were able to achieve between 4 and 6 MPa. Most previous research suggested that the permanent wilting point was 1.5 MPa (Veihmeyer 1950; Leeper 1964). The extraordinary values reported here are higher than the highest previously attributed to plant water extraction (3.6 MPa) which occurred under Brigalow (*Acacia harpophylla* F. Meull. ex. Benth.) near Meandarra in Queensland, Australia (Tunstall and Connor 1981). The greater soil water suction in the present study was likely to be due to a vigorous root system in the absence of root disease and the slow development of water stress resulting in maintained coordination of metabolic processes associated with photosynthesis (Harding *et al.* 1990a,b) and hence sustained transpiration throughout grain filling.

Reduction of soil water reserves to greater than 1.5 MPa suction have been reported for wheat crops (Fischer and Kohn 1966b, Angus *et al.* 1980, Ritchie 1981) though the actual magnitude of the soil water suction is rarely measured. Generally the only measurement is the 1.5 MPa suction over the soil profile, or so called lower limit of plant extractable water. It is possible that some of these earlier studies would also have found extremely high soil water suction directly attributable to the action of the crop, had the measurements been performed. Indeed in the study of Angus *et al.* (1980) on a heavy clay soil, a wheat crop was reported to extract a mean of 80mm of water in excess of the 1.5MPa "lower limit" in a depth of 0.90m. The nature of the soil water release curve is such that increasing soil water suction results in smaller and smaller decreases in water content. It is difficult to understand, therefore, how crops could derive much benefit from extracting the small amounts of water from the soil at suctions between 4 to 6 or even 8 MPa.

CHAPTER 3

NITROGEN AND CARBON REDISTRIBUTION DURING GRAIN FILLING IN WHEAT

Mountain or river or shining star,
There's never a sight can beat-
Away to the sky line stretching far-
A sea of ripening wheat.

Song of the Wheat
A. B. Paterson

3.1 INTRODUCTION

There are many interacting and complex factors which determine the final grain yield of wheat. Considerable discussion as to whether grain yield is limited by source (ie, carbon assimilation) or by sink (ie., maximum grain growth rate). However, in most biological systems, there is not one limiting factor, but many co-limiting processes (Rees *et al.* 1993) and these will be different in contrasting environments.

A considerable body of knowledge built up over the past thirty years (Fischer and Kohn 1966c, Spiertz and Ellen 1978, Austin *et al.* 1980b, Blacklow and Incoll 1981, Siddique *et al.*1989) indicates that grain yield is positively related to leaf area duration. Under terminal drought conditions the greater leaf area duration is expected to have limited benefits due to little water being available for transpiration and thereby growth. Work by Gallagher *et al.* (1976) indicated that the remobilisation of pre-anthesis reserves to the grain increased under terminal drought conditions. This contribution was large, whether it was determined as an amount of remobilisable dry matter or as a proportion of yield.

Rawson and Evans (1971) concluded that approximately one third of the decrease in stem weight during grain filling was due to respiration. Austin *et al.* (1977) estimated respiratory loss from the stems of six varieties of wheat during the post-anthesis period to be 62% of the decrease in stem weight. In contrast ^{14}C labelled studies with wheat indicate that post-anthesis respiratory losses from the above-ground dry matter are usually minor (Stoy 1963 ≈10%, Wardlaw and Porter 1967 ≈10%, Austin *et al.* 1977 ≈8%, Austin *et al.* 1980a ≈11%). In addition there is little evidence to suggest that stem respiration relies on stem reserves alone. Indeed, work by Winzeler *et al.* (1990), found that once laid down, water soluble carbohydrates were not turned over, while Bell and Incoll (1990) concluded that most respiration during grain filling used current rather than stored assimilate.

Because most of the protein available for grain filling is part of the photosynthetic machinery of a wheat crop (Evans and Seemann 1989), nitrogen redistribution during leaf senescence may be accompanied by the loss of photosynthetic capacity (Gregory *et al.* 1981). Under conditions of nitrogen inadequacy and/or post-anthesis water stress, which limits soil nitrogen mineralisation and hence nitrogen uptake (Clarke *et al.* 1990), there is a trade-off between nitrogen available for grain growth and photosynthesis (Sinclair and de Wit 1975). Under such conditions water soluble carbohydrate reserves are mobilised sooner and become a source of assimilates whose importance depends on the severity of such stress. Indeed, Blacklow and Incoll (1981) found that wheat grown at high nitrogen status continued to take up nitrogen after anthesis and net photosynthesis continued until the end of grain-filling. The wheat at low nitrogen status,

however, ceased nitrogen uptake and net photosynthesis halfway through grain filling and subsequent grain growth relied on remobilisation of WSC and protein. Water soluble carbohydrates are remobilised at times when the supply of assimilates from current photosynthesis cannot meet the demand of the developing grain. Hence, depending on the level of nutrition and/or environmental stress the contribution of water soluble carbohydrates reserves have been estimated to contribute from 5-10% of grain yield under favourable conditions (Wardlaw and Porter 1967) and up to 74% in dry conditions (Gallagher *et al.* 1975).

The aim of the experiments detailed in this chapter was to examine the effects of water, carbon and nitrogen dynamics on yield formation by separating the effects of these factors on sink development (kernel number) and source supply (grain filling). The biomass and nitrogen status of wheat crops at anthesis were manipulated through different rates and times of nitrogen application to examine different physiological responses of the wheat crops and determine which may increase the risk of haying-off. An additional aim was to determine the cost, in respiratory terms, using ^{14}C as a tracer, of retranslocating pre-anthesis stored assimilates to the grain.

3.2 MATERIALS AND METHODS

3.2.1 Cultural Conditions

The Barellan site was 320 km west of Canberra (146°39' long, 34°12' lat, 170 m a.s.l.) (Appendix 1) on the property of Neville and Pamela Semmler in the western half of the New South Wales wheat belt (long term annual rainfall 436 mm). Experiments were conducted in a flat 50 hectare paddock. The soil is a red brown earth, Dr 2.33 (Northcote *et al.* 1971) with a texture contrast at 30-40 cm. The Ginninderra site was 17 km north-west of Canberra (149° 06' long, 35° 12' lat, 600 m a.s.l.) (Appendix 1) on the Ginninderra Experiment Station, CSIRO in the southern tablelands of NSW (long term annual rainfall 706 mm.). The experiment was conducted in a two hectare trial site with a 1 percent slope. The soil is a yellow podzolic soil Gn 3.85 (Northcote *et al.* 1971). Further details of the trial sites and the cultural conditions were described in Chapter 2, Section 2.2.1

3.2.2 Experimental design and observations

The experimental design was described in Chapter 2 Section 2.2.2. Experiments at Barellan are referred to as BAR91 and BAR92 and the experiment at Ginninderra as GES92. In addition to the observations described in Chapter 2, observations were made in this part of the project to explain carbon and nitrogen between DC65 and maturity. A harvest at the late milk stage (DC78) at BAR91 divided grain filling into two periods which are referred to in the text as early and late grain filling. Similarly, at BAR92, harvests at the mid milk stage (DC75) and the early dough stage (DC83), divided grain filling into three periods which are referred to as early, mid and late grain filling.

Two of the crops at BAR92, 80SW and the 240SW, were selected at DC30 for detailed study of carbon redistribution, particularly by labelling with ^{14}C. The crops supplied with 80 kg N ha^{-1} were chosen in preference to control crops to represent low nitrogen status because they were more representative of a commercial crop showing mild nitrogen deficiency symptoms in contrast to the gross nitrogen deficiency exhibited by the control crop.

3.2.3 Meteorological data

Meteorological data for the experimental sites are presented in Chapter 2, Section 2.2.3.

3.2.4 Crop growth and soil measurements

Crop growth was monitored as detailed in Chapter 2, Section 2.2.4.

At BAR92 selected treatments were labelled with ^{14}C on two occasions at DC39 (September 8) and DC55 (October 1) using a method similar to that of Angus *et al.* (1972). The same area of crops were labelled on both occasions. For labelling with ^{14}C, 1 m^2 areas of crop (subplot) were enclosed in polyethylene chambers. Between 0.5 and 0.6 mCi of $^{14}CO_2$ was evolved from $Ba^{14}CO_3$ in a small, open glass vial suspended in front of an oscillating circulation fan by addition of 50% lactic acid. The reaction was allowed to proceed for approximately 10 minutes after which hot water was injected into a larger vial surrounding the reaction vial with the aim of driving the reaction to completion. Any $^{14}CO_2$ remaining in the chamber was vented to the atmosphere between 15-20 minutes after the addition of the lactic acid.

Plant harvests were taken from the subplots at DC65, DC75, DC81 and DC87. At each harvest 5 random grab samples which comprised 20-35 fertile spikes per sub-plot were taken from the low and high nitrogen crops and separated into spikes, flag leaf, second and third leaves, rest of leaf, and stem plus sheath. Samples were placed in a fan-forced dehydrator at 70°C until dry. Grain was threshed from the spikes, the grain redried and weighed and kernel weights calculated from the average of 3 lots of 100 kernel weights. After the DC87 harvest the remaining ^{14}C-labelled plant material was gathered and either disposed of through systems approved by the site radiation authorities or passed onto colleagues for soil microbiological research.

3.2.5 Plant sample analysis

Plant samples were ground using a Wiley mill to pass a 1.0 mm sieve. Nitrogen concentration was determined using a semi-micro-Kjeldahl method (Heffernan, 1985) and analysed for NH_4^+ (AOAC 1984). The same samples were used to determine water soluble carbohydrate (WSC) levels in the stems since less than a 1% in WSC difference has been shown between oven drying and freeze drying samples (Judel and Mengel 1982, Kiniry 1993). Carbohydrates were extracted from 0.1g of plant material by extracting once with 8 ml of 80% ethanol (v/v) at 80 °C followed by two extractions with 8 ml of distilled water at 60 °C. Extraction time was 60 minutes for each after which tubes were centrifuged and the extracts progressively combined. This procedure was considered to extract 99% of the total free and combined fructose (Borrell *et al.* 1989). Total carbohydrates in the extract were analysed by the anthrone method of Yemm and Willis (1954) using fructose as a standard.

The counts per minute of ^{14}C in each sample were determined by direct counting of 15-30 mg samples of the powdered material (O'Brien and Wardlaw 1961) using an ICN Tracerlab planchet sample counter. A sub-set of samples were randomly selected from each sampling time and 0.1 g digested in 16 ml of an organic solubilizer (Nuclear Chicago solubilizer) at 50^{0}C for 72 hrs with intermittent shaking. After cooling, equal quantities of digestion mixture and a scintillation cocktail of 0.15 g 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) and 12.0 g 2,5-diphenyloxazole (PPO) per litre of toluene were mixed and ^{14}C counted in a Beckman liquid scintillation counter, Model LS 6800. Quenching was calculated for each digest by spiking samples with a known activity of ^{14}C sucrose and recounting. Counting efficiency for the direct counting method was then estimated for each plant part at each harvest and counts per minute of all samples corrected accordingly. The results are expressed as disintegrations per minute (DPM) per unit of ground area.

The specific activity of ^{14}C in WSC extracts was determined by mixing 0.40 ml of extract with 3.60 ml of a scintillation cocktail of 670 ml of toluene and 330 ml of TritonX100 containing 75 mg l^{-1} 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) and 6.0g l^{-1} 2,5-diphenyloxazole (PPO) and placing in the Beckman liquid scintillation counter with a locally determined quench correction program installed. Quench correction was checked by spiking randomly selected samples with ^{14}C sucrose as above. ^{14}C content per unit of ground area was calculated as the product of the dry weight of WSC and the specific activity of ^{14}C in the WSC extracts.

3.2.6 Crop water use

Crop evapotranspiration was measured at all sites and partitioned into transpiration and soil evaporation components for BAR91 and BAR92 and described in Chapter 2, Sections 2.2.5, 2.2.6, and 2.2.7.

3.3 RESULTS

3.3.1 Weather conditions

Weather conditions were summarised in Chapter 2, Section 2.3.1.

3.3.2 Biomass production

The increases in biomass with time for selected crops at BAR91 and BAR92 are presented in Figure 3.1a and 3.1b respectively. In both years there were approximately exponential increases in biomass up to DC65. The exception to this was the nitrogen starved control crop at BAR92. Final biomass for the control crop was lower at BAR92 than BAR91 but similar for 80 kg N ha^{-1}. At BAR91 post-anthesis water stress severely limited net biomass production. The greatest increase in biomass during early grain filling was in the control crop. The next most rapid increase was by the crop supplied with nitrogen at DC30 and the slowest growth by crops fertilised at DC15 or DC10. The more rapid growth by all fertilised crops during late grain filling was due to 21 mm of rain.

At BAR92 linear increases in biomass continued during early and mid grain filling. Crop growth rate decreased for all crops in late grain filling and some crops lost biomass. The post-anthesis increase in biomass was greatest at the high rate of nitrogen and lowest for the control crop. Late application of nitrogen promoted greater post-anthesis biomass production than equivalent rates of early-applied nitrogen.

3.3.3 Changes in non-grain biomass

Figures 3.2a and 3.2b present the change in non-grain biomass with time at BAR91 and BAR92 respectively. Up to DC65 these curves are the same as those for biomass presented in Figures 3.1a and 3.1b. At BAR91 the non-grain biomass of all crops decreased between DC65 and maturity. Early application of nitrogen resulted in greater decreases in the non-grain biomass than later applications, which in turn caused more of a decrease than occurred in the control crop. During early grain filling the non-grain biomass of the control crop did not change while that of nitrogen fertilised crops decreased. Early application of nitrogen resulted in greater decreases than late application. The decrease in non-grain biomass during mid grain filling was similar for all crops.

The non-grain biomass of all crops decreased between DC65 and maturity at BAR92. In contrast to the data from BAR91, however, non-grain biomass increased in all nitrogen

Figure 3.1 Biomass production curves from DC10 to DC87 for a) control (O), 80SW (■), 80MT (▲), 80LT (▼) and 200ST (◆) at BAR91 and b) control (O) and 80SW, 240SW (□,■), 80MT, 240MT (Δ,▲), 80LT, 240LT (∇,▼) at BAR92. Curves were fitted by eye, arrow denotes mean anthesis date, bars indicate the l.s.d., (P = 0.05).

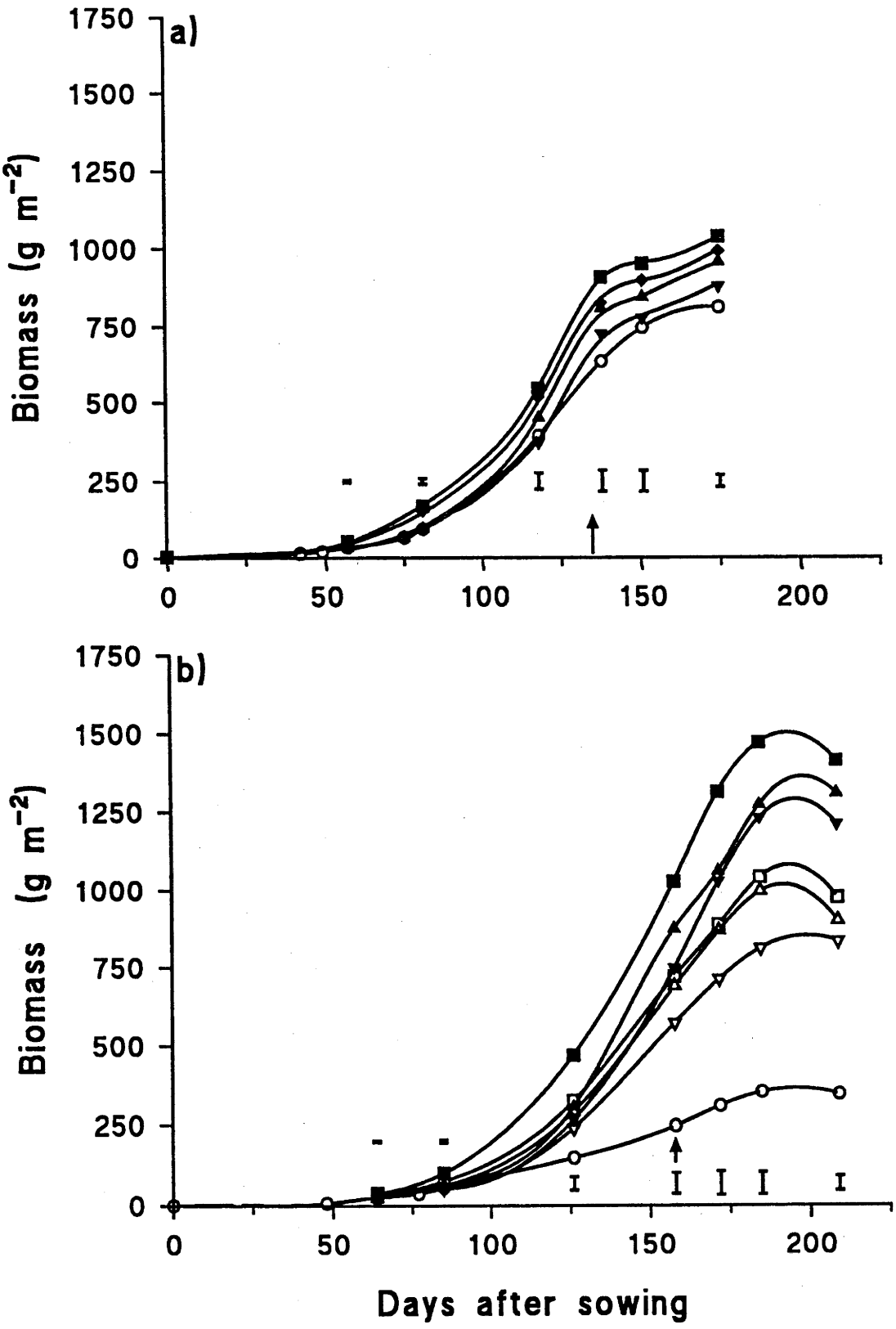
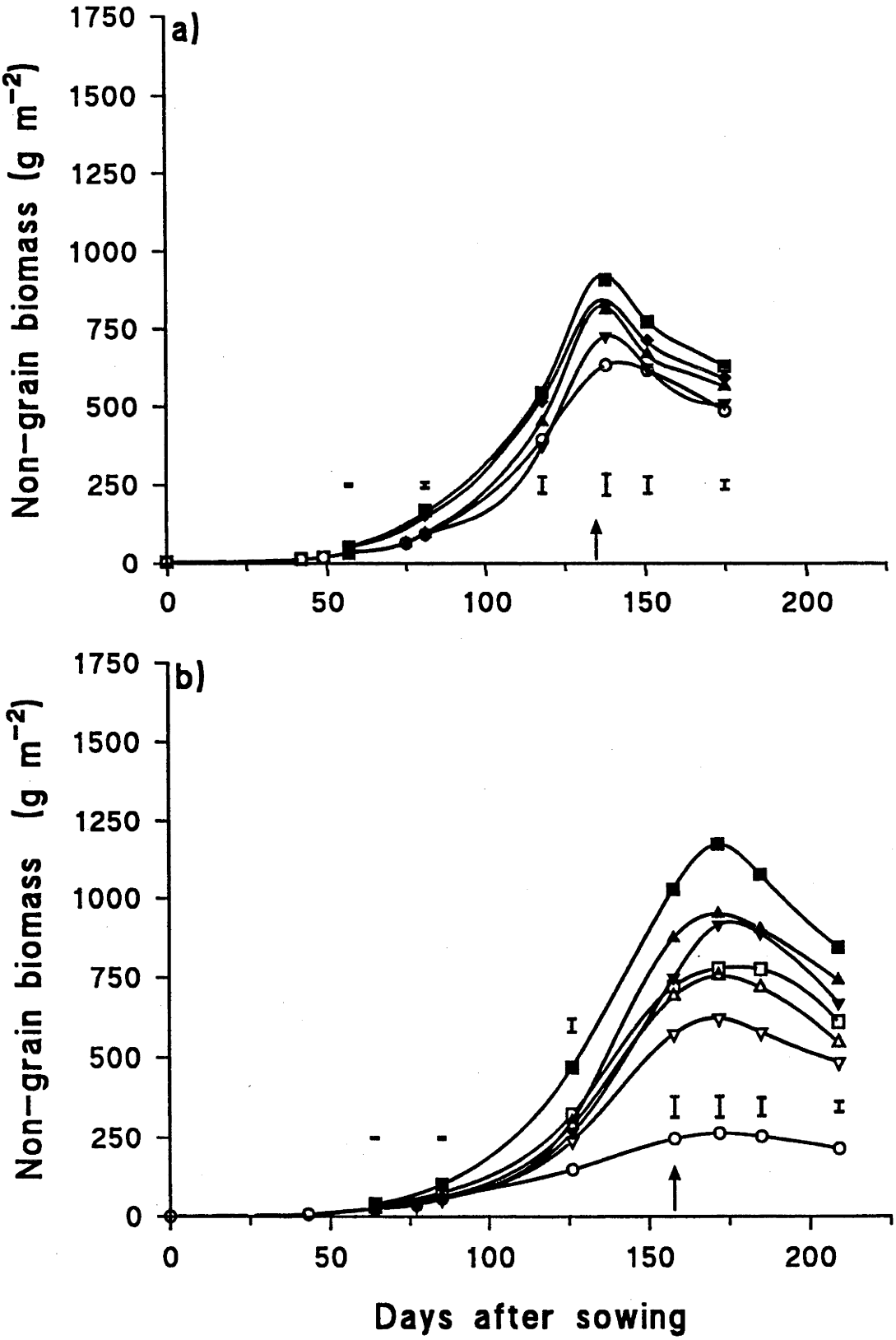


Figure 3.2 Non-grain biomass curves from DC10 to DC87 for a) control (O)80SW (■), 80MT (▲), 80LT (▼) and 200ST (◆) at BAR91 and b) control (O) and 80SW, (□,■), 80MT, (Δ,▲), 80LT, (∇,▼) at BAR92. Curves were fitted by eye, arrow denotes mean anthesis date, bars indicate the l.s.d., (P = 0.05).



fertilised crops but not the control crop during early grain filling. The non-grain biomass decreased for all crops during mid and late grain filling. Increasing rates of nitrogen resulted in greater decreases in non-grain biomass than low rates of nitrogen and control crops. Non-grain biomass tended to decrease to a greater extent in response to early compared to late application of nitrogen.

3.3.4 Nitrogen uptake

Nitrogen uptake at BAR91 reflected the rate of nitrogen applied and the time it was applied. The uptake of nitrogen was greater for early than for late application (Figure 3.3a). There was a net decrease of nitrogen from the biomass of fertilised crops during early grain filling suggesting that losses occurred. No rain fell at the time and crops showed symptoms of water stress. In contrast, the control crop took up nitrogen during this period. During late grain filling, when 21mm of rain fell thereby reducing water stress, losses of nitrogen from the biomass were minimal.

At BAR92 nitrogen uptake also reflected the rate and timing of nitrogen application (Figure 3.3b). Delayed nitrogen fertiliser application naturally delayed fertiliser uptake but faster rates of nitrogen uptake for later applications led to distinct groupings of treatments by DC65 based on the rate of nitrogen applied. In contrast to the advantage of early application at BAR91, the applications of nitrogen at DC15 resulted in more nitrogen uptake than for nitrogen applied at DC10. Crops at all levels of nitrogen continued to take up nitrogen after DC65 in contrast to losses experienced in the dry season at BAR91. The control and 80 kg N ha^{-1} crops took up less nitrogen at BAR92 than equivalent treatments at BAR91.

3.3.5 Grain yield formation

Grain growth at BAR91 was slower in the control crops and those with late nitrogen application (Figure 3.4a). The lower grain yields were associated with fewer kernels, as discussed in Chapter 2. At BAR92 grain growth was also faster in response to early-applied nitrogen than to late-applied nitrogen (Figure 3.4b) but yield was not related to kernel number as it was at BAR91. Greater amounts of nitrogen resulted in faster grain growth at BAR92 and in this case it was associated with increasing kernel number. Figure 3.4b shows that at final harvest at BAR92 grain yield had fallen into three distinct groups associated with the amount of nitrogen applied with little effect of the time of application.

Figure 3.3 Nitrogen uptake curves from DC10 to DC87 for a) control (O), 80SW (■), 80MT (▲), 80LT (▼) and 200ST (◆) at BAR91 and b) control (O) and 80SW, 240SW (□,■), 80MT, 240MT (Δ,▲), 80LT, 240LT (∇,▼) at BAR92. Curves were fitted by eye, arrow denotes mean anthesis date, bars indicate the l.s.d., (P = 0.05).

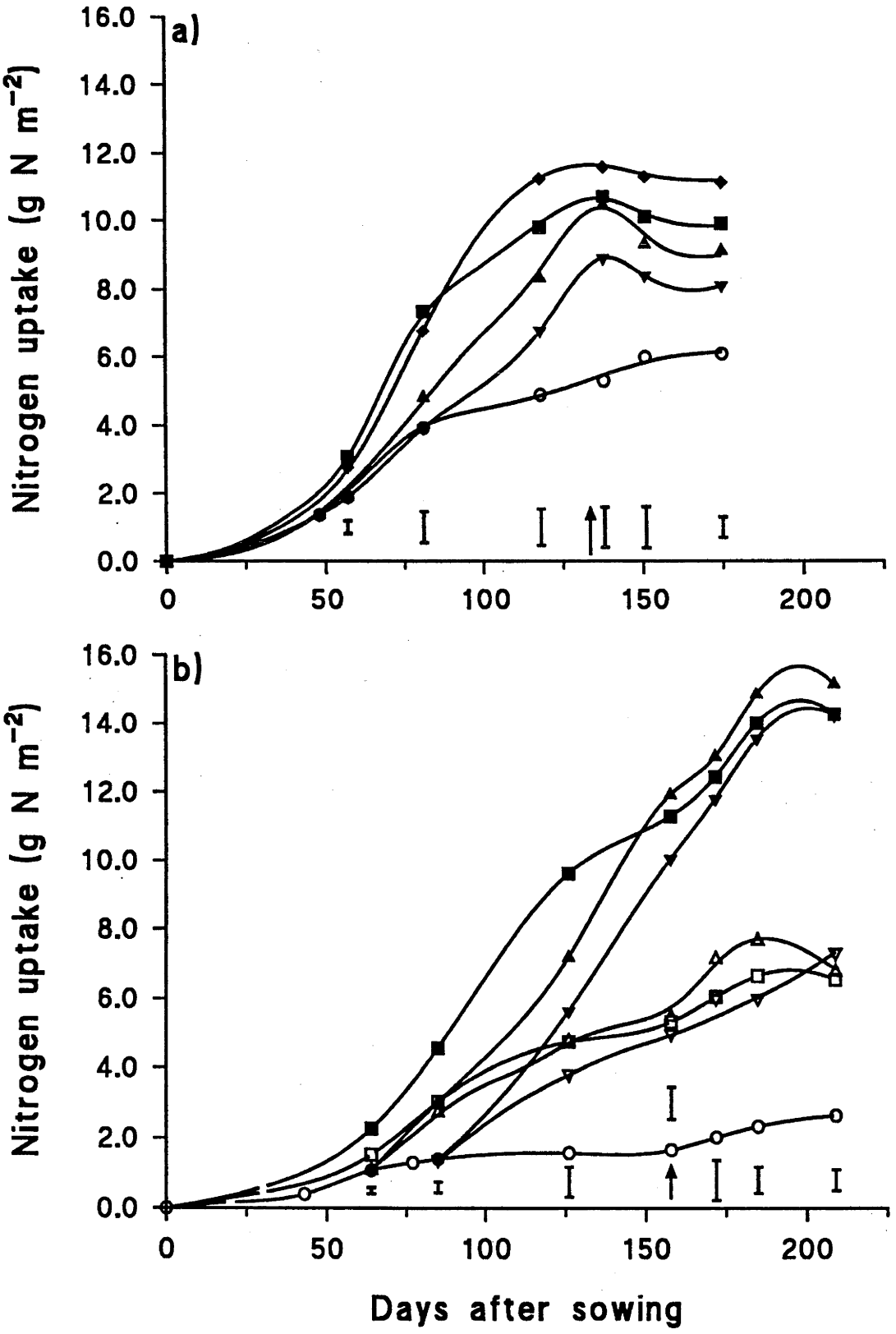
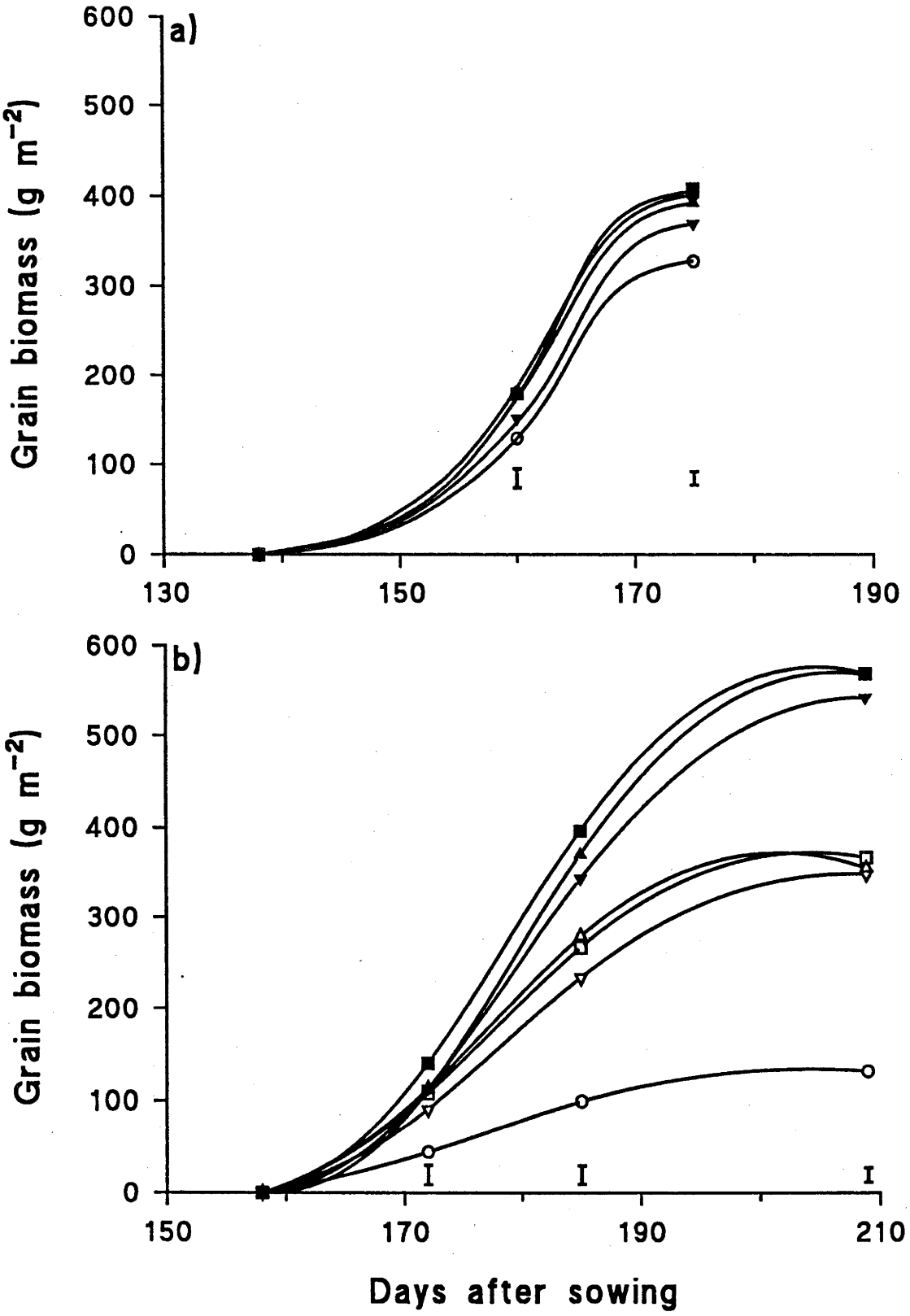


Figure 3.4 Grain biomass versus days after sowing for a) control (O), 80SW (■), 80MT (▲), 80LT (▼) and 200ST (◆) at BAR91 and b) control (O), 80SW, 240SW (□,■), 80MT, 240MT (Δ,▲), 80LT, 240LT (∇,▼) at BAR92. Curves were fitted by eye, bars indicate the l.s.d., (P = 0.05).



3.3.6 Post-anthesis transpiration efficiency

3.3.6.1 *BAR91*

During early grain filling at BAR91 soil evaporation constituted a small proportion of evapotranspiration reflecting the dry soil surface under the crops (Table 3.1). During late grain filling in which there was 21 mm of rain, however, more than half the rainfall was lost as soil evaporation for the control crop and that with late applied nitrogen. In the same period less than half the rain was lost to soil evaporation when at least some of the nitrogen was applied at DC10. Evapotranspiration was higher during late grain filling in all crops except the 80SW crop, but due to the greater soil evaporation, transpiration was similar or less than during early grain filling.

Table 3.1 Estimated evapotranspiration (ET), soil evaporation (E_S), Transpiration (T) and water use efficiency for biomass (W_{ET}^B) and transpiration efficiency for biomass (W_T^B) for periods during grain filling at BAR91.

Rate of N	Components of evapotranspiration (mm)			Efficiency (g m^{-2} mm^{-1})	
(kg N ha^{-1})	ET	E_S	T	W_{ET}^B	W_T^B
			DC69 to DC78		
control	26	2.5	23	4.4	4.9
80SW	32	1.6	30	1.2	1.2
80LT	26	1.9	24	2.1	2.2
200ST	28	1.5	27	2.3	2.5
(l.s.d. P=0.05)	n.s.	n.a.	n.s.	1.9	2.1
			DC78 to DC87		
control	33	12.6	20	2.1	3.5
80SW	29	9.1	20	2.8	4.2
80LT	35	10.7	25	3.0	4.4
200ST	34	8.6	25	2.8	3.8
(l.s.d. P=0.05)	n.s.	n.a.	n.s.	n.s.	n.s.
			DC69 to DC87		
control	59	15	44	3.0	4.1
80SW	61	11	50	2.2	2.7
80LT	61	13	49	2.6	3.3
200ST	62	10	52	2.6	3.1
(l.s.d. P=0.05)	n.s.	n.a.	n.s.	n.s.	n.s.

n.s. not significant, n.a. not applicable.

During early grain filling at BAR91 water use efficiency closely reflected transpiration efficiency due to the low soil evaporation. Water use efficiency and transpiration efficiency were greater for control than for crops supplied with additional nitrogen, due to less water stress as a result of lower evapotranspiration before DC65 (Chapter 2). Water use efficiency and transpiration efficiency were lowest for the 80SW crops which had used the most water before DC65.

During late grain filling water use efficiency and transpiration efficiency increased for crops which had received additional nitrogen but decreased for the control crop. This contrasting response was because the high nitrogen-status crops had retained green area which apparently resumed photosynthesis after the late rain, while the control crop whose photosynthetic tissues were senescing due to demand for nitrogen by the grain, was unable to respond to the rain and so had low water use efficiency and transpiration efficiency.

Water use efficiency over the whole grain filling period tended to be greater in the control crop than for nitrogen fertilised crops reflecting the lower water stress of control crops. Control crops also had the highest transpiration efficiency while the 80SW crops had the lowest.

3.3.6.1 *BAR92*

Components of evapotranspiration, water use efficiency for biomass and transpiration efficiency for biomass during grain filling at BAR92 are presented in Table 3.2. During early grain filling, evapotranspiration was lowest for the control crop and greatest at the high rate of nitrogen. In contrast soil evaporation decreased with increasing nitrogen and was greater with late than early application of nitrogen. Transpiration of the control crop was approximately half that of the 80 kg N ha^{-1} crops and only a quarter of the 240 kg N ha^{-1} crops. Water use efficiency reflected the increasing evapotranspiration and greater biomass production (Figure 3.1b) in response to higher rates of nitrogen application. Transpiration efficiency was similar across crops except for the 80LT crop which was significantly lower than other crops.

During mid grain filling, evapotranspiration was highest for the control crop and lowest at the high rate of nitrogen application. Late application of nitrogen tended to promote higher evapotranspiration than early application. Soil evaporation decreased with increasing nitrogen and was greater with late than early application. Transpiration was similar for crops during this period, except the 240SW crop which had low transpiration. Variation in water use efficiency reflected the variation in biomass production (Figure

Table 3.2 Estimated evapotranspiration (ET), soil evaporation (E_S), Transpiration (T) and water use efficiency for biomass (W_{ET}^B) and transpiration efficiency for biomass (W_T^B) for periods during grain filling at BAR92.

Rate of N	Components of evapotranspiration (mm)			Efficiency (g m^{-2} mm^{-1})	
(kgN m^{-2})	ET	E_S	T	W_{ET}^B	W_T^B
			DC65 to DC75		
control	39	20	19	1.9	4.1
80SW	44	9	35	3.6	4.5
240SW	63	3	60	4.4	4.6
80LT	56	11	44	2.5	3.1
240LT	64	6	59	4.3	4.8
(l.s.d. P=0.05)	11	n.a.	11	1.6	n.s.
			DC75 to DC83		
control	52	23	29	0.9	1.8
80SW	43	11	31	3.9	5.9
240SW	34	5	29	4.5	5.4
80LT	49	14	35	1.9	2.5
240LT	42	8	35	4.8	5.9
(l.s.d. P=0.05)	11	n.a.	n.s.	1.9	3.0
			DC83 to DC87		
control	60	34	26	-0.1	-0.4
80SW	52	20	32	-1.1	-1.5
240SW	51	12	39	-1.2	-1.6
80LT	65	24	41	0.4	0.7
240LT	57	15	42	-0.1	0.1
(l.s.d. P=0.05)	n.s.	n.a.	n.s.	n.s.	n.s.
			DC65 to DC87		
control	151	77	74	0.7	1.4
80SW	138	40	97	2.0	3.0
240SW	148	21	127	2.6	3.1
80LT	169	49	120	1.6	2.2
240LT	164	29	135	2.9	3.5
(l.s.d. P=0.05)	20	n.a.	20	1.1	n.s.

n.s. not significant, n.a. not applicable.

3.1b). Greater water use efficiency was positively associated with increased nitrogen fertilisation and negatively associated with evapotranspiration. Transpiration efficiency increased with increasing rates of nitrogen fertiliser except for the 80LT crop which was similar to the control. As at BAR91, water use efficiency and transpiration efficiency of crops supplied with additional nitrogen tended to increase during mid grain filling, while those of the control crop decreased.

During late grain filling, evapotranspiration tended to be lowest for crops supplied with nitrogen at DC10. Soil evaporation followed the same pattern of early and mid grain filling, with the highest rate for the control crop and a trend for higher rates with late nitrogen application than with early application. Transpiration tended to increase with increasing rates of fertiliser and delayed application. Water use efficiency and transpiration efficiency were mostly negative due to the continued use of water and apparent decrease in total biomass for most treatments during late grain filling (Figure 3.1b). Water use efficiency and transpiration efficiency reflected the decreases in biomass and tended to be greater when nitrogen was applied late.

3.3.7 Post-anthesis biomass and carbon dynamics of ^{14}C labelled crops

The biomass and ^{14}C content of leaves are shown in Figure 3.5 for crops of low and high nitrogen status at BAR92. The decreases in biomass and ^{14}C content between DC65 and maturity show similar patterns for both the low and high nitrogen crops. The decrease in ^{14}C from the flag, combined second and third leaves and the rest of the leaves mirrored the decrease in leaf biomass at low nitrogen application. At high nitrogen application the decrease in ^{14}C of the flag and combined second and third leaves was faster than the decrease in biomass of these leaf layers during mid grain filling but had a similar pattern during early and late grain filling periods.

The decrease in stem WSC (Figure 3.6b) agreed well with the decrease in stem biomass (Figure 3.6a) at low and high nitrogen. The only exception was during early grain filling when stems increased in biomass to a greater extent than the extra accumulation of WSC. As discussed in Chapter 2, this discrepancy was thought to be due to stem elongation and lignification. The decrease in the ^{14}C content of stem WSC (Figure 3.6b) agreed well with the decrease in stem ^{14}C (Figure 3.6a) at low and high nitrogen. There was good agreement between the change in stem WSC and the decrease in stem WSC ^{14}C during mid grain filling but not in early or late grain filling (Figure 3.6b). During early grain filling, stem WSC increased in contrast with the downward trend in stem WSC ^{14}C. During late grain filling stem WSC reserves fell proportionally more than ^{14}C of stem WSC.

Figure 3.5 Leaf biomass (open symbols, solid line) and leaf ^{14}C (closed symbols, dashed line) versus days after sowing at BAR92 for flag leaves (O,●), penultimate plus previous leaf (□,■), rest of leaves (Δ,▲) and total leaf (∇,▼) for a) 80SW and b) 240SW. Curves were fitted by eye, bars indicate the s.e.m. Anthesis and maturity harvests at 158 and 209 days after sowing respectively.

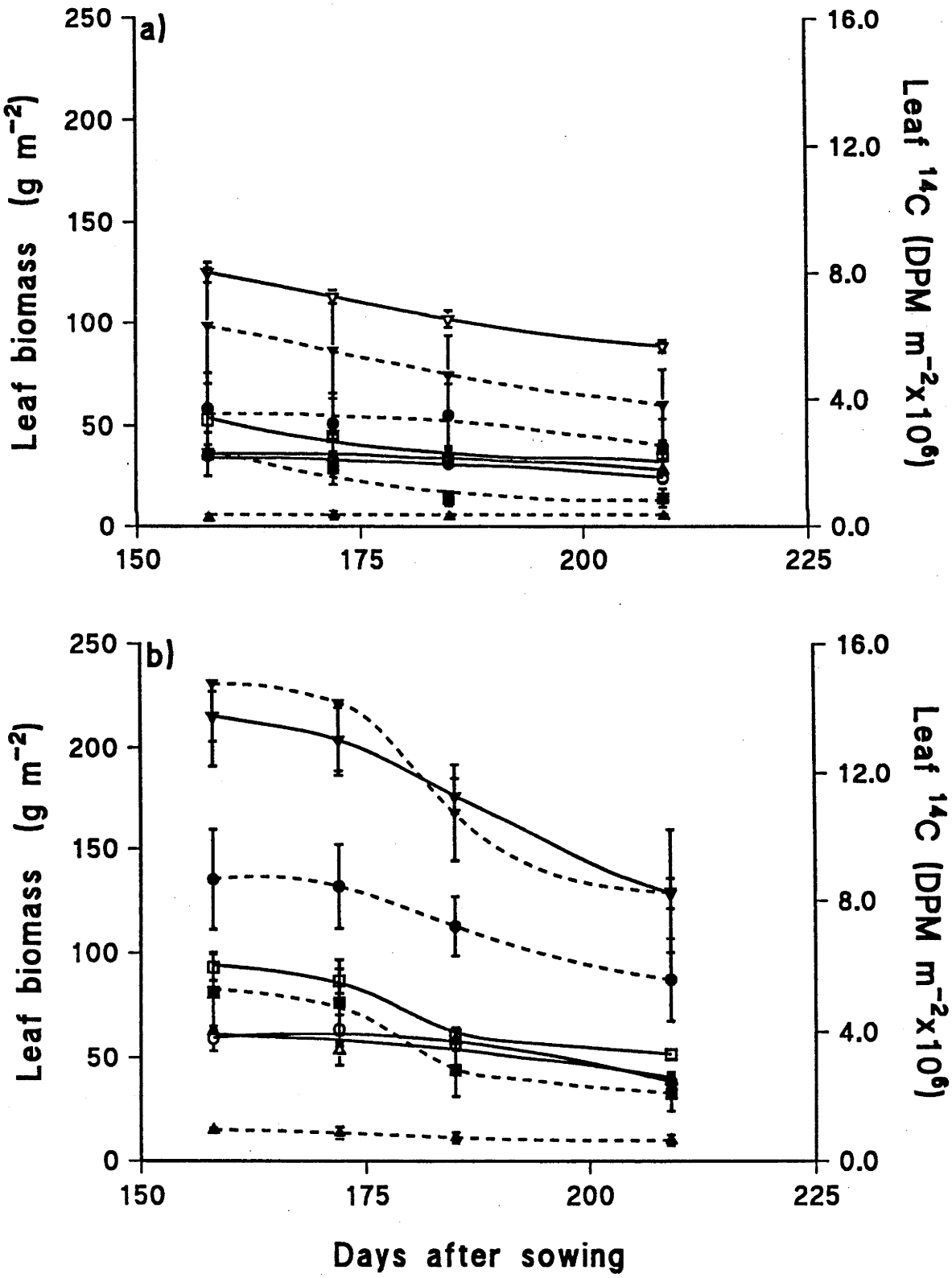


Figure 3.6 a) Stem biomass (open symbols, solid line) and stem ^{14}C (closed symbols, dashed line) and b) stem WSC reserves (open symbols, solid line) and stem WSC ^{14}C DPM (closed symbols, dashed line) versus days after sowing at BAR92 for 80SW (O,●),and 240SW (□,■). Curves were fitted by eye, bars indicate the s.e.m. Anthesis and maturity harvests as for Figure 3.5.

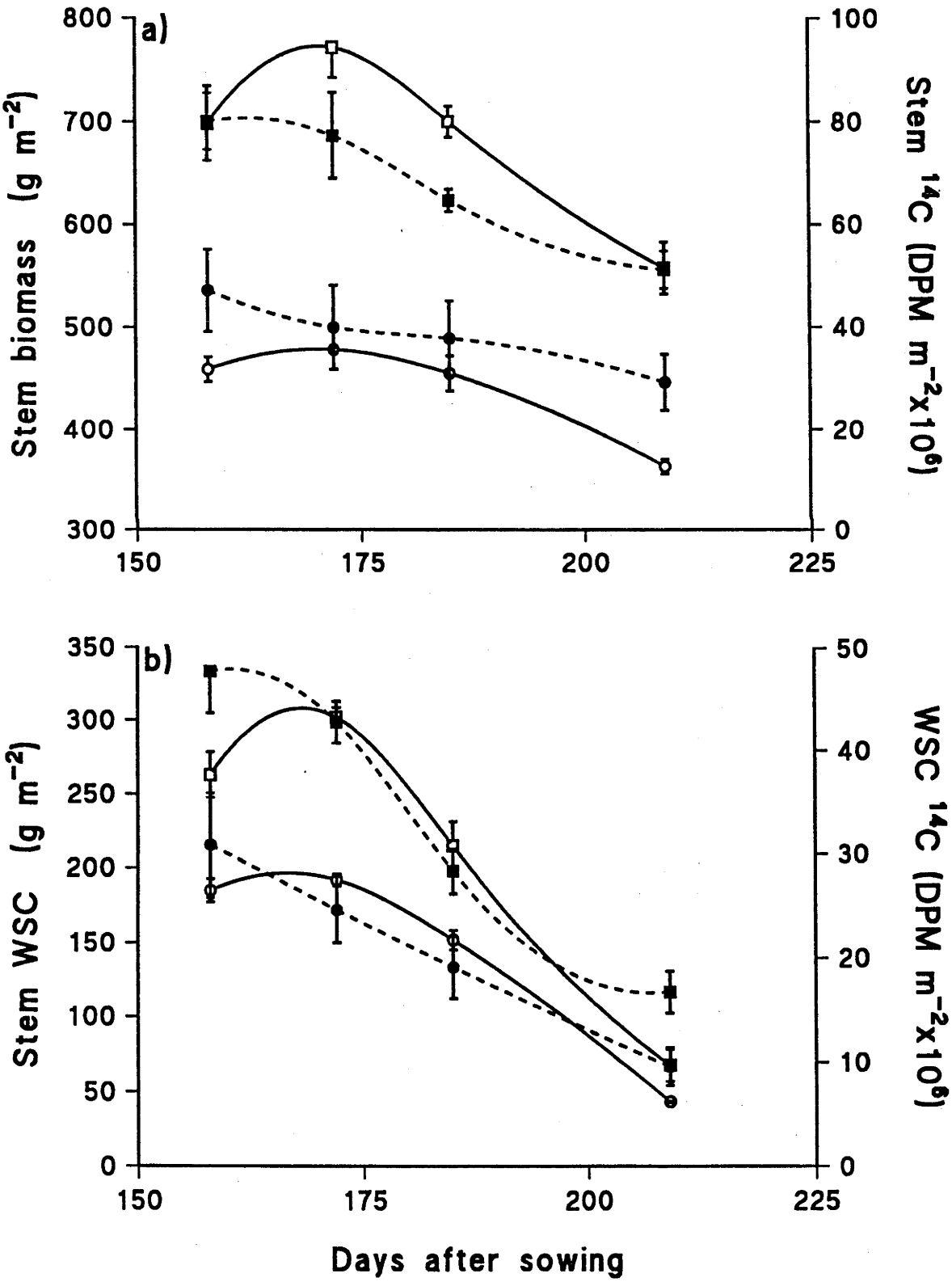
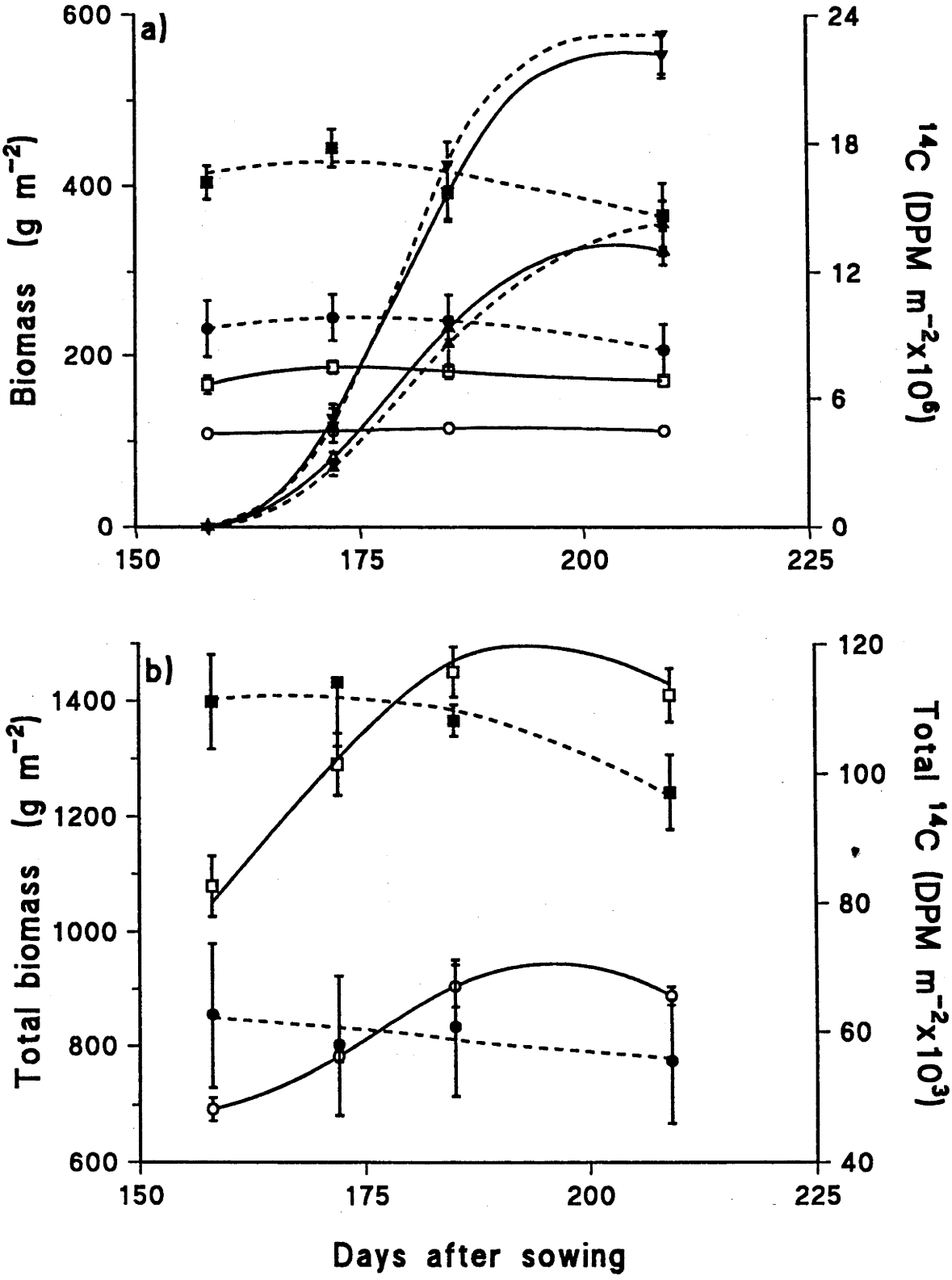


Figure 3.7 a) Biomass of non-grain spikes (O,□) and grain (Δ,∇) (solid lines) and ^{14}C content of non-grain spikes (●,■) and grain (▲,▼) (dashed lines) for 80SW and 240SW respectively and b) total above-ground biomass (open symbols, solid line) and ^{14}C DPM of total above-ground biomass (closed symbols, dashed line) versus days after sowing at BAR92 for 80SW (O,●),and 240SW (□,■). Curves were fitted by eye, bars indicate the s.e.m. Anthesis and maturity harvests as for Figure 3.5.



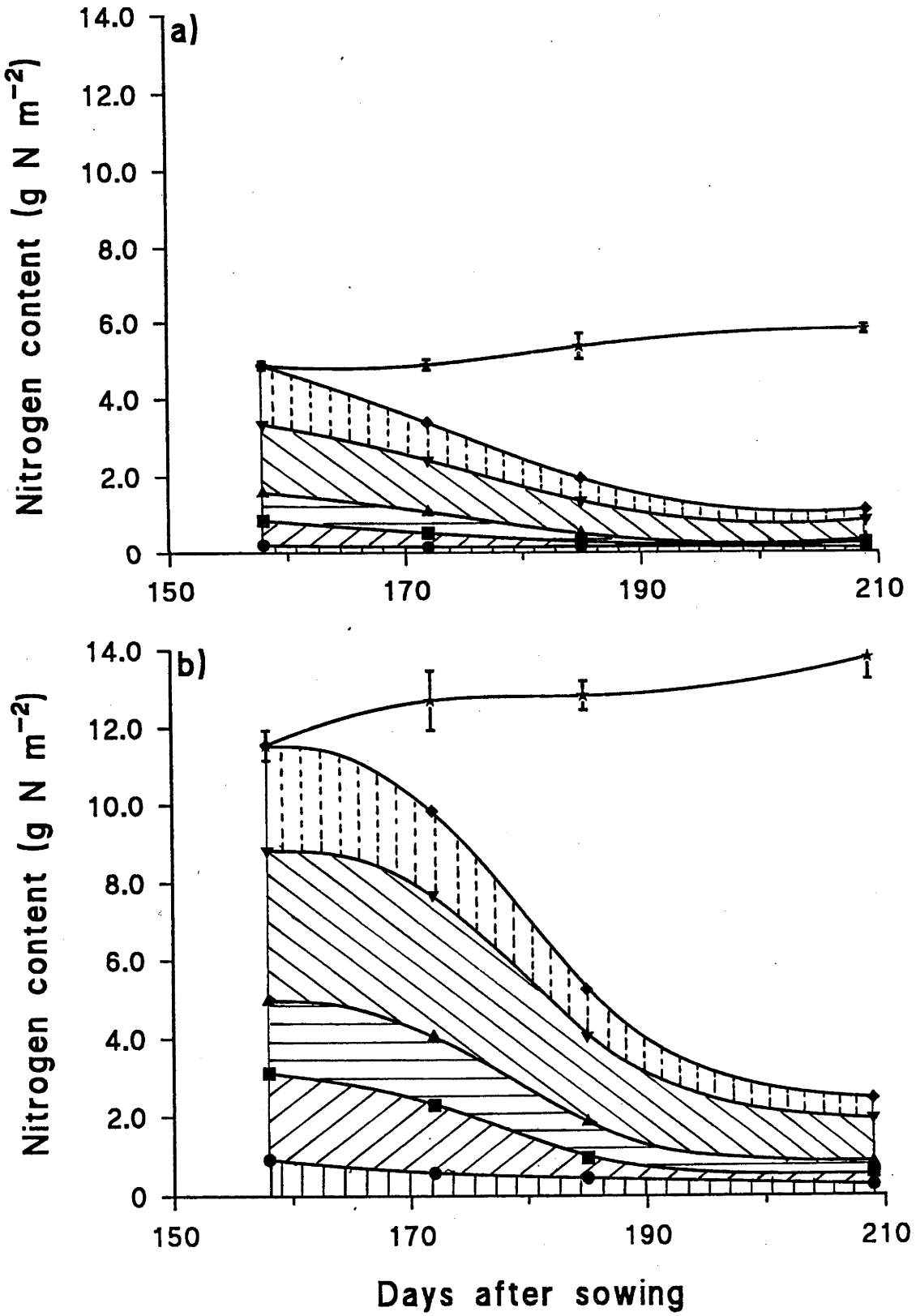
The increase in ^{14}C of the developing grain mirrored the grain growth of low and high nitrogen crops (Figure 3.7a) and shows that retranslocation continued over the whole grain filling period. There was significantly less grain biomass and ^{14}C throughout grain filling for the low nitrogen crop. Non-grain spikes did not change in biomass from DC65 to maturity while there appeared to be a slight decrease in ^{14}C, especially at high nitrogen. There was a net increase in the above-ground biomass for both crops between DC65 and maturity while the total crop decreased by approximately 10% in each crop over the same period (Figure 3.7b).

3.3.8 Post-anthesis nitrogen dynamics of ^{14}C labelled crops

The cumulative nitrogen uptake of above-ground plant parts between DC65 and maturity for low and high nitrogen are presented in Figures 3.8a and 3.8b respectively. At DC65 leaves contained 32% of the total nitrogen content of the low nitrogen crop while 43% was contained in the leaves of the high nitrogen crop. At low nitrogen flag leaves contained more nitrogen than other leaf layers while at high nitrogen the penultimate plus previous leaf contained the most nitrogen. Retranslocation of nitrogen resulted in a linear decline in nitrogen content of the non-grain plant parts of the low nitrogen crops during early and mid grain filling followed by decreased remobilisation in late grain filling. In contrast, at high nitrogen 40% of the grain nitrogen present at the end of early grain filling came from soil uptake resulting in a higher concentration of nitrogen remaining in the non-grain plant parts. During mid grain filling there was a rapid decline in non-grain nitrogen, coinciding with the linear growth phase of the grain (Figure 3.7a).

The retranslocation of protein during the rapid phase of grain growth explains the difference between crops in rate of decrease in ^{14}C from the leaves (Figures 3.5a, b). At maturity leaves made up only 6% of the crop nitrogen content, most of the DC65 nitrogen having been retranslocated to the grain. Both crops took up nitrogen between DC65 and maturity. Nitrogen harvest index at maturity was 0.81 and 0.82 for the low and high nitrogen crops respectively.

Figure 3.8 Cumulative nitrogen content of plant parts versus days after sowing at BAR92 for rest of leaves (vertical lines, ●), penultimate plus previous leaf (left to right upward diagonal lines, ■), flag leaves (horizontal lines, ▲), stem (left to right downward diagonal lines, ▼), non-grain spikes (dashed lines, ◆) and grain (open, ★) for a) 80SW and b) 240SW. Curves were fitted by eye, bars indicate the s.e.m. Anthesis and maturity harvests as for Figure 3.5.



3.4 DISCUSSION

3.4.1 Post-anthesis biomass dynamics and evapotranspiration

At BAR91, post-anthesis biomass production of crops receiving additional nitrogen was severely curtailed by the lack of soil water reserves and the absence of rain during early grain filling. The control crop grew more rapidly than fertilised crops after anthesis because of the additional 19 mm of soil water conserved before DC65. The factor most limiting its growth was therefore nitrogen deficiency rather than water stress. This assertion is corroborated by 21 mm less water use at maturity by the control crop compared to nitrogen fertilised crops. Post-anthesis water use for the control crop was similar to nitrogen fertilised crops, but occurred at a lower level of water stress and hence was likely to be used more efficiently than for the more water stressed nitrogen fertilised crops . Grain yield was less than for crops supplied with additional nitrogen, however, due to the lower kernel number. Crops supplied with additional nitrogen were able to capitalise on the 21 mm of rain during late grain filling through stimulation of photosynthesis due to their retention of green area. This benefit was expressed as extra grain yield, evidenced by the similar harvest indices between BAR91 and either BAR92 or GES92 in a wet season (Chapter 2, Table 2.9).

At BAR92 there was no visible water stress at any time during grain filling. The rate at which biomass was produced during early and mid grain filling was related to the nitrogen status of the crop (Figure 3.1b). Despite there being differences in biomass for a given level of nitrogen at DC65, post-anthesis biomass production was similar for the different times of fertiliser application. During late grain filling most crops lost biomass despite the continuation of grain growth. This decrease in biomass was associated with a rapid decrease in WSC of the non-grain biomass and may have been due to wasteful respiration (Schnyder 1993, and see discussion below).

The decrease in soil fertility of the site at Barellan between 1991 and 1992 is illustrated by the 69% and 44% lower nitrogen uptake of control and 80 kg N ha^{-1} crops respectively at DC65 for BAR92 compared to BAR91. The low plant establishment at BAR92 was also partly responsible for the low nitrogen uptake. The larger response in nitrogen uptake of 80 kg N ha^{-1} over control crops at DC65 at BAR92 than at BAR91 suggests that the low plant population did not limit nitrogen uptake.

Due to the favourable water status at BAR92 nitrogen uptake continued after DC65 in contrast to the loss of nitrogen at BAR91. The additional nitrogen and favourable water status allowed the high-nitrogen crops at BAR92 to maintain their photosynthetic area longer than at BAR91. The prolonged supply of current assimilate led to the

accumulation of WSC at the high rate of nitrogen during early grain filling (Chapter 2, Figure 2.1b; Figure 3.6b). Late fertiliser application resulted in greater post-anthesis growth than early application at both BAR91 and BAR92. The reason was probably due to greater photosynthetic capacity of biomass with a higher nitrogen concentration (Figure 3.9). Nitrogen concentration in the non-grain biomass was used as a surrogate measure of the post-anthesis photosynthetic capability since it has been shown that a good correlation exists between leaf area duration and nitrogen concentration of the biomass (Ellen and Spiertz 1980). The data from BAR92 show that higher rates of nitrogen and later application of nitrogen maintain the nitrogen concentration and hence the photosynthetic area for longer during grain filling if there is sufficient water supply. The transpiration rates of selected crops during the three periods of grain filling at BAR92 (Table 3.3) support the conclusion of greater photosynthetic area duration with additional nitrogen fertilisation and delayed application.

Figure 3.10 presents the relationship between nitrogen concentration of the biomass at DC65 and the post-anthesis change in biomass for the experiments BAR91, BAR92 and GES92. The differences between curves illustrate firstly, the dependence of post-anthesis growth and hence grain yield on water supply (Passioura 1977). Secondly, it shows the co-limitation of growth on nitrogen supply because at BAR92 and GES92 where there was an adequate post-anthesis water supply there was a positive relationship between growth and DC65 nitrogen concentration. In contrast at BAR91, where there was water stress during grain filling, there was reduced growth with increasing nitrogen concentration.

Table 3.3 Estimated transpiration rate of selected treatments during the three post-anthesis measurement periods and from anthesis to maturity at BAR92.

Rate of N	Transpiration rate (mm day^{-1})			
(kgN m^{-2})	DC65 - DC75	DC75 - DC83	DC83 - DC87	DC65 - DC87
control	1.4	2.2	1.2	1.5
80SW	2.5	2.4	1.4	1.9
240SW	4.1	2.4	1.7	2.5
80LT	3.2	2.7	1.8	2.4
240LT	4.2	2.7	1.8	2.7
(l.s.d. P=0.05)	0.9	n.s.	n.s.	0.5

n.s. not significant.

Figure 3.9 Nitrogen concentration of the non-grain biomass from DC40 to DC87 at BAR92 for control (O), 80SW, 240SW (□,■), 80MT, 240MT (Δ,▲), 80LT, 240LT (∇,▼). Curves were fitted by eye, arrow denotes mean anthesis date, bars indicate the l.s.d., (P = 0.05).

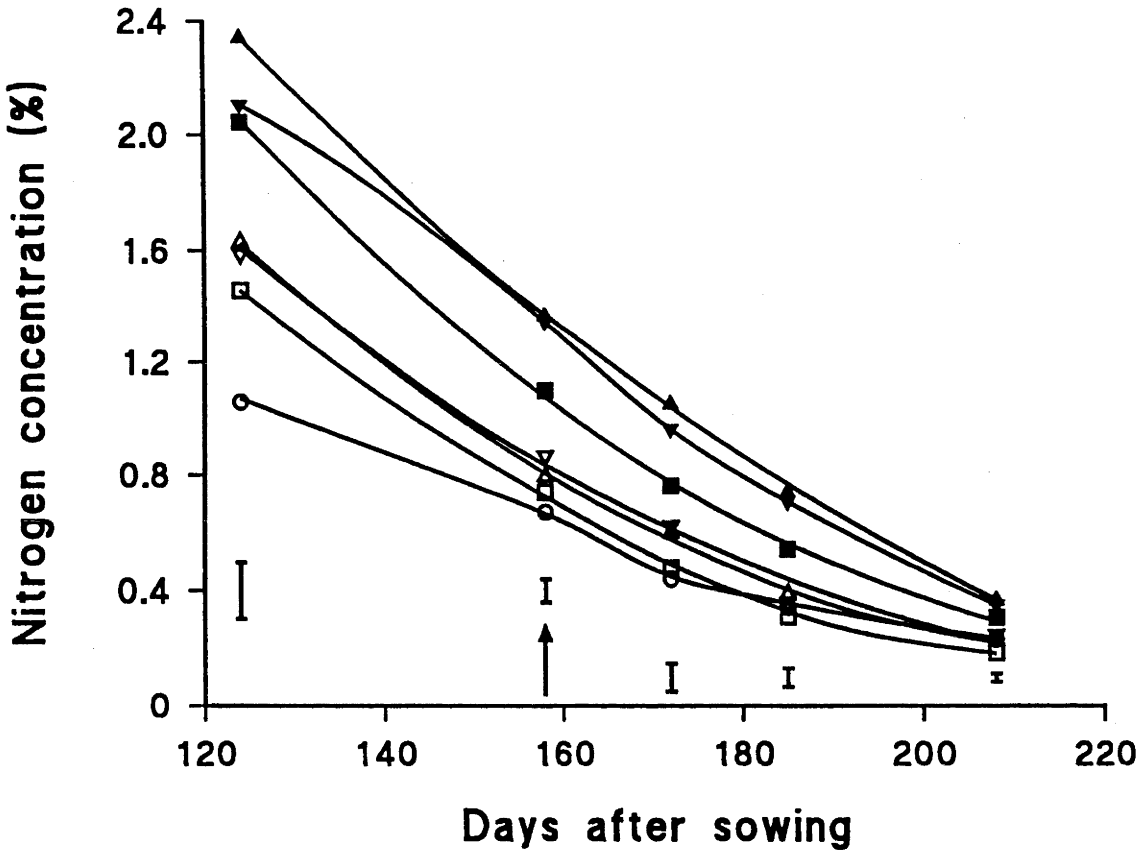
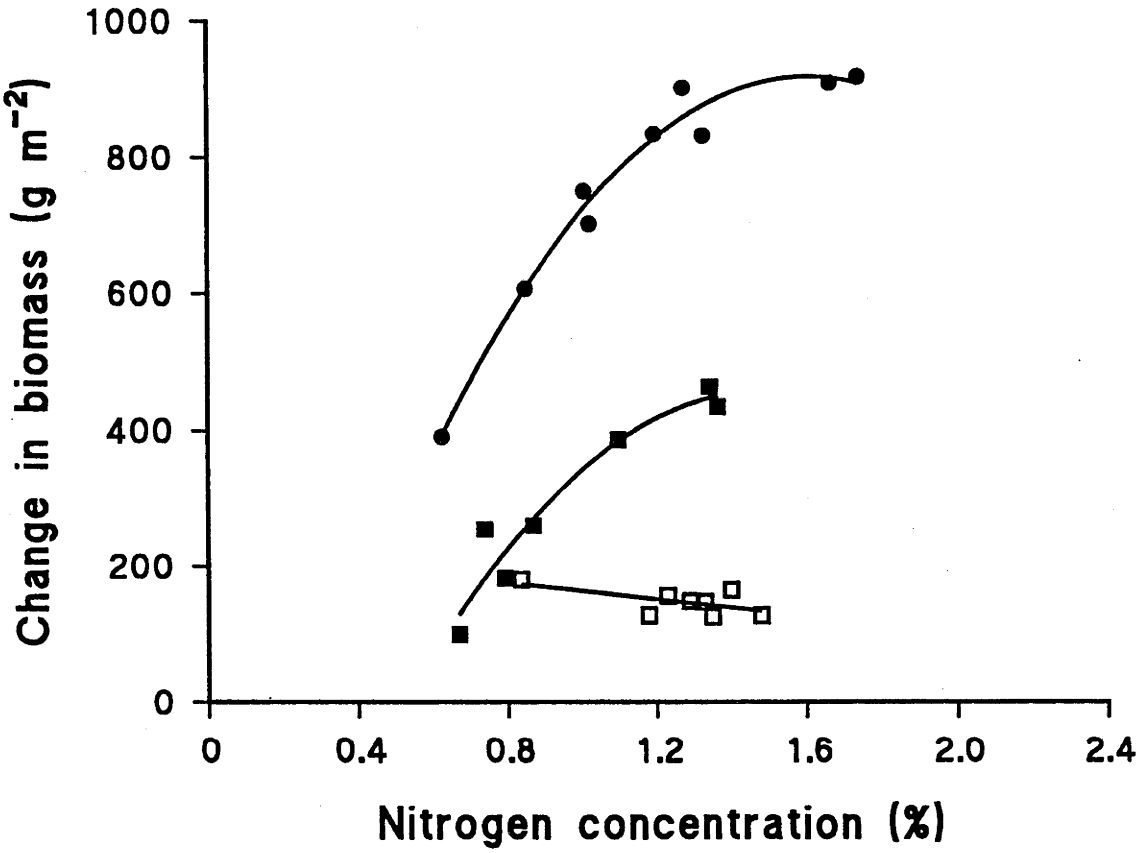


Figure 3.10 Relationship between nitrogen concentration of the biomass at anthesis with the change in total biomass from anthesis to maturity at GES92 (●, $y = -543x^2 + 1751x - 491$), BAR91 (□, $y = -61x + 224$) and BAR92 (■, $y = -501x^2 + 1482x - 639$). Curves are fitted regressions.



3.4.2 Determination of kernel number

3.4.2.1 *Anthesis biomass*

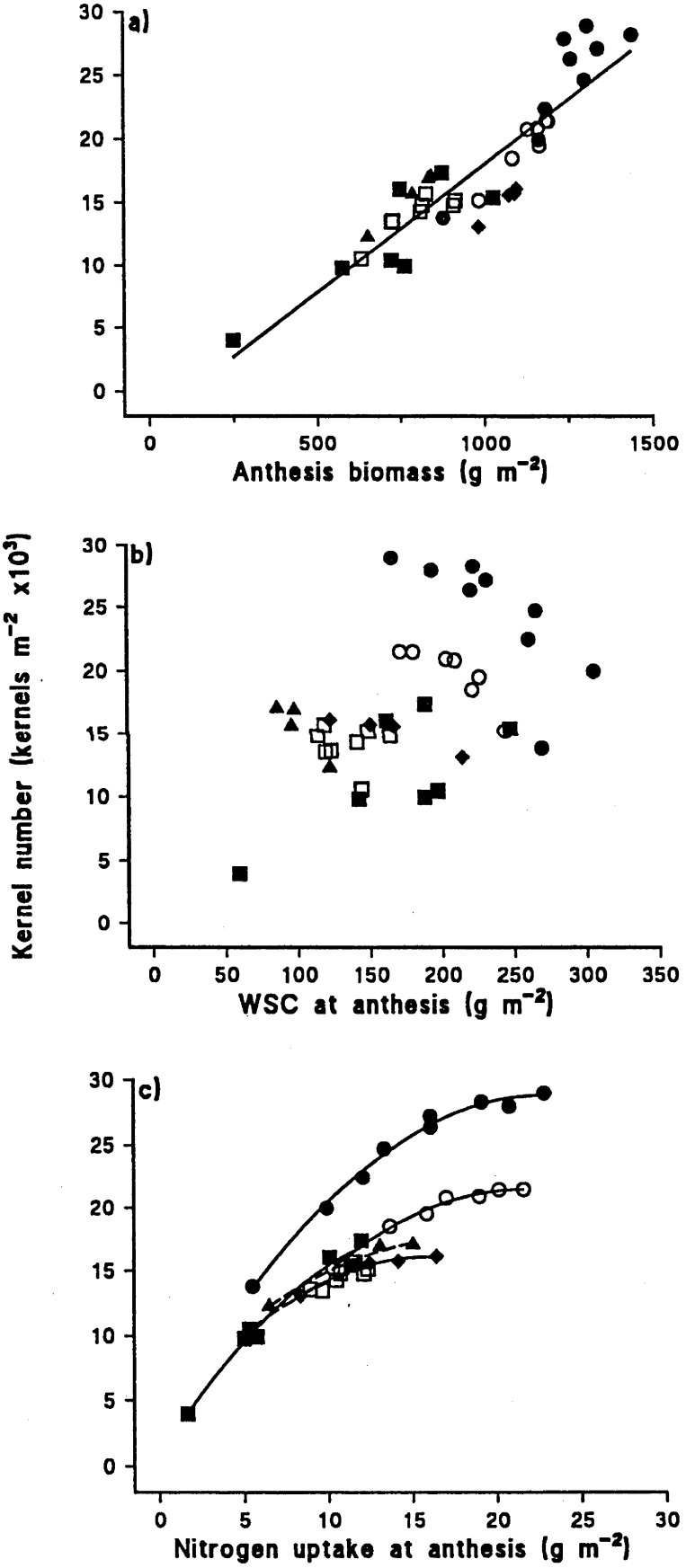
Consideration of the data presented in Figure 3.10 leads one to speculate that given more favourable post-anthesis water relations, the cluster of crops supplied with additional nitrogen from BAR91 could have achieved similar yield levels to the 240 kg N ha^{-1} crops from BAR92 with which they share similar nitrogen concentration. A similar nitrogen concentration of a lower DC65 biomass would, however, not result in the same yield as shown by the lower post-anthesis growth of crops at BAR92 compared to crops of similar nitrogen concentration at GES92. However, the control crop at BAR91 had a similar nitrogen concentration, biomass and nitrogen uptake to the 80 kg N ha^{-1} crops at BAR92 and achieved a similar grain yield. The DC65 biomass of 80 kg N ha^{-1} crops at BAR91 (728 - 910 gm^{-2}) was similar in range to the 240 kg N ha^{-1} crops at BAR92 (749 - 1029 gm^{-2}) which could, therefore, be expected to have a similar yield potential due to the strong positive relationship between kernel number (potential yield) and DC65 biomass (Fischer 1979, Fischer 1985, Stapper 1984). Indeed the kernel number at maturity of 80 kg N ha^{-1} crops at BAR91 (13500 - 14800 m^{-2}) were similar to the 240 kg N ha^{-1} crops at BAR92 (15400 - 17400 m^{-2}) and reflecting the slightly lower DC65 biomass at BAR91. However, delaying the application of 80 kg N ha^{-1} from DC10 to DC30 at BAR91 resulted in a fall in kernel number of only 9% compared to a 20% decrease in DC65 biomass. The same delay in nitrogen application of 240 kg N ha^{-1} at BAR92 increased kernel number by 4% while DC65 biomass fell by 27%. Clearly factors other than anthesis biomass affect the determination of kernel number.

A plot of kernel number (KNO) and DC65 biomass (DM_a) for the six field experiments presented in this thesis (Figure 3.11a) provides a similarly robust relationship to that of Fischer (1979) and Stapper (1984). The relationship is described by the equation:

$$KNO = 20DM_a - 2433 \qquad r^2 = 0.83 \qquad (P < 0.01; n=39) \qquad (3.1)$$

The gradient of 20 kernels m^{-2} per g m^{-2} increase in DC65 biomass is similar to the value of 22 kernels m^{-2} per g m^{-2} increase in DC65 biomass reported for a range of small-seeded wheat cultivars (Stapper 1984). The relationship is strong when all the sites are included but within most sites there is considerable variation. As described above, there is considerable variation in DC65 biomass at BAR92 which gives rise to similar kernel number. In addition, however, there is considerable variation in kernel number (10400 - 16100 m^{-2}) at a given DC65 biomass (723 - 749 gm^{-2}) between 80SW and 240LT crops, respectively.

Figure 3.11 Relationship between kernel number and a) anthesis biomass, b) anthesis WSC reserves and c) nitrogen uptake of anthesis biomass for the three sites of Chapters 2 and 3 GES92 (●), BAR91 (□), BAR92 (■) and the three sites of Chapter 4 GES91 (O), PUC91 (▲) and WAG91 (◆). Curves are fitted regressions.



Many workers have shown that assimilate supply just prior to and during anthesis is a major determinant of kernel number in wheat (Stockman *et al.* 1980; Kemp and Whingwiri 1980; Judel and Mengel 1982; Fischer 1985). In this thesis it is proposed that the amount of WSC present in the biomass at DC65 can be used as a measure of the assimilate supply since the deposition of fructans in wheat stems was found to occur when the concentration of mono- and disaccharides exceeded 130 g kg^{-1} of crop dry weight (Kühbauch and Thome 1989). This assertion is in agreement with the conclusion that WSC storage is not competitive with sink demand (eg. spike growth, peduncle elongation or grain growth) but accommodates excess photosynthate when supply exceeds demand (Schnyder 1993). Therefore, as assimilate supply increases so the deposition of WSC increases. Figure 3.11b shows that there was no correlation between kernel number and WSC at DC65 across the six sites studied. In fact four of the sites show distinct negative correlations between kernel number and WSC at DC65. Only at BAR92 was there a positive relationship, but even these data include considerable variation in WSC at DC65 for similar kernel numbers at a given level of nitrogen fertilisation. Stapper and Fischer (1990b) also found no correlation between kernel number and WSC at DC65 across a range of wheat cultivars.

The relationship between kernel number and nitrogen content of the biomass at DC65 (Figure 3.11c) is also weak for all experiments, with the data from GES92 apparently diverging from those at the other five sites. However, there are close relationships between kernel number and nitrogen content for the individual experiments. The distinct clustering of data for BAR92 is associated with the three rates of nitrogen, and points within a cluster represent the different times of nitrogen application.

3.4.2.2 *Anthesis spike biomass*

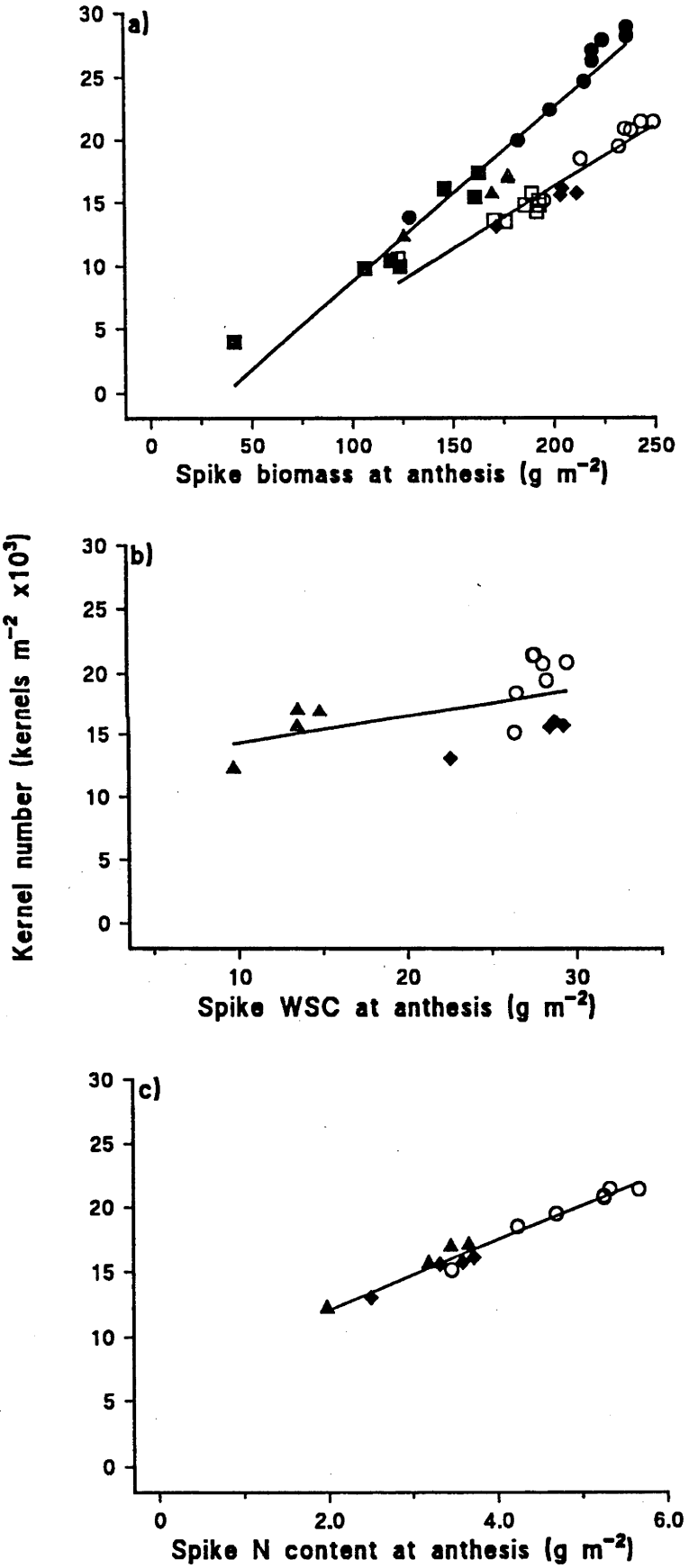
It is likely that the major determinant of kernel number is more directly related to attributes of the spike itself. Relationships between kernel number and spike biomass at DC65, spike WSC at DC65 and nitrogen in spikes at DC65 are presented in Figures 3.12a, 3.12b and 3.12c respectively. Spike biomass (SDM_a) has previously been shown (Thorne and Wood 1987) to be closely related to kernel number. In Figure 3.12a it is clear that the data for BAR92 and GES92 differ from those at the other experiments and two distinct relationships apply:

$$KNO = 138\ SDM_a - 5100 \qquad r^2 = 0.95 \qquad (P < 0.01;\ n=20) \qquad (3.2)$$

$$KNO = 99\ SDM_a - 3510 \qquad r^2 = 0.92 \qquad (P < 0.01;\ n=19) \qquad (3.3)$$

Reasons for the divergence are unclear. It is unlikely that equation 3.3 describes the effects of increased water stress as might be inferred by the lower gradient. It is unlikely

Figure 3.12 Relationship between kernel number and a) anthesis spike biomass at all sites from Figure 3.11 and b) anthesis spike WSC and c) anthesis spike nitrogen uptake at the three sites from Chapter 4. Curves are fitted regressions, symbols as for Figure 3.11.



because the water stressed site at PUC91 fits more appropriately into equation 3.2 and the well watered site at GES91 fits more appropriately into equation 3.3. In addition, crops which received increasing rates of nitrogen would be more stressed than control crops at DC65 at a dry site and yet there is no divergence from either line within a site. The reason for the divergence of equation 3.3 from equation 3.2 is, therefore, not likely to be due to reduced water potential of the crop affecting ovule or anther fertility, for example, but to reduced nitrogen uptake and transport to the spike prior to DC65 thereby limiting the number of kernels set despite an adequate number of fertile spikelets. All of the sites explained by equation 3.3 had dry periods for up to 4 weeks prior to DC65 which would have led to reduced nitrogen uptake due to the dry soil surface (Clarke *et al.* 1990). Data from the dry site at PUC91 may fit more appropriately into equation 3.2 due to the higher nitrogen concentration of the tissues as a result of the later sowing.

Spike WSC content had a positive correlation with kernel number in each experiment, in agreement with prior work (Stockman *et al.* 1980; Kemp and Whingwiri 1980; Judel and Mengel 1982; Fischer 1985). However, the relationship for all sites was weak but significant at P = 0.10. Work by Hendrix *et al.* (1986) found that 80% of the WSC present in the spikes at anthesis consisted of mono- and disaccharides (ie. not storage fructans) and that this level was relatively stable. Spike WSC concentration at anthesis may, therefore, be used as a measure of the assimilate supply to the spike, and as such bears no relationship with kernel number at PUC91 (77 -83 g kg^{-1}) and WAG91 (131 - 140 g kg^{-1}) but was negatively correlated with kernel number at GES91 (135 - 110 g kg^{-1} $r^2 = 0.74$; n = 7).

Figure 3.12c shows that there is a strong association between kernel number and nitrogen content of the spikes at DC65. It is possible that nitrogen content of the spikes at DC65 is a major determinant of kernel number in wheat. The relationships between kernel number and nitrogen uptake of the biomass at DC65 (Figure 3.11c) are non-linear due to a decrease in the proportion of total nitrogen contained in the spikes as nitrogen status increases (See Chapter 4). The relationship between kernel number and spike nitrogen content at DC65 (SNC_a) is described by the equation:

$$KNO = 2680\ SNC_a + 6770 \quad r^2 = 0.97 \qquad (P < 0.01;\ n=15) \qquad (3.4)$$

Nitrogen concentration of spikes was only determined separately for the three experiments from Chapter 4. The three sites presented include two varieties, range in sowing date by 4 weeks and vary in pre- and post-anthesis water stress such that grain yield and kernel weight range from 284 to 798 gm^{-2} and 18 to 37 mg respectively.

While the important effects that radiation, temperature and pre-anthesis water stress have in determining kernel number is recognised, it appears that the importance of nitrogen content of the spikes at DC65 has been neglected. It has been shown in this data set that assimilate supply was not positively correlated to kernel number. This was presumably due to deficiencies in supply of current photosynthate, if they occur, being bolstered by remobilisation of WSC reserves to buffer sinks against reduced supply (Fischer 1985, Bell and Incoll 1990) The present data implies that the wheat crop sets the kernel number at DC65 according to a minimal nitrogen allocation per kernel. From equation 3.4 it can be derived that for these data the value is 0.37 mg of spike nitrogen per kernel. Any additional nitrogen in the grain at maturity is dependent on retranslocation from the non-grain biomass and nitrogen uptake.

3.4.3 Interaction of time of nitrogen application with water supply and its effect on yield

In the terminal drought at BAR91, the late (DC30) application of nitrogen resulted in lower nitrogen uptake than the DC10 application due to a dry soil surface (Clarke *et al.* 1990). At BAR91 there was more than twice the level of mineral nitrogen remaining in the top 0.10m of the soil profile at maturity for the 80LT crop compared to the 80SW crop (data not shown). There was lower nitrogen uptake, reduced tiller survival and limited compensatory growth of the crop receiving late application of nitrogen. At DC65 there was reduced biomass and kernel set in crops fertilised at DC30 compared with those fertilised at DC10. The late fertilised crops also lost more water through soil evaporation than those fertilised at DC10, although there was little difference in evapotranspiration. The increased growth stimulated by the nitrogen uptake for the 80LT crop led to greater partitioning into structural biomass (Spiertz and Ellen 1978) and possible costs associated with nitrogen uptake and assimilation (Bänziger *et al.* 1994) which resulted in more reduced WSC storage at DC65 than for 80SW crops. Therefore grain yield was less when nitrogen was applied at DC30 than at DC10 due to the lack of sufficient compensatory post-anthesis assimilation.

In the wet post-anthesis conditions at GES92 the late (DC30) application of nitrogen resulted in similar or superior grain yields to those of crops fertilised at sowing. These results resemble those in which late application of nitrogen resulted in similar (Spiertz and van de Haar 1978) or superior (Ellen and Spiertz 1980) grain yields to those of crops fertilised at DC10. Greater yield response to DC30 application of nitrogen than DC10 application at GES92 was positively correlated with greater nitrogen uptake by DC65 but negatively correlated to WSC. Lower nitrogen uptake when nitrogen was applied at DC10 may have been due to ammonia volatilisation, or losses due to denitrification and leaching in wet conditions. Greater post-anthesis assimilation was

associated with higher nitrogen concentration at DC65 which has been shown to positively correlate with leaf area duration (Ellen and Spiertz 1980).

In circumstances of severe nitrogen deficiency in the wet season at BAR92 there was a reduction in yield potential due to tiller abortion prior to nitrogen application at DC30. However, there was compensation through more kernels per spike and/or late tillering similar to results obtained under irrigated conditions (Fischer 1993). Indeed, Stapper and Fischer (1990c) did not recommend optimum combinations of yield components for irrigated wheat due to compensation between yield components which resulted in similar yields across a range of genotypes. Despite having a lower biomass and nitrogen uptake at DC65, the 80LT crop set a similar kernel number at DC65 to the 80SW crop. Although the 80LT crop had a similar number of spikes at maturity as the DC10 application of nitrogen almost a third of its spikes developed after the fertiliser application. Late fertiliser application was again negatively correlated to WSC reserves at DC65. Crops with delayed fertiliser application were therefore able to achieve similar grain yield as those fertilised early through greater post-anthesis assimilation, as evidenced by higher nitrogen concentration throughout grain filling and greater post-anthesis transpiration.

3.4.4 Post-anthesis ^{14}carbon and nitrogen dynamics of low and high nitrogen crops

For the low nitrogen crop at BAR92 the decreases in total leaf ^{14}C and the ^{14}C content of individual leaf layers reflect the decreases in total leaf biomass and the biomass of individual leaf layers. However, at high nitrogen the decrease in ^{14}C of total leaf and the upper two leaf layers did not reflect the biomass decrease as well as it did for low nitrogen. The decrease in ^{14}C at either low or high nitrogen does correspond well, however, with the decrease in leaf nitrogen (Figure 3.8) indicating that protein is the major component in leaves which remains labelled with time. This is not surprising since it has been shown that more than 70% of current assimilate has left a leaf within 24 hours (Stoy 1963, Rawson and Evans 1971) and WSC rarely exceeds 5% of the leaf dry weight (Spiertz and Ellen 1978, Schnyder 1993). Leaf biomass decreases to a far greater extent than the combined decrease in protein and WSC (assumed decrease in WSC of 5% of the DC65 leaf biomass). Leaf fall is not expected to contribute significantly to the decrease in leaf biomass due to the careful sampling employed in subplots. Chapter 4 discusses another possible remobilisable reserve to account for decreases in leaf biomass in addition to protein and WSC.

In the low nitrogen crop, leaf nitrogen decreased linearly during early and mid grain filling (Figure 3.8a) due to the demand for nitrogen by the developing grain in the

absence of any significant nitrogen uptake. The decrease in nitrogen of the leaves reduced leaf area duration and thereby grain yield. Leaf nitrogen was also retranslocated to the grain in the high nitrogen crop during early grain filling but was mostly delayed until mid grain filling because some of the grain demand was supplied by soil uptake. Surprisingly, nitrogen is remobilised from the flag leaves during early grain filling despite there being sufficient levels in lower leaves and the stem to sustain the grain demand. The slow retranslocation of leaf nitrogen by the high nitrogen crop led to a longer leaf area duration and greater post-anthesis assimilation than the low nitrogen crop.

Decreases in ^{14}C of stem WSC reflect the decrease in total stem ^{14}C but not as well with the change in WSC between DC65 and maturity. Obviously WSC ^{14}C did not increase along with WSC during early grain filling because the ^{14}C was labelled prior to DC65. However, the possible explanation for the decrease in WSC ^{14}C during WSC deposition after DC65 is less straightforward. Wardlaw and Porter (1967) also observed decreases in ^{14}C of WSC while deposition of WSC was occurring after DC65. It is possible that stem WSC was being respired but no significant loss of total crop ^{14}C was measured during early and mid grain filling in this study or from anthesis to maturity in the study of Wardlaw and Porter (1967). A possible explanation is that WSC was being remobilised from some of the stem internodes while it was being deposited in others, though the physiological reasons for such a process are unclear. During late grain filling the ^{14}C content of stem WSC falls to a lesser degree than the stem WSC but this can be readily explained by the continued remobilisation of WSC or other stem components not labelled with ^{14}C.

Losses of ^{14}C from the above-ground shoots of approximately 10% between DC65 and maturity are similar to those previously reported (Stoy, 1963 ≈10%, Wardlaw and Porter 1967 ≈10%, Austin *et al.* 1977 ≈8%, Austin *et al.* 1980a ≈11%). Most of the ^{14}C fixed is lost in the first 24 hours, but from several days after labelling to maturity losses are usually minor (Austin *et al.* 1977, Austin *et al.* 1980a). This indicates that respiration during grain filling uses current rather than stored assimilates (Bell and Incoll 1990) which is at odds with the suggestion that up to 33% of stem weight loss could be attributed to respiration (Rawson and Evans, 1971). During early and mid grain filling at BAR92, loss of total crop ^{14}C (Figure 3.7b) appears to be minimal despite retranslocation of 60% of the final grain ^{14}C in the low nitrogen crop and 74% at high nitrogen by the end of mid grain filling (Figure 3.7a). These data indicate that losses of ^{14}C associated with remobilisation, retranslocation and synthesis into grain starch and protein are minimal. In the present study losses of ^{14}C appear to be greatest during late grain filling when grain yield formation is slowing down. In the same period the largest fall in stem WSC reserves occurs and total biomass decreases.

The above evidence suggests that wasteful respiration (Fischer 1979; Schnyder 1993) of soluble reserves in excess of the sink demand occurred in the final measurement period. The source of the reserves which were wastefully respired cannot be determined from this analysis though stem WSC are likely to be the source of the majority of the assimilate since the fall in ^{14}C is greatest in stem WSC. Therefore if it assumed that the majority of the loss of ^{14}C DPM was due to wasteful respiration, then the costs associated with the retranslocation of pre-anthesis stored assimilates are minor. This finding is in agreement with other work (eg. Bell and Incoll 1990, Palta *et al.* 1994) and with results presented in Chapter 5.

CHAPTER 4

"HAYING-OFF", NEGATIVE GRAIN YIELD RESPONSE OF DRYLAND WHEAT TO NITROGEN FERTILISER

Better than cattle and better than sheep
In the fight with drought and heat;
For a streak of stubbornness, wide and deep,
Lies hid in a grain of wheat.

Song of the Wheat
A. B. Paterson

4.1 INTRODUCTION

The inclusion of a legume based pasture phase in cereal rotations dramatically increased grain yield and protein levels of wheat in southern Australia during the 1950s (Donald 1960). By the 1960s there was concern that the legume based leys had built up soil nitrogen levels to such an extent that wheat yields would be adversely affected (Pugsley 1963; Storrier 1965b; White *et al.* 1978). The term 'haying-off' was coined by farmers in the 1890's (Colwell 1963a) and adopted by researchers (Jensen 1914) to describe premature ripening of wheat crops. Haying-off was described as premature cessation of grain filling and presumed to be due to exhaustion of soil moisture by the vigorous vegetative growth of crops stimulated by high soil nitrogen levels (Willis 1959; Storrier 1965b). Obvious features of haying off are a tall dark coloured biomass, small and/or pinched grain of a high protein content, and low yields. Haying off can also be induced by the use of nitrogen fertiliser (Storrier 1965; Russell 1967; Angus *et al.* 1989; McDonald 1991; Johnston and Fowler 1991). Stanford and Hunter (1973) could not explain negative grain yield responses to nitrogen fertiliser at each of five sites in central and southeastern Pennsylvania.

Haying off has been attributed to high levels of available soil nitrogen stimulating early vegetative growth and increasing water use, thereby depleting soil water reserves and inducing water stress in the crop (McDonald 1989). If the stress occurs early, grain yield is reduced due to decreased tiller survival (Barley and Naidu 1964; Fischer and Kohn 1966c) and reduced grain set per spike (Barley and Naidu 1964) but kernel weight may not be affected (Barley and Naidu 1964). The term haying-off is not used in this situation. It is typically used to refer to post-anthesis drought that may not reduce tiller survival or grain set per spike (Fischer and Kohn 1966c) but dramatically reduces kernel weight and hence harvest index and yield (McDonald 1991; Frederick and Marshall 1985).

There is evidence that other factors acting independently of water stress may also contribute to haying off. Increased incidence of disease with high rates of nitrogen fertiliser have been reported to decrease kernel weight and grain yield (Roth *et al.* 1984). Fischer and Kohn (1966c) concluded that haying-off was unlikely to be caused by an increase in evapotranspiration due to the small increase in leaf area in response to nitrogen fertiliser. They concluded that the probable cause was increased shoot numbers and reduced shoot size leading to an inability to compete for scarce resources. Even in the absence of apparent water stress the addition of nitrogen fertiliser can reduce grain yield by reducing kernel weight (Dann 1969; Lipsett and Simpson 1973). Studies in the field (Storrier 1965b) and in a controlled environment (Fischer 1980) also showed

reductions in kernel weight with the addition of nitrogen fertiliser in the absence of grain filling water stress.

These studies show that the interaction of soil mineral nitrogen with water stress is not likely to be the only cause of haying off as proposed in earlier studies. The effects of nitrogen on the pattern of growth and the physiological responses of wheat have received little attention and further work is warranted (McDonald 1989). The aim of the field experiments presented in this chapter was to determine the physiological responses to increasing soil mineral nitrogen which contribute to, or predispose a wheat crop to haying off. Measurements on wheat grown at sites with differing mean temperatures, evaporative demand and grain filling water status were expected to highlight physiological responses to nitrogen under different environmental conditions. The three experiments chosen for presentation in this chapter illustrate very clearly the progression from positive to negative yield responses to nitrogen.

4.2 MATERIALS AND METHODS

4.2.1 Cultural conditions

Experiments were sown at three sites in late May or June of 1991. The sites were at Ginninderra on the CSIRO Experiment Station, Pucawan on the property of Ray and Gloria Rodway, and Wagga Wagga on the grounds of Charles Sturt University. A map is presented in Appendix 1 and brief details are presented in Table 4.1 Experimental sites were chosen following a breakcrop (Angus *et al.* 1991; Kirkegaard *et al.* 1994) to minimise the detrimental effects of soil-borne disease on nitrogen response (McDonald 1991).

Table 4.1: Sites, sowing dates and paddock history of field experiments

Site	Trial designation	Sowing date	Previous 5 years paddock history
Ginninderra	GES91	21/06/91	Peas, Pasturex2, Fallow, Linola
Pucawan	PUC91	19/06/91	Wheat, Pasturex3, canola
Wagga Wagga	WAG91	26/05/91	Pasturex4, canola

4.2.1.1 *Ginninderra*

Wheat cv. Janz was sown at CSIRO Ginninderra Experiment Station, Australian Capital Territory, using a 14 row Shearer disk drill. The site was 17 km north-west of Canberra (149° 06' long, 35° 12' lat, 600 m a.s.l.) (Appendix 1) in the southern tablelands of New South Wales (long-term annual rainfall 706 mm.). The experiment was conducted in a two hectare trial site with a 1% slope. In 1990 the site had been used for a Linola breeding trial. The area was scarified in May 1991 and sown to wheat on June 21 at a rate of 85 kg ha^{-1} with 21.5 kg P ha^{-1} in the form of double superphosphate. The soil is a yellow podzolic soil Gn 3.85 (Northcote *et al.* 1971). Nitrogen treatments on wheat were Control, 40, 80, 160, 200 and 240 kg N ha^{-1}. Nitrogen was applied at the five leaf stage (DC 15) for the 40 kg N ha^{-1} treatment and split between sowing (DC 10) and the start of stem elongation (DC 30) for the 80, 160, 200 and 240 kg N ha^{-1} treatments. The experiment was sprayed with dicamba and MCPA on September 16 to control broad-leaf weeds. Spray irrigation was applied on November 1 (24 mm) and 14 (31 mm).

4.2.1.2 *Pucawan*

The site at Pucawan is 250 km west of Canberra (147°21' long., 34°23' lat., 272 m a.s.l.) (Appendix 1) in the middle of the New South Wales wheat belt (long-term annual rainfall

550 mm). Experiments were conducted in a flat 40 hectare paddock which had been sown to canola in the previous season and produced a yield of 2.5 t ha^{-1}.

The stubble was grazed and scarified once before sowing. The sowing implement was a 10 row 3 point linkage-mounted cone seeder with a fertiliser box attached. Wheat cv. Janz was sown on June 19, 1991 at a rate of 65 kg ha^{-1} (170 seeds m^{-2}) with triadamefon double superphosphate supplying 21 kg P ha^{-1}. The soil is a red earth Gn 2.12 (Northcote *et al.* 1971) with little textural change at any depth in the profile to 1.80 m. Nitrogen treatments were control, 40, 80, and 120 kg N ha^{-1} applied at either sowing (DC10) or the start of stem elongation (DC30) and two split treatments of 80/80 and 80/120 kg N ha^{-1}. Two blocks of the experiment were sprayed with diclofopmethyl and fenoxapropethyl to control monocotyledonous weeds on August 17. The fungicides triadamefon and benomyl were sprayed prophylactically on September 5 and triadamefon was sprayed on October 31.

4.2.1.3 *Wagga Wagga*

Wheat cv. Matong was sown at Wagga Wagga, New South Wales, using a 33 row Shearer trash seeder. The site is 250 km west south west of Canberra (147° 10'; long, 35 ° 03' lat, 210 m a.s.l.) (Appendix 1) in the southern half of the NSW wheat belt (long-term annual rainfall 579 mm). The paddock was 15 ha in area with a 2% north-westerly slope and had 2.5 tonne of canola ha^{-1} harvested from it in 1990. The stubble was grazed and wheat direct drilled on May 26 at a rate of 60 kg ha^{-1} with Starter DAP supplying 25 kg P ha^{-1} and 23 kg N ha^{-1}. The soil is a red earth, Gn 4.12 (Northcote *et al.* 1971). The paddock was sprayed with dicloropmethyl on June 27 and with MCPA and Diflufenican on July 23. Nitrogen treatments were Control, 40, 80, and 120 kg N ha^{-1} applied at either DC 10 or DC 30 and two split treatments of 80/80 and 80/120 kg N ha^{-1}.

4.2.2 Experimental design

All experiments were 4-replicate, randomised complete block designs. At PUC91 plots were sown 1.8 m wide and 15 m long while at other sites experiments were superimposed over the bulk area of wheat with plots running at 90° to the direction of sowing. Plots were either 3 m x 20 m or 2.5 m x 24 m and were marked by spraying out narrow pathways using the non-selective herbicide glyphosate. Nitrogen fertiliser in the form of urea was topdressed just before the anticipated arrival of rain and on all but one occasion rain commenced within 12 hours. Statistical analysis of data was conducted on the rate of nitrogen supplied and did not include time of application as a factor because of its minor impact on yield and nitrogen uptake. Previous work (Shimshi and Kafkafi, 1978; Strong, 1986; Fischer *et al.* 1993) has also shown little change in the yield

response when nitrogen was either applied at sowing, tillering or split between the two times. Therefore all data are presented as rate of applied nitrogen fertiliser unless otherwise stated.

4.2.3 Meteorological data

All sites had rain gauges installed prior to sowing. Rainfall was measured at each visit to the sites and a small amount of liquid paraffin was placed into the gauge to prevent evaporation of subsequent rainfall until the gauge was next read. There was a weather station 300 m from GES91, 500 m from WAG91 and at Temora Agricultural Research and Advisory Station, 14km east of PUC91. Measurements taken at each station included maximum and minimum temperatures, 9am and 3pm wet and dry bulb temperatures, solar radiation, precipitation, windrun, and Class A pan evaporation. At WAG91 and PUC91 daily maximum vapour pressure deficit was calculated using 9am wet and dry bulb temperatures and daily maximum temperature. At Ginninderra 3pm wet and dry bulb temperatures were used due to the possible influence of sea breezes on the 9am measurements. Data for each site are summarised in Tables 4.2 (GES91), 4.3 (PUC91), and 4.4 (WAG91).

4.2.4 Crop growth and soil measurements

4.2.4.1 *All sites*

Soil sampling, crop establishment and crop monitoring were conducted up to anthesis (DC65) as detailed in Chapter 2, Section 2.2.4.

At DC65 quadrats were harvested at the soil surface to estimate above-ground dry matter production. One quadrat of 0.30 m x 8 rows was harvested from each end of the plot, bulked, weighed and subsampled. Subsamples were counted for fertile shoots, separated into spikes, stem (including sheath) and leaves, placed in separate bags and oven dried at 70°C. Above-ground biomass was estimated on a per unit area basis. Samples were ground in a Wiley mill to pass a 1 mm sieve and used to determine water soluble carbohydrate (WSC) levels in the stems, spikes and leaves. Less than a 1% difference in WSC has previously been shown between oven-drying and freeze-drying samples (Judel and Mengel 1982, Kiniry 1993). Carbohydrates were extracted from 0.1g of plant material by extracting once with 8 ml of 80% ethanol (v/v) at 80 °C followed by two extractions with 8 ml of distilled water at 60 °C. Extraction time was 60 minutes for each after which tubes were centrifuged and the extracts progressively combined. This procedure was considered to extract 99% of the total free and combined fructose (Borrell *et al.* 1989). Total carbohydrates in the extract were analysed by the anthrone

method of Yemm and Willis (1954) using fructose as a standard. Nitrogen concentration was determined using a semi-micro-Kjeldahl method (Heffernan 1985) and analysed for NH_4^+ (AOAC 1984). Neutral detergent fibre (NDF, estimate of cell walls) and acid detergent fibre (ADF, estimate of cellulose and lignin) determinations were carried out on leaf samples from WAG91 and above ground biomass from GES91. Methods were the same as Goering and Van Soest (1970) except that 200mg of plant dry matter were refluxed in 50ml of neutral detergent or acid detergent for determination of NDF and ADF respectively.

At physiological maturity (DC86) or soon after, ten random grab samples were taken from each plot to estimate harvest index. Above ground biomass at maturity was calculated from quadrats, harvested as at DC65. Harvest index samples were separated into spikes and straw and oven dried at 70°C and weighed. Spikes were threshed and the glumes, awns and rachis placed with the straw (collectively termed non-grain biomass). The grain was redried at 70°C and weighed. Kernel weight was calculated from the average of 3 lots of 100 kernel weights. Grain was ground using a Cyclotec mill with a 0.5 mm sieve and the non-grain dry matter ground in a Wiley mill to pass a 1 mm sieve. Water soluble carbohydrate remaining in the non-grain biomass was estimated as described above. Nitrogen concentration in the grain and straw was analysed by near infrared reflectance spectroscopy using locally determined calibrations as detailed in Chapter 2, Section 2.2.4.1. NDF and ADF determinations were carried out on leaf samples from WAG91 and non-grain biomass from GES91.

When harvest ripe (DC92), 10 row plots were trimmed at each end, one outside row cut down with a brush cutter, the length measured and the inner 8 rows harvested. Plots running 90° to the direction of sowing had the ends trimmed, the length measured and a width of 1.32m harvested down the middle of each plot to avoid edge effects. The components of yield measured directly from the grab sample were harvest index and kernel weight. Kernels per spike, spike density and kernel number per unit area were then determined from the combination of harvest index and machine harvest samples. Samples harvested from DC86 to DC92 are referred to as maturity samples.

As soon as possible after harvest, soil cores were collected and analysed for determination of soil water content, mineral nitrogen and soil water suction as described in Chapter 2, Section 2.2.4.

4.2.4.2 *Pucawan*

At DC65 samples were separated into spikes, stems plus sheaths, green leaves and dead leaves. Specific Leaf Area (SLA), Leaf Area Index (LAI) and Green Area Index (GAI)

were estimated for selected crops. Soil sampling was undertaken at DC65 for determination of soil water content. Crops sampled were control and 80 kg N ha^{-1} applied at DC10.

4.2.4.3 *Wagga Wagga*

At DC65 control plots were soil sampled to a depth of 1.7m and the volumetric soil water content determined. At maturity samples were separated into grain, non-grain spikes, stems plus sheaths and leaves. Grain, non-grain spikes, stems plus sheaths and leaves were analysed for nitrogen concentration using a semi-micro-Kjeldahl method (Heffernan 1985) and were analysed for NH_4^+ (AOAC 1984) while only the latter three plant parts were analysed for WSC as described in Section 4.2.4.2.

4.2.5 Radiation Interception

Interception of radiant energy by the crop canopies was estimated for each plot by placing a 1 m line quantum sensor (Decagon, Sunfleck ceptometer) horizontally on the ground beneath the canopy 45° to the row direction. The mean of six to eight measurements were taken from each plot. Estimates of incident radiation were obtained at regular intervals during the measurement of canopy interception by holding the sensor horizontal above the crop. All measurements were taken in bright sunshine within one and a half hours either side of solar noon. From the incident and crop canopy measurements the proportion of incident radiation intercepted by the canopy was determined for each plot. Measurements were made in conjunction with dry matter sampling dates provided light conditions were favourable.

4.2.6 Crop water use and transpiration efficiency

Crop evapotranspiration, ET, was calculated using the moisture budget equation:

$$ET = P - \Delta S - R - D \qquad (4.1)$$

where P is the precipitation during the given period, ΔS is the change in total moisture in the soil profile, R is the surface runoff and D is the drainage beyond the depth of measurement. There was no evidence that either runoff or drainage occurred at any of the sites and these were assumed to be zero. Thus, for sites at GES91, PUC91 and WAG91, seasonal ET could be calculated.

Crop ET was partitioned into components of soil evaporation, E_S, and transpiration, T, using a model based on radiation penetration (Section 4.2.5) to the soil surface under

crop canopies and evaporation from bare soil (Cooper *et al.* 1983). Crop water use efficiency, W_{ET}, and transpiration efficiency, W_T, for above-ground biomass (W_{ET}^B, W_T^B) and grain yield (W_{ET}^G, W_T^G) were calculated based on ET and T respectively. Water use efficiency was calculated as the ratio of dry matter produced to water used from DC10 to maturity. Crop water use efficiency, W_{ET} and transpiration efficiency, W_T from DC10 to DC65 and DC65 to maturity could be calculated at PUC91 and WAG91.

4.2.7 Carbon isotope discrimination (Δ)

A short description of theory and the methods of analysis of carbon isotope discrimination are given in Chapter 2, Section 2.2.8.

4.3 RESULTS

4.3.1 Weather conditions

A summary of the climatic averages and weather conditions for the year 1991 for GES91, PUC91 and WAG91 are given in Tables 4.2, 4.3 and 4.4 respectively. Maximum temperatures were above average for all but one month at GES91 while only the May/June temperatures were above average for PUC91 and WAG91. Monthly mean minimum temperatures were close to average for all months except June which was double the mean at all sites. April to December rainfall was below the mean at all sites. At GES91, spray irrigation was applied on two separate occasions in November (55 mm total) which brought the total to above average for the season. At WAG91 rainfall was 18% below average and 30% below at PUC91. Winter rainfall at PUC91 and WAG91 was above average due to a wet June while spring was very dry. The onset of terminal drought corresponded to the grain filling period at these sites and coincided with a sharp rise in ET and VPD at all sites in October.

Maximum temperatures were generally highest at PUC91, followed by WAG91 and GES91. Minimum temperatures, however, were comparable at WAG91 and PUC91 with GES91 remaining the coldest. Class A pan evaporation was greatest at GES91 during winter and early spring but comparable to WAG91 and PUC91 during the rest of the season. Mean daily maximum vapour pressure deficit (VPD) was least at GES91 and comparable at PUC91 and WAG91. The monthly range in daily maximum VPD was also least at GES91 and greatest at WAG91 during grain filling.

Table 4.2. Monthly observed and derived weather data at GES91.

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
				Maximum temperature (°C)					
Mean[a]	19.3	14.7	11.3	10.3	11.8	14.5	18.0	21.5	25.1
1991	20.7	16.8	14.1	11.1	11.6	15.0	21.9	22.7	24.2
				Minimum temperature (°C)					
Mean[a]	6.5	3.5	1.2	0.2	1.5	3.5	6.1	8.4	11.0
1991	5.8	3.1	5.4	1.2	1.7	3.3	6.8	8.3	11.2
				Rainfall (mm)					
Mean[a]	56	55	39	59	63	69	73	65	58
1991	16	32	107	109	71	74	21	26(55)[b]	58
				Class A Pan Evaporation (mm)					
Mean[a]	87	53	38	40	58	86	124	162	204
1991	126	67	56	54	94	112	179	216	218
				Vapour pressure deficit (k Pa)[c]					
1991	1.6	1.1	0.6	0.5	0.7	0.9	1.7	2.0	2.1
Range	0.3-3.2	0.2-2.0	0.1-1.0	0.1-0.9	0.3-1.2	0.2-1.7	0.8-2.5	0.9-3.9	0.4-4.9

[a] Long-term mean (32 years) Ginninderra Experiment Station

[b] 26 mm rainfall, 55 mm spray irrigation

[c] Mean daily maximum vapour pressure deficit

Table 4.3. Monthly observed and derived weather data at Temora Research Station and PUC91

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
				Maximum temperature (°C)					
Mean[a]	22.8	17.8	14.1	13.0	14.7	17.7	22.1	26.1	29.6
1991	23.0	19.7	15.6	12.6	13.7	17.4	24.2	26.8	28.1
				Minimum temperature (°C)					
Mean[a]	9.2	6.0	3.1	1.9	3.0	4.5	7.7	10.5	13.6
1991	7.3	5.8	7.9	3.9	2.2	4.3	8.0	10.1	12.3
				Rainfall (mm)					
Mean[a]	49	53	37	48	53	46	56	44	47
1991[b]	7	17	93	44	37	53	13	9	29
				Class A Pan Evaporation (mm)					
Mean[a]	106	59	34	38	56	80	134	200	253
1991	110	69	38	41	55	67	135	219	236
				Vapour pressure deficit (k Pa)[c]					
1991				Not available at time of printing.					
Range									

[a]Long-term mean (60 years) Temora Research Station

[b]Actual data for PUC91

[c] Mean daily maximum vapour pressure deficit

Table 4.4. Monthly observed and derived weather data at Wagga Wagga, Soil Conservation and Charles Sturt University

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
				Maximum temperature (°C)					
Mean[a]	22.1	17.0	13.5	12.4	14.2	16.9	21.3	25.2	29.1
1991	22.9	19.3	15.5	11.9	12.6	16.0	22.6	26.1	27.9
				Minimum temperature (°C)					
Mean[a]	10.3	7.3	4.0	3.0	4.5	5.9	8.9	11.3	14.6
1991	7.8	5.1	8.0	3.9	4.0	5.8	8.5	10.1	13.9
				Rainfall (mm)					
Mean[a]	47	57	44	55	56	53	62	42	41
1991[b]	6	12	120	73	50	65	11	14	24
				Class A Pan Evaporation (mm)					
Mean[a]	108	58	34	35	53	76	125	192	258
1991	127	70	39	33	44	78	149	230	239
				Vapour pressure deficit (k Pa)[c]					
1991[b]	1.9	1.3	0.6	0.6	0.7	0.9	1.8	2.5	2.3
Range[b]	1.2-2.9	0.6-2.2	0.1-1.5	0.2-1.2	0.3-1.1	0.5-1.7	0.7-3.6	1.5-4.9	0.8-4.7

[a]Long-term mean 46 years) Wagga Wagga Soil Conservation.

[b] Actual figures at trial site Charles Sturt University.

[c] Mean daily maximum vapour pressure deficit

4.3.2 Growth, shoot density and grain yield

Established seedling density was within recommended limits for each district. Density was 180 plants m^{-2} at GES91, 130 plants m^{-2} at PUC91 and 138 plants m^{-2} at WAG91 respectively.

The application of nitrogen fertiliser stimulated biomass production through an increase in tiller density. This was apparent at PUC91 and WAG91 by DC30 (Table 4.5). At DC65 there were significant responses in spike density to the applied nitrogen fertiliser at all sites and significant biomass responses at GES91 and PUC91. However, the

Table 4.5. Effect of nitrogen fertiliser on the growth and grain yield of wheat at GES91, PUC91, WAG91.

	DC30		DC65		DC87		
N rate (kgN ha^{-1})	Dry wt[a] ($g\ m^{-2}$)	Shoot density[a] (m^{-2})	Dry wt ($g\ m^{-2}$)	Spike density (m^{-2})	Dry wt ($g\ m^{-2}$)	Spike density (m^{-2})	Grain yield ($g\ m^{-2}$)
			GES91				
0	167	1056	986	515	1366	500	607
40	-	-	1086	603	1578	557	723
80	-	-	1167	627	1615	606	729
120	-	-	1132	606	1619	570	765
160	-	-	1163	633	1683	604	781
200	-	-	1194	646	1670	620	777
240	-	-	1188	644	1676	615	798
(l.s.d. P=0.05)			97	70	131	46	78
			PUC91				
0	106	499	653	372	995	355	420
40			-	-	1052	394	446
80	139	631	787	459	1074	415	458
120			836	485	1097	426	463
160			-	-	1084	425	444
200			843	525	1069	453	432
(l.s.d. P=0.05)	11	53	58	43	ns	ns	22
			WAG91				
0	218	549	984	377	1086	372	374
40	286	607	-	-	1158	380	366
80	291	618	1075	421	1148	382	345
120	316	691	1092	416	1163	376	328
160			-	-	1132	389	283
200			1097	420	1106	379	284
(l.s.d. P=0.05)	42	58	ns	31	ns	ns	30

[a]These figures for N applied at sowing

biomass response was not significant at WAG91 though the trend remained. At physiological maturity the biomass and spike density responses were significant at GES91 but not at the other sites (Table 4.5). At PUC91 there was a trend of increasing spike density with applied nitrogen, though biomass plateaued at high rates of nitrogen. The increasing trend of biomass at WAG91 was similar to PUC91 but spike density was unaltered by applied nitrogen. There was a loss of spikes between DC65 and maturity at all sites with the greatest loss being at PUC91 and WAG91 and small losses at GES91.

Grain yield was greatest at GES91 and least at WAG91. There was a positive curvilinear response with decreasing returns to applied nitrogen at GES91, a parabolic response curve at PUC91, initially positive but finally negative and a linear decline in grain yield with applied nitrogen at WAG91 (Figure 4.1).

4.3.3 Yield components

Applied nitrogen led to significantly more kernels per square metre at all sites. The largest increase occurred at GES91 and the lowest at WAG91 (Table 4.6). The number of kernels per spike also increased significantly with higher rates of nitrogen at all sites but kernel weight decreased. The reduction in kernel weight had a range of 5 mg at GES91 and 11 mg at WAG91. Harvest index increased with higher rates of nitrogen at GES91, had a non-significant downward trend at PUC91 and declined dramatically at WAG91.

4.3.4 Retranslocation of non-grain biomass to grain

Figures 4.2, 4.3 and 4.4 show the grain yield, total dry weight and the apparent retranslocation from non-grain biomass between DC65 and maturity in relation to the biomass at DC65 for GES91, PUC91 and WAG91. Apparent retranslocation is defined as the decrease in the non-grain biomass between DC65 and maturity Chapter 2, Section 2.4.2) At GES91, increasing biomass at DC65 resulted in increased grain yield, increases in biomass and a greater apparent retranslocation. At PUC91 increasing DC65 biomass initially led to increases in grain yield but finally a yield depression, a smaller change in total biomass and greater apparent retranslocation. Finally, at WAG91 greater DC65 biomass was associated with a dramatic decline in grain yield, ultimately no change in total biomass and similar amounts of apparent retranslocation from the non-grain biomass.

Table 4.6 Effect of nitrogen fertiliser on grain yield components of wheat at GES91, PUC91, WAG91.

N rate kg N ha^{-1}	Kernels m^{-2} ($x10^3$)	Kernels $spike^{-1}$	Kernel wt (mg)	Harvest index
		GES91		
0	15.19	30.2	40.1	0.44
40	18.52	33.2	39.1	0.46
80	19.52	32.2	37.7	0.45
120	20.81	36.6	36.8	0.47
160	20.92	34.8	37.4	0.46
200	21.45	34.6	36.3	0.47
240	21.49	35.0	37.1	0.48
(l.s.d. *P*=0.05)	1.91	3.2	1.6	0.01
		PUC91		
0	12.29	34.7	34.2	0.42
40	14.23	36.2	31.4	0.42
80	15.67	38.2	29.3	0.43
120	16.94	40.0	27.4	0.42
160	17.17	40.4	25.9	0.41
200	17.08	38.2	25.4	0.40
(l.s.d. *P*=0.05)	1.26	3.7	1.9	ns
		WAG91		
0	13.10	35.2	28.6	0.34
40	15.02	39.7	24.4	0.32
80	15.62	40.9	22.2	0.30
120	15.76	41.9	20.9	0.28
160	16.18	41.6	17.6	0.25
200	16.14	42.6	17.6	0.26
(l.s.d. *P*=0.05)	1.37	2.7	1.9	0.02

n.s. - not significant

Figure 4.1 Responses of grain yield (closed symbols) and protein (open symbols) response curves at 12% water content nitrogen fertiliser applied for a) GES91, b) PUC91, c) WAG91. Curves are fitted regressions, bars indicate the l.s.d. (P=0.05).

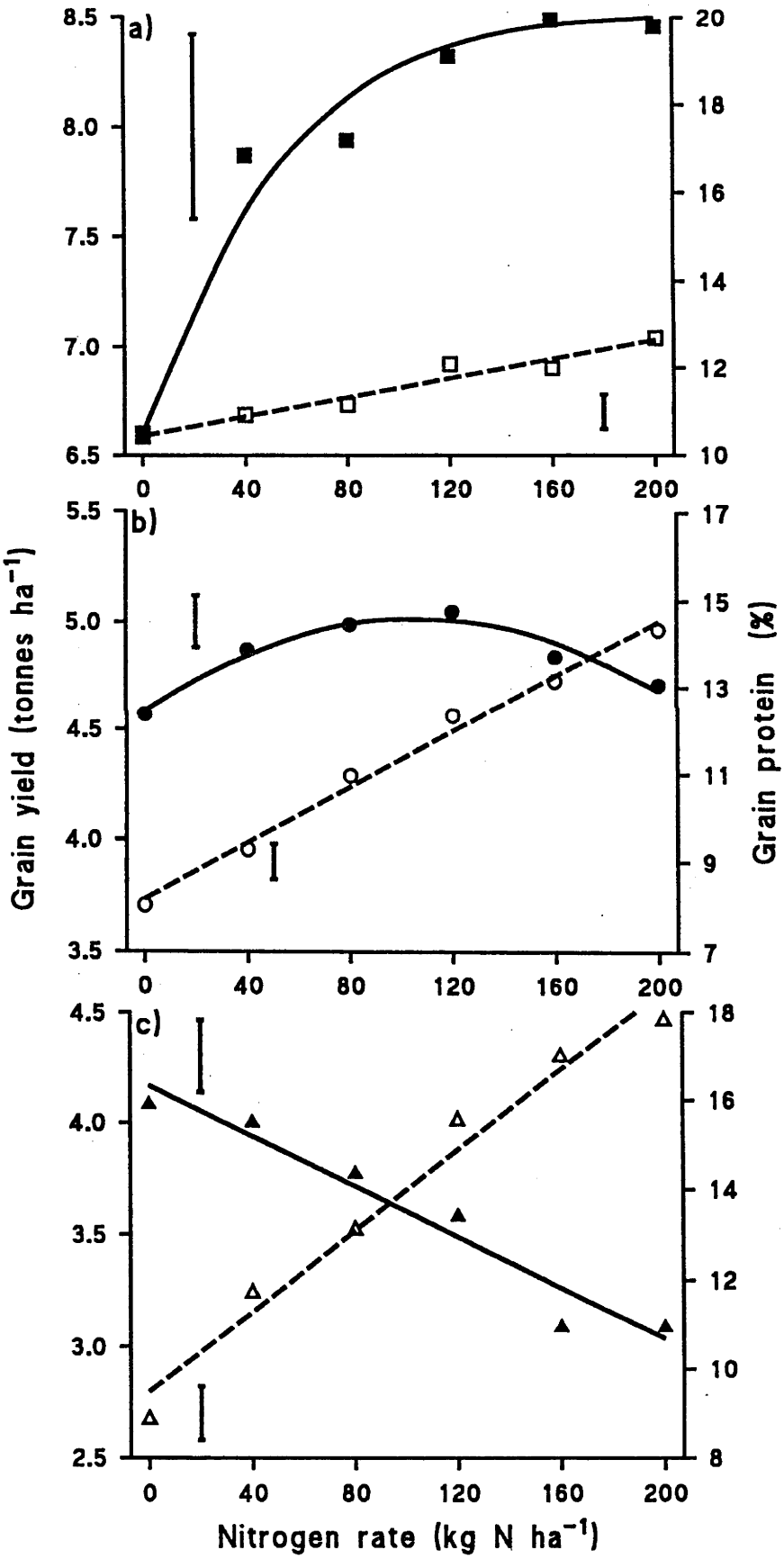


Figure 4.2 Relationship between anthesis biomass and a) grain yield, b) change in total biomass and c) apparent retranslocation between anthesis and maturity for GES91. Curves are fitted regressions, bars indicate the l.s.d. (P=0.05), absence of a bar indicates non-significance.

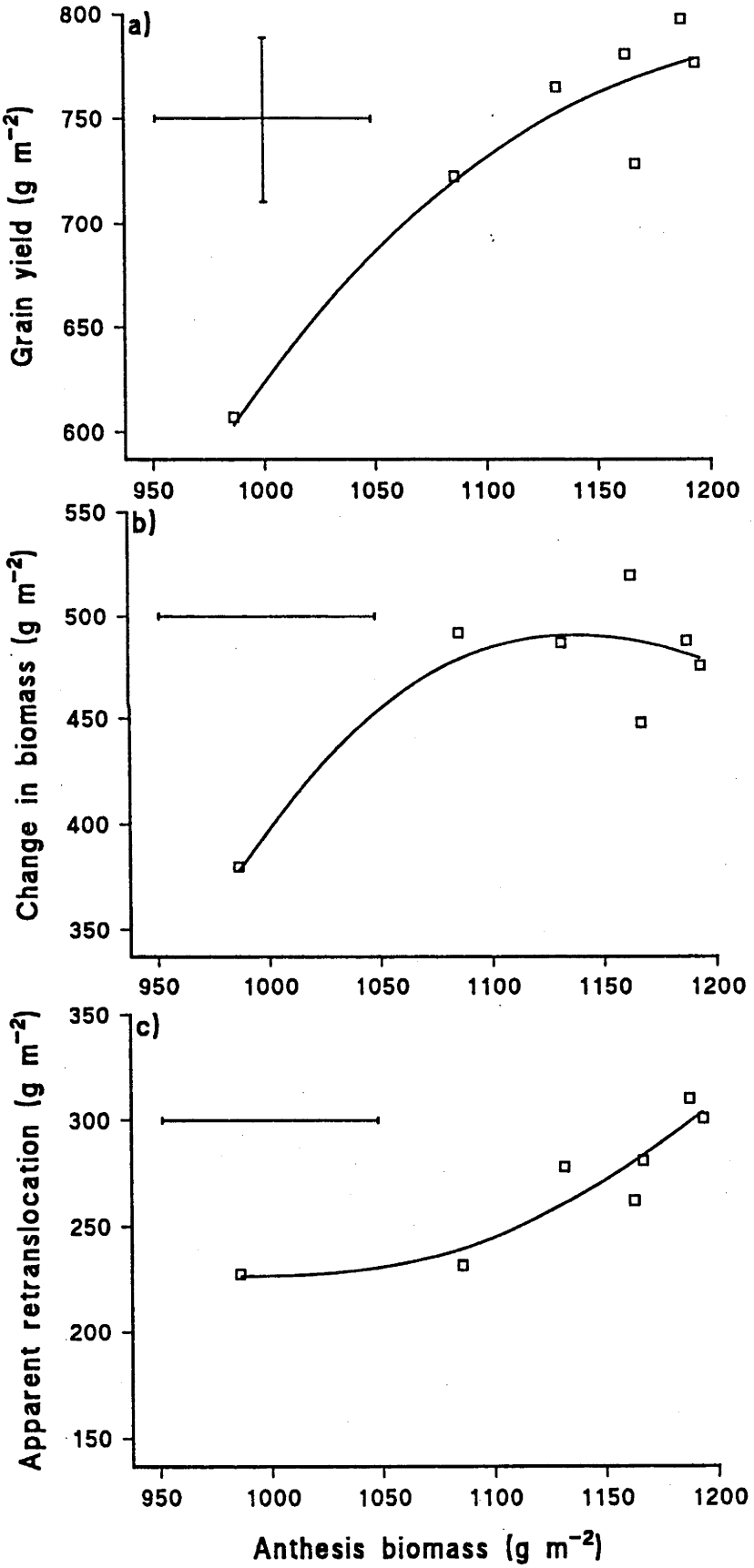


Figure 4.3 Relationship between anthesis biomass and a) grain yield, b) change in total biomass and c) apparent retranslocation between anthesis and maturity for PUC91. Curves are fitted regressions, bars indicate the l.s.d. (P=0.05).

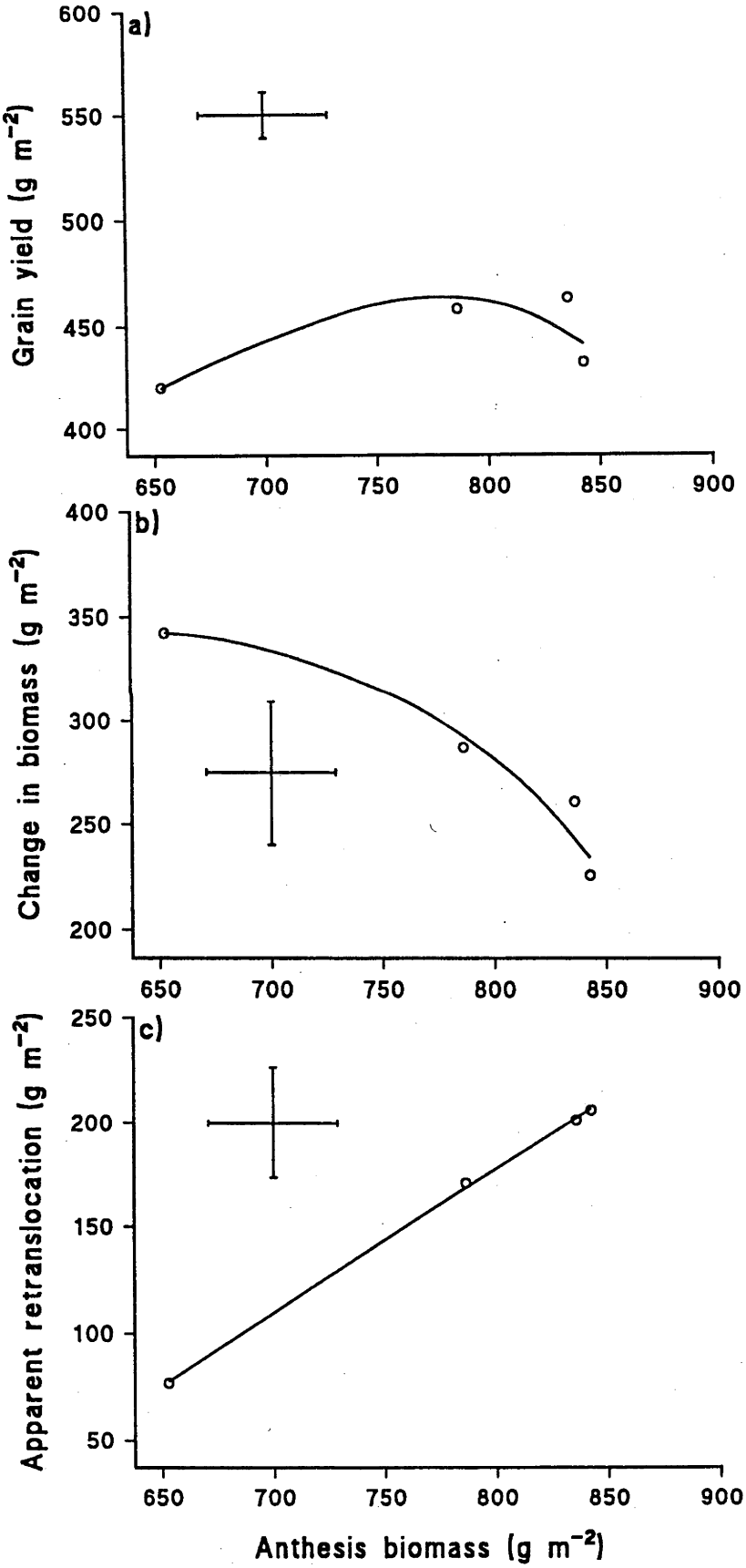
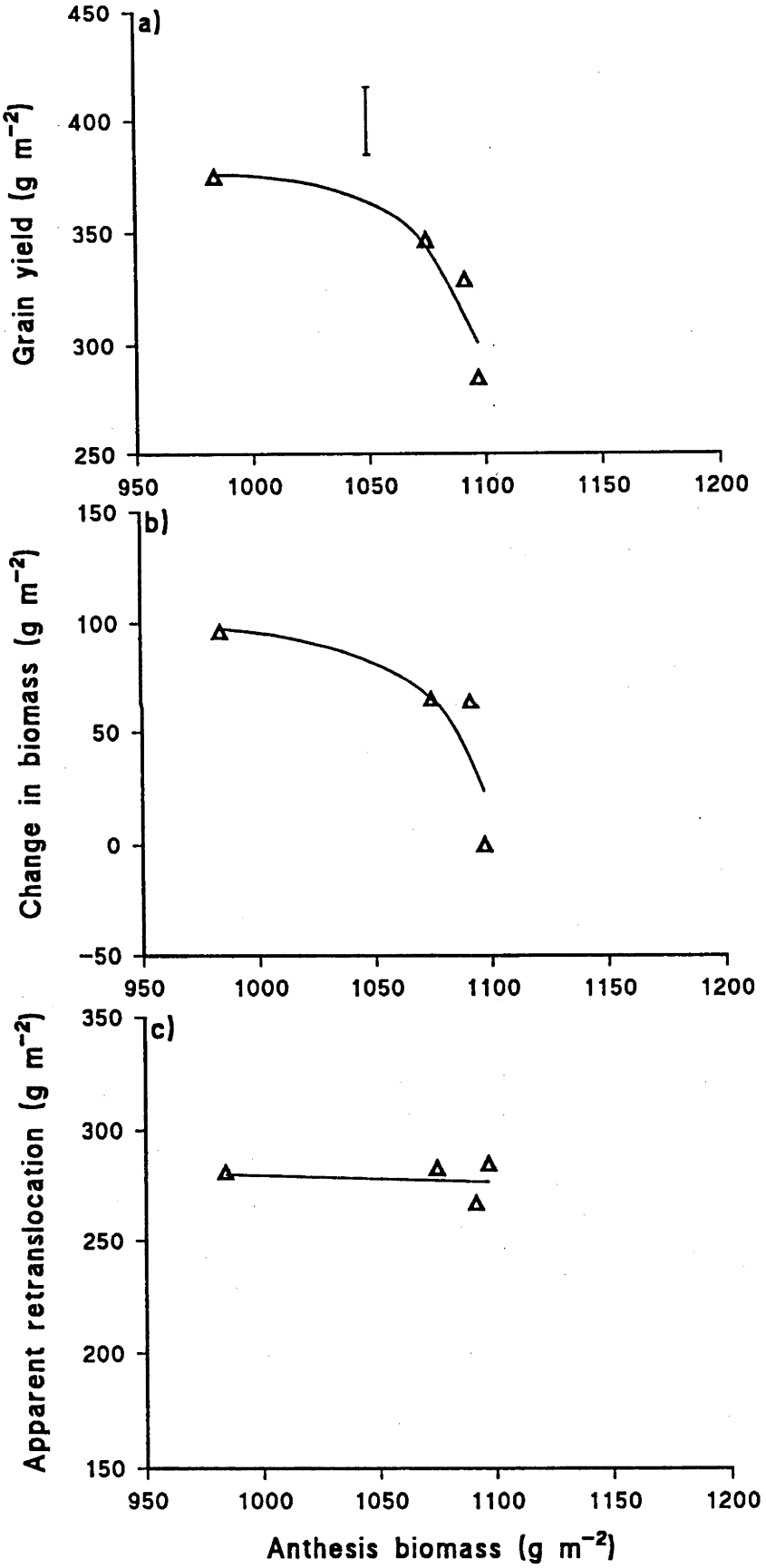


Figure 4.4 Relationship between anthesis biomass and a) grain yield, b) change in total biomass and c) apparent retranslocation between anthesis and maturity for WAG91. Curves are fitted regressions, bars indicate the l.s.d. (P=0.05), absence of a bar indicates non-significance.



4.3.5 Dry matter distribution

The dry matter distribution between spikes, leaves and stems at DC65 in relation to rate of nitrogen fertiliser is given for the three sites in Table 4.7. The biomass of spikes increased at each site with nitrogen fertilisation though as a proportion of the biomass it remained relatively constant. Leaf biomass almost doubled at each site increasing from 16% of total biomass for controls at GES91 to 29% for 200 kg N ha^{-1} at PUC91. Stem biomass increased significantly with nitrogen fertilisation at PUC91, showed a positive trend at GES91 and a negative trend at WAG91. As a proportion of the total biomass stem biomass declined in response to nitrogen at each site. Individual stem weight decreased with the addition of nitrogen at each site despite increases in crop height (data not shown) resulting in falls of up to 24% in stem linear density (Table 4.7). Stem linear density is defined as the mass of stem plus sheath in grams per metre of length. It is a better estimate of relative differences in stem weight because height differences are accounted for.

Table 4.7 Dry weight of spikes, leaves and stems at anthesis for GES91, PUC91 and WAG91.

N rate (kg N ha^{-1})	Spikes (g m^{-2})	Leaves (g m^{-2})	Stems[a] (g m^{-2})	Total (g m^{-2})	Stem wt anthesis (g $stem^{-1}$)	Linear density (g m^{-1})
			GES91			
0	196	159	632	986	1.23	1.80
40	214	206	667	1086	1.11	1.57
80	233	228	706	1167	1.13	1.57
120	239	232	662	1132	1.10	1.54
160	236	262	665	1163	1.05	1.46
200	250	262	681	1194	1.06	1.45
240	244	280	664	1188	1.03	1.42
(l.s.d. P=0.05)	24	30	n.s.	97	0.08	0.11
			PUC91			
0	126	147	380	653	1.02	1.54
80	170	210	407	787	0.89	1.29
120	178	227	432	836	0.89	1.30
200	178	247	418	843	0.80	1.17
(l.s.d. P=0.05)	14	27	27	58	0.07	0.10
			WAG91			
0	172	196	617	984	1.64	1.76
80	204	258	614	1075	1.46	1.46
120	212	275	604	1092	1.45	1.37
200	205	311	581	1097	1.38	1.35
(l.s.d. P=0.05)	19	39	n.s.	n.s.	0.09	0.09

[a] Stem includes leaf sheath.

Table 4.8 shows the dry matter distribution of different plant parts for WAG91 at maturity. Non-grain spikes constituted approximately the same proportion of biomass at each nitrogen level as they did at DC65. Leaf biomass contributed between 11 and 15% of total biomass at maturity, leaf biomass falling by 40-49% between DC65 and maturity for control and high nitrogen respectively. In contrast to the results at DC65 there was significantly more stem biomass for nitrogen fertilised crops than controls due to greater individual stem weight. The greater individual stem weight was a result of a taller crop as shown by similar values of stem linear density.

Table 4.8 Dry weight and total water soluble carbohydrates present in above-ground plant parts at maturity for WAG91.

N rate (kg N ha^{-1})	Grain ($g\ m^{-2}$)	Non-grain spikes ($g\ m^{-2}$)	Leaves ($g\ m^{-2}$)	Stems[a] ($g\ m^{-2}$)	Total ($g\ m^{-2}$)	Stem wt. (g $stem^{-1}$)	Linear density ($g\ m^{-1}$)
				Biomass			
0	374	164	118	423	1079	1.14	1.22
80	345	188	140	465	1139	1.22	1.22
120	328	190	162	474	1154	1.26	1.24
200	284	186	159	468	1096	1.24	1.21
(l.s.d. P=0.05)	28	13	27	37	n.s.	0.07	n.s.
				Water Soluble Carbohydrates ($g\ m^{-2}$)			
0		6.9	3.6	9.8	20.3		
80		5.6	4.0	10.3	19.9		
120		5.1	4.0	9.0	18.2		
200		4.9	4.1	8.6	17.6		
(l.s.d. P=0.05)		n.s.	n.s.	n.s.	n.s.		

[a] Stem includes leaf sheath.

4.3.6 Water soluble carbohydrates

Table 4.9 shows the WSC present in spikes, leaves, stems and total above-ground biomass at DC65 for GES91, PUC91 and WAG91. The amount of WSC present in spikes increased slightly with nitrogen fertilisation contributing from 8% of the total for control crops at PUC91 to 14% for those crops receiving 200 kg N ha^{-1} at WAG91. Leaves contained approximately half the level of WSC as spikes but stems contributed between 70% and 85 % of the total above-ground reserves. The amount of WSC in the stems at DC65 was negatively correlated with nitrogen fertilisation at all sites. Water soluble carbohydrates were as high as 32% of stem weight for controls at GES91 and as low as 14% of stem weight for 200 kg N ha^{-1} at WAG91. The amount of WSC left in the non-grain biomass at maturity was approximately 10% of the DC65 level except at PUC91 where the remainder ranged from 29% to 17% with increasing nitrogen fertilisation.

Table 4.9 Water soluble carbohydrates present in spikes, leaves and stems at anthesis and in non-grain biomass at maturity for GES91, PUC91 and WAG91.

N rate	Anthesis ($g\ m^{-2}$)				Maturity ($g\ m^{-2}$)
($kg\ N\ ha^{-1}$)	Spikes	Leaves	Stems[a]	Total	Non-grain
			GES91		
0	26.4	13.2	204.7	244.4	30.8
40	26.6	12.8	182.4	221.9	24.5
80	28.4	13.2	185.0	226.6	24.8
120	28.2	11.7	170.0	209.9	19.2
160	29.6	11.6	163.0	204.3	15.4
200	27.6	10.3	143.2	181.1	14.5
240	27.7	11.2	133.2	172.1	12.8
(l.s.d. P=0.05)	n.s.	n.s.	16.5	19.8	9.4
			PUC91		
0	9.7	8.8	103.8	122.3	34.3
80	13.5	6.9	75.2	95.5	17.2
120	14.9	6.7	76.8	98.4	16.0
200	13.6	7.5	64.9	86.0	15.4
(l.s.d. P=0.05)	2.2	1.4	12.5	13.5	3.6
			WAG91		
0	22.6	10.0	181.5	214.1	20.3
80	28.5	11.6	126.4	166.5	19.9
120	29.3	11.9	110.1	151.3	18.2
200	28.8	11.6	82.4	122.8	17.6
(l.s.d. P=0.05)	4.0	n.s.	19.7	20.9	n.s.

[a] Stems include leaf sheaths.

At WAG91, the amount of WSC at maturity was low in all plant parts (Table 4.8) with no significant differences between crops though there was a negative trend between total WSC and nitrogen fertilisation.

4.3.7 Plant nitrogen dynamics

At DC30 wheat with applied nitrogen fertiliser had taken up more nitrogen than control crops (Table 4.10). This was also the case at DC65 and DC87 with increasing rates of nitrogen fertiliser leading to greater uptake of nitrogen at all sites. There was uptake of nitrogen for the lower rates of fertiliser between DC65 and maturity at GES91 but apparently only for the control at PUC91. There were small and non-significant losses of nitrogen from the above-ground biomass for other crops at PUC91 and all crops at WAG91. Apparent fertiliser recovery (AFR) decreased with increasing rates of nitrogen at all sites, with the crops at GES91 having the greatest uptake and the largest decline in AFR. The allocation of nitrogen per kernel increased with increasing rates of nitrogen fertiliser at all sites but plateaued at the higher rates of nitrogen at WAG91. At all sites grain protein increased with increasing rates of nitrogen (Figure 4.1) due to greater nitrogen allocation and concomitant decrease in kernel weight. The largest increase in grain protein was at WAG91 where there was a negative grain yield response. Nitrogen harvest index remained relatively stable at PUC91, declined slightly at GES91 and fell by 25% at WAG91.

The amount of total above-ground nitrogen contained in leaves increased with the addition of nitrogen at all sites (Table 4.11). The proportion of total above-ground nitrogen contained in leaves also increased due to an increase in leaf biomass and nitrogen concentration. In contrast, although the amount of nitrogen contained in spikes and stems at GES91 and PUC91 increased in response to nitrogen fertiliser, these plant parts contained a lower proportion of total above-ground nitrogen as the rate of nitrogen fertiliser increased. At WAG91 the proportion of total above-ground nitrogen contained in stems did not exhibit a clear trend.

Spikes contained a similar proportion of total above-ground nitrogen at each site while leaves contained less as a proportion at GES91 than at PUC91 which in turn was less than at WAG91. Stems at GES91 contained a greater proportion of total above-ground nitrogen than at PUC91 and WAG91.

Table 4.10 Nitrogen uptake during the season, apparent fertiliser recovery (AFR), grain protein, nitrogen harvest index (NHI) and nitrogen allocation per kernel (NAK).

N rate	N uptake ($g\ m^{-2}$)			AFR	Grain Protein[a]	N HI	NAK
kg N ha^{-1}	DC30	DC65	DC87	%	%		(mg $kernel^{-1}$)
			GES91				
0	5.67	10.28	13.01	-	10.4	0.86	0.73
40	-	13.66	16.22	80	10.9	0.86	0.75
80	-	15.85	16.97	50	11.2	0.84	0.74
120	-	17.02	19.24	52	12.1	0.84	0.78
160	-	18.98	19.85	43	12.0	0.83	0.79
200	-	20.15	21.08	40	12.7	0.82	0.81
240	-	21.59	21.43	35	12.8	0.84	0.84
(l.s.d. P=0.05)	-	1.92	2.61	n.a.	0.8	0.01	0.07
			PUC91				
0	4.21	6.47	7.85	-	8.0	0.75	0.48
40	-	-	9.51	42	9.3	0.76	0.51
80	6.42	11.80	11.26	43	11.0	0.78	0.56
120	-	13.05	12.82	41	12.3	0.78	0.59
160	-	-	13.19	33	13.1	0.78	0.60
200	-	15.01	13.99	31	14.3	0.78	0.63
(l.s.d. P=0.05)		1.22	0.93	n.a.	0.8	0.02	0.04
			WAG91				
0	5.80	8.35	7.63	-	8.8	0.76	0.44
40	7.74	-	10.01	60	11.7	0.75	0.50
80	8.96	12.45	11.57	49	13.1	0.69	0.51
120	10.46	14.14	13.62	50	15.5	0.65	0.57
160	-	-	13.68	38	17.0	0.62	0.52
200	-	16.42	15.50	39	17.8	0.57	0.55
(l.s.d. P=0.05)	1.50	1.27	1.19	n.a.	1.2	0.03	0.04

n.a. - not applicable, [a] Grain protein % at 12% moisture

Table 4.11 Nitrogen content of spikes, leaves and stems at anthesis for GES91, PUC91 and WAG91.

N rate		($g\ N\ m^{-2}$)		
($kg\ N\ ha^{-1}$)	Spikes	Leaves	Stems[a]	Total
		GES91		
0	3.46	2.20	4.62	10.28
40	4.24	2.38	6.04	13.66
80	4.70	4.15	7.00	15.85
120	5.26	4.52	7.24	17.02
160	5.26	5.67	8.05	18.98
200	5.67	6.04	8.44	20.15
240	5.33	7.75	8.51	21.59
(l.s.d. P=0.05)	0.81	1.08	1.03	1.92
		PUC91		
0	1.98	2.12	2.37	6.47
80	3.19	4.83	3.78	11.80
120	3.45	5.45	4.14	13.05
200	3.66	6.52	4.83	15.01
(l.s.d. P=0.05)	0.31	0.69	0.36	1.22
		WAG91		
0	2.50	2.90	2.95	8.35
80	3.32	5.11	4.02	12.45
120	3.59	5.93	4.62	14.14
200	3.72	7.12	5.59	16.42
(l.s.d. P=0.05)	0.34	0.68	0.46	1.27

[a] Stem includes leaf sheath.

4.3.8 Water use and water use efficiency

Seasonal water use (ET), varied between sites due to differences in rainfall and soil water storage at DC10 (Table 4.12). Positive yield response to applied nitrogen at PUC91 resulted in increased water use. However, at WAG91 where there was a negative yield response to nitrogen, there was a decrease in total ET. Figure 4.5 shows the soil water suction over the soil profile at DC10 and maturity for PUC91 and WAG91 for two rates of nitrogen. Rainfall after maturity at PUC91 and WAG91 resulted in a decreased soil water suction at the three points of measurement in the top of the soil profile. The results show that at PUC91 the application of 80 kg N ha^{-1} resulted in a greater soil water suction over the whole profile at maturity. This was equivalent to the extraction of an extra 25 mm of soil water.

Table 4.12 Evapotranspiration for the whole season (ET), between sowing and anthesis, (ET_{SA}), between anthesis and maturity (ET_{AM}), and water use efficiency for final biomass (W_{ET}^{B}) and grain (W_{ET}^{G}) for GES91, PUC91 and WAG91.

N Rate (kg N ha^{-1})	ET (mm)	ET_{SA} (mm)	ET_{AM} (mm)	W_{ET}^{B} (g m^{-2} mm^{-1})	W_{ET}^{G} (g m^{-2} mm^{-1})
		GES91			
0	456	-	-	3.1	1.3
240	476[a]	-	-	3.6	1.7
(l.s.d. P=0.05)	n.a.			n.a.	n.a.
		PUC91			
0	317	227	90	3.2	1.3
80[b]	342	260	82	3.2	1.3
200[c]	≈342	>260	<82	≈3.1	≈1.3
(l.s.d. P=0.05)	11	5	n.s.	n.s.	n.s.
		WAG91			
0	384	321	63	2.8	1.0
200	374	>321	<53	2.9	0.8
(l.s.d. P=0.05)	6	n.a.	n.a.	n.s.	0.1

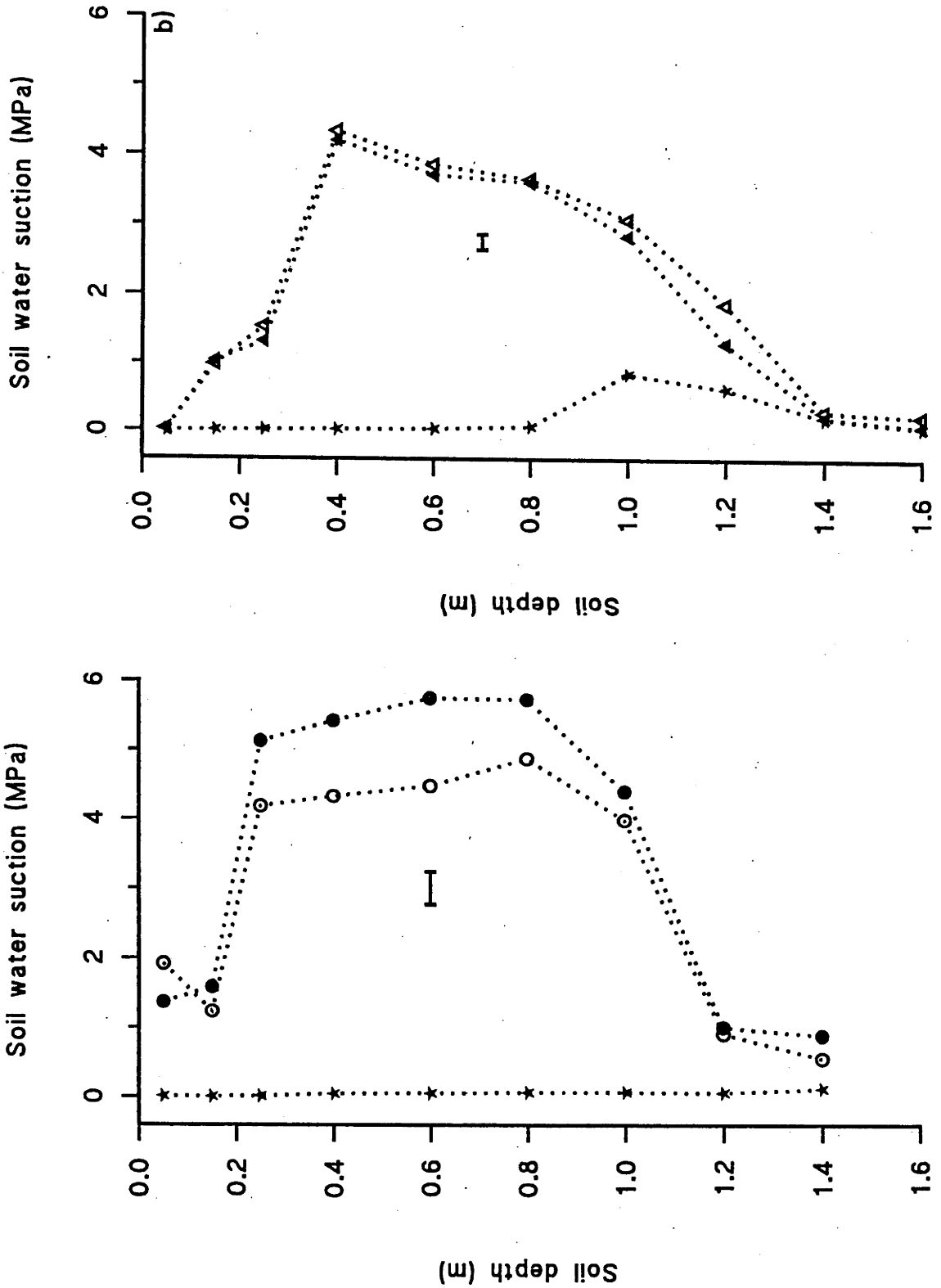
[a] Estimated water use based on measurements in the same paddock, under similar conditions in 1992 (Chapter 2).

[b] 80 kg N ha^{-1} applied at DC10

[c] Not included in ANOVA.

n.a. not applicable

Figure 4.5 Soil water suction in relation to depth versus at sowing and maturity for a) PUC91 at DC10 (★),control (O), and 80 kg N ha^{-1} (●), b) WAG91 at sowing (★),control (Δ), and 200 kg N ha^{-1} (▲). Bars indicate the l.s.d. (P=0.05).



In contrast, at WAG91 the soil under control and 200 kg N ha^{-1} crops did not differ in soil water suction to 0.8m in depth but in deeper layers, the suction was greater for control plots. This resulted in the high nitrogen crops leaving behind 10 mm more soil water below 0.8 m than the control crops. In the terminal drought conditions at PUC91 an increase in nitrogen fertiliser resulted in greater water use to DC65 and less water available for grain filling. Table 4.12 shows that water use efficiency for total above ground biomass, (W_{ET}^{B}) was similar at all sites and rates of nitrogen except at the high rate at GES91. Water use efficiency for grain (W_{ET}^{G}) increased with the positive yield response at GES91, was stable at PUC91 and became negative with the negative yield response at WAG91. Soil evaporation was greatest at GES91 (Table 4.13) due to a combination of more frequent rainfall events and greater Class A pan evaporation from June to October (Table 4.2). The estimate of transpiration efficiency (W_{T}^{B}) is stable at WAG91 and GES91 but is lower with nitrogen fertilisation at PUC91 and varies considerably between sites. Transpiration efficiency for grain (W_{T}^{G}) decreased in response to nitrogen fertilisation at PUC91 and WAG91 but there was a positive trend at GES91.

4.3.9 Carbon isotope discrimination (Δ)

Determination of carbon isotope discrimination (Δ) was carried out on samples from PUC91 and WAG91 at DC30. Results are presented in Figure 4.6 The variety Janz at PUC91 showed a non significant decrease in Δ with the addition of 80 kg N ha^{-1} while the Δ of Matong at WAG91 increased linearly with nitrogen fertilisation applied at DC10.

Table 4.13 Evapotranspiration (ET), Soil evaporation (E_S) and Transpiration (T) and Transpiration efficiency for final biomass (W_T^B) and grain (W_T^G) at GES91, PUC91 and WAG91.

Rate of N	ET	E_S	T	T_{SA}	T_{AM}	W_T^B	W_T^G
kg N ha^{-1}	(mm)	(mm)	(mm)	(mm)	(mm)	(g m^{-2} mm^{-1})	(g m^{-2} mm^{-1})
			GES91				
0	456	235	221	-	-	6.2	2.7
200	476[a]	207	269	-	-	6.2	3.0
(l.s.d. P=0.05)	n.a.	n.a.	n.a.	-	-	n.a.	n.a.
			PUC91				
0	317	133	184	106	79	5.5	2.3
80[b]	342	118	224	150	74	4.9	2.0
200[c]	≈342	115	≈227	>152	<76	<4.7	1.9
(l.s.d. P=0.05)	11	n.a.	11	5	n.s.	0.4	0.2
			WAG91				
0	384	99	285	235	50	3.8	1.3
200	374	78	296	>252	<45	3.7	1.0
(l.s.d. P=0.05)	6	n.a.	6	n.a.	n.a.	n.s.	0.1

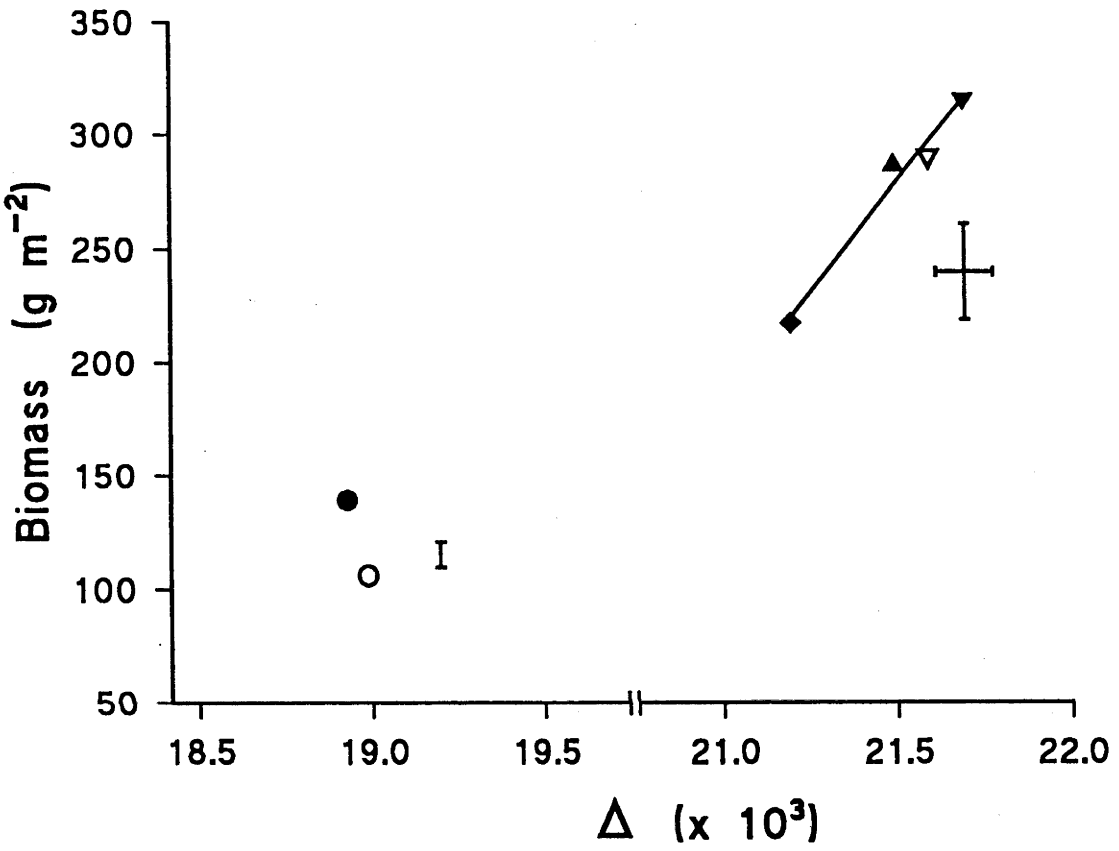
[a] Estimated water use based on measurements in the same paddock, under similar conditions in 1992 (Chapter 2).

[b] 80 kg N ha^{-1} applied at DC10

[c] Not included in ANOVA.

n.a. not applicable

Figure 4.6 Relationship between biomass at DC30 and carbon isotope discrimination, (Δ), for crops with nitrogen applied at DC10 for cv. Janz control (O) and 80SW (●)at PUC91 and for cv. Matong control (◆), 40SW (▲), 80SW (▽) and 120SW (▼) at WAG91. Bars indicate the l.s.d (P=0.05), absence of a bar indicates non-significance.



4.4 DISCUSSION

4.4.1 Biomass production and retranslocation

The application of nitrogen fertiliser increased nitrogen uptake (Table 4.10) and biomass production at DC65 (Table 4.5) at all sites, as reported in previous studies (Barley and Naidu 1964, Dann 1969, Morgan 1988, Angus et al. 1991, McDonald 1992). Increases in biomass were associated with a greater number of tillers per square metre.

In the present study, greater biomass to DC65 resulted in vastly different grain yield and protein responses at the three sites (Figure 4.1). At GES91, all levels of increasing nitrogen led to increased yield, while at WAG91 all additions of nitrogen led to decreases in yield. At PUC91 low rates of nitrogen fertiliser led to yield increases but application of nitrogen above 120 kg N ha^{-1} decreased yield. Positive grain yield responses to nitrogen fertiliser were associated with greater spike density, more kernels per spike and hence more kernels per square metre but also with a reduction in kernel weight. Harvest index went up at GES91 but exhibited a downward trend at PUC91. Negative grain yield responses were also associated with increases in spike density, kernels per spike and more kernels per square metre whereas harvest index declined due to a dramatic fall in kernel weight. The crops at WAG91, therefore, represent a clear example of haying-off.

Such negative yield responses have in the past been attributed to greater post-anthesis water stress (Colwell 1963a, Storrier 1965b, Taylor 1965b, Fischer and Kohn 1966c) caused by the high water use to produce biomass up to DC65 leaving little in the soil for grain filling. This lack of soil water reduces photosynthesis and hence assimilates for grain filling (Fischer 1979, 1980). In addition to the reduced soil water available for grain filling with nitrogen fertilisation (Table 4.12), Figure 4.5 illustrates that haying-off appears to reduce the final soil water extraction in contrast to greater extraction for positive yield responses. This novel result is corroborated by data extracted from Kirkegaard *et al.* (1994) which showed that wheat following peas hayed-off and extracted less soil water than wheat following other break crops. In their experiment the soil profile of wheat following peas contained the same amount of water at DC10, but at least twice as much mineral nitrogen as wheat following other break crops.

That differences in vegetative growth do not translate to grain yield may be due to increased competition for limiting resources in denser crops (Fischer and Kohn 1966c). At each site in this study there was a reduction in spike density between DC65 and maturity as has been observed under irrigation (Stapper and Fischer 1990a) and dryland

conditions (Barley and Naidu 1964; E A Koetz pers. comm.). This reduction in spike density was likely due to inter-culm competition and resulted in an overestimation of fertile spikes at DC65. It is possible that the competition for resources with increasing nitrogen was so great at WAG91, due to the greater number of kernels set and lack of current assimilate, that roots were starved of carbohydrate and died prematurely. This may explain the reduced water extraction with high nitrogen and is supported by the work of Hurd *et al.* (1979) with tomatoes. In this study, the fruit set of well fertilised plants created a sink of such magnitude that competition for carbon resulted in the cessation of root growth and some root death during fruit filling. In contrast, the roots of plants which had the fruit load reduced by flower thinning, continued to grow and no root death was observed.

Water stress was not apparent at any stage at GES91 and nitrogen fertilised crops produced significantly more biomass by maturity than control crops (Table 4.5). This contrasts with PUC91 where differences in biomass were not significant at maturity despite the greater biomass at DC65 for the fertilised crops. The response at WAG91 was also different because water stress was so severe that there were no significant differences in biomass at DC65 or maturity. At PUC91 water stress was first observed at DC65 with leaf rolling being worst at high rates of nitrogen. This was corroborated by 33 mm extra water use to DC65 (Table 4.12). At WAG91 visual differences in water stress between crops were first observed 3 weeks prior to DC65. Net biomass production between DC65 and maturity ranged from 380 - 488 g m^{-2} at GES91, 342 - 226 g m^{-2} at PUC91 and 102 - 9 g m^{-2} at WAG91 for control crops and those supplied with the highest rate of nitrogen respectively. This range in post-anthesis growth further highlights the difference in water stress between sites, and the differential water stress due to nitrogen fertilisation at each site. Indeed, 23% of rain from DC10 to maturity fell after DC65 at GES91 while 13% fell at PUC91 and only 7% at WAG91

The impact of terminal water stress also affected retranslocation of assimilates accumulated before anthesis. The simplest estimate of the amount retranslocated is by the method of Gallagher *et al.* (1976) which assumes that the decrease in weight of the non-grain biomass between DC65 and maturity equates to net retranslocation. In this study it is termed 'apparent retranslocation'. Apparent retranslocation was calculated for the three sites and graphed against biomass at DC65 (Figures 4.2, 4.3, 4.4) since the two were positively correlated. Previous studies have shown increasing DC65 biomass associated with increases in the amount of non-grain biomass apparently retranslocated to the grain (Midmore *et al.* 1984; and Angus *et al.* 1991).

An increase in drought severity between sites does not increase the magnitude of apparent retranslocation to the grain though the proportion of final grain yield appears to

increase markedly. Apparent contribution of retranslocation to grain yield ranged from 37-39 % at GES91 up to 73-97 % at WAG91 while the late sown PUC91 ranged from 15-47%. Angus *et al.* (1991) reported apparent retranslocation of between 84-124 % of grain yield with increasing DC65 biomass in an experiment in which yields increased with applied nitrogen. The proportion of grain yield accounted for by apparent retranslocation is surprisingly high considering the values at WAG91 where crops hayed off.

4.4.1.1 *Decrease in leaf weight between anthesis and maturity*

The problem with the method of Gallagher *et al.* (1976) is that it does not take into account tissue formed before DC65 that is lost in the post-anthesis period, such as leaf fall (Barley and Naidu 1964, Austin *et al.* 1980a) or saprophytic decay of lower leaves (Bidinger *et al.* 1977). This error is likely to overestimate the pre-anthesis contribution to grain filling. Austin *et al.* (1977) conclude that leaves do lose weight but that the export of dry matter is difficult to quantify because dead leaves are fragile and tend to drop off the plant. The loss of leaf material due to no rain or disease was estimated to be 100 g m^{-2} , or 50 % of the DC65 leaf dry matter by Bidinger *et al.* (1977) while Austin *et al.* (1977) consider this proportion too high and estimate leaf fall to be 20% for a leaf biomass of 150 g m^{-2}.

Conversely, calculating retranslocation in this way does not take into account non-grain tissues formed after DC65 that are measured at maturity but not at DC65, thereby decreasing the magnitude of apparent retranslocation. This error is likely to be greatest in the absence of water stress due to the continued growth of the stem after DC65 (Bonnet and Incoll 1992a; Borrell *et al.*1989, 1993) and cell-wall thickening and lignification (Stoy 1965; Pearce *et al.* 1988) which lead to an increase in structural biomass thereby reducing apparent retranslocation.

In the present study, leaf biomass almost doubled from 16 to 29% of the total above ground biomass at DC65 for the control and the highest rate of nitrogen (Table 4.7). Individual spike weight did not vary significantly and hence spike biomass increased due to a greater spike density. As a proportion of the total biomass, however, spike biomass remained relatively constant and did not increase by more than 2%. Stem biomass increased by 10 % with nitrogen fertilisation at PUC91 but was not significantly different at either GES91 or WAG91. Stem biomass did not vary greatly due to increases in spike density being countered by a decrease in individual stem weight. This was despite a taller crop with nitrogen fertilisation and it resulted in up to 24% reduction in stem linear density (Table 4.7). The contribution of stems to total biomass fell from 64% to 50% with increasing nitrogen fertilisation. Increases in total biomass with nitrogen

fertilisation are, therefore, largely due to the greater leafiness of the high nitrogen crop, as shown by Stirzaker (1983). Because leaves are fragile organs it is not surprising that losses can occur due to saprophytic decay, leaf fall in the field and during sampling procedures and that these losses are likely to be greater with increased leafiness.

Table 4.8 shows biomass distribution at the final harvest for WAG91. In contrast to the DC65 harvest, leaves made up only between 11-15% of the biomass. Between DC65 and maturity leaf biomass fell by 40 % in control and 49% (77-151 g m^{-2}) at the highest rate of nitrogen. Non-grain spike biomass fell by less than 10%. Individual stem weight fell to a greater extent for control crops than fertilised ones, resulting in high nitrogen stems weighing more than control stems in contrast to the DC65 harvest. Linear density was not significantly different at maturity. The decrease in stem biomass for control crops was almost double that at high nitrogen.

Assuming that WSC (Kiniry 1993) and protein (Bell and Incoll 1990) are the two major sources of dry matter for export from senescing leaves between DC65 and maturity, a budget can be determined to estimate the decrease in dry matter associated with retranslocation and respiration. The decrease in WSC and protein between DC65 and maturity suggests that approximately 13% (21-40 g m^{-2}) of DC65 leaf biomass was retranslocated for the treatment extremes at WAG91 (Table 4.14). Approximately two thirds of the decrease in leaf biomass remains unaccounted for (57-112 g m^{-2}). Is it possible that leaf fall, saprophytic decay and poor sampling procedures could result in such losses? Close examination of several studies (Spiertz and Ellen 1978, Spiertz and van de Haar 1978, Blacklow and Incoll 1981) which report WSC and nitrogen levels for leaves at DC65 and maturity help answer this question.

Reported sampling procedures in these studies were precise and careful and so it is unlikely that poor sampling resulted in leaf loss. Saprophytic decay or leaf fall were also unlikely to have contributed significantly as each experimental crop was sprayed with fungicides to prevent leaf diseases and frequent sampling during grain filling meant that fallen leaves, if any, would be picked up at sampling times and not have had sufficient time to decay. In these studies between 30-40% (61-73 g m^{-2}) of the DC65 leaf biomass had been remobilised or lost by maturity. A budget of leaf WSC and protein between DC65 and maturity suggests that retranslocation and respiration accounts for between 33-51 % (27-33 g m^{-2}) of the decrease in leaf biomass. High nitrogen crops tended to account for more of the decrease in weight.

This brief analysis illustrates that the unexplained loss of leaf biomass during grain filling is a general problem and at best the decrease in WSC and protein can only account for 50% of this decrease in leaf weight. These combined data (Spiertz and Ellen 1978,

Spiertz and van de Haar 1978, Blacklow and Incoll 1981) account for a mean of 38% of the decrease in leaf biomass for control and 45% for high nitrogen crops and were used to calculate estimate A of maturity leaf biomass at WAG91 (Table 4.14). Estimates for the maturity leaf biomass range from 141 to 222 g m^{-2}.

Table 4.14 Leaf biomass, protein and WSC at anthesis and maturity and estimates of maturity leaf biomass and leaf fall at WAG91.

	($g\ m^{-2}$)								
	DC65			Maturity			Estimate of maturity leaf		Leaf
Rate of N kg N ha^{-1}	Leaf biomass	Leaf Protein	Leaf WSC	Leaf biomass	Leaf Protein	Leaf WSC	A[a]	B[b]	fall[d]
0	196	18	10	118	4	4	141	130	17
80	258	32	12	140	6	4	176	172	34
120	275	37	12	162	9	4	189	183	25
200	311	45	12	159	12	4	222	207	55
(l.s.d.[c])	19	4	2	27	2	n.s.	n.a.		n.a.

[a] Estimate A based on WSC and protein budget

[b] Estimate B based on 33% of anthesis biomass retranslocated

[c] l.s.d. P = 0.05

[d] Uses mean of estimates A and B in calculation.

n.s. not significant; n.a. not applicable

A review of the literature provided 6 studies in which sampling techniques were considered of sufficient standard to minimise leaf losses (Spiertz and Ellen 1978; Spiertz and van de Haar 1978; Blacklow and Incoll 1981; Austin *et al.*1977; Austin *et al.*1980b; Bell and Incoll 1990). The mean decrease in the leaf biomass between DC65 and maturity was 33 ±2% of the DC65 leaf biomass. This figure is calculated from 20 estimates of the decrease in leaf biomass. It was applied to the data from WAG91 to calculate estimate B of the maturity leaf biomass (Table 4.14). This method estimates maturity leaf biomass to range from 130 to 207 g m^{-2} with increasing nitrogen fertiliser.

There is surprisingly good agreement between these two estimates for the maturity leaf biomass. The unexplained loss of leaf biomass at WAG91, therefore, ranges from 17 g m^2 for low nitrogen crops to 55 g m^{-2} at the highest rate of nitrogen (Table 4.14), or 9 to 18 % of DC65 leaf biomass. This range is similar to the estimate of 30 g m^{-2} by Austin *et al.* (1977) but is far less than 100 g m^{-2} reported by Bidinger *et al.* (1977). If the mean of

the maturity leaf biomass estimates is used to recalculate a WSC and protein budget (Table 4.15), and leaf fall calculated in Table 4.14 is included, then significant amounts of leaf biomass (41 to 56 g m^{-2}) remain unaccounted for (Table 4.15).

Table 4.15 Change in leaf biomass, protein and WSC (g m^{-2}) between anthesis and maturity for at WAG91

Rate of N (kg N ha^{-1})	Anthesis biomass	Maturity estimate[a]	Change in biomass	Change in protein	Change in WSC	Unknown decrease
0	196	125	-61	-14	-6	41
80	258	174	-84	-26	-8	50
120	275	186	-89	-28	-8	53
200	311	214	-97	-33	-8	56
(l.s.d. P=0.05)	19	n.a.	n.a.	n.a.	n.a.	n.a.

[a] Mean of estimates A and B from Table 4.14

n.s. not significant; n.a. not applicable

Mineral elements make up a small proportion of leaf biomass (Masle *et al.* 1992) and as such are unlikely to contribute significantly to the unknown decrease in leaf weight. Is it possible that some of the more accessible cell wall components are mobilised between DC65 and maturity and contribute to grain filling? Work by Ballard *et al.* (1990) with an ungrazed ryegrass pasture would suggest that this is the case. Hemicellulose, calculated as the difference between Neutral Detergent Fibre (NDF, estimate of cell walls) and Acid Detergent Fibre (ADF, estimate of cellulose and lignin), falls from 31% of the top leaf dry weight (56% of NDF)at DC65 to 20% of the leaf dry weight (32% of NDF) at the end of grain-filling. In absolute terms, due to the decrease in neutral detergent solubles (estimate of cell contents), hemicellulose fell by 60% while the dry weight of other cell wall components showed minimal change. Interestingly, hemicellulose did not decrease to the same extent in leaf sheaths and stems. Pearce *et al.* (1988) also showed that hemicellulose decreased between DC65 and maturity for wheat stem, sheaths and leaves. Organ dry weights are not presented by Pearce *et al.* (1988) but the fall in NDF of leaves between anthesis and maturity can be accounted for by the decrease in hemicellulose. The decrease in hemicellulose from 56% of NDF at DC65 to 35% of NDF at maturity is similar to that reported by Ballard *et al.* (1990). The level of stem hemicellulose did not change while leaf sheath hemicellulose declined slightly. An important question regarding this previously unaccounted for decrease in weight remains - how efficient is this mobilisation of hemicellulose and does it contribute to grain growth or is it lost through respiration? There is no reason to expect that the hydrolysis of the predominantly β-1-4 and β-1-3 bonding of sub-units in hemicellulose (Carpita and

Gibeaut 1993) would require any more energy than the hydrolysis of β-2-1 and β-2-6 bonding in fructans of wheat (Bancal *et al.* 1992). Once liberated, the sugar sub-units of hemicellulose could be translocated to the grain as efficiently as those of fructans.

Table 4.16 shows the NDF and ADF analyses for leaves at DC65 and maturity for WAG91. Hemicellulose present in the leaves at DC65 ranged from 22% of leaf dry weight (35% of NDF) for control crops to 20% of dry weight (34% of NDF) at the highest rate of nitrogen. At maturity hemicellulose content had only fallen slightly and made up from 21% of leaf dry weight (31% of NDF) for control crops to 19% of dry weight (28% of NDF) at the highest rate of nitrogen. While the concentration of hemicellulose in the leaves at maturity was similar to those reported by Ballard et al. (1990) and Pearce *et al.* (1988), the concentration at DC65 is almost half that previously reported.

If the decrease in hemicellulose between DC65 and maturity is calculated from Tables 4.15 and 4.16 the estimates account for less than 50% of the unknown decrease in leaf biomass in Table 4.15. Calculation of leaf ADF biomass at DC65 and maturity results in an apparent decrease in leaf ADF biomass (data not shown). This is highly unlikely given that cellulose and lignin are immobile, and it is more probable that leaf fall was underestimated in Table 4.14. Leaf fall was probably underestimated because the experiments from the literature on which the estimates were based, were well watered throughout. That is, retranslocation from leaves in the absence of water stress is likely to be greater than for severely water stressed leaves, most of which were senescing at DC65, for WAG91. Indeed, leaf nitrogen concentration at maturity fell to 0.7% at high nitrogen in the absence of water stress (Spiertz and Ellen 1978) but only fell to 1.2% for high nitrogen at the water stressed site WAG91.

If leaf ADF biomass is assumed to be constant for leaves between DC65 and maturity, then new estimates of maturity leaf biomass can be calculated and retranslocation of WSC, protein and hemicellulose and leaf fall can be estimated (Table 4.17). Apparent retranslocation of leaf dry matter is therefore estimated to amount to between 11-19% of DC65 leaf biomass at WAG91. A budget of WSC, protein and hemicellulose provides a larger estimate than retranslocation of dry matter for control crops but underestimates it at high nitrogen. Hemicellulose appears to constitute approximately 30% of the dry matter moved out of the leaves.

Table 4.16 Concentrations of NDF, ADF and by difference hemicellulose in the leaves at anthesis and maturity for nitrogen treatments at WAG91.

	Nitrogen rate (kg N ha^{-1})				
Analysis	0	80	120	200	l.s.d.[a]
			Anthesis		
NDF (% DW)	62.8	60.3	60.1	58.9	1.0
ADF (% DW)	40.8	39.2	40.2	38.6	n.s.
Hemicellulose (% DW)	22.0	21.1	19.9	20.3	n.s.
Hemicellulose (% NDF)	35.0	35.0	33.1	34.5	n.s.
			Maturity		
NDF (% DW)	65.9	67.1	67.5	66.0	n.s.
ADF (% DW)	45.4	46.2	47.3	47.2	1.5
Hemicellulose (% DW)	20.5	20.9	20.2	18.8	n.s.
Hemicellulose (% NDF)	31.0	31.0	29.9	28.5	n.s.

[a] (l.s.d. $P = 0.05$)

Table 4.17 Leaf biomass at DC65, maturity leaf biomass estimate based on leaf ADF and a budget of WSC, protein and hemicellulose at WAG91.

			(g m^{-2})					
Rate of N (kg N ha^{-1})	Leaf biomass		Leaf fall[b]	Apparent retrans-location	Change in WSC	Change in protein	Change in hemi-cellulose	Estimated retrans-location
	DC65	Maturity[a]						
0	196	175	57	21	-5	-13	-7	25
80	258	219	79	39	-6	-22	-8	36
120	275	235	73	40	-6	-24	-6	36
200	311	253	94	58	-6	-26	-16	47

[a] Estimate of maturity leaf biomass based on constant ADF biomass between anthesis and maturity.

[b] By difference of estimated maturity leaf biomass and that measured at maturity

The high nitrogen crops at WAG91 experienced greater water stress than control crops from 3 weeks prior to DC65 and by DC65 a significant proportion of the leaf biomass was dead. This contrasts to the leaf material of Ballard et al. (1990) and Pearce *et al.* (1988) which was green at DC65 and had been grown under well watered conditions. It is likely that most of the hemicellulose at WAG91 had already been remobilised from the leaves by DC65. This contention is supported by the NDF and ADF data for total above ground biomass at DC65 and non-grain biomass at maturity, for the well-watered site at GES91 (Table 4.18).

Table 4.18 Concentrations of NDF, ADF, and hemicellulose in the anthesis and maturity non-grain biomass and decrease in hemicellulose between anthesis and maturity for GES91.

	Nitrogen rate (kg N ha^{-1})							
Analysis	0	40	60	120	160	200	240	l.s.d.[a]
				Anthesis				
NDF (% DW)	59.7	61.5	60.1	61.3	62.3	62.7	62.2	1.7
ADF (% DW)	35.4	36.5	36.6	36.3	36.9	37.3	37.5	0.8
Hemicellulose (% DW)	24.3	25.0	24.5	25.0	25.4	25.4	24.7	n.s.
Hemicellulose (% NDF)	40.7	40.5	40.1	40.8	40.8	40.5	39.8	n.s.
				Maturity				
NDF (% DW)	79.7	82.4	82.8	82.6	82.2	82.0	81.6	1.9
ADF (% DW)	53.0	54.4	54.4	54.1	54.6	54.6	55.0	0.7
Hemicellulose (% DW)	26.7	28.0	28.4	28.5	27.5	27.5	26.6	n.s.
Hemicellulose (% NDF)	33.5	33.9	34.3	34.5	33.5	33.5	32.6	n.s.
	Decrease in hemicellulose between anthesis and maturity							
Hemicellulose (g m^{-2})	38	34	36	41	47	57	61	n.s.

[a] (l.s.d. $P = 0.05$)

In the absence of post-anthesis water stress NDF increased from 60-63% of the DC65 biomass to 80-83% of the non-grain biomass at maturity due to the decrease in cell contents. Despite the decrease in non-grain biomass between DC65 and maturity the biomass of NDF (data not shown) increased. The continued growth of the stem after DC65 (Bonnett and Incoll 1992; Borrell *et al.* 1989, 1993) and cell-wall thickening and lignification (Stoy 1965; Pearce *et al.* 1988), which lead to an increase in structural biomass, would account for the increase in biomass of NDF. ADF increased as a proportion of NDF (data not shown) between DC65 and maturity which means, by difference, that hemicellulose decreases. Hemicellulose made up 24-25% of DC65

biomass (40-41% of NDF) and 27-29% of non-grain biomass at maturity (33-34% of NDF). If the fall in hemicellulose of 33-61 g m^{-2} from the non-grain biomass between DC65 and maturity represents rertranslocation, then it constitutes approximately 5-8% of the grain yield.

If it is assumed that most of the hemicellulose remobilised comes from the leaves (Pearce et al. 1988; Ballard *et al.* 1990) then the decrease in hemicellulose was similar in magnitude to an estimate of the combined decrease in protein and WSC which agreed with estimates derived from the above publications and at similar proportions would account for the unexplained leaf biomass from Spiertz and Ellen (1978), Spiertz and van de Haar (1978) and Blacklow and Incoll (1981).

Heyland (1959), investigating the structural carbohydrates of spring wheats and rye in relation to lodging resistance during the grain filling period, found that hemicellulose content of the straw (leaves, leaf sheaths and stem) increased prior to anthesis. The author postulated that the hemicellulose was a storage of reserves for use during the grain filling period. It appears that hemicellulose can act as a significant, previously disregarded, carbohydrate reserve for grain filling though the environmental conditions which affect its synthesis and remobilisation are unclear.

4.4.1.2 *Water soluble carbohydrate reserves*

Another problem with the method of Gallagher *et al.* (1976) is that it has led to the assumption that greater biomass at anthesis equates with higher pre-anthesis assimilate reserves (eg. Fischer 1979). This assumption comes about through the indirect evidence that apparent retranslocation increases with greater anthesis biomass (Gallagher *et al.* 1975,1976; Angus *et al.* 1991; Palta and Fillery 1995a). In addition Campbell *et al.* (1983) have showed that for wheat plants grown in pots under controlled environment conditions, WSC retranslocation to grain increased at high nitrogen status.

However, work by Spiertz and Ellen (1978) and Spiertz and van de Haar (1978) using different nitrogen treatments, and Stapper and Fischer (1990b) using different genotypes, showed that this is not the case for well managed crops. Greater biomass at DC65, whether due to nitrogen fertilisation or genotypic variation, was associated with an increase in spike density and a reduction in WSC. This reduction was as a percentage of stem mass or per unit ground area. This reduction of WSC with increased biomass is most likely due to a greater proportion of assimilates being used for structural materials (Spiertz and van de Haar 1978; Stapper and Fischer 1990b; Schnyder 1993) and as carbon skeletons in additional protein (Austin *et al.* 1977) and higher respiration rates due to the greater biomass (Spiertz and Ellen 1978) and higher nitrogen concentration of

tissues (Spiertz and van de Haar 1978, Pearman *et al.* 1981, McCree 1983, Amthor 1989).

These findings explain the reduction in individual stem weight and linear density with increasing nitrogen at DC65 for GES91, PUC91 and WAG91 (Table 4.7) and are corroborated by the WSC data presented in Table 4.9. Although additional nitrogen promoted leaf area development and green area duration and hence the photosynthetic potential, the content of WSC fell from 32 to 14% of the stem dry weight with increasing nitrogen. Stems contained far greater reserves than either spikes or leaves. Water soluble carbohydrates ranged from 25 to 10% of the total above-ground biomass at DC65.

The decrease in water soluble carbohydrates from stem internodes during grain-filling parallels the decrease in dry matter, accounting for 96 - 100% of stem weight loss (Austin *et al.* 1977, Austin *et al.*1980a). When the leaf sheaths are included in stem samples, as in the present study, a smaller proportion of the decrease in weight of stems is attributable to the decrease in WSC (Kühbauch and Thome, 1989). Schnyder (1993) estimates much of the material not accounted for by WSC is probably accounted for by the mobilisation of protein. The retranslocation of WSC and protein between DC65 and maturity from stems at WAG91 accounted for between 96 - 80% of the decrease in weight for control and high nitrogen respectively. As already mentioned, significant amounts of hemicellulose are remobilised from the sheaths of ryegrass (Ballard *et al.* 1990) and wheat (Pearce *et al.* 1988) between DC65 and physiological maturity. A significant proportion of the unaccounted for decrease in stem biomass at WAG91 may be due to remobilisation of hemicellulose.

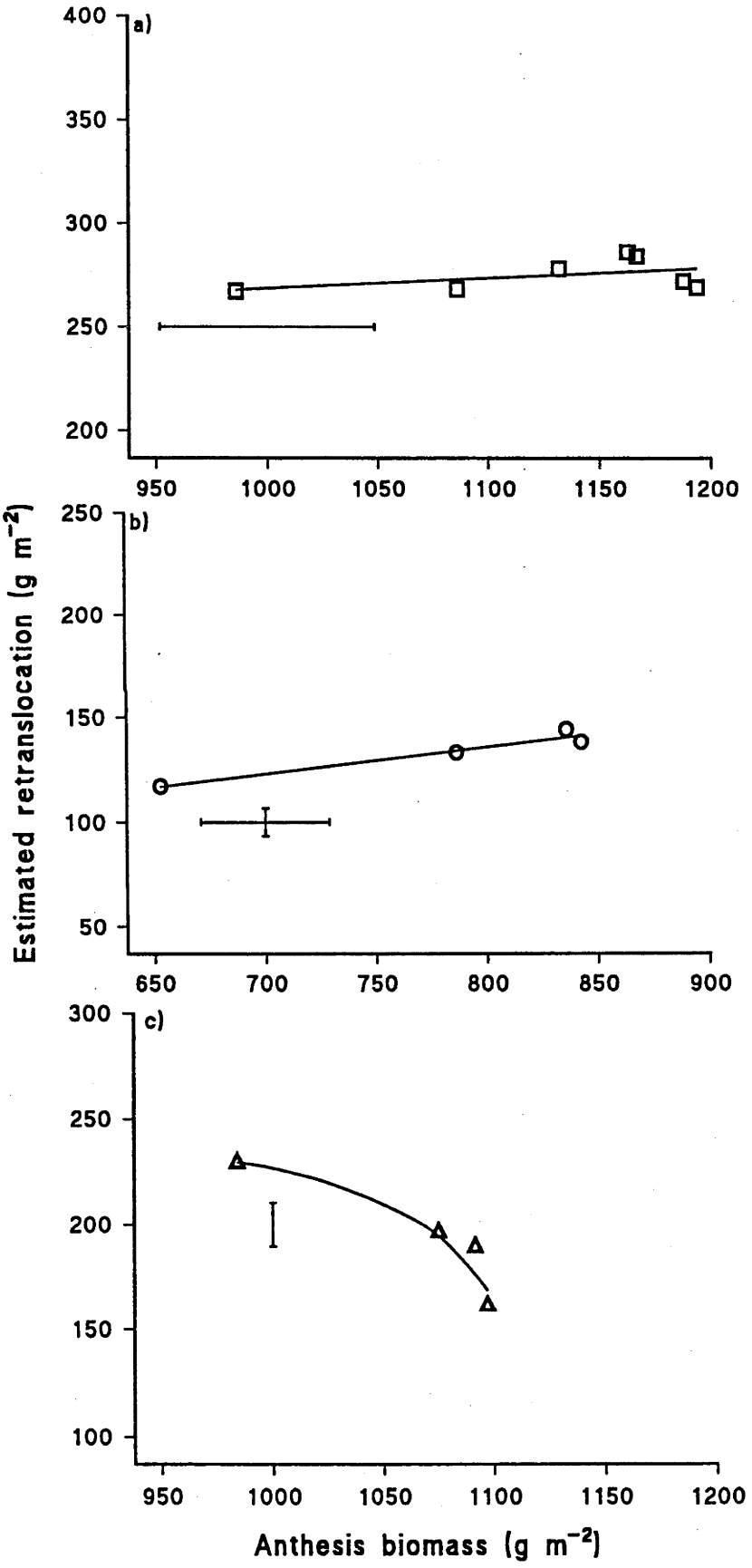
The implication of reduced WSC with increasing biomass is that the high nitrogen crop has a greater dependence on current assimilate to achieve its potential yield. This is corroborated by the number of kernels per gram of available WSC at DC65. Values are similar for GES91 and WAG91 ranging from 61 kernels g WSC^{-1} for control crops to 131 kernels g WSC^{-1} for the highest rate of nitrogen. The late sown PUC91 is even more dependent on current assimilate with values ranging from 100 to 198 kernels g WSC^{-1}. Spiertz and Ellen (1978) propound that at high nitrogen levels stem reserves are less important because of the availability of more assimilates produced by the prolonged photosynthetic activity of the green tissues. This may be the case in the non-water limited European environment, but in the event of terminal drought the benefits of longer green area duration are limited by water stress and WSC reserves constitute a greater proportion of final grain yield.

At GES91 no water stress was observed during grain filling with grain yield ranging from 607 to 798 g m^{-2} with 0 to 240 kg N ha^{-1}. Water soluble carbohydrates utilised between DC65 and maturity ranged from 214 to 159 g m^{-2} (Table 4.9). Work by Winzeler *et al.* (1990) showed that once laid down, water soluble carbohydrates were not turned over. This finding is supported by Wardlaw and Porter (1967) and Bell and Incoll (1990) who showed that most respiration during grain filling used current rather than stored assimilate. If it is assumed that nitrogen lost from the non-grain biomass between DC65 and maturity is transported to the grain, then this would account for 53 g m^{-2} protein for control crops and 113 g m^{-2} at the highest rate of nitrogen. Hemicellulose in the non-grain biomass decreased from between 38-61 g m^{-2}. Thus, by difference, an estimate of net assimilation during grain filling was 302 g m^{-2} in control crops and 465 g m^{-2} at the highest rate of nitrogen.

In contrast to the non-water limited site at GES91 grain yield at WAG91 fell from 374 g m^{-2} to 284 g m^{-2} with 0 to 200 kg N ha^{-1}. Water soluble carbohydrates utilised between DC65 and maturity ranged from 194 g m^{-2} to 105 g m^{-2}. Nitrogen in the above-ground biomass declined in all crops between DC65 and maturity so protein retranslocation is assumed to be the protein present in grain. This accounts for 36 g m^{-2} and 55 g m^{-2} in controls and 200 kg N ha^{-1}. Hemicellulose from the leaves was a minor contributor and is estimated to contribute between 7 g m^{-2} and 16 g m^{-2} to grain filling. Leaf sheaths may also contribute hemicellulose as already mentioned but levels in this plant part were not determined. The grain yield accounted for by an estimate of net assimilation, therefore, was 137 g m^{-2} for control crops and 108 g m^{-2} for the highest rate of nitrogen. The estimate of net assimilation reflects the degree of post-anthesis water stress. The decrease in WSC with increasing nitrogen had no apparent detrimental effect on the grain yield at GES91 but with increasing post-anthesis water stress at PUC91 and WAG91 lower reserves limited the buffering capacity of the high nitrogen crops against a lack of post-anthesis assimilation.

As already seen, rates of nitrogen which give positive yield responses, result in greater soil water extraction, to meet the demand for transpiration. With corrections due to leaf fall made to the final at WAG91, retranslocation from non-grain biomass can be estimated from a budget of WSC and protein (Figure 4.7c). Samples from GES91 and PUC91 could not be corrected for leaf fall, but sampling losses would be lower due to damp conditions at the maturity sampling which reduces leaf fragility (Figure 4.7a,b). It can be seen from this figure that increasing DC65 biomass does not always lead to proportional increases in mobilisable reserves available for grain filling as has often been assumed. This means that a freely tillering, high nitrogen crop is more dependent on post-anthesis assimilation to reach potential yield. Severe post-anthesis water stress, such as at WAG91, can result in limited retranslocation to grain and a reduction in net

Figure 4.7 Relationship between estimated retranslocation between anthesis and maturity versus biomass at DC65 for a) GES91, b) PUC91, c) WAG91. Bars indicate the l.s.d (P=0.05), absence of a bar indicates non-significance.



assimilation leading to lower grain yields. This could also be the case if green area duration was limited by nitrogen deficiency or foliar disease.

4.4.2 Nitrogen dynamics of above-ground biomass

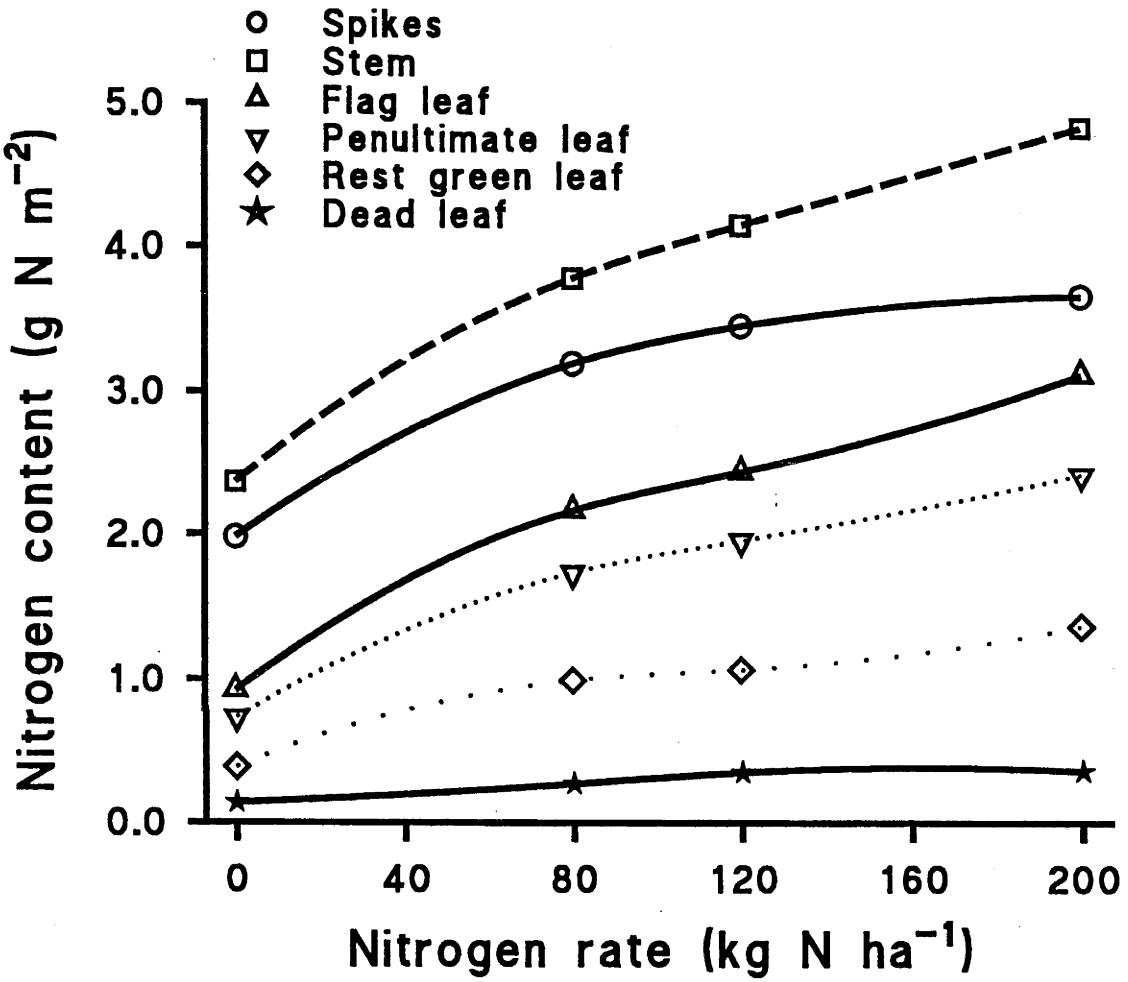
A characteristic of responses to nitrogen fertilisation in semi-arid environments is the large season to season, and site to site variability due mainly to wide fluctuation in rainfall amount and distribution. At each site, uptake of supplementary nitrogen resulted in greater biomass production to DC65, and higher tissue nitrogen concentrations stimulating increases in spike density. Only at GES91, in the absence of water stress, were significant differences in biomass maintained at maturity. The post-anthesis nitrogen economy is equally as important as the carbon economy due to the dependence of current assimilate supply on adequate levels of nitrogen to maintain photosynthetic capacity.

Apparent fertiliser recovery (AFR) decreased with additional rates of fertiliser at each site and varied considerably between sites. Except for the 40 kg N ha^{-1} crop, AFR was similar at WAG91 and GES91 reflecting the absence of water stress during the pre-anthesis period permitting the uptake of nitrogen from moist topsoil (Gregory *et al.* 1981; Clarke *et al.* 1990). By comparison AFR at PUC91 was low, possibly because the late sowing date compressed the time interval available for nitrogen uptake (DC10 to DC65 120 days PUC91, 143 days GES91, 149 days WAG91). In addition, the relatively low nitrogen status of the site (total N, 0-0.1m depth PUC91 0.14%, GES91 0.19%, WAG91 0.15%) may have led to competition for soil mineral nitrogen between the crop and the soil microflora.

The high proportion of total nitrogen uptake contained in stems at DC65 at GES91 and the relatively low proportion contained in stems at WAG91 reflects the degree of water stress at that time. The higher concentration of nitrogen in stems at GES91 represented recent uptake and storage. The similar nitrogen concentrations of leaves at GES91 and WAG91 for equivalent fertiliser rates indicates the importance of maintaining adequate levels of nitrogen in the leaves to maximise the potential source of current assimilate in the event of reduced nitrogen uptake due to water stress.

Late sowing at PUC91 resulted in a leafier crop of higher nitrogen concentration. At PUC91, nitrogen content of the three green leaf layers at DC65 more than doubled from the control crops to those with the highest rate of nitrogen (Figure 4.8). The nitrogen content of the leaves increased to a greater extent than other plant parts leading to a greater proportion of the total plant nitrogen present in the leaves.

Figure 4.8 Nitrogen content of leaf strata, stem and spikes at anthesis as affected by rate of nitrogen fertiliser for cv. Janz at PUC91. The data are not cumulative. The l.s.d. (P = 0.05) are, 0.14 dead leaf, 0.23 rest green leaf, 0.26 penultimate leaf, 0.44 flag leaf, 0.36 stem, 0.31 spike.



There was a net loss of nitrogen from the above-ground biomass between DC65 and maturity from all crops at WAG91 and all but the control plots at PUC91. Post-anthesis losses of nitrogen are likely to occur as a result of deamination of amino acids for the use of carbon skeletons in grain filling (Wetselaar and Farquhar (1980); Austin *et al.* 1977). Data from Barley and Naidu (1964) suggest that losses of nitrogen can occur when there is a lack of current assimilate caused by increased post-anthesis water stress of nitrogen fertilised crops and later flowering varieties. In contrast, at GES91 there was post-anthesis uptake of nitrogen from the soil, the magnitude of which diminished with increasing rates of nitrogen. In the absence of water stress, mineralisation of nitrogen in the topsoil supplied sufficient mineral nitrogen for uptake to meet the simultaneous demands for grain filling and maintenance of photosynthetic area. Indeed, it was observed (data not shown) that high nitrogen plots maintained some green flag leaves to physiological maturity.

Nitrogen harvest index (NHI) was higher at GES91 than at either PUC91 or WAG91 presumably due to the lack of water stress enabling continued retranslocation of amino acids up until the completion of grain filling. The high values of NHI at GES91 are similar to those reported by Blacklow and Incoll (1981) exhibiting a slight decrease with the addition of nitrogen due to the incomplete remobilisation from the leaves at high nitrogen. Intermediate water stress at PUC91 reduced NHI to levels comparable with Bulman and Smith (1994) and Spiertz and Ellen (1978). The addition of nitrogen fertiliser resulted in slight increases in NHI at PUC91, presumably due to a lack of post-anthesis nitrogen uptake as a result of the dry soil surface (Clarke *et al.* 1990). This resulted in the need to remobilise existing reserves of nitrogen to meet the demand for nitrogen by the developing grain. As water stress became severe in high nitrogen crops during grain filling it is likely that carbon became limiting and the deamination of leaf proteins resulted in a decrease in non-grain nitrogen further increasing NHI. At WAG91, water stress was so severe that even control crops lost nitrogen from the above-ground biomass and grain-filling ceased prematurely, as evidenced by the low kernel weight and high protein concentration. NHI decreased dramatically with increasing rates of nitrogen fertiliser probably because the severe water stress inhibited metabolism associated with protein remobilisation.

Nitrogen allocation per kernel (NAK), increased with additional nitrogen fertilisation at GES91 and PUC91. At WAG91, NAK initially increased at low nitrogen but plateaued at high nitrogen. As with NHI, NAK reflects the degree of water stress at each site. Post-anthesis nitrogen uptake and/or cool, moist growing conditions allowed complete remobilisation of nitrogen reserves to the grain at GES91 while at WAG91 severe water stress limited remobilisation of nitrogenous compounds resulting in the lowest values of NAK among the three sites and a dramatic decrease in NHI as already discussed.

Despite the low values of NAK, grain protein concentration remained high at WAG91 due to low kernel weights.

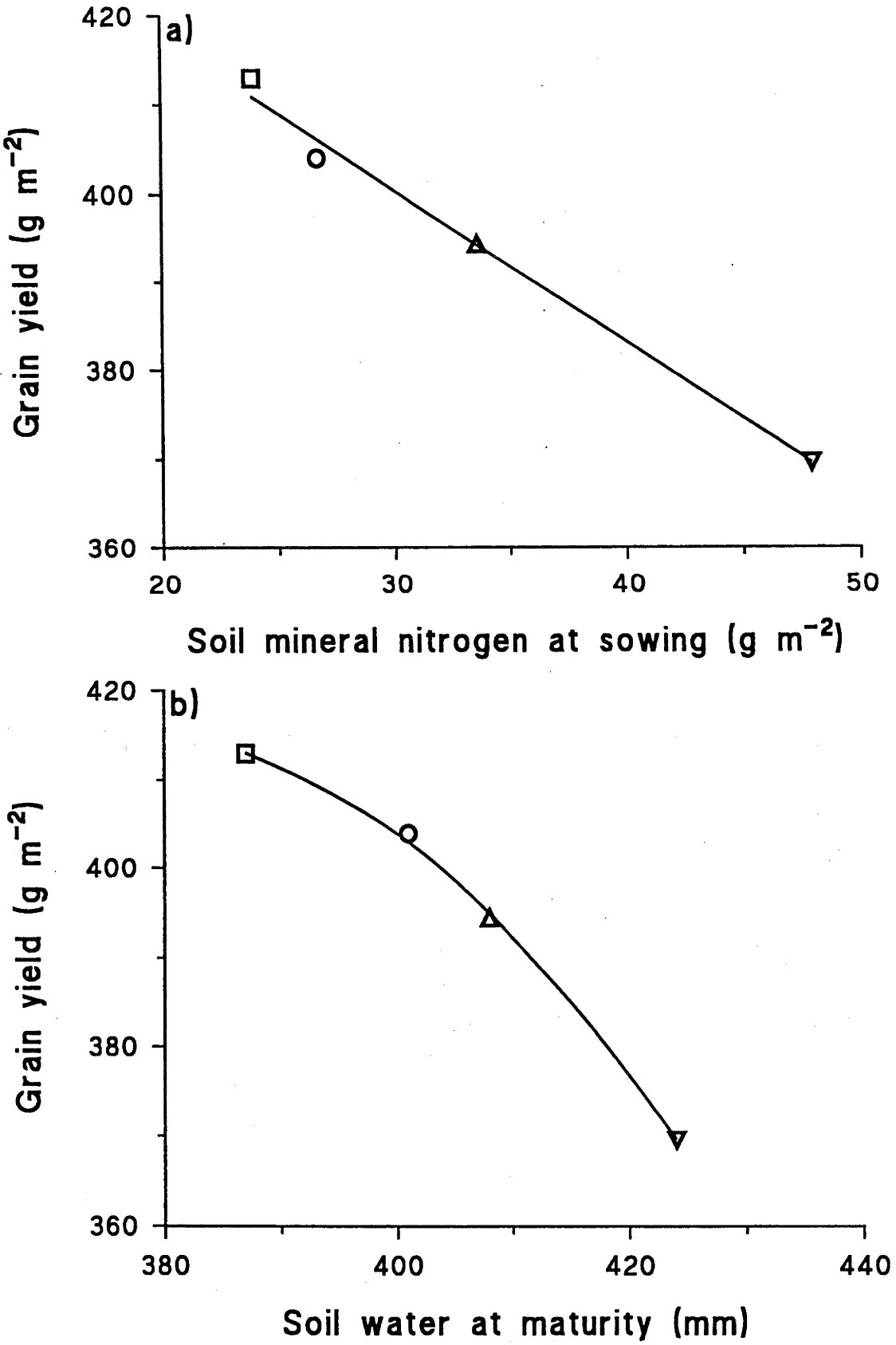
4.4.3 Water use and transpiration efficiency

As already discussed in Section 4.4.1, nitrogen fertilisation leads to a greater extraction of soil water under terminal drought conditions except in the event of haying-off. The large negative yield response to nitrogen fertiliser at WAG91 resulted in 10 mm less soil water extraction between the depths of 0.8 and 1.7 m. In the study by Barley and Naidu (1964), application of nitrogen resulted in a similar bell shaped yield response curve to that at PUC91. The highest rate of nitrogen produced the same yield as that of the control crop (224 g m^{-2}) but less than that of the crop of intermediate nitrogen status (269 g m^{-2}). Assuming an average bulk density for soil between 0-0.75m of 1.6 g cm^{-3} the highest rate of nitrogen had extracted 12mm more water than the control crop by maturity despite the lack of yield difference. In contrast there was a negligible difference in water extraction between the intermediate and the highest rates of nitrogen despite a yield reduction of 45 g m^{-2}.

Another example of haying-off leading to poor soil water extraction is shown in Figure 4.9 using data extracted from Kirkegaard *et al.* (1994) which shows the relationship between grain yield and soil mineral nitrogen at DC10 and the relationship between grain yield and soil water at maturity for wheat following various breakcrops. There is a linear decrease in grain yield with increasing soil mineral nitrogen at DC10 similar to the relationship at WAG91 for grain yield and nitrogen fertiliser rate. In contrast to the crops reported by Barley and Naidu (1964), those which hayed off extracted significantly less water from the soil than those which did not. For example, wheat following mustard used 98 mm of soil water between DC65 and maturity, while wheat after peas only used 57 mm. Kernel weight was low for all treatments and fell significantly from 23.3 mg for wheat following canola to 21.1 mg for wheat following peas.

Additional soil water extraction by 80 kg N ha^{-1} crops at PUC91 amounted to an additional 25 mm of water available over the whole season and resulted in a positive grain yield response of 38g m^{-2}. Due to the higher water use to DC65 this translated to 8 mm less water used during grain filling than for controls. However, the drier soil at DC65 and maturity, for 80 kg N ha^{-1} crops relative to controls indicates that the fertilised crop was always more water stressed while extracting the final 82 mm than controls were while extracting the final 90 mm. In addition, growth and maintenance respiration were likely to be greater for fertilised crops than controls. This results from a greater biomass, higher nitrogen concentration and higher tissue temperatures (Loomis and Connor 1992) due to differential water stress. It is not surprising, therefore, that transpiration

Figure 4.9 Relationship between grain yield at 12% moisture content with a) soil mineral nitrogen (0-2.0m) present at sowing and, b) soil water present (0-2.0m) at maturity for wheat following mustard (□), canola (O), linseed (Δ), and peas (∇). (Data extracted from Kirkegaard *et al.* 1994).



efficiency decreased with additional fertiliser due to an increase in the ratio of respiratory losses to current assimilation and the low reserves of WSC at DC65.

This reduction in transpiration efficiency in response to nitrogen is contrary to the findings of work investigating the interaction of nitrogen fertilisation with transpiration efficiency in pots. When spaced plants are given nitrogen fertiliser, the specific leaf area (SLA) of new leaves decreases (become thicker) and hence photosynthetic capacity per unit leaf area increases, which in turn positively influences transpiration efficiency (Evans 1983). In wheat crops, however, addition of nitrogen fertiliser causes a large increase in tillering and hence leaf area but in contrast to spaced plants, SLA of crops increases (Fischer 1993). This means that the addition of nitrogen can have a self diluting effect due to increases in biomass and SLA, possibly reducing transpiration efficiency. By DC65, despite a higher nitrogen concentration in the flag leaf of a fertilised crop, Gregory *et al.* (1981) found, in the absence of water stress, that net photosynthetic rates were similar to low nitrogen crops at DC65 and midway through grain filling.

The carbon isotope discrimination data for PUC91 indicate that there was no difference in transpiration efficiency up to DC30. This means that changes in photosynthetic capacity due to an increase in nitrogen concentration and SLA with nitrogen fertilisation were balanced by higher stomatal conductance. However transpiration efficiency up to DC65, estimated using the method of Cooper *et al.* (1983), was lower with applied nitrogen, falling from 6.2 to 5.4 g m^{-2} mm^{-1}. Post-anthesis transpiration efficiency was less than that prior to DC65 but was still lower in fertilised crops, falling to 4.3 g m^{-2} mm^{-1} for control and 4.0 g m^{-2} mm^{-1} for nitrogen fertilised crops. Due to the late sowing, organ nitrogen concentrations were higher than in other experiments. As discussed in Chapter 2, Section 2.4.3, greater respiration rates of leaves at higher nitrogen concentrations (Winzeler *et al.* 1989, Stoy 1965) and the decrease in photosynthetic rates at temperatures above 20°C may increase the ratio of respiration to gross photosynthesis resulting in a decrease in transpiration efficiency.

The Δ of the wheat variety Matong grown at WAG91 exhibited a different response to nitrogen fertilisation than the variety Janz which was grown at PUC91 (Figure 4.6). In Matong there was a positive linear relationship between Δ measured at DC30 and the DC30 biomass. This relationship indicates that not only did nitrogen fertilised crops use more water from DC10 to DC30 due to increased biomass production, but that nitrogen fertilised crops were more profligate in their water use (lower transpiration efficiency) as hypothesised by Barley and Naidu (1964). At WAG91, it is likely that photosynthetic capacity increased due to an increase in the nitrogen concentration of the biomass at DC30 in response to nitrogen fertilisation. The decrease in the transpiration efficiency is

presumably due, therefore, to a greater increase in stomatal conductance than that required to balance the increase in photosynthetic capacity. These data raise the possibility that some wheat varieties may be more prone to haying-off than others due to their more profligate water use at high nitrogen levels.

4.4.4 Summary

Haying-off, then, is caused by water stress during grain filling due to high water use prior to DC65. The lack of water available for post-anthesis transpiration reduces the availability of current assimilate during grain filling. That yield can fall to levels below that of crops receiving no additional nitrogen is due to the counter-intuitive decline in water soluble carbohydrate reserves with increasing biomass. Brief periods of high temperature and hot dry winds are not necessary to induce haying-off as suggested by Fischer (1979) and are not likely to cause haying-off in the absence of water stress in all but extreme cases (Azzi 1956, Fischer 1980). Further reductions in grain yield are, however, likely to occur with heat shock in conjunction with water stress due to damage to photosynthetic machinery (Harding *et al.* 1990a,b), increased respiration during the heat shock, a dramatic decrease in photosynthetically active tissues post heat shock and concomitant reductions in kernel weight (Stone and Nicolas 1995).

CHAPTER 5

A CONTROLLED ENVIRONMENT STUDY TO SEPARATE THE EFFECTS OF NITROGEN, DROUGHT AND HIGH TEMPERATURE ON HAYING-OFF IN WHEAT

"The crops are done; ye'll have your work
To save one bag of grain;
From here way out to Back-o'-Bourke
They're singin' out for rain."

Said Hanrahan
P.J Hartigan
(pen name- John O'Brien)

5.1 INTRODUCTION

"Most generally, haying-off is regarded as the phenomenon in which cereal crops fail to yield grain in accordance with their vegetative potential" (Taylor 1965b). Early research attributed the negative yield response to premature cessation of grain filling due to exhaustion of soil water by the vigorous vegetative growth of crops stimulated by high soil nitrogen levels (Willis 1959, Storrier 1965b). The belief that water stress is the major factor contributing to haying-off is maintained to the present (Fischer 1979, McDonald 1989, Simpson 1992). Fischer and Kohn (1966c), however, indicated that haying-off by crops of high nitrogen status was not likely to be caused by an increase in evapotranspiration because there was only a small increase in leaf area in response to nitrogen. Their explanation of the reduced yield was poor survival of the additional small shoots. Storrier (1965b), Dann (1969) and Lipsett and Simpson (1973) found that kernel weight was reduced by addition of nitrogen, even when post-anthesis moisture was adequate. Dann (1969) concluded that other factors apart from water stress were also involved in haying-off.

Russell (1967) found that haying-off did not occur in drought years but in seasons when hot dry weather caused water stress during heading. Gardener (1972) reported that crops which hayed-off wilted severely after anthesis on days of high vapour-pressure deficit. Fischer (1979) hypothesised that the sudden onset of terminal drought may not allow full retranslocation of available assimilate to the developing kernels. Azzi (1956) described the effects of high temperature and associated Sirocco winds on the wheat crop during grain filling and credited it with the cause of haying-off known as 'hot spell damage' in Italy. Indeed, high vapour pressure deficit events associated with high temperatures and dry winds are common in various cereal growing regions of the world during grain filling and are considered responsible for grain shrivelling. Damage is claimed to occur with only brief periods of atmospheric stress and even under wet soil conditions (Fischer 1980). By imposing 6 hours of high temperature (46 °C) atmospheric stress to well watered plants 12 or 20 days after anthesis Fischer (1980) found that kernel weight was reduced by 30% in nitrogen fertilised plants but only 18% in controls.

Sofield *et al.* (1970), working under controlled environment conditions concluded that kernel weight decreased as temperature rose above 20°C due to the failure of rate of grain-filling to compensate for the decreased duration of grain filling. Jenner (1991a,b) imposed several days of high temperature to spikes isolated in small cuvettes while the rest of the plant was maintained at ambient temperature. This treatment was commenced at 20 days after anthesis and reduced the rate of grain filling on return to ambient conditions and reduced final kernel weight. Hawker and Jenner (1993), also heating

spikes only, implicated damage to the enzyme, soluble starch synthase, as the bottleneck responsible for reduced rates of grain filling.

Harding *et al.* (1990 a,b) found that two days of heat shock was enough to reduce photosynthetic rates by 17%. In the first paper the authors found that heat shock increased the rate of thylakoid breakdown which disrupted the senescence process. They concluded that this effect would be particularly damaging during heat shock. In the second paper they concluded that heat-shock damage to the photosynthetic apparatus and diminished sink capacity may be equally important in reducing grain yield. In these experiments the roots of heat stressed wheat plants were maintained at cooler temperatures than shoots to avoid the hastened senescence of shoots caused by high root temperatures (Kuroyanagi and Paulsen 1988). It is not clear that these experiments involve representative heat treatments and cultural conditions applying to a field crop. In particular there is no clear evidence that high-temperatures are involved in haying-off. In order to separate the effects of water stress and high-temperature heat shock, a controlled-environment experiment was conducted in which both stresses were applied independently.

The aims of the experiment presented in this chapter were to; a) simulate the growth conditions of field-grown wheat, at two rates of nitrogen, as closely as possible and inflict a 'natural' high temperature and high vapour pressure deficit event (heat shock), to the above-ground plant part parts once kernel number had been set. b) to determine the effect of the interaction of heat shock and water stress with nitrogen fertilisation on physiological processes during grain-filling c) to determine whether these conditions are responsible for or contribute to haying-off in wheat.

5.2 MATERIALS AND METHODS

5.2.1 Preparation of soil and tubes

The experimental soil was a red-brown earth Dr 2.32 (Northcote *et al.* 1971), typical of the NSW Riverina collected from the Temora Agricultural Research Station using a back-hoe and separated into 7 layers; 0-10, 10-30, 30-50, 50-70, 70-90, 90-110, 110-140 cm. At the time of sampling, the paddock had been cultivated ready for cropping after five years of subterranean clover based annual pasture. The soil was sieved and wet up to facilitate packing into tubes. Prior to packing the 0-10 cm and 10-30 cm layers were put into 20 cm deep troughs and planted to Indian mustard, in order to deplete the mineral nitrogen level and inhibit the growth of soil-borne pathogenic fungi of wheat (Angus *et al,* 1994). The Indian mustard plants were harvested at flowering and the soil resieved.

The tubes consisted of 1.2 m lengths of white UV stable PVC pipe 10.3 cm internal diameter (8.41×10^{-3} m^2). End caps were glued in place, a drainage hole drilled and disks of shade cloth placed inside to prevent soil washing out the bottom. Soil was packed into the tubes to bulk densities detailed in Table 5.1 which also presents pH and mineral nitrogen levels at the time of packing. Tubes were wet up to the drained upper limit (Ritchie 1981) and covered and allowed to stand for 3 weeks. Each tube was then weighed and this weight not exceeded during the experiment to prevent drainage. Single superphosphate was placed at a depth of 5 cm at a rate of 32 kg P ha^{-1} . Seeds of wheat cv. Janz were selected between 45-50 mg each for an average weight of 48 mg and germinated in petri dishes. After germination seeds were planted at a depth of 2 cm, one per tube (equivalent to a plant density of 119 plants m^{-2}) and kept indoors at 17°C until emergence at which time they were shifted outside on July 11.

Tubes were placed into two insulated boxes with holes cut in the top so as to control root temperatures independent of shoot temperatures. Each box was 1.2 m long, 1.2 m wide, 1.1 m high and contained 36 tubes located in holes equally spaced at 0.185 m centre to centre. Raven® door and window weather strip was glued to the outside of each tube 0.1 m below the top to seal the box when all the tubes were in position. Copper heating/cooling coils with attached heat-exchanging veins were positioned in the box 0.10 m below the top. Refrigerated water circulated through the coils to maintain a constant 12 ±1 °C inside the boxes. Air circulation inside the boxes relied on convection currents set up by placing the cooling coils near the top of each box. These precautions were taken to ensure a realistic differential between soil and air temperature because of

studies by Kuroyanagi and Paulsen (1988) which showed that growth and senescence of shoots and grain were influenced more by root temperatures than shoot temperatures.

Table 5.1 Soil characteristics of 1.2 m long reconstituted soil tubes

Depth in field (cm)	Depth in tube (cm)	Bulk density ($g\ cm^{-3}$)	pH[a]	Mineral N ($g\ m^{-2}$)
0 - 10	0 - 16.5	1.42	4.97	8.3
10 - 30	16.5 - 35.5	1.52	4.93	3.9
30 - 50	35.5 - 51.0	1.53	5.63	2.9
50 - 70	51.0 - 69.5	1.51	6.15	3.6
70 - 90	69.5 - 86.0	1.52	6.58	1.7
90 - 110	86.0 - 97.5	1.53	6.81	1.2
110 - 140	97.5 - 115	1.61	6.92	1.0

[a] pH measured in 0.01 Molar $CaCl_2$

5.2.2 Cultural conditions

At DC12 the high nitrogen treatment was imposed by supplying each plant with 160 kg N ha^{-1}, as a dilute urea solution and control plants with an equivalent volume of water. A mulch of 2 cm of clear Alkathane® beads was placed on the soil to minimise soil evaporation. Up to DC16, rainfall was supplemented with irrigation to supply the plants' water requirements. After this stage, possible differences between plants in leaf and stem flow of water during rainfall events warranted the use of a manually operated rainout shelter and plant water requirements were met by irrigation alone. Tubes were weighed regularly at 2-week intervals or earlier to coincide with harvests and /or treatment events. Irrigation was supplied on an individual-tube basis determined by the water use since the previous weighing. Corrections were made for estimated plant fresh weight. After each weighing, tubes were randomised in the boxes and a shade cloth surround put in place to minimise any edge effects on growth. The whole experiment was enclosed in bird netting to prevent the attack of parrots on the plants.

At DC50 an additional 80 kg N ha^{-1} was added to each of the high nitrogen tubes and the equivalent volume of water to the low nitrogen plants. At DC55 and DC60 plants were labelled with $^{14}CO_2$ according to the method of Angus *et al.* (1972). Plants were enclosed in a polyethylene chamber and between 0.5 and 0.6 mCi of $^{14}CO_2$ was evolved from $Ba^{14}CO_3$ in a small, open glass vial suspended in front of two oscillating circulation

fans by addition of 50% lactic acid. The reaction was allowed to proceed for approximately 10 minutes after which hot water was injected into a larger vial surrounding the reaction vial with the aim of driving the reaction to completion. Any $^{14}CO_2$ remaining in the chamber was vented to the atmosphere between 20 minutes after the addition of the lactic acid. Mean anthesis date was October 28. A water stress treatment was set up just prior to DC65 by allowing half the tubes to gradually dry to 53% of estimated plant-available water. This stress was gradual and was not intended to limit plant growth but to get tubes into a situation where stress could be initiated rapidly.

At dawn 13 days after anthesis (DC72), tubes were weighed and watered as necessary to 30% and 77% of estimated plant available water for water stressed and non-stressed plants respectively. At this stage some of the high nitrogen plants were partially detillered and defoliated to have the same number of spikes and approximately the same green leaf area as low nitrogen plants to determine if high nitrogen concentration predisposes tissues to damage by water stress or heat shock. At dawn the next day half of all tubes were put into one of the insulated boxes in a glasshouse and the root-cooling system reconnected as before. The glasshouse cooling system was controlled manually to mimic the diurnal rise and fall in air temperature. Vapour pressure deficit was maintained as high as possible by allowing only cooler dry air to be blown into the glasshouse. Two additional fans on stands were used to circulate the air in the glasshouse. Over 3 days maximum temperatures in the glasshouse reached 35 °C while outside the maxima were 25 °C. Soil/root temperature in the glasshouse rose to a mean of 14 °C while outside maintained a mean of 11 °C over the three days. Atmospheric CO_2 concentration was the same inside the glasshouse as outside due to the inflow of fresh dry air from the modified glasshouse cooling system. Light levels were reduced by an average of 9% inside the glasshouse but still reached a maximum of 1800μEm^{-2} at solar noon. Tubes were weighed at dawn each day and at night on the third day and then watered to the soil water contents measured prior to heat shock. Water stressed plants were subsequently watered at half the rate of the well watered plants until maturity.

5.2.3 Plant harvesting

Plant harvests were taken at DC65, DC72, DC81 and DC90. At DC65, 3 plants of each of the low and high nitrogen were taken, separated into spikes, green leaf, dead leaf and stem (stem plus leaf sheath). Samples were dried in a dehydrator at 70° C. At DC72 the same number of plants were taken and treated as at DC65, except spikes were separated into non-grain spike and developing grain. At DC81 and DC90, plants were separated as at DC72 and each of the eight treatments were sampled.

5.2.4 Experimental design

The experiment was a 2x2x2 factorial design with nitrogen, water and heat shock were the three factors. Where the three way interaction was not significant in an analysis of variance, superscripts have been included in the tables to indicate which main effects and/or two way interactions were significant. There were three replicates of each treatment. Tubes were randomly assigned to positions in the boxes following each weighing, with the exception that tubes could not spend two consecutive periods in the border positions Nitrogen treatments were assigned randomly. Two weeks prior to anthesis low and high nitrogen tubes were categorised into high, medium and low rate of water use. Tubes were randomly assigned to treatments, one from each category of water use, into plus or minus water stress and heat shock.

The day prior to the commencement of water stress and heat shock 12 of the high nitrogen plants, encompassing plus or minus water stress and heat shock, were detillered and defoliated as detailed in Section 5.2.2. These plants were in addition to the other high nitrogen plants and were prepared so as to have a similar leaf area and spike number as low nitrogen plants to test the hypothesis that high tissue nitrogen concentration predisposes wheat plants to detrimental effects of high temperature and high VPD. These are termed the detillered and defoliated (DTDF) plants.

5.2.5 Meteorological data.

The experimental site was at CSIRO's Black Mountain Laboratories, Australian Capital Territory. A small weather station was set up at the site to measure maximum and minimum temperatures, 9am and 3pm wet and dry bulb temperatures and precipitation. Class A pan evaporation and long-term mean weather data were obtained from Canberra Airport. Daily maximum vapour pressure deficit was calculated using 3 pm wet and dry bulb temperatures and daily maximum rather than 9 am measurements to avoid the possible influence of sea breezes on the 9am measurements. Data for the site are summarised in Table 5.2.

5.2.6 Non-destructive plant measurements

Anthesis date was recorded for each tube and stage of development recorded at each plant harvest. The mean number of green leaves per spike was determined on six occasions during grain filling and the senescence of stems and spikes was scored on five occasions over 14 days prior to maturity. Scoring of stress levels were made on all tubes on four occasions during the 3 days of heat shock as a non-destructive means of estimating water stress severity.

Leaf stomatal conductance was measured at DC60 using a Delta-T Steady State porometer (model AP4). CO_2 assimilation rates and stomatal conductance were measured at DC69 and DC73 using a LiCOR portable photosynthesis chamber.

Table 5.2 Monthly observed and derived weather data at CSIRO, Black Mountain 1993 and Canberra airport.

	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
				Maximum temperature (°C)					
Mean[a]	19.7	15.2	12.0	11.1	12.7	15.9	19.2	22.4	25.9
1993	21.3	16.4	11.7	11.6	14.4	16.9	19.0	23.4	27.2
				Minimum temperature (°C)					
Mean[a]	6.7	3.1	0.9	-0.2	0.9	3.0	5.9	8.5	11.2
1993	6.5	1.6	-0.3	3.3	3.2	5.8	7.5	10.4	14.0
				Rainfall and irrigation (mm)					
Mean[a]	52	48	38	43	48	52	66	63	53
1993[b] i)	98	59	44	41	9	53	265	171	52
ii)							210	125	27
iii)							302	302	96
iv)							260	195	27
				Class A Pan Evaporation (mm)					
Mean[a]	106	68	47	53	78	109	158	195	252
1993[c]	98	59	44	42	71	93	143	173	203
				Vapour pressure deficit (k Pa)[d]					
1993[c]	1.5	1.0	0.6	0.6	0.9	0.9	1.2	1.5	2.0
Range	0.7-2.3	0.3-1.7	0.2-0.9	0.1-1.2	0.4-1.3	0.3-1.8	0.1-1.9	0.6-3.2	0.5-4.4

[a]Long-term mean (55 years) Canberra airport.

[b]Rainfall includes tube irrigation. i)Low N, well watered; ii)Low N, water stress; iii)High N, well watered; iv)High N, water stress;

[c]1993 Canberra airport

[d] Mean daily maximum vapour pressure deficit

5.2.7 Plant sample analysis

Plant samples were ground using a Wiley mill to pass a 1.0 mm sieve taking suitable precautions not to contaminate myself or preparation areas with ^{14}C. Nitrogen

concentration was determined using a semi-micro-Kjeldahl method (Heffernan, 1985) and analysed for NH_4^+ (AOAC 1984). Water soluble carbohydrate levels were determined using the methods described in Chapter 3, Section 3.2.8. The ^{14}C counts per minute of each sample was determined by direct counting of 15-30 mg samples of the powdered material (O'Brien and Wardlaw 1961) using an ICN Tracerlab planchet sample counter. A sub-set of samples were randomly selected from each sampling time and 0.1000-0.1100g digested in 16 ml of an organic solubilizer (Nuclear Chicago solubilizer) at 50^0C for 72 hrs with intermittent shaking. After cooling, equal quantities of digestion mixture and a scintillation cocktail of 0.15g 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) and 12.0g 2,5-diphenyloxazole (PPO) per litre of toluene were mixed and ^{14}C counted in a Beckman liquid scintillation counter, Model LS 6800. Quenching was calculated for each digest by spiking samples with a known activity of ^{14}C sucrose and recounting. Counting efficiency for the direct counting method was then estimated for each plant part at each harvest and all samples corrected accordingly. The results are expressed as disintegration's per minute (DPM) per plant part.

The specific activity of ^{14}C in WSC extracts was determined by mixing 0.40 ml of extract with 3.60 ml of a scintillation cocktail of 670 ml of toluene and 330 ml of TritonX100 containing 75 mg l^{-1} 1,4-bis[2-(5-phenyloxazolyl)]-benzene (POPOP) and 6.0 g l^{-1} 2,5-diphenyloxazole (PPO) and placing in the Beckman liquid scintillation counter with a locally determined quench correction program installed. Quench correction was checked by spiking randomly selected samples with ^{14}C sucrose as above.

5.3 RESULTS

5.3.1 Weather

A summary of long term mean weather conditions for the Canberra airport weather station, and the 1993 conditions at the experimental site CSIRO, Black Mountain is given in Table 5.2. Maximum and minimum temperatures were higher at CSIRO in 1993 than the long-term means. Rainfall plus irrigation shows a sharp increase in October when tubes were rewatered to 80% of water holding capacity after greater than expected rates of water use. Differences in irrigation during October, November and 13 days of December reflected the greater size of the nitrogen fertilised plants and the imposed water stress. Evaporation in 1993 was lower than the long-term mean in all months. Mean daily maximum VPD exhibited a gradual increase associated with increasing maximum temperatures. Values were low throughout the season without a large range in values until November. VPD was similar to the values reported for 1991 in Chapter 4 except in October and November when maximum values and the range remained low. The high value of 4.4 kPa reported in December occurred after final harvest. Weather conditions during the commencement of water stress and the imposition of heat shock are presented in Table 5.3. Maximum temperatures inside the glasshouse were 10 °C higher than ambient while minimums were the same or slightly higher. Maximum VPD in the glasshouse was double the level outside, which created a far greater evaporative demand on glasshouse plants. As mentioned in Section 5.2.2 the air temperature inside the insulated box containing the soil tubes increased slightly for the plants in the glasshouse but differences were small compared to air temperatures experienced by the above-ground plant parts.

Table 5.3 Weather conditions and root temperature (inside the soil box) for the day prior to, days during, and the day after imposition of heat shock, outside and inside (in parenthesis) glasshouse.

	November				
	11th	12th	13th	14th	15th
$Temp_{max}$ (°C)	21.0	25.0 (35.5)	25.0 (34.5)	26.0 (36.0)	24.5
$Temp_{min}$ (°C)	9.0	8.0	16.0 (17.9)	14.5 (17.5)	14.0
VPD_{max} (k Pa)	1.4	1.9 (4.1)	1.3 (3.3)	2.1 (4.2)	1.0
Root Temp. (°C)	11.0	10.0 (13.0)	11.0 (15.0)	13.0 (15.0)	12.0

5.3.2 Growth, grain yield and yield components

The application of nitrogen fertiliser stimulated dry weight accumulation to anthesis and was associated with an increase in culm weight, spikes per plant, green area of each plant and plant height, while specific leaf area decreased (Table 5.4). Stem linear density at anthesis was greater for high nitrogen plants despite the increase in height.

Figure 5.1 shows the change in grain yield, above-ground dry weight and stem dry weight between anthesis and maturity for plants in the eight treatments. Added nitrogen led to significantly more growth at each harvest while the effects of water stress and heat shock were smaller and generally not consistent. There was an interaction of nitrogen and water stress shown by a reduction of dry matter production and increased fall in stem weight in response to water stress at high nitrogen but no such effects of water stress at low nitrogen.

Table 5.4 Effect of nitrogen fertiliser on the growth and growth attributes of wheat plants (cv. Janz) at anthesis.

Attribute	Low N	High N	l.s.d. P = 0.05)
Dry weight (g plant^{-1})	18.9	25.0	2.9
Spike number	7.4	8.4	0.3
Stem wt (g plant^{-1})	1.7	2.1	0.2
Plant height (m)	0.55	0.61	n.s.[b]
Stem linear density (g m^{-1})	3.0	3.5	n.s.[b]
Green area[a] (cm^{-2} plant^{-1})	650	892	80
Specific leaf area (cm^2 g^{-1})	174	148	14

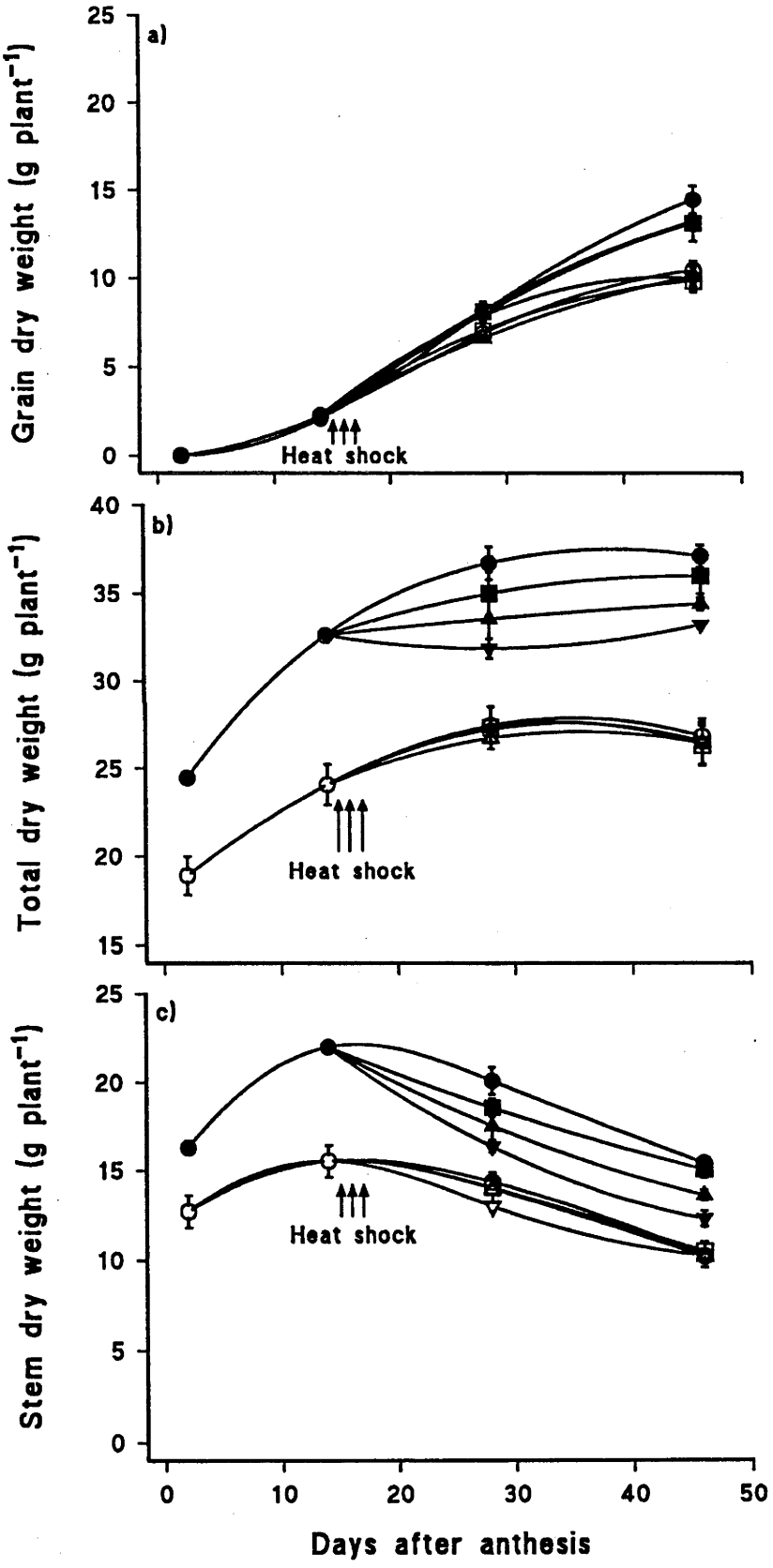
[a] one surface only of spikes, stems and leaves.

[b] Significant l.s.d. P = 0.10

Figure 5.1 shows the high nitrogen plants grown with no water or temperature stress achieved the greatest final yield but there was little variation in grain yield for a given level of nitrogen. Total dry weight for high nitrogen plants, however, progressively decreased with the imposition of heat shock, water stress and the combination of both. The increase in dry weight from prior to the heat shock to maturity ranged from 4.5g to 0.6g respectively. In contrast there appears to be very little response in total dry weight to heat shock and water stress in low-nitrogen plants.

The responses of stem dry weight growth to the treatments were similar to those of total dry weight growth except that maximum stem weight occurred close to DC72 compared with DC81 for maximum dry weight. The decrease in stem dry matter from the time of

Figure 5.1. Changes in a) grain dry weight, b) total dry weight and c) stem dry weight after anthesis for low nitrogen (open symbols) and high nitrogen (closed symbols) wheat plants. (O,● well watered, no heat shock; □,■ well watered, heat shock; Δ,▲ water stress, no heat shock; ∇,▼ water stress, heat shock). Curves are smoothed, bars indicate the s.e.m.



maximum stem weight to maturity was 5.2g plant^{-1} for low nitrogen plants and between 6.6 to 9.7g plant^{-1} for high nitrogen plants.

There was a significant increase in the number of kernels per plant with nitrogen fertiliser application (Table 5.5). Water stress and heat shock had no effect on kernel number. There was a downward trend in kernel weight for high nitrogen plants but not at low nitrogen. There was no consistent effect of any treatment on harvest index though water stress tended to increase it while heat shock tended to decrease it.

Table 5.5 Effect of nitrogen fertiliser, water stress and heat shock on the yield characteristics of tube wheat plants (cv Janz).

Treatment	Kernels per plant	Kernel weight (mg)	Harvest index (%)	Grain protein[b] (%)
-N W C[a]	281	37.2	0.39	7.5
-N W H	278	35.7	0.37	7.6
-N D C	264	38.1	0.38	7.8
-N D H	266	37.5	0.38	7.7
+N W C	358	41.1	0.39	9.9
+N W H	327	39.9	0.36	10.8
+N D C	338	39.3	0.38	10.8
+N D H	342	38.8	0.40	10.9
l.s.d. (P = 0.05)	n.s.[c]	n.s.	n.s.	n.s.[c]

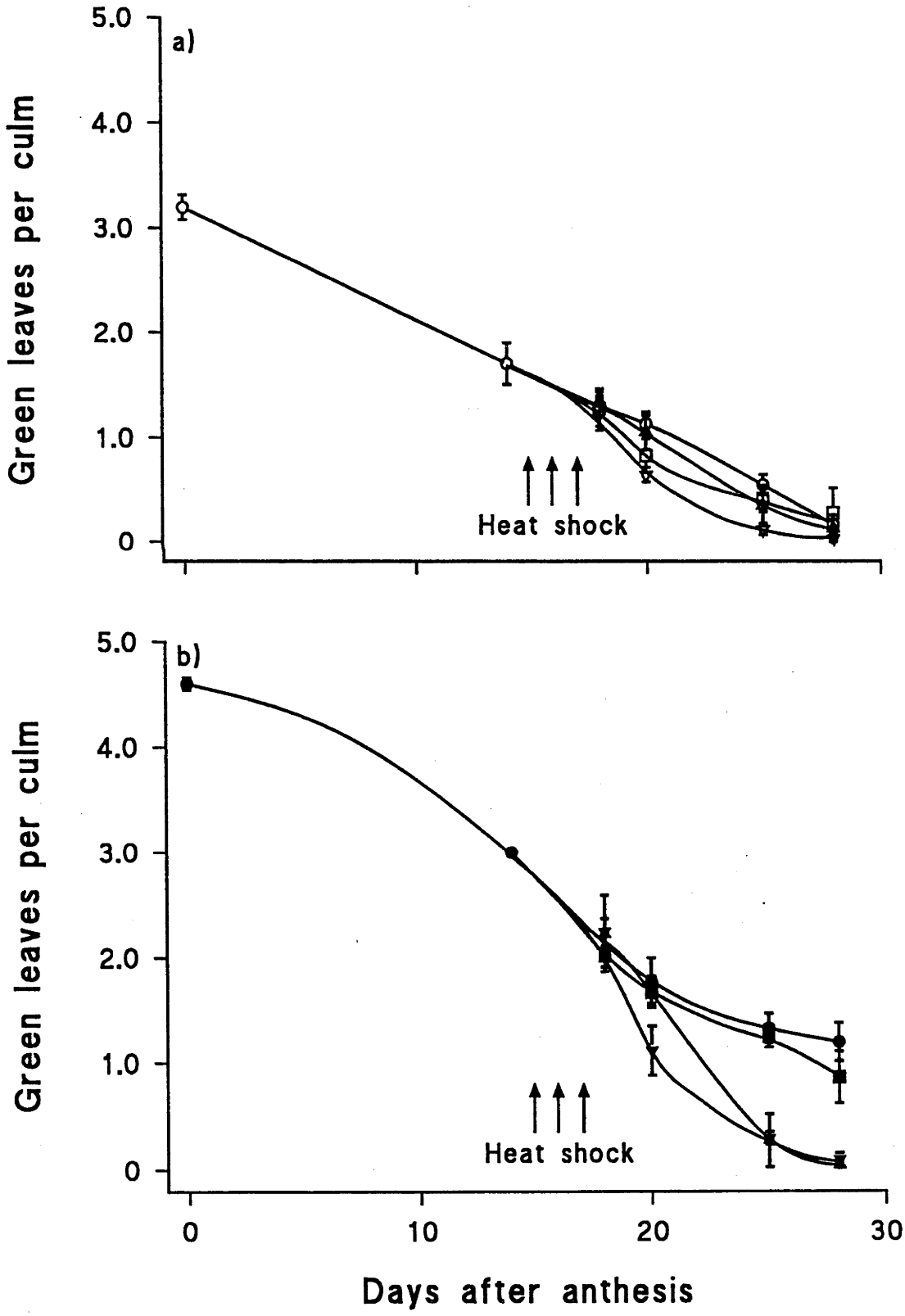
[a] (N= nitrogen, W=well watered, D=drought or water stress, C=cool or ambient, H= heat shock)

[b] Grain protein expressed at 12 % moisture

[c] Main effect of nitrogen significant $P < 0.05$

The mean number of green leaves per fertile spike was greater for high nitrogen plants than low nitrogen plants between DC65 and the day after the completion of heat shock (Figure 5.2). Water stress treatments and to a lesser extent heat shock resulted in a faster rate of senescence of green leaves for low or high nitrogen plants. By three days after heat shock the greatest loss of green leaf area was for combined water stressed and heat shocked plants relative. By twenty eight days after anthesis (DAA) all the low nitrogen plants, the high nitrogen water stress and combined high nitrogen, water stressed and heat shocked plants had essentially lost all their green leaf area. The leaves of high nitrogen water stressed and heat shocked plants had a peak senescence rate immediately following heat shock of almost 0.6 leaves spike^{-1} or (75 cm^{-2} plant^{-1}) per day compared to 0.1 leaves spike^{-1} (12 cm^{-2} plant^{-1}) per day and 0.1 leaves spike^{-1} (19 cm^{-2} plant^{-1}) per day for low and high nitrogen control plants respectively.

Figure 5.2. Changes in the number of green leaves per culm between anthesis and maturity for a) low and b) high nitrogen wheat plants. (Symbols as for Figure 5.1). Curves are fitted by eye, bars indicate the s.e.m.



5.3.3 Gas exchange

Flag leaf conductance measured using a Delta-T porometer seven days before anthesis was 0.274 mol m^{-2} sec^{-1} for low nitrogen plants and 0.330 mol m^{-2} sec^{-1} for high nitrogen plants. These values were double those measured on flag leaves using a LiCOR portable photosynthesis chamber six days after 50% anthesis, (DC69), prior to the commencement of water stress and heat shock (Table 5.6). Following the period of heat shock plants in all treatments were rewatered to determine if damage to the photosynthetic apparatus had reduced potential assimilation rates and conductance. At DC73 (18 DAA) the conductance for non-stressed, non-shocked plants returned to levels comparable with those before anthesis. Conductance fell by 73% following water stress and heat shock.

Assimilation rates were greater for high than low nitrogen plants at DC69. At DC73 there were significant reductions in assimilation rate due to either heat shock or water stress. Assimilation rates progressively decreased following the imposition of heat shock, water stress and their combination though the three way interaction was not significant. Values of instantaneous water use efficiency (WUE) at DC73 were almost half those at DC69. WUE was greater in the high nitrogen plants at both growth stages. At DC73 water stress had little effect on WUE but there was a significant negative effect of heat shock.

5.3.4 Water soluble carbohydrates

At DC65 and DC72 the concentration of WSC in the stems was not significantly different between nitrogen treatments, though levels did tend to be higher in low nitrogen plants. Reserves per plant, however, were significantly greater for high nitrogen plants at both times (Table 5.7). By the DC81 harvest, reserves of WSC reflected the damage to photosynthetic apparatus as assessed at DC73. Reserves per plant were remobilised more slowly for well watered plants, especially high nitrogen plants that encountered no heat shock, reflecting the longer leaf area duration and hence the greater contribution of current assimilate to grain filling. Conversely where water stress and/or damage to photosynthetic apparatus had led to reduced photosynthesis, mobilisation of stem WSC commenced earlier to meet the demand for assimilate by the developing kernels. WSC comprised 16% of stem weight for low nitrogen plants at maturity. While there was no difference in WSC between treatments at low nitrogen there was a trend towards less WSC in response to increasing stress at high nitrogen, from 19% of stem weight for unstressed plants down to 16% for water stressed and heat shocked plants.

Table 5.6 Stomatal conductance, assimilation rate and instantaneous water use efficiency of flag leaves five days prior to the commencement of water stress and heat shock (DC69) and two days after the completion of heat shock (DC73) and following rewatering.

Treatment[a]	Conductance mol m^{-2} sec^{-1}	Assimilation µmol CO_2 m^{-2} sec^{-1}	Water use efficiency µmol CO_2 mmol H_2O^{-1}
		DC69	
-N[a]	0.139	11.5	2.59
+N	0.166	14.9	2.94
l.s.d. (P = 0.05)	0.026	2.0	0.20
		DC73	
-N W C	0.238	10.9	1.52
-N W H	0.184	8.9	1.41
-N D C	0.171	9.1	1.67
-N D H	0.063	2.9	1.35
+N W C	0.273	15.9	1.98
+N W H	0.228	12.1	1.75
+N D C	0.160	10.0	1.84
+N D H	0.054	3.2	1.56
l.s.d. (P = 0.05)	n.s.	n.s.	n.s.
-N	0.164	8.0	1.49
+N	0.179	10.3	1.78
l.s.d. (P = 0.05)	n.s.	1.5	0.13
W	0.234	12.3	1.70
D	0.117	6.4	1.62
l.s.d. (P = 0.05)	0.032	1.5	n.s.
C	0.211	11.7	1.78
H	0.139	6.9	1.54
l.s.d. (P = 0.05)	0.032	1.5	0.13

[a] Code as for Table 5.5.

5.3.5 Water use and transpiration efficiency

Transpiration efficiency measured by changes in plant weight and water use from sowing to anthesis ($W_T{}^B{}_{S\text{-}A}$) was greater for high nitrogen than low nitrogen plants (Table 5.8). From anthesis to maturity, however, high nitrogen led to decreased transpiration efficiency ($W_T{}^B{}_{A\text{-}M}$) while water stress increased it. For the period between anthesis and maturity transpiration efficiency fell by 28% and 41% for low and high nitrogen plants respectively, relative to pre-anthesis values.

Table 5.7. Effect of nitrogen, water stress and heat shock on stem WSC (g plant^{-1}) and stem linear density (g m^{-1}, in parenthesis) during grain filling.

Low nitrogen				High nitrogen				
W[a]		D		W		D		l.s.d.
C	H	C	H	C	H	C	H	(P = 0.05)
				Anthesis DC65				
5.8				7.3				1.3
(3.0)				(3.5)				n.s.[b]
				DC72				
7.3				9.9				1.7
(3.1)				(3.6)				n.s.[b]
				DC81				
5.9	5.6	5.7	4.6	8.4	7.3	6.7	6.3	n.s.[c]
(2.8)	(2.8)	(2.7)	(2.6)	(3.4)	(3.2)	(2.9)	(2.9)	n.s.[d]
				Maturity				
1.6	1.7	1.6	1.7	3.0	2.7	2.6	2.0	n.s.[e]
(2.1)	(2.1)	(2.0)	(2.0)	(2.6)	(2.5)	(2.4)	(2.2)	n.s.[d]

[a] Code as for Table 5.5.

[b] Significant at P = 0.10

[c] Main effects of nitrogen, water stress, and heat shock significant P < 0.05

[d] main effects of nitrogen and water stress significant P < 0.05

[e] Main effect of nitrogen significant P < 0.05, main effect of water stress and the interactions between nitrogen and water stress and heat shock significant P = 0.10

This decrease in transpiration efficiency was corroborated by the data for instantaneous leaf photosynthesis, conductance and water use efficiency obtained using the LiCOR model 6200 (Table 5.6). Towards the end of anthesis, at DC69, transpiration efficiency was almost double the level measured during grain filling, 11 days later. Transpiration efficiency from sowing to maturity (W_T^B) was less than from sowing to anthesis reflecting the post-anthesis decline in efficiency. The main effects of nitrogen and water stress increased transpiration efficiency from sowing to maturity. Transpiration efficiency for grain (W_T^G) was increased by nitrogen fertilisation and water stress, though values did not vary greatly reflecting the similar harvest index of treatments.

Table 5.8. Transpiration efficiency for biomass production (W_T^B) between sowing and anthesis ($W_T^B{}_{S-A}$), anthesis to maturity ($W_T^B{}_{A-M}$), and sowing to maturity ($W_T^B{}_{S-M}$), and for grain (W_T^G).

($g\ litre^{-1}$)
($g\ m^{-2}\ mm^{-1}$)

Low nitrogen				High nitrogen				
W[a]		D		W		D		l.s.d.
C	H	C	H	C	H	C	H	($P = 0.05$)
Sowing to anthesis ($W_T^B{}_{S-A}$)								
6.0				6.7				0.5
Anthesis to maturity ($W_T^B{}_{A-M}$)								
3.8	3.7	4.9	5.0	3.9	3.4	4.6	4.2	n.s.[c]
Sowing to maturity ($W_T^B{}_{S-M}$)								
4.9	5.0	5.7	5.5	5.2	5.0	6.0	5.8	n.s.[b]
Transpiration efficiency for grain (W_T^G)								
1.9	1.9	2.2	2.1	2.0	1.8	2.3	2.3	n.s.[c d]

[a] Code as for Table 5.5.

[b] Main effects of nitrogen and water stress significant $P < 0.05$

[c] Main effect of water stress significant $P < 0.05$

[d] Main effect of nitrogen significant $P = 0.05$

Daily water-use varied greatly in response to the treatments during the three days of heat shock (Table 5.9). During the first two days, the three treatments of nitrogen, heat shock and water stress independently affected water use with high nitrogen and heat shock increasing water use while water stress decreased water use. On day three, as water stress became more severe, differences in water use between treatments increased. In the case of the low nitrogen, unstressed plants, water use increased on day three due to the higher temperature and greater VPD. In contrast, the water use of water stressed plants fell on day three, the greatest decrease being for the water stressed, heat shocked, high nitrogen plants. The water use of these plants fell by 75% relative to the well watered, heat shock and high nitrogen plants while the same comparison for low nitrogen plants resulted in a 47% decrease. In the days following the heat shock treatment, differences in mean daily water use associated with the main effects of nitrogen, water stress and heat shock were evident. Nitrogen increased mean daily water use while water stress and heat shock decreased it.

Table 5.9. Water use (ml plant^{-1} day^{-1}) prior to, during and following three days of heat shock.

	Low nitrogen				High nitrogen				
	W[a]		D		W		D		l.s.d.
Date	C	H	C	H	C	H	C	H	(*P*=0.05)
Prior to heat shock (10/11 November)	40		30		65		63		n.s.[c]
During heat shock (12/13 November)	53	90	47	77	85	130	78	93	n.s.[d]
During heat shock (14 November)	59	94	34	50	92	151	40	37	20
After heat shock (15/19 November[b])	53	49	38	31	83	76	45	34	n.s.[d e]

[a] Code as for Table 5.5.

[b] Includes 15th to 17th November when all plants were well watered

[c] Main effect of nitrogen significant $P < 0.05$, water stress significant $P = 0.10$

[d] Main effects of nitrogen, water stress, and heat shock significant $P < 0.05$

[e] Interaction of nitrogen and water stress significant $P < 0.05$

5.3.6 ^{14}Carbon budget between anthesis and maturity

The decreases in ^{14}C from the stem WSC reserves between anthesis and maturity reflect the decreases in ^{14}C from the stem in both low and high nitrogen plants (Figure 5.3 a,b). The increase in ^{14}C of the grain can be entirely explained by the decrease in ^{14}C of stem WSC or the total stem. The ^{14}C of the non-grain spikes was similar for low and high nitrogen plants and did not change significantly between anthesis and maturity (Figure 5.4a). The decrease in ^{14}C from the leaves of high nitrogen plants was greater than for low nitrogen plants (Figure 5.4b), presumably due to greater retranslocation of protein to the grain. Indeed, at DC72 the decrease in ^{14}C for low and high nitrogen plants from the stem and leaves was matched by the gain in ^{14}C of the spike including grain. Figure 5.4c shows a small loss of ^{14}C from the total above-ground plants during the later part of grain filling but the amount was not statistically significant.

The harvest index for ^{14}C increased with the progression of grain filling and was always greater for low nitrogen than for high nitrogen (Table 5.10). Heat shock had the initial effect of stimulating retranslocation of ^{14}C to the grain, presumably due to the increased metabolic activity with higher temperatures and damage to the photosynthetic apparatus resulting in a lack of current assimilate to sustain grain growth. At maturity the effect of heat shock was not significant though a trend remains at high nitrogen. Water stress

Figure 5.3. Changes in ^{14}C content between anthesis and maturity for a) low nitrogen stems (solid line) and WSC (dashed line), b) high nitrogen stems (solid line) and WSC (dashed line) and c) grain for low (open symbols, dashed line) and high nitrogen (closed symbols, solid line) wheat plants. (Symbols as for Figure 5.1). Curves are smoothed, bars indicate the s.e.m.

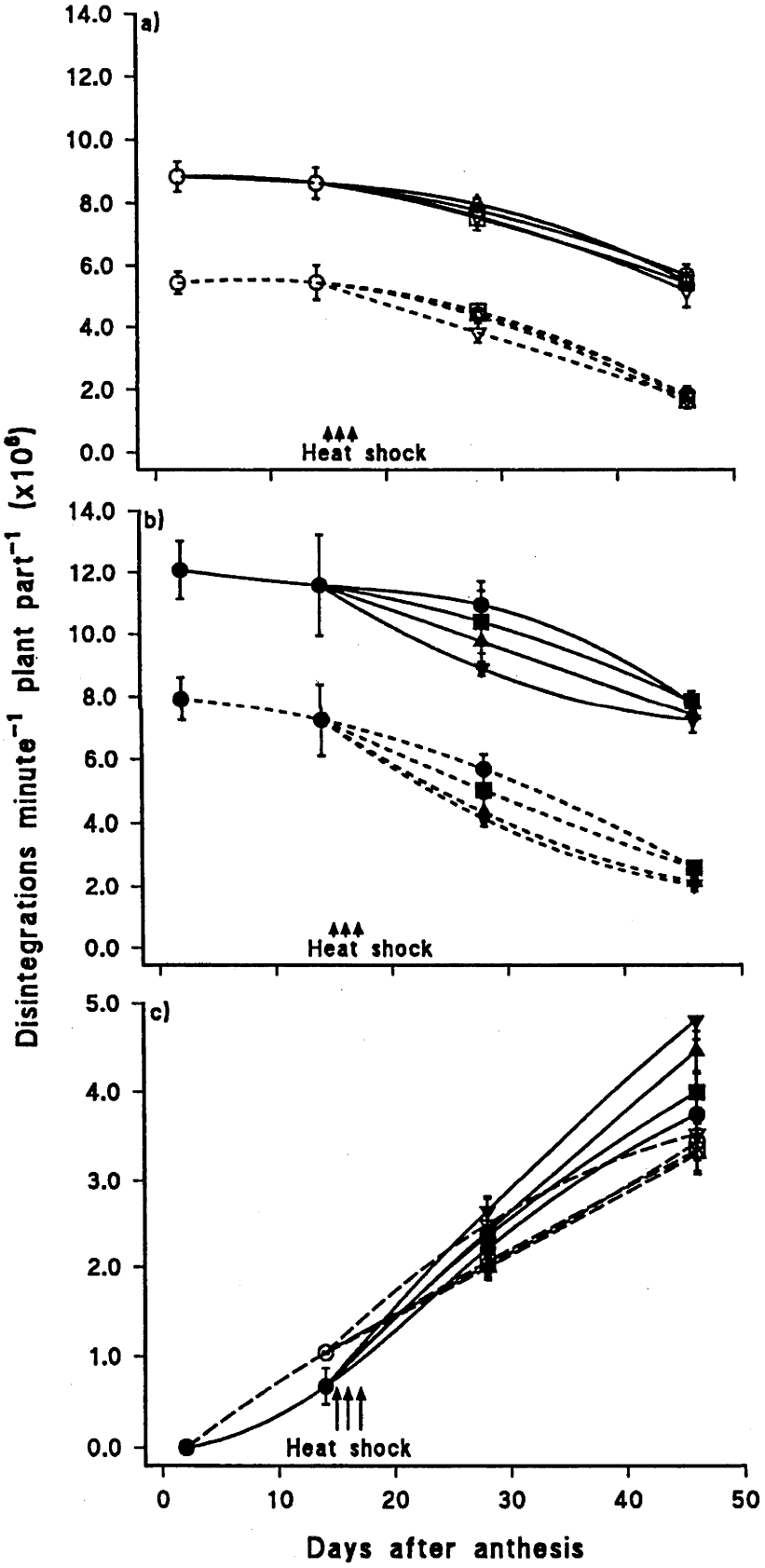
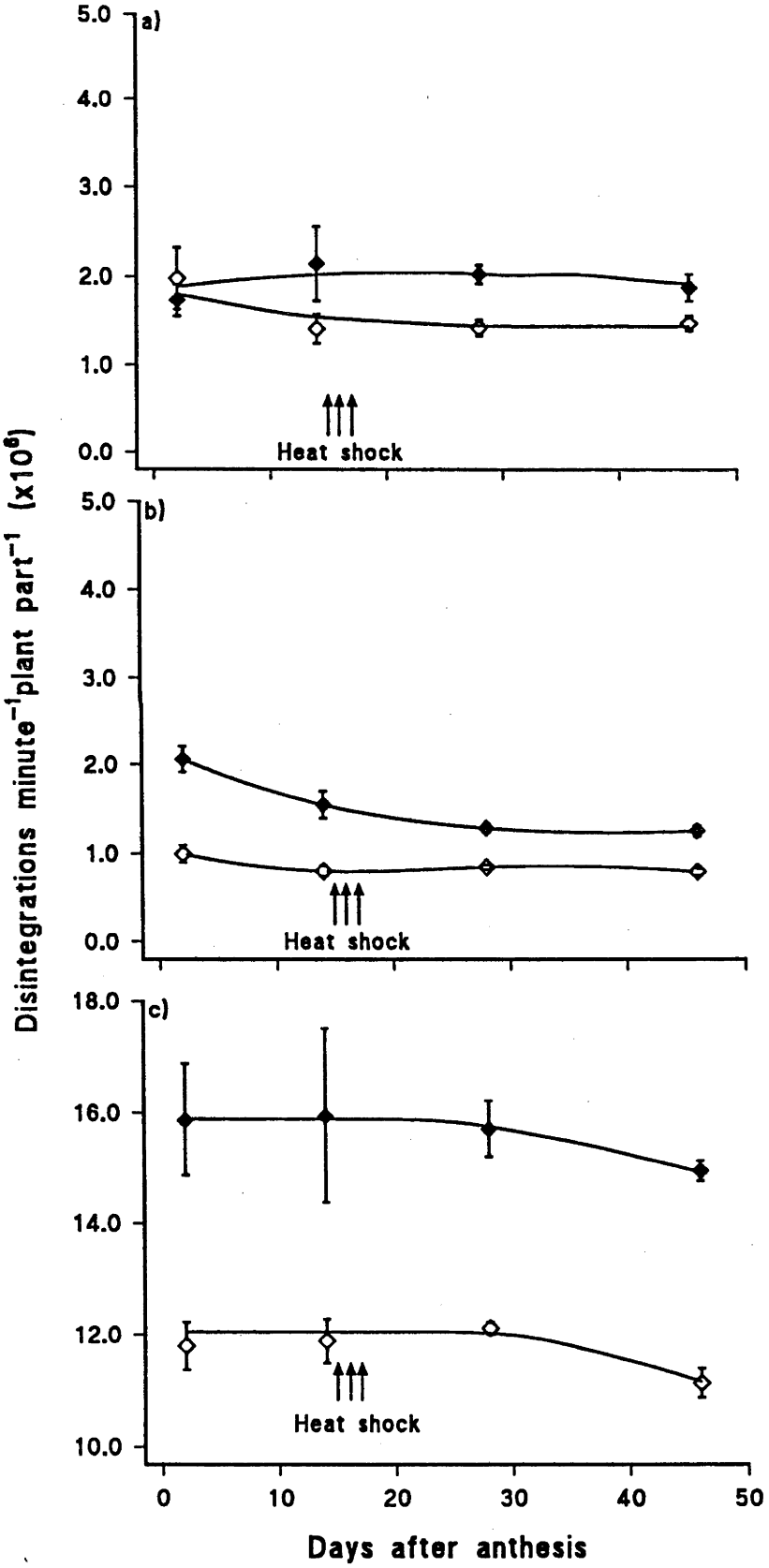


Figure 5.4. Changes in ^{14}C content between anthesis and maturity for a) non-grain spikes, b) leaves and, c) total above ground dry matter for the means of low nitrogen (open symbol) and high nitrogen (closed symbol) wheat plants. Curves are fitted by eye, bars indicate the s.e.m.



resulted in a significant increase in ^{14}C harvest index especially at high nitrogen, the interaction of nitrogen and water significant at $P < 0.10$.

Table 5.10. Harvest index of ^{14}C for all treatments between DC72 and maturity.

Low nitrogen				High nitrogen				
W[a]		D		W		D		l.s.d.
C	H	C	H	C	H	C	H	($P = 0.05$)
			DC72					
0.09				0.04				0.03
			DC81					
0.17	0.17	0.16	0.20	0.13	0.15	0.15	0.18	n.s.[b]
			DC90					
0.30	0.30	0.30	0.32	0.26	0.27	0.30	0.31	n.s.[c]

[a] Code as for Table 5.5.

[b] Main effects of nitrogen, water stress, and heat shock significant $P < 0.05$

[d] Main effects of nitrogen and water stress significant $P < 0.05$

5.3.7 Nitrogen dynamics

High nitrogen plants had taken up more nitrogen than low nitrogen plants by anthesis and both low and high nitrogen plants took up nitrogen between anthesis and maturity (Table 5.11). Apparent fertiliser recovery was 61%. Surprisingly there was no discernible difference in uptake between water and heat shock treatments within each level of nitrogen. Nitrogen contained in the stems, leaves and grain is shown in Figure 5.5 a,b,c. Leaves and stems of high-nitrogen plants contained more nitrogen at each harvest from anthesis to maturity. Water stress stimulated a decrease in nitrogen from leaves and stem of high nitrogen but not of low nitrogen plants. Heat shock tended to stimulate the decline in nitrogen content of stems but to inhibit the decline in leaves. Nitrogen harvest index (NHI) was greater for low nitrogen plants at DC72 and DC81 (Table 5.12). At maturity, however, NHI was greater for high nitrogen plants indicating a delayed retranslocation to the grain.

Figure 5.6 shows the nitrogen contained in the grain and the contribution from non-grain spikes, stem and leaves for low and high nitrogen plants between anthesis and maturity This figure shows that stems contribute approximately the same proportion of nitrogen to the grain in low and high nitrogen plants. Non-grain spikes contributed almost half the proportion of grain nitrogen at high nitrogen than at low nitrogen while leaves

Figure 5.5. The nitrogen content of a) leaves, b) stems and c) grain of wheat plants between anthesis and maturity. (Symbols as for Figure 5.1). Curves are smoothed, bars indicate the s.e.m.

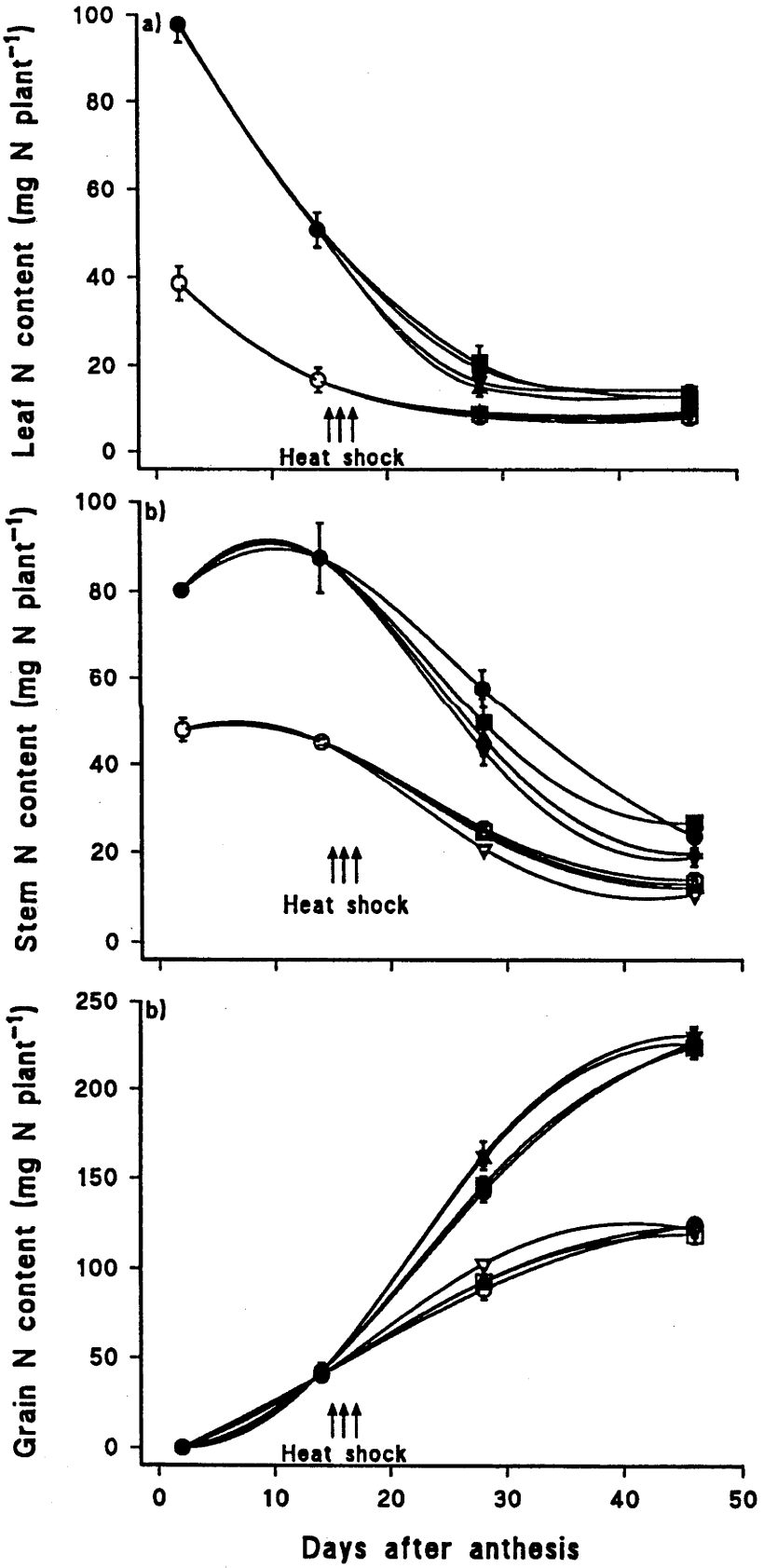
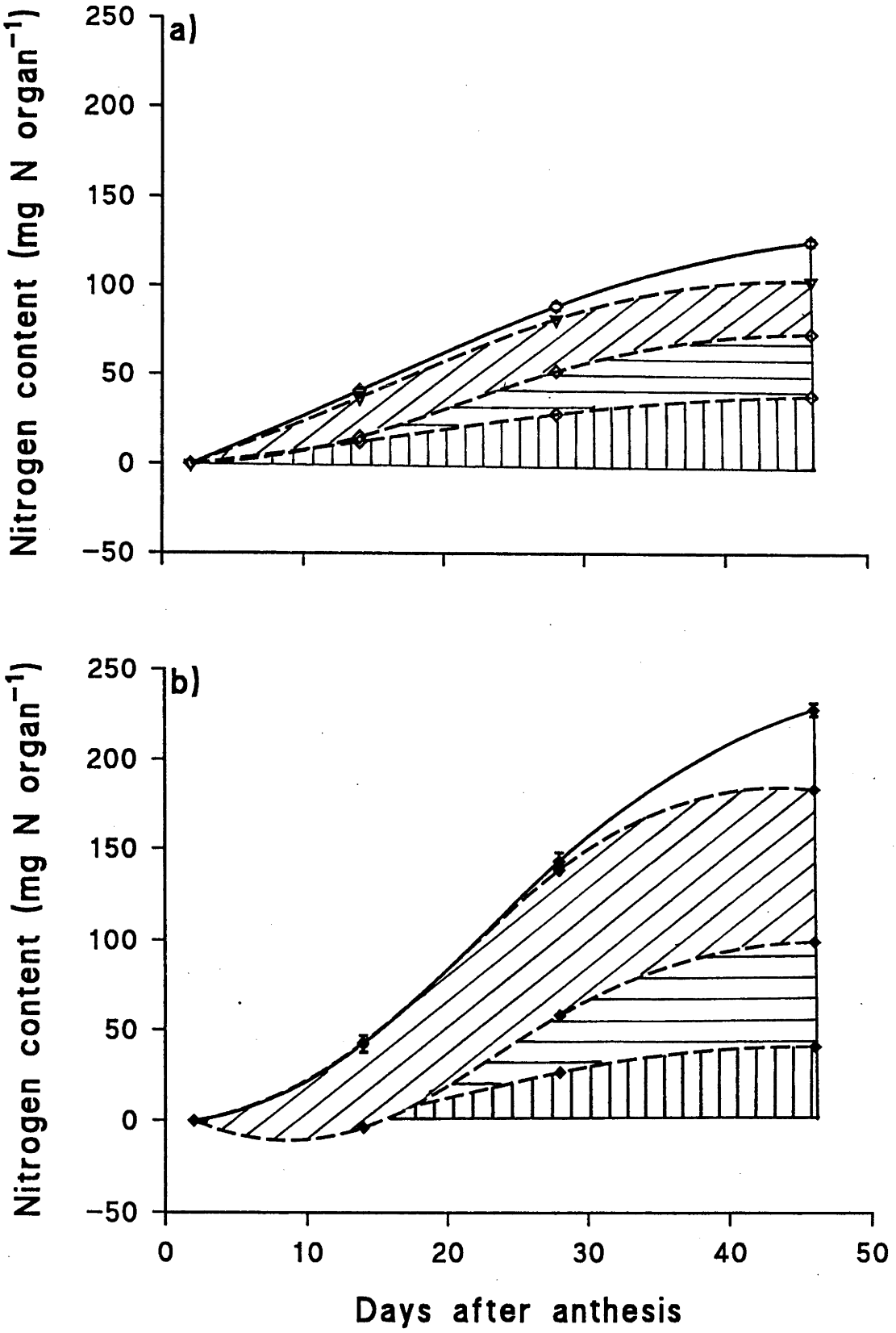


Figure 5.6. Nitrogen content of grain (solid line) and estimated cumulative contribution to grain from non-grain spikes (vertical lines), stems (horizontal lines) and leaves (diagonal lines) for the mean of all a) low nitrogen and b) high nitrogen tube plants. Curves are smoothed, bars indicate the s.e.m. for grain only.



contributed more than one third of grain-nitrogen at high nitrogen and only a quarter at low. The discrepancy between total nitrogen content of the grain and the net contribution from the above-ground organs suggests that there was uptake of nitrogen directly into grain from the soil or senescing roots.

Table 5.11 Nitrogen content of above-ground dry matter from anthesis to maturity

(mg N plant^{-1})								
Low nitrogen				High nitrogen				
W[a]		D		W		D		l.s.d.
C	H	C	H	C	H	C	H	(P = 0.05)
			Anthesis					
131				229				21
			DC72					
134				215				22
			DC81					
140	143	143	148	245	241	246	245	n.s.[b]
			DC90					
154	148	153	148	274	275	269	273	n.s.[b]

[a] Code as for Table 5.5.

[b] Main effect of nitrogen significant P < 0.05

Table 5.12. Nitrogen harvest index for all treatments between DC72 and maturity.

Low nitrogen				High nitrogen				
W[a]		D		W		D		l.s.d.
C	H	C	H	C	H	C	H	(P = 0.05)
			DC72					
0.30				0.19				0.04
			DC81					
0.64	0.65	0.65	0.70	0.59	0.61	0.66	0.67	n.s.[b]
			DC90					
0.82	0.81	0.82	0.83	0.83	0.82	0.84	0.85	n.s.[b]

[a] Code as for Table 5.5.

[b] Main effects of nitrogen and water stress significant P < 0.05

5.3.8 Detillered and defoliated high nitrogen plants

Grain yield, total dry weight and stem dry weight at maturity for detillered and defoliated high nitrogen (DTDF) plants are presented in Table 5.13. DTDF plants yielded more than low nitrogen plants. The imposition of water stress and heat shock resulted in a negative trend in grain yield as for intact high nitrogen plants but yield remained above low nitrogen plants. Total dry weight and stem dry weight followed a similar downward trend as for intact high nitrogen plants. Detillering reduced sink size from 341 to 286 kernels per plant which was comparable to 272 kernels for low nitrogen plants. There was a non-significant increase in kernel weight for DTDF plants compared to low nitrogen plants. DTDF plants showed a similar downward trend in stem WSC at maturity as that for intact high nitrogen plants, the concentration falling from 20-15%.

Stomatal conductance and assimilation rates for DTDF plants at DC73 were 30-50% higher than for intact high nitrogen plants though similar detrimental effects of water stress and heat shock were evident (Table 5.14). Instantaneous transpiration efficiency, however, was at levels comparable to the intact plants.

Table 5.13. Effect of water stress and/or heat shock on the grain yield and physiological attributes of detillered and defoliated (DTDF) plants at maturity.

Treatment	Dry weight ($g\ plant^{-1}$)	Grain yield ($g\ plant^{-1}$)	Kernel weight (mg)	Stem weight ($g\ plant^{-1}$)	Stem WSC ($g\ plant^{-1}$)
+N W C[a]	30.2	11.9	39.7	12.2	2.5
+N W H	29.9	11.9	39.5	11.7	2.1
+N D C	27.9	11.0	41.1	11.1	1.9
+N D H	26.6	10.7	39.0	10.3	1.5
l.s.d. ($P = 0.05$)[b]	n.s.[c]	n.s.[c]	n.s.[c]	n.s.[c]	n.s.[c]

[a] Code as for Table 5.5.

[b] ANOVA includes low nitrogen plants.

[c] Main effect of nitrogen significant $P < 0.05$

Table 5.14 Stomatal conductance, assimilation rate and instantaneous water use efficiency of flag leaves following the completion of heat shock and rewatering for detillered and defoliated (DTDF) plants.

Treatment	Conductance mol m^{-2} sec^{-1}	Assimilation μmol CO_2 m^{-2} sec^{-1}	Water use efficiency μmol CO_2 mmol H_2O^{-1}
+N W C[a]	0.358	20.4	1.97
+N W H	0.384	18.5	1.70
+N D C	0.247	17.1	2.00
+N D H	0.081	4.7	1.46
l.s.d. (P = 0.05)	0.048	3.3	0.20

[a] Code as for Table 5.5.

Final nitrogen uptake of DTDF plants was midway between low nitrogen and intact high nitrogen plants and also showed little variation caused by water stress and heat shock treatments. Nitrogen harvest index, however, was higher in DTDF plants (data not shown) than intact high nitrogen plants presumably due to the reduced supply of nitrogen for grain filling caused by defoliation. Grain nitrogen concentration was 1.6 % for DTDF plants compared to 1.7 % for high nitrogen plants.

The harvest index for ^{14}C of DTDF plants followed a similar trend to high nitrogen plants (data not shown). Measured assimilation rates for control DTDF plants (Table 5.14) were greater than for low nitrogen controls (Table 5.6), leading to a greater supply of current assimilate for grain filling. Although the DTDF plants had 15 % more total ^{14}C, the ^{14}C in the grain was the same due to greater retranslocation for Low nitrogen controls. Damage to the photosynthetic apparatus due to the combination of heat shock and water stress (Table 5.14, 5.6) resulted in a greater reliance on soluble reserves for grain filling indicated by an increase in the ^{14}C harvest index from 0.26 to 0.34 for DTDF plants. The magnitude of this increase in ^{14}C harvest index for DTDF plants was similar to the intact high nitrogen plants.

5.4 DISCUSSION

5.4.1 Biomass and water use

The application of nitrogen fertiliser stimulated dry weight accumulation and increases in tillering and plant height as in the field experiments. The trends exhibited in Figure 5.1 indicate that high nitrogen plants reacted differently to post-anthesis water stress and heat shock than low nitrogen plants. In high nitrogen plants, water stress and heat shock limited growth in the 2 weeks after the commencement of treatments. This effect was due to assimilation being limited by water stress and/or damage to the photosynthetic apparatus by heat shock as shown in Table 5.6. Heat shock under well watered conditions had a limited effect on assimilation rate probably due to the cooling of the leaves by transpiration (Stone and Nicolas, 1994). The extent of cooling can be inferred from the greater daily water use of the well watered heat shocked plants relative to the water stressed heat shock plants, especially on the final day of heat shock (Table 5.9). The 20% reduction in assimilation rate after heat shock was similar to that reported by Harding *et al.* (1990a,b) who conclude that damage to photosystem II was responsible for reduced assimilation. Water stress had a greater effect on assimilation, even after rewatering, suggesting some damage which limits assimilation (Morgan 1984). The combination of water stress and heat shock had a synergistic effect because the low transpiration rate (Table 5.9) probably resulted in the leaves heating up to levels above air temperature (Fischer 1980; Dunnin *et al.* 1991). Increased damage to the photosynthetic apparatus during combined water stress and heat shock reduced assimilation rates by 80% when plants were rewatered.

The rate of grain growth, however, did not decrease following heat shock as has been observed previously (Jenner 1991b, Jenner 1994). This may have been due to heat tolerance of the variety used (Stone and Nicolas 1995) or to artefacts in the experimental conditions under which heat shock is imposed in most published work in this area. Plants are usually detillered to leave the main stem only (Wardlaw 1994). Roots may be subjected to the same heat shock temperatures as the above-ground parts (Stone and Nicolas 1994; Wardlaw 1994) despite the findings of Kuroyanagi and Paulsen (1988) who reported the hastened senescence of shoots caused by high root temperatures. In addition plants are often taken from ambient and placed directly into 35-40°C chambers without acclimation or only spikes are heat shocked upsetting source/sink ratios.

The shortfall in assimilate caused by the damage to photosynthetic tissues of high nitrogen plants was made up by the remobilisation of stem reserves as indicated by differences in the decrease of stem weight (Figure 5.1c). The levels of WSC in the stem and stem linear density (Table 5.7) corroborate this conclusion with the agreement

between the decreases in stem weight and stem WSC being as good as for the field crops (Chapter 3, Figure 3.6) or in studies by Austin *et al* (1977) and Borrell *et al* (1989). The increase in WSC between anthesis and DC72, however, was approximately half the increase in stem weight. This observation agrees with the data from Chapter 3 where less than half the increase in stem biomass between DC65 and DC75 was attributable to the deposition of WSC. Similarly Kühbauch and Thome (1989) found that WSC only accounted for 13% of the increase in stem weight in the first 13 days after anthesis. It has been observed subsequently (data not shown) that the stems of cv. Janz, grown under adequate water supply, continue to elongate until approximately DC73. Indeed, in other studies, maximum crop height was not reached until 11 - 17 days after anthesis (Bonnet and Incoll 1992; Borrell *et al* 1989, 1993). Hence the increase in stem weight, not accounted for by WSC, was assumed to be structural material.

In contrast to the high nitrogen plants there was no apparent difference in plant dry weight from anthesis to maturity between water stressed or heat shocked plants at low nitrogen (Figure 5.1b). This was despite damage to the photosynthetic apparatus in the leaves of similar magnitude to that of high nitrogen plants (Table 5.6). Grain growth rates were similar for treatments at low nitrogen, though there was some indication of increased rate of development of the water stressed and heat shocked plants at the DC81 harvest. This was corroborated by a lower stem weight at the same harvest indicating more rapid remobilisation of WSC to the grain. In contrast to the high nitrogen plants there was no difference in the remobilisation of WSC from the stem as indicated by the decrease in stem weight. As for the high nitrogen plants WSC levels correlate well with stem weight gain/loss except in the 2 week period from anthesis to DC72.

The results indicate that under high nitrogen conditions, damage to photosynthetic tissues caused by water stress and/or heat shock limited the amount of current assimilate available for grain filling. This contention was supported by the more rapid loss of green leaves following the commencement of water stress and/or heat shock treatments (Figure 5.2b). Greater leaf area duration has been positively correlated with increased grain yield (Fisher and Kohn 1966c, Siddique *et al.* 1989). Due to the large sink size of treatments at high nitrogen and the damage to photosynthetic tissues of the leaves caused by water stress and/or heat shock treatments there was a shortfall in assimilate supply to the developing grain which was made up by a more rapid remobilisation of stem WSC. Judel and Mengel (1982) showed that when assimilate supply was reduced by shading, 33% more WSC was remobilised from the stems than for control plants and grain yield fell by only 17%. Pheloung and Siddique (1991) found that decreased photosynthesis in response to water stress also caused a faster rate of WSC mobilisation in wheat and barley crops. Conversely, high nitrogen plants that received no water stress or heat shock remobilised less of their stem WSC reserves (Table 5.7) due to a greater supply of

current assimilate. In low nitrogen plants, demand for nitrogen in the grain was such that remobilisation from the leaves was occurring regardless of water stress and/or heat shock and these treatments only served to hasten the process slightly (Figure 5.2a). Hence damage to the leaves was of little consequence for the low nitrogen plants because stem WSC reserves and current assimilate delivered by the spike itself was sufficient to meet the demand for assimilate by the developing grain. Araus *et al.* (1993) found that most of the current assimilate present in the grain came from spike photosynthesis under both well watered or terminal drought conditions. In the well watered conditions, penultimate and lower leaves started to senesce soon after anthesis and the flag leaf and sheath had senesced completely by 28 days post anthesis suggesting that nitrogen deficiency existed and that demand for nitrogen by the grain led to premature senescence of the leaves resulting in the majority of post-anthesis assimilation being carried out by the spikes. This was also the case for the low nitrogen plants in the present study. If leaves had contributed significantly to grain yield then differences in grain yield or remobilisation of WSC should have been evident due to water stress and/or heat shock as it was in high nitrogen plants. Work by Blum (1986) showed that the photosynthetic apparatus of the awns was more stable at high temperatures than for other spike tissues or leaves of wheat. Assuming that respired carbon was derived from current assimilate (Bell and Incoll 1990), the contribution of pre-anthesis stem WSC and whole shoot protein amounted to 4.8 g plant^{-1} (Table 5.15) or 48% of the final grain yield. Assuming that the non-protein decrease in leaf weight was WSC and hemicellulose translocated to the grain, leaves contributed a further 0.5 g plant^{-1}. Post-anthesis storage and remobilisation of WSC in stems amounted to 1.5 g plant^{-1} . Net assimilation from the day before the commencement of water stress and/or heat shock to maturity, therefore, was estimated to have contributed only 3.3 g plant^{-1} or 33% of grain yield for all low nitrogen treatments. This net assimilation all took place before DC81 after which low nitrogen plants began to lose total dry matter. Blacklow and Incoll (1981) also found that for a low nitrogen crop grain yield continued to increase after net assimilation had ceased, sustained by remobilisation of WSC and protein.

Damage to the photosynthetic tissues by water stress and heat shock and the impact that these have on the carbohydrate dynamics of grain filling for high nitrogen plants are evident in Table 5.15. The high nitrogen control plants contributed the least pre-anthesis reserves to grain yield of 6.6 g plant^{-1} or 45% of grain yield. High nitrogen, water stressed and heat shocked plants had the highest estimated contribution of pre-anthesis reserves to grain yield of 7.5 g plant^{-1} or 56% of grain yield. The estimated post-anthesis assimilation of high nitrogen, water stressed and heat shocked plants was 5.8 g plant^{-1} compared to the high nitrogen control plants (8.0 g plant^{-1}) reflecting the damage to their photosynthetic tissues.

Table 5.15. Estimates of pre anthesis retranslocation, including WSC and protein, to the grain and their contribution to grain yield per plant.

Treatment	Non protein leaf weight loss	Shoot protein retranslocation	Pre-anthesis Stem WSC	Grain yield	Contribution of soluble reserves to grain yield
		(g plant^{-1})			(%)
-N W C[a]	0.52	0.65	4.2	10.4	52
-N W H	0.53	0.65	4.1	9.8	54
-N D C	0.42	0.65	4.2	10.1	52
-N D H	0.35	0.67	4.1	10.0	52
+N W C	1.08	1.17	4.3	14.5	45
+N W H	1.28	1.15	4.6	13.1	54
+N D C	1.17	1.20	4.7	13.2	54
+N D H	0.94	1.21	5.3	13.2	56

[a] Code as for Table 5.5.

5.4.2 Relating the controlled environment data to field studies

In contrast to the field work of Chapter 4, individual stem weight and stem linear density increased with additional nitrogen while specific leaf area decreased. The concentration of WSC in the stems was not significantly different between nitrogen treatments prior to the imposition of water stress and heat shock but there were greater total reserves in the high nitrogen plants due to a greater individual stem weight and extra spikes. Campbell *et al.*(1983) also found increased amounts of WSC with increased nitrogen in spaced wheat plants in contrast to field work (Spiertz and Ellen 1978, Blacklow and Incoll 1981). Contrary to the field experiments at Ginninderra, Pucawan and Wagga Wagga, low and high nitrogen plants did not differ in sink demand per gram of stem WSC present at anthesis, containing 47 and 45 kernels g WSC^{-1}, respectively. Corresponding values are approximately 30% greater in the field for low nitrogen crops but 300% greater for high nitrogen crops. It is of little surprise then, that investigations into haying-off with spaced plants such as Elliott (1965), with a range of water stress treatments, cannot induce negative grain yield responses to nitrogen.

The accumulation of dry matter showed a similar response to nitrogen in tube plants as did the field crops but plant morphological responses were reversed for some important attributes. Wardlaw (1994), however, concludes that single culms, although not as sensitive to high temperature, are representative of the canopy response. The reduced

sensitivity may, in no small part, be due to the buffering capacity of relatively high levels of stem WSC in spaced plants.

The immediate physiological responses to water stress and heat shock, are therefore, likely to be the same for spaced plants as in the field but the response in grain yield is likely to be more stable for spaced plants due to the buffering capacity of higher levels of WSC. Work by Palta and Fillery (1995a,b) in Western Australia involved the addition of nitrogen fertiliser to a low tillering variety of wheat (cv. Kulin). Despite severe post-anthesis water stress, the crop at the highest rate of nitrogen did not hay-off and still yielded 20% higher than crops receiving no nitrogen. Similarly, the high nitrogen plants in the tube experiment did not hay-off, the combined water stress and heat shock plants yielding 31% above the low nitrogen plants.

For Palta and Fillery (1995a) spike density ranged from 166 to 209 m^{-2} which resulted in individual stem plus leaf weights (stem weight was not reported) at anthesis from 1.97 to 2.75g respectively. In the tube experiment above, nitrogen treatments were randomised resulting in mean spike density per unit of ground area of 234 m^{-2}. Individual stem plus leaf weight at anthesis was 2.06g for low nitrogen and 2.66g for high nitrogen plants. This comparison highlights the similarities between the experimental field plots of Palta and Fillery (1995a,b) and the current tube experiment, illustrating that field crops with a low spike density behave as spaced plants. To contrast the difference in apparent stem reserves between the mean stem plus leaf weight at anthesis of an open crop (Palta and Fillery 1995a) and spaced plants (this chapter) and the mean of the well managed crops at GES91, PUC91 and WAG91 was 1.70g (15% decrease) for low nitrogen and 1.62 g (40% decrease) for high nitrogen. Austin *et al* (1977) acknowledged that there were likely to be differences in remobilisation of stem reserves between spaced plants and the high population density of field grown plants but only considered plants of high nutrient status and thus postulated less remobilisation than in the field due to better light relations and greater post-anthesis assimilation.

5.4.3 ^{14}Carbon dynamics during grain filling.

The decrease in ^{14}C from the stem WSC fraction correlated well with the decrease in ^{14}C from stems for both high and low nitrogen plants (Figure 5.3). Between anthesis and DC72 the concentration of ^{14}C in the stem and WSC decreased due to the increase in non-labelled stem structural materials and WSC. The amount of ^{14}C present in the stem did not change. At DC72 low nitrogen grain contained more ^{14}C than in high nitrogen plants due to an initially faster rate of remobilisation of nitrogenous compounds to the grain as indicated by the higher NHI for low nitrogen plants (Table 5.10). Between DC81 and maturity the decrease in ^{14}C from the stem paralleled the decrease in dry

matter from the stem and accounted for the increase in ^{14}C in the grain. The harvest index of ^{14}C at DC81 (Table 5.10) was significantly greater for treatments which reduced current assimilation; low nitrogen, water stress or heat shock. The grain, therefore, was being buffered against a decrease in supply of current assimilate to maintain grain growth rates. Bidinger *et al* (1977) also found a higher harvest index of ^{14}C at any stage of grain filling for droughted compared to irrigated crops. When current assimilation can no longer supply the spike's requirements, reserves are mobilised and stem dry matter begins to decrease (Bell and Incoll, 1990). In fact, in the 14 days from DC72 to DC81, kernel growth rate increased by 7% due to heat shock in the absence of water stress and 19% due to water stress and heat shock. The increase in kernel growth rate due to heat shock still evident at DC81 was in contrast to the result reported by Jenner (1991b), that growth rate was greater during heat shock but rapidly fell to rates lower than controls when returned to ambient temperatures. Hence there was no significant reduction in final kernel weight in this study as has been reported previously (Jenner 1991a,b; Hawker and Jenner, 1993; Jenner, 1994). This apparent disagreement may be due to heat tolerance (Stone and Nicolas 1995) of the variety used in the current work and/or to differences in the severity of treatments imposed.

Stone and Nicolas (1995) reported large variation in the response of kernel weight to heat shock from no effect to large significant reductions. Treatments in the present study were designed to simulate hot, windy days of high VPD often experienced in the Australian wheat belt during grain filling. The treatments imposed by Jenner, (1991a,b); Hawker and Jenner, (1993) entailed placing only the spike directly into 35 °C from 21 °C for continuous or at best alternating 12 hour square wave periods of heat shock and lower temperature. Even so, significant decreases in final kernel weight were only achieved after 7 days of treatment (Jenner, 1991b) or 4 days (Hawker and Jenner, 1993). When heat shock treatments designed to simulate naturally occurring conditions were imposed on a range of wheat genotypes at a similar time to the current experiment 10 days after anthesis grain yield and kernel weight was significantly reduced in only one variety (Stone and Nicolas 1994). However as already mentioned significant variation in response of grain yield and kernel weight to heat shock was found among 75 diverse genotypes (Stone and Nicolas 1995). In these studies roots and shoots were subjected to the heat shock treatment. The significant reduction found in kernel weight, which contrasts to the present study, may be due to the hastened senescence of shoots caused by high root temperatures (Kuroyanagi and Paulsen 1988) and/or the higher maximum temperature (40 °C) during the heat shock period than in the present study (35 °C).

For the same environmental conditions imposed, the effect of water stress and/or heat shock are likely to be worse for a field crop than for spaced plants due to the reduction in WSC reserves per kernel present at anthesis as discussed earlier. Final kernel weight

was not significantly reduced in the present study due to soluble reserves buffering against any shortfall in current assimilate as corroborated by an increase in ^{14}C harvest index with water stress or heat shock and a decrease with high nitrogen.

Losses of ^{14}C from the above-ground shoots of approximately 5% (Figure 5.4) between anthesis and maturity are similar to those previously reported (Stoy, 1963 ≈10%, Wardlaw and Porter 1967 ≈10%, Austin *et al* 1977 ≈8%, Austin *et al* 1980a ≈11%, Bell and Incoll (1990) ≈5%, Palta *et al* 1994 ≈6%,). Penning de Vries, (1972) calculated the cost of remobilisation of carbohydrate such that 1 g of remobilised hexose or sucrose will yield 0.85 or 0.94 g of starch respectively. Remobilised nitrogenous compounds, arising from the hydrolysis of 1 g of leaf or stem protein would be expected to yield approximately 0.84 g of grain protein (Austin *et al* 1980a). The losses from the above studies, therefore, fall within the expected range for an estimated conversion efficiency for soluble dry matter of 84 - 94 %. These findings support the results of Winzeler *et al* (1990) who concluded that once laid down WSC reserves were not turning over. Indeed most of the ^{14}C fixed is lost in the first 24 hours but losses from several days after labelling to maturity are minor (Austin *et al* 1977, Austin *et al* 1980a). This indicates that respiration during grain filling uses current rather than stored assimilates which is at odds with the suggestion that up to 33 % of the decrease in stem weight could be attributed to respiration (Rawson and Evans, 1971). However, most of the above mentioned studies were conducted under well watered conditions and so it seems reasonable to assume that if the supply of current assimilate was severely limited by drought stress then respiratory demands would be met by stored assimilates. Treatments were not severe enough in the present study to show significant differences in respiration but water stressed and heat shocked plants contained, on average, 7% less ^{14}C than control plants at maturity. Palta *et al* 1994 found that respiratory losses of pre-anthesis labelled ^{13}C was greater for rapid severe water stress (7%) than for slowly developing water stress (4%) imposed during grain filling. Losses of ^{14}C, therefore, could be expected to be dependent on the amount of soluble dry matter retranslocated and thus subject to losses, and the supply of current assimilate for respiration. If plants are not harvested soon after physiological maturity there is always the possibility that losses of ^{14}C during grain-filling can be overestimated due to the possibility of wasteful respiration of WSC left behind in the stem (Schnyder 1993).

5.4.4 Nitrogen dynamics of above-ground biomass

All plants took up nitrogen between anthesis and maturity and there was no difference in uptake between water or heat shock treatments. This was in contrast to the field situation where a dry soil surface limits nitrogen uptake (Clarke *et al* 1990). It was physically impossible to allow water stressed tubes to dry down slowly due to the fact

that spaced plants were 3 - 4 times larger than for crop plants exploiting the same soil volume. The water stress treatment received water at half the rate of control plants which were watered *ad libitum*. This meant that the surface soil of water stressed plant tubes received enough water to allow significant nitrogen mineralisation and similar uptake to control plants.

The onset of senescence of leaves between anthesis and DC72 results in transport of nitrogen directly to the grain (Figure 5.6a) for low and high nitrogen plants while high nitrogen plants temporarily stored remobilised nitrogen, in excess of the grain demand, in the stem (Figure 5.6b, van Keulen and Seligman, 1987). This was corroborated by stems not contributing to grain nitrogen until ≈10 days after anthesis for low nitrogen and ≈18 days after anthesis for high nitrogen (Figure 5.6). Nitrogen remaining in the non-grain spikes was greater at high nitrogen at DC81, but tended to be less with water stress or heat shock (data not shown). The nitrogen dynamics of stems followed similar trends to those shown for stem biomass and stem ^{14}C. The higher NHI of high nitrogen plants experiencing water stress compared with high nitrogen controls was a result of greater remobilisation of nitrogen from the stems and leaves. The rapid retranslocation of nitrogen in response to water stress for high nitrogen leaves corroborated the earlier findings of damage to photosynthetic apparatus and faster senescence of leaves. In contrast, the lack of any effect of water stress or heat shock on nitrogen retranslocation from leaves of low nitrogen plants was because nitrogen was already moving to developing grain regardless of imposed treatments. At the imposition of water stress and heat shock 73 % of the nitrogen had already retranslocated from the leaves of low nitrogen plants. This compared with 56% of leaf nitrogen having been remobilised for the leaves of high nitrogen plants. There was a trend for reduced remobilisation of leaf nitrogen from subject to heat shock in contrast to a trend for greater remobilisation from stems. This may have been due to desiccation and sudden death of distal portions of green leaves making those areas of leaf inaccessible to catabolism by the plant (Clarke *et al* 1990). High final leaf nitrogen concentration, the high proportion of the final biomass made up by leaves and a decline in NHI for plots which hayed-off at WAG91 (Chapter 4) and for water stressed compared to irrigated treatments of Giunta *et al* (1995) support this hypothesis.

5.4.5 Water use and transpiration efficiency

The mean transpiration efficiency for all plants was greater from sowing to anthesis (6.4 g litre^{-1}) than from anthesis to maturity (4.2 g litre^{-1}). This decline was not as marked as the 90% reduction in water use efficiency between the periods from sowing to heading and from heading to maximum ear weight found by Giunta *et al* (1995) which was improbably attributed to a small increase in the mean VPD. It was more likely that this

reduction in water use efficiency was due to a reduction in photosynthetic capacity as a result of remobilisation of leaf nitrogen and retranslocation to the grain (Gregory *et al* 1981; Sinclair and Horie 1989). Between DC69 and DC73 in the present study, instantaneous TE for high nitrogen plants fell from 2.94 μmol CO_2 mmol H_2O^{-1} with an estimated leaf nitrogen concentration of 2.0% to 1.78 μmol CO_2 mmol H_2O^{-1} with an estimated leaf nitrogen concentration of 1.3%. The decrease in instantaneous TE was not due to an increase in leaf to air VPD because this was similar at both times (2.9 and 3.0 kPa respectively). These data indicate an uncoupling of photosynthetic capacity and stomatal control as nitrogen concentration decreased, such that stomates remain open during leaf senescence (Table 5.6), dramatically reducing instantaneous TE.

Long-term transpiration efficiency was greater for high nitrogen plants from sowing to anthesis supporting earlier findings of greater transpiration efficiency for high nitrogen treatments in spaced plants (Wong *et al* 1979; Morgan 1984). Increased transpiration efficiency with the addition of nitrogen was due to a higher leaf nitrogen concentration (Wong *et al* 1979) and a reduction in SLA thereby increasing photosynthetic capacity per unit of leaf area. Instantaneous TE was also greater for high nitrogen plants at DC69 and DC73. The latter result, however, was contrary to the long term transpiration efficiency measured between anthesis and maturity which shows a negative trend with the addition of nitrogen. This discrepancy between measurements was likely to be due to the long term transpiration efficiency using a measure of whole plant assimilation which includes losses due to respiration (Pearman *et al* 1981; Amthor 1989) and the costs of retranslocation (Austin *et al* 1977), while instantaneous TE uses a measure of leaf assimilation. The percentage drop in instantaneous transpiration efficiency between DC69 and DC73 was the same for both nitrogen treatments but the greater losses from high nitrogen plants due to respiration and retranslocation are likely to account for the negative trend. Heat shock also results in a negative trend in long term transpiration efficiency measured between anthesis and maturity due to damage to the photosynthetic apparatus (Harding *et al* 1990a,b) as already discussed.

Long-term transpiration efficiency between anthesis and maturity was significantly higher for water stressed plants, whose leaves senesced more rapidly, compared to well watered plants. This result was in agreement with the work of Araus *et al* (1993) and Blum *et al* (1983) who found that when spikes were the major contributor to grain filling transpiration efficiency increased. In addition, transpiration efficiency can increase with water stress due to the maintenance of assimilation rates while stomates partially closed (Morgan 1984; van Herwaarden unpublished) This result was contrary, however, to the reduction in instantaneous transpiration efficiency due to water stress obtained after temporary rewatering at DC73. This apparent memory effect of previously water stressed plants has been observed before (Morgan 1984) and was attributed to the

apparent greater sensitivity of mesophyll photosynthetic capacity to water stress than stomatal control which results in reduced transpiration efficiency upon rewatering.

There was a trend for greater transpiration efficiency (32% higher) in response to water stress at low nitrogen than well watered low nitrogen plants which was less than the response to water stress at high nitrogen (20%). This reduced response of high nitrogen plants was likely to be due to several factors including higher respiration rates, and greater sensitivity of mesophyll photosynthetic capacity to water stress than stomatal control (Morgan 1984).

5.4.6 Detillered and defoliated high nitrogen plants

DTDF high nitrogen plants reacted differently to the stress and/or shock treatments than the low nitrogen plants of similar spike number, leaf area and kernel number, but similarly to the intact high nitrogen plants. When photosynthetic area was significantly damaged by water stress and/or heat shock, then the grain yield of DTDF plants decreased but did not fall below the level of low nitrogen plants whose leaf lamina senesced early due to demand for nitrogen by the developing grain. DTDF control plants are able to achieve greater grain yield than low nitrogen plants because their superior leaf area duration and higher assimilation rate (Table 5.7) supplied more current assimilate to the grain. This compensatory increase in photosynthetic activity post-anthesis as a result of defoliation has been observed by others (Aggarwal *et al* 1990) and was corroborated by the upward trend in kernel weight and the reduced utilisation of stem WSC in the absence of water stress and/or heat shock. High nitrogen concentration of the plant tissues does not appear to confer greater susceptibility of tissues to water stress or heat shock than low nitrogen concentration. Inhibition of photosynthesis due to water stress and/or damage to photosynthetic apparatus due to heat shock showed the same percentage decrease at low or high nitrogen status. However the reduction in assimilation at high nitrogen was compensated for by the greater soluble reserves and DTDF plants yielded more than low nitrogen plants. This conclusion corroborates the earlier discussion as to why haying-off does not occur in sparse field crops.

5.4.7 Summary

Haying-off in the field is caused by differential water stress during grain filling due to greater water use prior to anthesis at high nitrogen. The lack of water for transpiration reduces the availability of current assimilation thereby limiting grain yield. At high spike densities, yield can fall to levels below nil nitrogen controls. This is due to a counter-intuitive decline in water soluble carbohydrate reserves with increasing biomass and the detrimental effects water stress and/or heat shock have on post anthesis transpiration

efficiency. Brief periods of high temperature and hot dry winds are not necessary to induce haying-off as Fischer (1979) indicated and are not likely to cause haying-off in the absence of water stress in all but extreme cases (Azzi 1956, Fischer 1980). Further reductions in grain yield are likely to occur for heat shock in conjunction with water stress due to damage to photosynthetic apparatus (Harding *et al.* 1990a,b), the uncoupling of photosynthetic capacity and stomatal control (Morgan 1984), greater respiration at high nitrogen concentration (Pearman *et al* 1981) and a dramatic decrease in green leaf area post heat shock (van Herwaarden unpublished), resulting in spectacular reductions in kernel weight (Stone and Nicolas 1995, WAG91 Chapter 4) and hence grain yield.

The results of these experiments account for high levels of WSC in low plant density canopies and leads to speculation that while haying-off is a problem in the closed-canopy crops of the South-eastern wheatbelt, it is not recognised as a problem in the North-eastern or Western wheatbelt where the open-canopy crops are likely to contain higher levels of pre-anthesis stored WSC.

6 GENERAL DISCUSSION

6.1 INTRODUCTION

Wheat will continue to be Australia's most important cereal crop, both in terms of area sown and the value of production. On average, over the last 40 years, more than two-thirds of the crop has been exported, contributing from 12 to 25% of national rural export earnings (Hamblin and Kyneur 1993). More than 90% of the area sown to wheat in southern and eastern Australia is grown under dryland conditions, the main limitation to wheat production being the amount of rainfall during the growing season. Most of the wheat belt is classified as a Mediterranean environment with about 70% of the annual rainfall received from May to October.

In the past 25 years there has been a decline in the protein concentration in wheat delivered to Australian silos (Hamblin and Kyneur 1993). The decrease in protein concentration has been attributed to a decline in soil fertility due to increased cropping intensity and widespread nitrogen limitation (Angus and Fischer 1991). In contrast to other wheat-producing regions, wheat crops in Australia are usually grown with little or no nitrogen fertiliser. The mean rate of nitrogen fertiliser applied to wheat crops in Australia in 1987 was less than 11 kg N ha^{-1} (McLaughlin *et al.* 1992). Studies on research stations and commercial farms (Russell 1967; Taylor *et al.* 1974; Angus *et al.* 1989) and farm surveys (Martin *et al.* 1988; Wegener *et al.* 1988) have established that nitrogen deficiency of commercial wheat crops was widespread in the wheat belt. One of the reasons for this is that the yield response to nitrogen fertiliser is highly variable, ranging from strongly positive (Russell 1967; Angus *et al.* 1989; McDonald 1991) to negative (haying-off) (Colwell 1963; Storrier 1965a; Angus *et al.* 1989; McDonald 1991). The variability of the yield response has meant that farmers are reluctant to apply nitrogen. Furthermore, the pricing system for wheat rewards yield rather than protein.

Crop scientists commonly perceive that farmers' preoccupation with haying-off was unjustified after the early 1980s with the introduction of semidwarf varieties (Hamblin and Kyneur 1993). However, the belief among farmers that haying-off is still a problem stifles decision making with respect to nitrogen fertiliser application. The incidence of haying-off found in nitrogen fertiliser trials on commercial wheat crops in the late 1980s and early 1990s indicates that haying-off is not a thing of the past but will continue to plague farmers. Its occurrence is likely to increase in frequency as farmers manage nitrogen better through leguminous crops or pastures or through increased fertiliser applications. Indeed Angus *et al.* (1989) found that 15 of 33 wheat crops hayed-off in response to 40 kg N ha^{-1} in New South Wales while McDonald (1992) found that 9 of 10 crops he evaluated hayed-off in South Australia.

The aim of the work presented in the preceding chapters was to gain sufficient understanding of the physiological responses of wheat crops to applied nitrogen to explain the mechanisms of haying-off. Field experiments were established in environments which were expected to provide contrasts in water availability during grain filling. At each experiment increasing rates of nitrogen fertiliser were applied at different stages of crop development. The mechanism of haying-off was investigated by monitoring the carbon and nitrogen dynamics through measurements of biomass production, nitrogen uptake and water use during crop development.

In addition to the field studies, a controlled environment study was conducted to separate the effects of drought and high temperature on haying-off. Wheat plants were grown as a simulated crop (micro-canopy) at low and high nitrogen status, under post-anthesis drought and well-watered conditions, so as to assess the effect of a heat shock event on haying-off. The heat shock event was designed to be as realistic as possible so as to simulate that which can occur through spring and summer in the southeastern Australian wheatbelt. The micro-canopy was labelled with ^{14}C several times prior to anthesis to examine respiratory losses during grain filling.

6.2 DISCUSSION

6.2.1 Yield responses

The application of nitrogen fertiliser increased nitrogen uptake and biomass production to anthesis in all experiments. This has also been found in other studies (eg. Barley and Naidu 1964; Dann 1969; Morgan 1988; Angus et al. 1991; McDonald 1992). Increases in biomass were associated with a greater shoot density. It was found that even delaying fertiliser application from DC10 to DC30 in a wet season did not result in a yield penalty (BAR92) and in fact resulted in a yield advantage (GES92). However, under terminal drought conditions yield declined when fertiliser application was delayed to DC30 because of reduced nitrogen uptake and post-anthesis growth due to dry soil conditions.

In this study, greater biomass production to anthesis resulted in vastly different grain yield responses. These were a function of rainfall distribution. In the wettest environment (GES92) where 41% of the rain from DC10 to maturity fell after anthesis (DC65), there were positive grain yield responses to nitrogen fertiliser. These were associated with greater spike density, more kernels per spike and hence greater kernel number but a reduction in kernel weight. Harvest index either increased or declined slightly.

Negative grain yield responses were also associated with increases in spike density, kernels per spike and greater kernel number. In contrast, however, harvest index decreased due to a dramatic fall in kernel weight. At this site (WAG91) more than 90% of the rain from DC10 to maturity fell prior to DC65 and grain filling occurred under water stress conditions. Despite greater water stress of high nitrogen crops from prior to anthesis onwards, kernel number continued to increase in response to nitrogen fertilisation.

6.2.2 Determination of kernel number

Radiation, temperature and pre-anthesis water stress have important effects in determining kernel number (Fischer and Stockman 1980; Fischer 1985;). However, data presented in Chapter 3 indicate that nitrogen content of the spikes at DC65 is a better predictor than these factors.

Figure 3.12c shows that there was a strong correlation ($r^2 = 0.95$; n = 15) between kernel number and nitrogen content of the spikes at DC65. It is possible that nitrogen content of the spikes at DC65 is the major determinant of kernel number in wheat. The three sites presented include two varieties, a range in sowing date of 4 weeks and differences in pre and post-anthesis water stress such that grain yield and kernel weight range from 284 to 798 gm^{-2} and 18 to 37 mg respectively. The 'special effect' of nitrogen which predisposes a wheat crop to haying-off (Dann 1969; Fischer 1979) may be that improved nitrogen status of a wheat crop results in a greater kernel number (larger yield potential, greater sink demand) despite increased water stress at anthesis. This comparison suggests that it is the content of nitrogen in the spikes rather than carbon supply that determines kernel number in this environment.

Spike WSC content had a positive correlation with kernel number in each experiment, in agreement with prior work (Kemp and Whingwiri 1980; Judel and Mengel 1982; Stockman *et al.* 1983; Fischer 1985). However, the relationship using data from all sites was weak. For WSC concentrations at PUC91 (77 -83 g kg^{-1}) and WAG91 (131 -140 g kg^{-1}) there was no relationship between kernel number and WSC while over a wider range of WSC at GES91 (110 - 135 g kg^{-1}) there was a negative correlation ($r^2 = 0.74$; n = 7).

6.2.3 Problems with apparent retranslocation

Many studies have assumed that weight loss from non-grain biomass between anthesis and maturity equates with the retranslocation of pre-anthesis assimilates for grain growth

and losses due to respiration (Gallagher *et al.* 1975, 1976; Midmore *et al.* 1984; Angus *et al.* 1991; Palta and Fillery 1995a). This method calculates retranslocation from the non-grain above-ground biomass to the grain by assuming that weight loss between DC65 and maturity equates to net retranslocation plus respiration. In this study it was termed 'apparent retranslocation'. Retranslocation was also estimated from changes in WSC and protein in the biomass between DC65 and maturity. Here it is termed 'estimated retranslocation'. At the dry sites BAR91, PUC91 and WAG91, apparent retranslocation was generally greater than estimated retranslocation. At the wet sites of BAR92, GES91 and GES92, however, estimated retranslocation was generally greater than apparent retranslocation.

There are several sources of error which results in apparent retranslocation to either overestimate or underestimate retranslocation. It does not take into account tissue formed before DC65 that is lost in the post-anthesis period, such as leaf fall (Barley and Naidu 1964, Austin *et al.* 1980) or saprophytic decay of lower leaves (Bidinger *et al.* 1977). This error is likely to overestimate the pre-anthesis contribution to grain filling. Conversely, it does not take into account non-grain tissues formed after DC65 that are measured at maturity but not at DC65, thereby decreasing the magnitude of apparent retranslocation. This error is likely to be greatest in the absence of water stress due to the continued growth of the stem after DC65 (Bonnett and Incoll 1992a; Borrell *et al.*1989, 1993) and cell-wall thickening and lignification (Stoy 1965; Pearce *et al.* 1988) which lead to an increase in structural biomass, thereby reducing apparent retranslocation. This error is likely to underestimate the pre-anthesis contribution to grain filling. Austin *et al.*(1977) concluded that leaves do lose weight but that the export of dry matter is difficult to quantify because dead leaves are fragile and tend to drop off the plant. The loss of leaf material due to rain or disease was estimated by Bidinger *et al.* (1977) to be 100 gm^{-2}, or 50 percent of the anthesis leaf biomass, while Austin *et al.* (1977) considered this amount to be improbably high and estimated leaf fall to be 20 percent with a leaf biomass of 150 gm^{-2}.

Leaf loss (leaf fall from standing crop or losses during the sampling process) was estimated at WAG91 from a budget that included an estimate of leaf weight that involved calculation of the leaf cellulose and lignin as well as the mobilised WSC and protein. The cell wall biomass was estimated from the methods of Goering and Van Soest (1970) for determination of Neutral Detergent Fibre (NDF) and cellulose and lignin was estimated as the Acid Detergent Fibre (ADF, estimate of cellulose and lignin). The biomass of ADF in the leaves apparently decreased between DC65 and maturity. This is highly unlikely given that cellulose and lignin are immobile, and it is more probable that leaf fall was responsible. Leaf ADF biomass was assumed to be constant for leaves between DC65 and maturity, and estimates of maturity leaf biomass calculated. These revised

estimates of maturity leaf biomass indicate that leaf loss between anthesis and maturity ranged from 57 to 94 g m^{-2} (30 % of DC65 leaf biomass) for nitrogen applications from 0 to 200 kg N ha^{-1}.

Hemicellulose in the cell walls of leaves and leaf sheaths was identified as another possible source of pre-anthesis assimilate available for retranslocation to the grain. The hemicellulose content was determined as the difference between NDF and ADF content. Hemicellulose constituted approximately 30% of the dry matter exported from the leaves between DC65 and maturity at WAG91 and contributed up to 5% to yield.

6.2.4 Negative association between anthesis biomass and WSC

It is commonly assumed that greater biomass at anthesis equates with higher pre-anthesis assimilate reserves (eg. Fischer 1979). This assumption comes from the indirect evidence that apparent retranslocation increases with greater anthesis biomass (Gallagher *et al.* 1975, 1976; Angus *et al.* 1991; Palta and Fillery 1995a). Work with spaced wheat plants (Campbell *et al.* 1983) has added weight to this argument by reporting greater WSC retranslocation to grain at high nitrogen status. Plants in the high nitrogen treatment were also larger.

However, greater biomass at anthesis, whether due to nitrogen fertilisation (Spiertz and Ellen 1978; Spiertz and van de Haar 1978) or genotypic variation (Stapper and Fischer 1990b), was associated with an increased spike density and a reduction in WSC as a percentage of stem mass or per unit ground area. This reduction of WSC with increased biomass is most likely due to the use of carbon skeletons in additional protein (Austin *et al.* 1977), higher respiration rates for increased nitrogen concentration of tissues (Amthor 1989) and assimilates being used to a greater extent for structural materials (Spiertz and van de Haar 1978; Stapper and Fischer 1990b). The mass of WSC reserves retranslocated in high nitrogen crops between anthesis and maturity is less than in low nitrogen crops in both wet (Spiertz and van de Haar 1978; Austin *et al.* 1980b; Blacklow and Incoll 1981) and dry years (Spiertz and Ellen 1978).

The time when maximum WSC reserves are accumulated will also be important if they contribute to grain filling. For example if their highest value is accumulated early in grain filling then they are likely to contribute less to grain growth and they are also likely to be depleted earlier which may result in small grains. Under cool, well watered conditions maximum WSC reserves usually occur between 20-25 days after anthesis (Austin *et al.* (1980b; Blacklow *et al.* 1984; Bell and Incoll 1990; Bonnett and Incoll 1992). Under dryland conditions in a favourable spring in southeastern Australia, Borrell *et al.* (1989) found that maximum WSC reserves were achieved 14 days after anthesis. Austin *et al.*

(1980a) found maximum water soluble carbohydrate reserves for barley genotypes were achieved 5 days after anthesis in a dry year and 14 days after anthesis under more favourable conditions. Work by Rawson *et al.* (1977) indicates that pre-anthesis stress reduces reserves for subsequent retranslocation to the grain. In the present study, whether under water stress or well watered conditions, WSC content in the non-grain biomass of control crops or crops receiving 80 kg N ha^{-1} was highest at DC65 and declined thereafter. For high nitrogen crops growing under well watered conditions the 240SW at BAR92 achieved its highest store of WSC 14 days after DC65 while the 240LT crop reached its highest level at 28 days after DC65. On the other hand, under dry conditions high nitrogen crops achieved their highest levels of WSC at or before anthesis.

Simpson (1992) concludes that reduced photosynthetic capacity caused by drought stress or nitrogen deficiency results in stem WSC reserves being mobilised sooner and becoming an assimilate source of increased importance for grain growth. In this study it is suggested that the negative association between WSC with increasing anthesis biomass (nitrogen fertilisation), and the lower WSC content and earlier remobilisation of WSC due to water stress, indicate that a crop of high nitrogen status is vulnerable to haying-off if it encounters the onset of terminal drought close to anthesis.

6.2.5 Post-anthesis carbon and nitrogen dynamics

Under well watered conditions, Austin *et al.* (1977) found that most of the carbon for grain filling was assimilated after anthesis. Supplementary nitrogen is known to promote leaf area development (Spiertz and Ellen 1978, Spiertz and van de Haar 1978) and extend leaf area duration (Fischer and Kohn 1966c, Austin *et al.* 1980b, Spiertz and Ellen 1978, Blacklow and Incoll 1981). The high nitrogen crop studied by Blacklow and Incoll (1981) continued net photosynthesis until the cessation of grain filling. In contrast, their low nitrogen crop ceased net assimilation half way through grain filling and further grain growth was sustained by retranslocation of WSC and protein from the non-grain biomass.

Austin *et al.* (1980b), found that grain growth rates, whether in crops of low or high nitrogen status, exceeded crop growth rates by 109 and 82 percent respectively between 20 and 45 days after anthesis. Bell and Incoll (1990) also found that in a well managed crop the demand for assimilate exceeded current supply during the linear growth phase of the grain and stem reserves were mobilised to support maximum grain growth rate. Spiertz (1977) found that during grain filling in wheat WSC reached a peak of 30 percent of stem weight 28 days after anthesis at 10 °C. At 25 °C, WSC peaked at anthesis and decreased thereafter. Plants also contained 60 percent less WSC at anthesis. This was

due to depressed photosynthetic rates at 25 °C while grain growth rates doubled between 10 and 25°C. A 50 percent reduction in grain yield between 10 and 25 °C was associated with a decrease in kernel weight. There is a significant body of literature reviewed by Amthor (1989) which show that the rate of respiration and other metabolic processes increase with increasing temperature. In addition respiration rate is stimulated by higher tissue nitrogen concentration (Amthor 1989).

The combination of a decline in photosynthetic rate and increased respiration with temperatures above 20 °C explain why positive yield responses to nitrogen in the cooler, wetter European environment are associated with an increase in kernel weight (Blacklow and Incoll 1981, Spiertz and Ellen 1978) while under warmer Australian conditions positive yield responses to nitrogen are associated with a decrease in kernel weight whether under dryland conditions (Angus *et al.* 1991; McDonald 1992; Frederick and Camberato 1994), or irrigated conditions (Strong 1986; Fischer 1993; J.F. Angus pers. comm.). Lower kernel weights associated with increasing nitrogen fertiliser have been observed in the absence of post-anthesis moisture stress (Storrier 1965; Lipsett and Simpson 1973) and attributed to an additional effect of nitrogen per se (Dann 1969, Fischer 1979).

The wheat crop attempts to buffer the developing grain against variations in assimilate supply so that grain growth can continue at its maximum rate at all times (Bell and Incoll 1990). However, photosynthetic rates and water soluble carbohydrate storage are likely to be limited by the warmer Australian conditions compared to the climate in the UK or the Netherlands and grain growth rates increase in response to the higher temperatures, which may result in daily shortfalls in supply during the linear grain growth phase. The greater dependence of high nitrogen crops on current assimilate due to the greater number of kernels per gram of non-grain biomass at anthesis (eg. 16 - 22 kernels g^{-1} m^{-2} at GES92) and reduced WSC reserves, results in a yield increase but reduced kernel weight under well watered conditions. Drought stress, which is also usually associated with higher temperatures, further reduces assimilate supply to the grain while increasing respiration. Haying-off occurs under these conditions when lower WSC reserves, reduced current assimilate supply and increased metabolic activity result in a dramatic decrease in kernel weight and harvest index.

In crops of low nitrogen status there is inevitably a trade-off between nitrogen available for photosynthesis and grain growth (Sinclair and de Wit 1975); photosynthetic rates decline as a linear function of the proportion of nitrogen lost from the leaf (Gregory et al. 1981). The proportion of grain nitrogen derived by mobilisation from the culm is often higher in dry years or dryland cropping regions of Australia as a result of low levels of mineral nitrogen available for uptake due to a dry surface soil (Simpson 1992). The

consequence of this is that under water or nitrogen limited conditions, crop photosynthesis is in decline when assimilate requirements for grain growth are at their highest (Gregory et al. 1981). The demand for assimilate is satisfied by rapid remobilisation of water soluble carbohydrates during the linear phase of grain growth (Spiertz and van de Haar, 1978).

6.2.6 The effects of heat shock

The detrimental effects of high temperature heat shock (>30 °C for up to 7 days) during grain filling on kernel growth rate and final kernel weight are well documented (Fischer 1980; Jenner 1991; Hawker and Jenner 1993; Harding *et al.* 1990a,b; Stone and Nicolas 1994, 1995). In most of these studies the cultural conditions consisted of plants grown in small soil volumes and subjected to high temperatures without acclimation. In the present study no significant decrease in kernel weight was found following water stress and/ or heat shock at either low or high nitrogen status. Disagreement with the previous studies was probably due to either the use of a heat tolerant variety and/or to the simulation of field conditions and thus slightly less extreme treatments in the present work. High nitrogen concentration of the plant tissues did not appear to confer greater susceptibility of tissues to water stress or heat shock than low nitrogen concentration. Inhibition of photosynthesis due to water stress and/or damage to photosynthetic apparatus due to heat shock did not result in differences in grain yield or remobilisation of pre-anthesis reserves at low nitrogen because leaves were senescing regardless of treatment, due to demand for nitrogen by the grain. At high nitrogen however, yield potential was greater, not due to greater WSC reserves per kernel which were almost identical at anthesis (21.3 mg kernel^{-1} at low N, 21.4 mg kernel^{-1} at high N), but due to the greater photosynthetic capacity of a larger leaf area with a higher nitrogen concentration. Reduced post-anthesis assimilation in response to post-anthesis drought and/or heat shock at high nitrogen resulted in a decline in grain yield, but remobilisation of pre-anthesis reserves increased. Grain yields remained higher than for low nitrogen plants. High nitrogen plants did not hay-off as expected because of greater WSC reserves of high nitrogen plants at DC65 in contrast to crops of a high yield potential in the field. Higher WSC reserves were attributed to better light relations due to reduced spike density.

The results of this study suggests that brief periods of high temperature and hot dry winds are not necessary to induce haying-off as indicated by Fischer (1979), and are not likely to cause haying-off in the absence of water stress in all but extreme cases (Azzi 1956, Fischer 1980). However, for heat shock in conjunction with water stress grain yield losses are likely to occur due to the uncoupling of photosynthetic capacity and stomatal control with water stress (Morgan 1984), damage to photosynthetic leaf tissues

with heat shock (Harding *et al.* 1990a,b), greater respiration with raised tissue temperature (Amthor 1989) and a dramatic decrease in green leaf area after heat shock (van Herwaarden unpublished). Compensation for the further decrease in supply of current assimilate is not possible due to low WSC reserves at high nitrogen and spectacular reductions in kernel weight (Stone and Nicolas 1995, WAG91 Chapter 4) and hence grain yield occur.

6.2.7 Water extraction

Negative yield responses to nitrogen fertiliser or haying-off have in the past been attributed to greater post-anthesis water stress (Colwell 1963, Storrier 1965, Fischer and Kohn 1966c) caused by the high water use to produce biomass up to anthesis, leaving little in the soil for grain filling. This lack of soil water reduces photosynthesis and hence assimilates for grain filling (Fischer 1979, 1981).

In addition to the reduced soil water available for grain filling for high nitrogen crops, haying-off appears to reduce the final soil water extraction which contrasts with greater extraction for positive yield responses, as shown by the 10 mm less soil water extracted by hayed-off crops at WAG91. This unexpected result is supported by data extracted from Kirkegaard *et al.* (1994) where hayed-off crops of wheat following peas extracted less soil water than wheat following the other break crops. At sowing, the soil profile (0 to 2 m) of wheat following peas contained the same amount of water but at least twice as much mineral nitrogen as other break crops. There was a linear decrease in grain yield with increasing soil mineral nitrogen at DC10 similar to the relationship at WAG91 between grain yield and rate of nitrogen fertiliser.

6.3 CONCLUSIONS

The results of these experiments lead to an improved understanding of haying-off. Firstly, high nitrogen status leads to greater kernel set at DC65. Secondly, high nitrogen status leads to decreased WSC reserves at anthesis. Provided there is little or no water stress, a high nitrogen crop can fill grain from current photosynthesis and call on WSC reserves during the periods of peak assimilate demand. However, in the event of water stress, and reduced current photosynthesis, the lack of WSC reserves and failure of the root system to extract available soil water results in haying-off. A low nitrogen crop does not face the same degree of water stress because lower anthesis biomass results in reduced water use more than does a high nitrogen crop. Greater yield at low than high nitrogen is achieved through greater current photosynthesis and greater reserves of pre-anthesis WSC.

This model also accounts for high levels of WSC in low plant density canopies such as in spaced plants. It is speculated that the reputation of tall varieties for haying-off is explained by low pre-anthesis reserves of WSC due to the greater assimilate demand by larger stems. It also leads to speculation that while haying-off is a problem in the closed-canopy crops of southeastern Australia, it is unlikely to be a problem in the northeastern or western wheatbelt where crops are thinner and are more likely to contain higher levels of pre-anthesis stored WSC and lower grain numbers.

In the southeastern wheatbelt haying-off is likely to be a continuing problem with current freely tillering varieties. Haying-off occurs when crops of a high nitrogen status reach anthesis with low levels of soluble carbohydrate and subsequently encounter severe water stress. Results from this thesis indicate that the problem could be reduced by reducing the amount of structural biomass produced by DC65 and opening the wheat canopy after DC30 to improve the light relations of lower leaves. Breeding of low-tillering cultivars would produce lower biomass with greater reserves of WSC than current cultivars when grown at high nitrogen status. Lower biomass at anthesis would result in savings of soil water available for post-anthesis transpiration. Compensation by other yield components is expected to account for reduced spike density, though a fine balance would need to be maintained so that yield potential is not sacrificed when water supply is adequate.

Opening up of the canopy of wheat grown at high nitrogen status through reduced tillering or progressively reducing leaf size after DC30 (as in barley cultivars) would improve the light relations of lower leaves and hence improve their carbon balance. This improved canopy efficiency and reduced growth of structural material would result in higher WSC content at high nitrogen than at low nitrogen as it did in the micro-canopy of the controlled environment study and the herbicide induced low tillering crops at BAR92.

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APPENDIX 1 **MAP OF EXPERIMENTAL SITES**