THE USE OF CARBON ISOTOPE DISCRIMINATION
IN SCREENING WHEAT GENOTYPES
FOR EFFICIENT USE OF WATER

A Thesis Submitted for the Degree of
Doctor of Philosophy
at the Australian National University

by

ANTHONY GERARD CONDON

November 1988

STATEMENT

The work presented in this thesis is my own. Specific contributions and co-operative work with others are referred to in the acknowledgements.

AG Condon

A.G. Condon.

Plant Environmental Biology Group
Research School of Biological Sciences
Australian National University
Canberra

Much of the work appearing in Chapter 2 has been published as:

> Condon, A.G., R.A. Richards and G.D.Farquhar (1987) "Carbon isotope discrimination is positively correlated with grain yield and dry matter production in field-grown wheat". *Crop Sci.*, 27: 996-1001.

ACKNOWLEDGEMENTS

I would like to thank Professor I.R. Cowan for the opportunity to carry out this work in the Plant Environmental Biology Group. I would also like to thank Dr. W.J. Peacock, Chief, CSIRO Division of Plant Industry, for allowing me to use the Division's facilities.

I am especially grateful to Drs. Graham Farquhar and Richard Richards for their helpful and enthusiastic supervision. Their encouragement and guidance are greatly appreciated.

Thanks are also due to Drs. John Passioura, Tony Fischer, Peter Bremner, Howard Rawson, Kerry Hubick and Susanne von Caemmerer for helpful discussions and comments on the work.

For assistance with mass spectrometry, I would like to thank Dr. Kerry Hubick, Mr. Derek Millar and Mr. Zarko Roksandic. Thanks also to Drs. Howard Rawson, Susanne von Caemmerer and Chin Wong for advice on gas-exchange work.

Staff of the CSIRO Division of Plant Industry provided willing assistance in field and glasshouse studies. My special thanks to Mr. Bernie Mickelson for his untiring work in the field.

Field facilities were generously provided at Yanco and Wagga Wagga by the New South Wales Department of Agriculture. Mr. Alan Semmler, "Glenora", Moombooldool, provided generous assistance with land and equipment.

Special thanks to Leonie Hoorweg to her patient and accurate typing.

Finally, I would especially like to thank my wife, Lesley, for her assistance in various more tedious aspects of the work and for her encouragement and patience during the research programme and the writing of this thesis.

This work was carried out while I held a Commonwealth Postgraduate Research Award.

ABSTRACT

Carbon isotope discrimination, Δ, has been proposed as a measure of the ratio of intercellular to atmospheric partial pressures of CO_2, p_i/p_a. In previous work on droughted wheat plants grown in the glasshouse, Δ was shown to be negatively correlated with plant water-use efficiency (i.e. the amount of dry matter produced per unit water transpired), as predicted by theory. The aim of the work presented in this thesis was to assess the usefulness of Δ as a selection criterion for improved crop water-use efficiency in wheat breeding programmes. The ultimate objective of such selection would be to improve grain yield under water-limited conditions.

The experimental programme addressed both the physiological rationale for the use of Δ as a selection criterion for improved water-use efficiency and also its reliability and use in breeding.

Material from a range of sources was evaluated for genotypic variation in Δ. This material included recently released cultivars and advanced lines from breeding programmes based in different parts of the Australian wheat belt. Large numbers of genotypes were grown together in the field in replicated experiments at several sites. Genotypic variation in Δ was substantial, the range in Δ being up to 2.5×10^{-3}. Genotype x environment interaction for Δ was generally non-significant, except when more extreme environments were contrasted. Estimates of broad-sense heritability for Δ ranged from 41% to 80% with a mean of 63%. This value was similar to the broad-sense heritability for harvest index in these experiments.

These results indicate that Δ is under relatively strong genetic control. They also indicate that, if selection based on Δ was shown to be worthwhile, there is already considerable variation in Δ present among conventional, agronomically acceptable wheat genotypes, that could be utilised to improve crop water-use efficiency.

Ideally, selection for traits which may enhance yield under water-limited conditions should have minimal impact on yield potential under well-watered conditions. Field studies conducted at two sites in 1984 provided an opportunity to determine the relationships between grain yield and Δ and biomass production and Δ under conditions where pre-anthesis water supply was largely non-limiting. These relationships were found to be positive. For a relatively small increase in Δ, there were substantial increases in both grain yield and biomass production.

Although water use was not measured, the slopes of these relationships indicated that, under these growing conditions, relationships between crop water-use efficiency and Δ were probably not strong, and possibly positive as well. To examine possible causes for this, several genotypes were selected spanning a range of Δ values. Experiments using these genotypes were conducted at the leaf, single plant and crop canopy levels.

Gas-exchange studies were done on flag leaves of well-watered plants of 14 genotypes to determine the relationship between p_i/p_a and Δ and to characterise genotypic variation in p_i/p_a. Positive correlations were observed between p_i/p_a measured in flag leaves and discrimination measured in plant dry matter. Linear regressions fitted to the data on p_i/p_a and Δ fell close to the simplified theoretical relationship, $\Delta = (4.4 + 22.6p_i/p_a) \times 10^{-3}$.

Among the genotypes used, variation in p_i/p_a was attributed, approximately equally, to variation in stomatal conductance and to variation in photosynthetic capacity.

Approximately 45% of the observed variation in Δ was not explained by variation in p_i/p_a. This result indicates that, while genotypic variation in Δ may provide a reasonable estimate of genotypic variation in long-term mean p_i/p_a, there may also be considerable variation in p_i/p_a and, by inference, plant water-use efficiency, that is not accounted for by variation in Δ.

Container studies were performed in the glasshouse using plants of 16 genotypes, to examine the relationship between plant water-use efficiency and Δ. These studies revealed substantial genotypic variation in plant water-use efficiency. Genotypic variation in plant water-use efficiency was negatively correlated with Δ in both well-watered and terminally water-stressed plants. Water-use eficiency under water stress was also negatively correlated with Δ measured in well-watered plants. Relationships between plant water-use efficiency and Δ were reasonably strong, variation in Δ accounting for between 30% and 55% of the observed variation in water-use efficiency.

In the experiments on well-watered plants, the response of plant water-use efficiency to a change in Δ, though relatively large, was approximately 35% less than the change in leaf transpiration efficiency

(i.e. the ratio of the instantaneous rates of CO_2-assimilation and transpiration) that would be expected, for a similar change in Δ, in a well-stirred gas-exchange cuvette at constant leaf-to-air vapour pressure difference. This result was attributed largely to the greater influence of boundary layer conductance on transpirational water loss at the whole-plant level. The influence of boundary layer conductance on transpirational water loss is likely to be even greater for field canopies.

Field experiments were conducted over two seasons to determine the relationship between water-use efficiency and Δ for a range of genotypes grown in small plots under rainfed conditions. In one experiment, crop evapotranspiration was partitioned into transpiration and direct soil evaporation using a model based on the proportion of incident radiation reaching the soil surface.

Under the growing conditions of high winter/spring rainfall followed by terminal water stress, significant genotypic variation was observed in canopy water-use efficiency (i.e. the amount of above-ground dry matter produced per unit water transpired), but there was no relationship between canopy water-use efficiency and Δ.

For the well-watered conditions up to anthesis, variation in canopy water-use efficiency resulting from genotypic differences in stomatal conductance was less than expected from leaf gas-exchange theory. This was attributed to the increased influence of boundary layer conductance on transpirational water loss from field canopies. There was no effect on canopy water-use efficiency of reduced photosynthetic capacity. This may have been partly due to the increased influence of boundary layer conductance on canopy transpiration, and partly due to possible effects of genotypic variation in dry matter distribution on the ratio of above-ground to total dry matter and on the relative extent of respiratory carbon losses.

Genotypes with high Δ values accumulated dry matter more rapidly up to anthesis, the period of lowest evaporative demand. This was associated with greater early radiation interception by high Δ lines. The greater early radiation interception by these lines also reduced direct soil evaporation.

Water deficits were largely confined to the post-anthesis period. Water-use efficiency during this phase of crop growth was also independent of variation in Δ, but was strongly correlated with post-anthesis dry matter production. Greater dry matter production during this phase was favoured in low Δ genotypes that had conserved soil water in the period up to anthesis. However, there was also substantial variation in post-anthesis dry matter production among high Δ lines that had used considerably more water at anthesis.

These field results indicate that in southern Australia, where winter/spring rainfall is common and evaporative losses may be relatively high, selection for improved crop water-use efficiency based on low Δ would be inappropriate. Rather, given the consistent positive relationships observed between grain yield and Δ, selection for high Δ could be much more useful in this environment. In the northern wheat belt, where evaporative losses are less important and crops utilise more stored soil moisture, conservation of soil moisture through selection for low Δ may be more appropriate.

CONTENTS

CHAPTER 1

INTRODUCTION, LITERATURE REVIEW AND STATEMENT OF EXPERIMENTAL OBJECTIVES

1.1 INTRODUCTION

> "The determining factor in the cultivation of wheat in New South Wales is the rainfall ... 6 inches of rain must actually pass through a crop in order to produce 20 bushels (per acre) ... It is sufficient to say that the rainfall during the growing season in most ... wheat districts is not sufficiently large to allow 6 inches, or even 4 1/2 inches, of moisture to pass through the crop during the growing period. The first impression produced by such a statement is, perhaps, surprise that wheat-growing should have become such an extensive industry as it has."

The above quotation from "The Farmers' Handbook", published by the NSW Department of Agriculture earlier this century (Synnott, 1944), outlines the limitation imposed by inadequate rainfall on wheat production in most areas of Australia and in other semi-arid areas of the world. It also introduces the concept of "water-use efficiency", suggesting the grain yield attainable for a certain amount of transpired water.

Efforts to improve this efficiency have been the direct or indirect aim of agricultural researchers and plant breeders for centuries (Stanhill, 1985). In recent years, considerable emphasis has been placed on agronomic means of improving the efficiency of water use in cropping systems. In terms of cost to the farmer, however, the cheapest way to increase water-use efficiency is through improved varieties. As reviewed by Richards (1982) and by Garrity et al. (1982) in the same volume, attempts to improve yields in water-limited environments by varietal improvement have followed two general approaches: an "empirical" or "performance" approach on the one hand, and an "analytical" or "physiological" approach on the other.

The empirical approach uses yield as the selection criterion, allowing the environment to determine the combination of traits which lead to maximum yield. With respect to breeding for water-limited environments, philosophies differ as to which is the best environment in which to select. Some researchers favour selection under the dry environment in which the crop is to be grown (e.g. Hurd, 1976), whereas others have found wetter environments more efficient (e.g. Roy and Murty, 1976). In the former case it may be a peculiarity of the test environment, such as the availability of substantial amounts of water at depth, which favours selection in that environment. In the latter it may be that the yield advantage enjoyed by some genotypes in wetter environments is so large that the yield constraint must become very severe before that advantage is eliminated (e.g. Fischer and Wood, 1979).

The analytical approach seeks to identify specific physiological or morphological traits that will enable the plant to better withstand the constraints imposed by an inadequate water supply. A short list of traits proposed or investigated in the recent past could include morphological traits such as deep or extensive root systems (Kramer, 1969; Hurd, 1976); root systems that ration the uptake of water (Richards and Passioura, 1981); awns (Evans et al., 1972); a restricted tillering habit (Islam and Sedgely, 1981); erect leaves (Innes and Blackwell, 1983); glaucousness (Richards, 1984); and few stomata (Jones, 1977). Metabolic traits investigated include proline accumulation (Singh et al., 1973) and abscisic acid accumulation (Quarrie, 1980), while physiological traits such as osmotic adjustment (Morgan, 1983); stomatal sensitivity (Jones, 1976); and the magnitudes of plant water potential (Fischer and Sanchez, 1979) and osmotic potential (Keim and Kronstad, 1981) have also been investigated.

In this thesis, a recently proposed physiological trait, carbon isotope discrimination (Farquhar and Richards, 1984), is assessed for its usefulness to plant breeders as a possible selection criterion for improved water-use efficiency in wheat

Both Richards (1982) and Garrity et al. (1982) point out that, to date, little progress has been made using the analytical approach. They suggest that this is due to two main reasons. Firstly, few traits have been demonstrated to confer a yield advantage. One factor contributing to this lack of yield advantage may be that many traits have been proposed as a result of studies on plants native to arid regions. Turner (1981) suggests that too many such traits are concerned with survival rather than production, and, as such, have slowed progress towards the identification of positive traits that contribute to yield enhancement under water-limited conditions. Another factor contributing to the lack of yield advantage may be that many of the proposed traits, especially metabolic and physiological ones, represent short term responses to rapidly imposed stress that do not integrate over the whole season to give increased yield (Passioura, 1986). The second major reason for the lack of success of the analytical approach is that, for many of the proposed traits, especially those based on metabolic or physiological characters, efficient and rapid screening techniques have not been developed, thus limiting their usefulness to breeders.

To overcome these shortcomings, Garrity et al. (1982) proposed five critical stages in the assessment of physiological traits, viz.

1. Development of a hypothesis concerning the potential contribution of a particular trait;
2. Search for genotypic variability for the trait
3. Development of a rapid and efficient screening method;
4. Elucidation of the mode of trait inheritance and selection of an appropriate breeding method to incorporate it into agronomically superior genotypes;
5. Documentation of a strong causal relationship between high levels of a trait and improved yield performance in water-limited environments.

To these five may be added another: an assessment of the likely impact of trait selection on yield potential under well-watered conditions. In many rainfed cropping environments, the extent of water limitation may vary substantially from year to year, and average yields may be influenced more by the higher yields obtained in relatively favourable seasons.

This framework forms the basis for the study on carbon isotope discrimination and water-use efficiency presented in this thesis. In the literature review that follows, the hypothesis relating carbon isotope discrimination to water-use efficiency will be examined. The review will focus on constraints on efficient use of water in the Australian wheat-growing environment since field experiments will be conducted in that environment. The review will consider the efficient use of water by crops in terms of processes at both the plant and crop level. In so doing, previous attempts and philosophies behind yield improvement in water-limited environments will be summarised. The concluding sections will consider in some detail the physiology of carbon isotope discrimination and the proposed relationship between carbon isotope discrimination and water-use efficiency. Following the literature review, the specific objectives of the experiments performed in this study will be presented in terms of the framework outlined above.

1.2 LITERATURE REVIEW

1.2.1 The Australian Wheat-growing Environment.

Climatic constraints on wheat production in Australia have been reviewed by Nix (1975). These constraints could conveniently be divided into those associated with rainfall and those associated with temperature. Wheat is grown in a broad band from the south-west corner of Western Australia, through south-eastern Australia and into southern Queensland (Figure 1.1). This band is bounded roughly by mean winter (May to October) rainfall totals of 41 to 150 cm, the upper limit being more associated with terrain than rainfall as such (Nix, 1975).

A feature of the rainfall constraint is its marked seasonality, this seasonality being linked with the sun-controlled shifts in the position of the mid-latitude high pressure belt. In winter, the high pressure cells move north bringing dry conditions to the northern parts of the wheat belt and allowing cool, moist air to flow in over southern Australia from the Southern Ocean. Thus, most of the rain in the southern parts of the wheat belt, and particularly in Western Australia and South Australia, falls during the winter. In summer, the highs move southward bringing dry, stable conditions to southern Australia. The northern part of Australia receives most of its rainfall during this season as warm, moist, unstable air flows in from the north and east. In eastern Australia, the rainfall distribution is more complex because of the contribution to rainfall from moist air masses moving in from the Tasman and Coral Seas which tends to blur the dominance of the seasonal shifts described above.

Because of these patterns of rainfall distribution, the contribution of "current rainfall" to wheat production in Australia varies considerably from one end of the wheatbelt to the other. This is illustrated in Figure 1.1, which also shows the <u>average</u> proportion of the wheat crop's water supply that is contained in the soil at the time of sowing (Passioura, 1986). Significant contributions to fallow water storage from summer rainfall are essentially restricted to the eastern and particularly the north-eastern sector of the wheatbelt. In the south

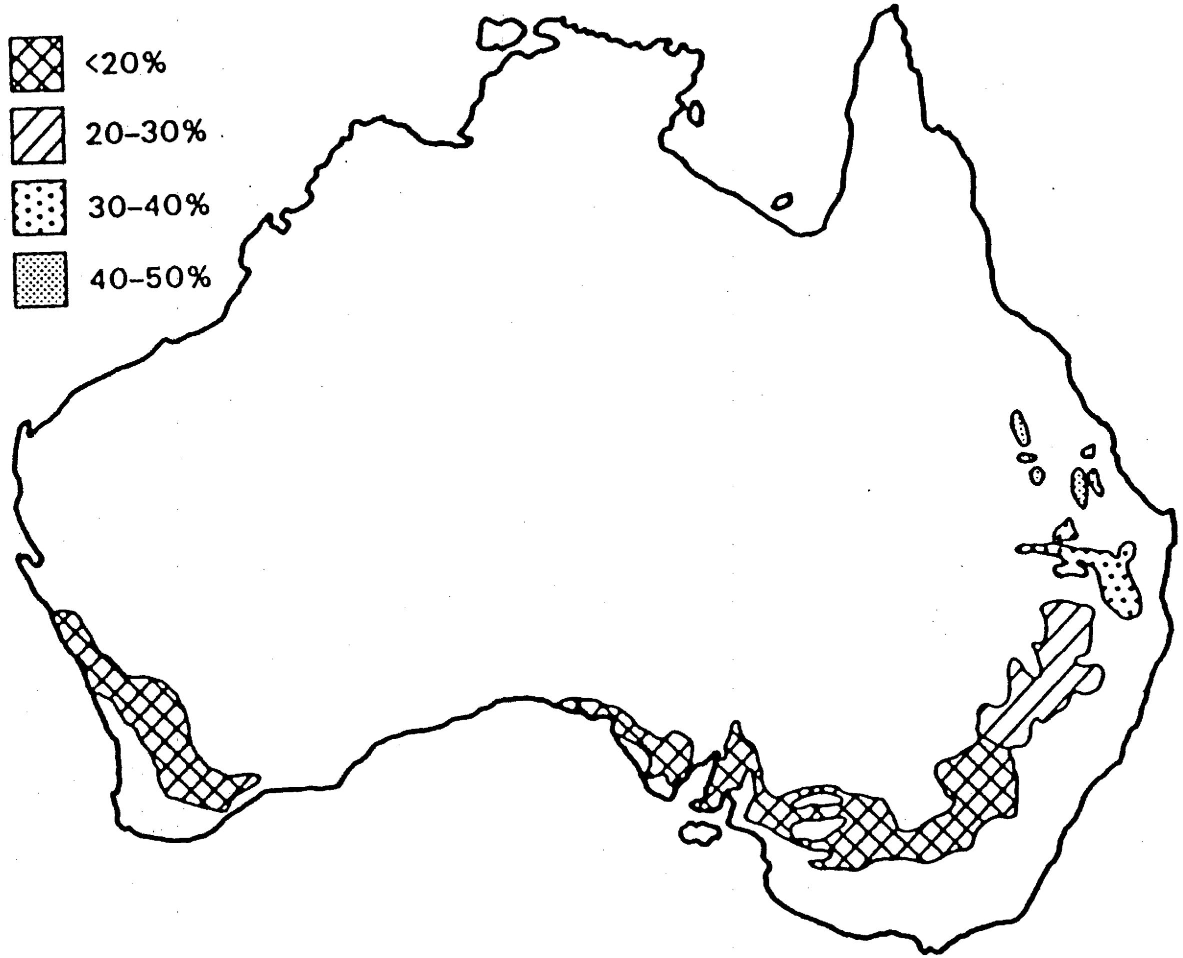


Fig 1.1 The Australian wheat belt classified according to the proportion of a crop's water supply that is contained in the soil, on average, at the time of sowing. (from Passioura, 1986).

and west, significant fallow moisture must be derived from the previous winter period, except when good "opening" rains are received shortly before sowing.

The varying contribution of "current rainfall" to wheat production as one moves from the western to the northern parts of the wheatbelt is reflected in the conclusions of studies aimed at determining causes of variation in wheat yields in different regions. In South Australia, Cornish (1950) and Russell (1968) found winter rainfall (June-August) to be a major determinant. In central New South Wales, spring rainfall (September-October) was more important (Seif and Pederson, 1978), while in Queensland, Waring et al. (1958) found a strong correlation between available soil moisture at planting and final yield.

Apart from its marked seasonality and the consequent variation in stored moisture, the other characteristic of rainfall in the Australian wheatbelt is its temporal variability. This is illustrated by studies of yield trends for wheat in Australia over the past forty to fifty years (McWilliam, 1986). On average, severe droughts reduced national yields by between 30 and 50% one in five years. This is a fairly simplified view, however, since rarely does drought affect the whole of the wheatbelt at the same time. Different parts of the wheatbelt experience drought at different times and of differing severity.

While insufficient rainfall is a perennial, if variable, constraint on yield levels in Australia, yield levels have been increasing over the last fifty years or so. As well as revealing the regular depredations caused by drought in Australia, the study by McWilliam reveals an upward trend in yield of about 11kg/ha/yr, the trend being much the same for the "drought" years as for the remainder. Better adapted varieties are considered to have contributed approximately 40% of this upward trend (Antony and Brennan, 1988).

There are two major temperature constraints on wheat production in Australia. One is frost. The risk of crop damage due to frost increases as the crop approaches anthesis, with the stage of growth and position of the ear within the canopy being important in determining the susceptibility of the crop (Single, 1961). There are few reports of frost damage in the literature. Those appearing are generally associated with sowing time trials in which early sowing results in early ear emergence (e.g. Doyle and Marcellos, 1974; Woodruff and Tonks, 1983).

The other major temperature constraint is that associated with increasing temperatures at about anthesis and then into grain-filling. This increase in temperature coincides with a rapid increase in evaporative demand due to the greater vapour pressure deficit and radiation load as summer approaches. The increase in evaporative demand, together with a rapidly declining soil water status, invariably leads to water deficits during the later phases of crop development. The production of the crop therefore becomes a compromise between yield loss due to frost if it flowers too early (Doyle and Marcellos, 1974) or from moisture stress and high temperature if too late. The constraints imposed by this compromise are perhaps best illustrated by studies aimed at optimising the sowing date, and hence flowering date, of crops in New South Wales. In northern New South Wales, Doyle and Marcellos (1974) concluded that increasing water stress would lead to reductions in relative grain yield of 9 to 13% for each week flowering was delayed beyond the end of the first week in October. Kohn and Storrier (1970) arrived at similar, though not so drastic, conclusions from studies in the south of the state.

The overlapping of the danger periods from frost on the one hand and drought on the other, has led to the determination of an "earliest safe ear-emergence date" for different areas of the wheat belt. This date is generally determined on the basis of meteorological records. Nix (1975) calculated it as the mean date of the last 0°C minimum screen temperature plus one standard deviation. Fischer (1979) was somewhat more conservative, using 2°C as his reference temperature.

Given the above-mentioned constraints, and despite the considerable variation in patterns of rainfall distribution and soil-water accumulation, Nix (1975) concluded that there are basic similarities in the seasonal crop water balance throughout the wheatbelt. During the vegetative phase, rainfall exceeds crop evapotranspiration, leading to soil-water recharge. The duration of this recharge phase is variable, depending on location and season, but generally crop water stress is unlikely to occur during the early vegetative phase. At some point during the reproductive phase, usually before ear emergence, crop evapotranspiration exceeds rainfall and soil-water depletion commences. As this depletion continues, there is a decline in the ratio of actual to potential evapotranspiration rates which signals the onset of stress. With the evaporative demand increasing, the decline in this ratio continues and stress becomes more severe as the crop moves through anthesis and into the grain-filling phase.

1.2.2 Analytical Approaches to Yield Improvement in Water-limited Environments

Marshall (1987) suggests that, while the empirical approach has led to progress in increasing wheat yields in Australia in recent decades, the environmental constraints have meant that progress has been relatively modest compared to the absolute gains made over the same period in more favourable environments. Consequently, breeders and physiologists remain interested in analytical approaches that may result in more rapid or substantial increases in yield.

Fischer (1981) further partitioned the analytical approach into the "black box" strategy, whereby attempts are made to proceed from yield differences to underlying process differences, and the "ideotype" strategy, whereby yield improvement is predicted on the basis of an understanding of a physiological process and its possible contribution to yield.

Examples of the "black box" strategy are the studies by Kaul (1969), Kaul and Crowle (1971), Jones (1977) and Keim and Kronstad (1981). In each case, studies were made of plant water relations parameters and also grain yield under drought. It was hoped to relate observed differences in yield to genotypic differences in the parameters measured. The significant correlations found were few and low. A more extreme example would be the work by Fischer and Wood (1979). In an intensive study of bread and durum wheats, barleys and triticales, they measured yield components, other aspects of crop development and growth, and morphological characters, as well as plant water relations. The measurements were taken over several years in wet environments and also a range of dry environments. Despite the breadth of this study, none of the traits measured, in either wet or dry environments, was considered a suitable criterion for selecting for yield under drought.

Passioura (1977), in espousing the "ideotype" strategy, suggested that further progress could be made by considering the following identity. He proposed that grain yield in water-limited environments (Y) could be considered simply as the product of three factors: the amount of water transpired (T); the water-use efficiency (W_T^*), i.e. the amount of above-ground dry matter produced per unit water transpired; and the harvest index (HI), i.e. the proportion of above-ground dry matter harvested as grain. Thus,

$$Y = T \times W_T^* \times HI \quad . \qquad (1.1)$$

Passioura (1977, 1986), Fischer and Turner (1978), Fischer (1979, 1981) and Richards (1982) have all extensively discussed this identity and their musings are reviewed below. Loomis (1983) and Sinclair et al. (1984) cover much the same ground but from slightly different perspectives. Rather than dealing with each component in the order in which it appears above, I will deal with water-use efficiency last since it leads naturally into discussion of the possible significance of carbon isotope discrimination as a selection criterion.

1.2.3 The Amount of Water Transpired by the Crop

The term "evapotranspiration", (ET), is widely used to describe the water loss from vegetated surfaces, and encompasses "evaporation" directly from the soil (E) and water lost through "transpiration" by the plants (T). The significance of the direct evaporation component has long been recognised. For example, quoting again from Synnott (1944),

> "... 6 inches of rain must actually pass through a crop in order to produce 20 bushels. This by no means signifies that only 6 inches of rain must fall during the growing period, because out of an actual fall of 6 inches during six months, 3 inches or more may be lost through evaporation or other causes".

The losses through "evaporation and other causes" may be put more formally in the following manner (Fischer and Turner, 1978):

$$T = P - R - E - D - S \quad , \qquad (1.2)$$

where T and E are as defined above, P is precipitation, R is runoff, D is drainage beyond the root zone and S is the change in soil water in the root zone not accounted for by T, E or D. Given that P is beyond our control, several possible means have been proposed whereby plants could be modified to increase T.

A more rapid approach to full ground cover will reduce E, since most of the loss from direct soil evaporation occurs while the crop is building up its leaf area (Ritchie and Burnett, 1971). This is especially so in those environments where a substantial proportion of the crop's water supply comes from current rainfall, such as in the southern and western parts of the Australian wheatbelt. Two recent estimates from this sort of environment (South Australia - French and Schulze, 1984; Syria - Cooper et al., 1983) give E over the season as being 30-40% of total ET in the former case and up to 60% in the latter. Clearly, T will also rise in an absolute sense as leaf area increases. So the benefit of reducing E by rapid canopy development must be weighed against the risk of too rapid exhaustion of the soil water supply, especially in those environments or seasons where current rainfall is a smaller component of seasonal ET (Passioura, 1977; Loomis, 1983).

The opportunities for reducing runoff by plant modification appear limited. Agronomic practices affecting the way the soil surface is maintained appear by far the easiest solutions (Unger and Stewart, 1983).

Development of genotypes with deeper or more extensive root systems would appear a reasonable means of increasing the proportion of soil water transpired by the crop, reducing that lost either to drainage or that left behind in the soil at maturity. Root characteristics are difficult to manipulate although some success with wheat (Hurd, 1974) and sorghum (Blum, 1979; Jordan and Miller, 1980) in breeding deeper rooted plants has been achieved. The greater yield under drought of wheat lines selected for high osmotic adjustment (Morgan and Condon, 1986) was associated with their ability to extract substantially more soil water, especially at depth, than lines with poor osmotic adjustment.

1.2.4 Harvest Index

Harvest index is the partitioning component of the yield identity, and is defined as that part of above-ground dry matter harvested as grain (Donald, 1962). The large yield increases in well-watered environments which accompanied the introduction of dwarfing genes into wheat have been largely attributed to increased HI. However, when semi-dwarf wheats are compared with older, tall varieties under drought conditions, this yield advantage diminishes, and is eliminated when water limitation is severe (Fischer and Wood, 1979).

Passioura (1976) concluded that HI and yield of wheat growing largely on stored water could be substantially increased by forcing the plants to save water for post-anthesis growth. Apart from the benefit to post-anthesis assimilation, he considers that two other factors may be of equal, if not greater, importance (Passioura, 1986). Conserving water for use after anthesis provides time for the plant to remobilise pre-(and post-) anthesis assimilate from stems and other organs and transfer it to the grain. Under well-watered conditions the contribution to grain yield of remobilized pre-anthesis assimilate is thought to be small (Thorne, 1974; Austin et al., 1977; Gifford et al., 1984) but with increasing stress this contribution increases (Bidinger et al., 1977; Richards and Townley-Smith, 1987). Secondly, the number of grains set per ear is strongly influenced by water stress in the period from shortly before ear emergence to anthesis (Salter and Goode, 1967; Morgan, 1980). Slow early use of soil water should be beneficial at this time as well.

Richards and Passioura (1981) explored an indirect means of reducing the transpiring surface of wheat, by selecting genotypes with either less seminal roots (the roots mainly concerned with extracting subsoil water) or with seminal roots with a main xylem vessel of smaller diameter. The object of this exercise was to increase resistance to flow of water into the aerial parts of the plant, causing self adjustment of leaf area. They found vessel diameter to be more easily manipulated genetically. Among isogenic lines differing in xylem vessel diameter, those lines with smaller diameter vessels had higher HI and grain yield when tested under severe drought (Richards, 1987).

Strategies for conserving water for post-anthesis use are likely to be more successful in those environments where the contribution of current rainfall is least, and where slow development of leaf area does not result in excessive E losses. In environments where current rainfall represents a major source of the crop's water supply, other means of maximising HI must be sought. Fischer (1981) concludes that, for this sort of environment, there will be an optimal amount of pre-flowering growth which will be less the lower the total supply of water available. This optimum arises for two reasons. Again, more dry matter at anthesis means less water (and assimilation) after anthesis and secondly, more dry matter at anthesis means more kernels to be filled after anthesis. Too many kernels could result in pinched grain if post-anthesis assimilate is insufficient to fill them.

Rapid expansion of leaf area in these environments will reduce E, but in several experiments where this was achieved through high levels of nitrogen fertilization, excessive leaf area development prior to anthesis resulted in poor yields due to low HI (Colwell, 1963; Fischer and Kohn, 1966b; Russell 1967; Angus et al., 1980; Cooper et al., 1983). In other words, the nitrogen response in terms of grain yield was less than that of dry matter. The poor grain yields were associated with a rapid decline in green leaf area after anthesis (Fischer and Kohn, 1966b). On the other hand, if leaf area development is too slow, there may be an increase in soil water levels at flowering but dry matter at anthesis may be too low to take advantage of this extra water (Fischer and Kohn, 1966a; Hamblin et al., 1982). It is difficult to envisage how genotypes might be selected to achieve an optimum anthesis dry matter other than by conventional, empirical means.

1.2.5 Water-use Efficiency

Passioura (1977) defined water-use efficiency (W_T^* in Equation 1.1) as the ratio of the total above-ground dry matter at final harvest to the total amount of water transpired. A more complete definition would include root dry matter also. Hence, W_T is defined as the ratio of total dry matter produced per unit water transpired. Defined either way, water-use efficiency is particularly tantalising to physiologists and breeders since, in a water-limited environment, an improvement in water-use efficiency represents "getting something for nothing", in this case extra dry matter for the same amount of water used.

Since early this century, attempts have been made to identify genotypic differences in water-use efficiency. These attempts usually took the form of experiments where plants were grown in sealed containers (e.g. Briggs and Shantz, 1913; Shantz and Piemeisel, 1927). The amount of water consumed and the increase in plant dry weight were measured over the course of the experiment, and the ratio determined. Inter- and intraspecific comparisons were made and substantial differences observed.

Since that time, container experiments comparing species and genotypes within species have remained a major means by which researchers have attempted to identify more "drought resistant" material. An example would be the series of experiments conducted during the 1960's and early 1970's, which attempted to identify better adapted species for the semi-arid rangelands of western U.S.A. Hunt (1962) found significant genotypic differences in water-use efficiency when he compared selected lines of *Elymus junceus* and *Agropyron intermedium* and their progeny. Both the "water requirement" (the inverse of water-use efficiency) and forage production were highly heritable. Dobrenz and coworkers studied water-use efficiency in several clones of *Panicum antidotale* and in different genotypes of alfalfa (Dobrenz et al., 1969a; Dobrenz et al., 1971). They found significant variation in both species. Bleak and Keller (1973) compared several *Agropyron* species and also *Elymus junceus* in container studies lasting almost two years. Several cuts were made to simulate grazing. Significant genotypic differences in "water requirement" were again found, the two extreme genotypes being different lines of *Agropyron sibiricum*. Container studies on crop plants continued also. For instance, Anderson and Read (1966) compared water-use efficiency in two varieties each of wheat, oats, barley and flax. They found genotypic differences in 2-row barley and oats, although the mean water-use efficiencies measured in 2-row and in 6-row barley and in wheat were all very similar. Passioura (1977), in pursuing his hypothesis regarding harvest index and available soil water at anthesis, grew several wheat varieties in tall containers and found significant varietal differences in water-use efficiency, ranging from 4 down to 3g of above-ground dry matter per kg of water used.

All these studies suggest that genotypic variation in water-use efficiency does exist. However, such studies are time consuming and labour intensive and thus of limited use in breeding programmes except perhaps in identifying possible parental material. For screening on a large scale, more efficient techniques are needed. In order to consider the worth of other techniques for improving water-use efficiency, it may be as well, at this stage, to consider water-use efficiency on a more fundamental basis, i.e. at the leaf level, to determine what might make one genotype more efficient in its water use than another.

1.2.6 An Analytical Approach to Improving Water-use Efficiency.

At the leaf level, the instantaneous water-use efficiency is the ratio of the rate of carbon fixation, A, to that of transpiration, T. Farquhar and Richards (1984, Equation 4) derived a simplified approximate expression for instantaneous water-use efficiency as follows,

$$A/T = \frac{g_c(p_a-p_i)}{g_w(e_i-e_a)} = \frac{p_a}{1.6v}(1 - p_i/p_a) \quad , \qquad (1.3)$$

where g_c and g_w are the conductances to diffusion of CO_2 and water vapour respectively; e_i and e_a are the intercellular and atmospheric vapour pressures, with v being the difference between them; and p_i and p_a are the intercellular and atmospheric partial pressures of CO_2. The factor 1.6 is the ratio of the diffusivities of water vapour and CO_2 in still air. Sinclair et al. (1984, Equation 3) describe A/T in a similar way.

Farquhar et al. (1988), extending this equation to the whole plant over the period of growth, defined plant water-use efficiency, W, as the number of moles of carbon in the plant divided by the number of moles of water transpired during the period of growth. By allowing for the proportion, ϕ, of carbon which is fixed during growth but which is lost due to respiration by the leaf at night and by other parts of the plant over the period of measurement, the following expression is obtained (Farquhar et al., 1988, Equation 2)

$$W = \frac{(1-\phi)\ p_a(1-p_i/p_a)}{1.6\bar{v}} \quad . \qquad (1.4)$$

In Equation 1.4, it is assumed that p_i is averaged with respect to conductance. As well, vapour pressure difference, $\bar{v}$, is also weighted by conductance. The weighting by conductance is needed to take into account that a particular vapour pressure difference causes a greater water loss when conductance is large than when it is small. If above-ground dry matter production only is being considered, then a factor, (1-r), would also appear in the numerator, with r representing the proportion of plant carbon in the roots.

Equation 1.4 suggests four possible means by which water-use efficiency could be improved, i.e. by reducing either ϕ, r, $\bar{v}$ or p_i/p_a.

1.2.6.1 Respiratory losses

The utilisation of carbon in respiratory processes represents a substantial proportion of the carbon fixed by plants. Estimates of this proportion vary across a range from about 0.3 to 0.5 depending on the species, time-scale of measurement and condition of the plant. Genotypic variation in the ratio of dark respiration to net photosynthesis has been observed in the leaves of some species (Heichel, 1971; Jones and Nelson, 1979) and could contribute to enhanced water-use efficiency. For example, Wilson (1975) identified differences in mature-leaf dark respiration rate among *Lolium perenne* genotypes and has selected for greater productivity on that basis (Wilson and Jones, 1982).

Gifford et al. (1984) noted that much of this productivity advantage in *Lolium* appeared to be associated with regrowth after defoliation, a situation unlikely to be encountered by cereal crops. For cereal crops during the later stages of ontogeny, leaves make up a relatively small proportion of the total dry matter. Respiratory losses from other plant parts may become more important than losses from leaves. Austin et al. (1977) observed substantial variation in respiratory losses from stems among wheat genotypes. Screening for a reduced proportion of respiratory losses from different organs or on a whole-plant basis, may identify useful parental material for breeding programmes.

Another "leak" in the system in terms of the trade of carbon for water is transpirational losses via the cuticle or from incomplete stomatal closure. In Equation 1.4 it is assumed that these losses are negligible. Such losses may be substantial under unfavourable circumstances, such as when hot, dry winds continue to blow at night or when water stress has resulted in stomatal closure during the day (Clarke and McCaig, 1982; Rawson and Clarke, 1988). Genotypic variation in excised leaf water retention, which could be taken as a measure of incomplete stomatal closure (Kirkham et al., 1980), was sought in durum wheats by Clarke and Townley-Smith (1986). They found both the heritability and the yield advantage associated with high retention to be variable.

1.2.6.2 Allocation of carbon to roots

Allocation of carbon to the roots has been discussed above in terms of increasing the amount of water available for transpiration. Passioura (1982) suggests that, in some circumstances, excessive investment in roots could represent a waste of carbon and water, given that water must be exchanged to fix that carbon and that, per gram dry weight, roots appear to respire more vigorously than shoots. Recent studies also indicate that there may also be substantial amounts of photosynthate exuded from roots (Clarkson, 1985). Taylor and Nguyen (1987) stress the need to define the minimum rooting densities necessary to extract soil water within reasonable time periods. However, selection for such root characteristics, especially the size of the root system, is a tedious process (Taylor and Nguyen, 1987).

1.2.6.3 Vapour pressure difference

The vapour pressure difference, v, between the leaf and air is subject to manipulation at the leaf level by various means. Characters which alter leaf surface morphology, such as pubescence and glaucousness (waxiness), may be effective in reducing the leaf to air vapour pressure difference by reducing the energy load on the leaf. The vapour pressure inside the leaf is regarded as being very close to the saturation vapour pressure at the leaf temperature. Pubescence and glaucousness, by increasing the reflectivity of the leaf surface, may serve to keep the leaf cooler than might otherwise be the case.

Richards (1984) compared wheat lines isogenic except for the presence or absence of glaucousness under drought conditions in the field. He found the glaucous lines to have an advantage in terms of both above-ground biomass and grain yield. Studies undertaken to explain this advantage (Johnson et al., 1983; Richards et al., 1986) found the ears of glaucous lines significantly cooler than those of the non-glaucous lines. This was associated with increased water-use efficiency at the plant level. Gas exchange studies of flag leaves and ears revealed no difference in water-use efficiency of flag leaves, but differences in water-use efficiency were apparent between the ears of glaucous and non-glaucous lines. Clawson et al. (1986) compared soybean lines isogenic except for different levels of pubescence. In one of the two backgrounds used, water-use efficiency (measured as above-ground biomass per unit evapotranspiration) was increased. There was no difference in the other background.

Another means of reducing the energy load on the leaf, which should be effective in increasing water-use efficiency when radiation levels are greater than necessary to saturate photosynthesis, is to change the angle of the leaf so that it is tangential to the sun's direct beam. In wheat this can be done by selecting for an erect leaf habit. Innes and Blackwell (1983) demonstrated improved water-use efficiency (on a biomass basis) in winter wheats selected on this basis. No significant differences were found in either water use or in grain yield. In several dicotyledonous species (notably many legumes), active leaf movements (heliotropism) may serve a similar purpose. Selection could be exercised to maximise this effect.

It is evident from the literature (and from a consideration of Equations 1.3 and 1.4) that the major environmental influence on water-use efficiency at the leaf (and crop) level is the leaf to air vapour pressure difference. Tanner (1981) and Tanner and Sinclair (1983) reviewed studies on water-use efficiency from the level of container-grown plants through to crop canopies and concluded that an equation of the form

$$B/T \approx k/(e^{*} - e_a) \quad , \qquad (1.5)$$

originally proposed by Bierhuizen and Slatyer (1965), could be used to adequately explain much of the variation observed between experiments. In Equation 1.5, B is the total dry matter per unit area, T the transpiration per unit area, $(e^{*} - e_a)$ approximates the leaf to air vapour pressure difference (e_a and e^{*} being the actual and saturation vapour pressures of the air), and k is a crop dependent coefficient.

In re-deriving Equation 1.5, Tanner and Sinclair (1983) incorporated a variety of factors into k, including variation in the efficiency of converting fixed CO_2 into biomass, variation in respiration, leaf temperature and in the ratio p_i/p_a. They concluded that by far the greatest amount of variation in water-use efficiency found for different crops could be accounted for by differences in the vapour pressure deficit during growth. Further, they suggested two avenues to significantly modify the water-use efficiency of crops. One was to bring about a large change in the ratio p_i/p_a (this will be discussed more fully below). The other was to grow crops in conditions where the vapour pressure deficit is lower. This second avenue was also favoured by Namken et al. (1974) and Stanhill (1985).

Keatinge and Cooper (1983) demonstrated the possibilities of such an approach in experiments with chickpea in northern Syria. In the past, winter sowing of chickpeas in that region had been restricted because of the damage caused by disease during the cool, humid winter months. Following the development of disease resistant varieties, they compared winter and spring sown crops and observed a vast improvement, of the order of 50%, in both yields and water-use efficiency with winter sowing.

For wheat in Australia, O'Leary et al. (1985) have suggested earlier sowing to achieve the same result. The study by Woodruff and Tonks (1983) in Queensland showed that under irrigation and in the absence of frost damage, the grain yield of a number of semidwarf wheats was highest when anthesis occurred in mid-winter. Under dryland conditions, they found yield could be reduced if excessive leaf area development caused soil water to decline too rapidly. In southern Australia, early sown "winter" type wheats have had a revival in popularity. Attempts to develop frost-tolerant spring wheats which can be sown and hence flower earlier (Fletcher, 1984), represent one possible means of improving the water-use efficiency of wheat in Australia.

1.2.6.4 Water-use efficiency and p_i/p_a

Returning to a consideration of Equation 1.4, and as suggested by Tanner and Sinclair (1983), it is recalled that water-use efficiency may also be improved by a reduction in the ratio p_i/p_a. There are at least two means by which this reduction may take place; either by reducing the conductance to diffusion of CO_2, i.e. stomatal closure, or, for the same conductance, increasing the capacity for carboxylation.

This is perhaps more apparent if we express instantaneous water-use efficiency at the leaf level in terms of resistances to diffusion (rather than the inverse, conductance, as in Equation 1.3). This is done in Equation 1.6, in which the gas-phase resistances ($1/g_w$ and $1/g_c$) are further divided into components of stomatal (r_s) and boundary layer (r_b) resistances to water vapour and stomatal (r_s') and boundary layer (r_b') resistances to CO_2. Hence:

$$A/T = \frac{(r_s + r_b)}{(r_s' + r_b')} \frac{(p_a - p_i)}{(e_i - e_a)} \quad . \qquad (1.6)$$

In Equation 1.6, both carbon fixation and transpiration are considered as diffusional processes. Carbon fixation may also be considered as a biochemical process, as in the following expression.

$$A = (p_i - \Gamma)/r_i \quad , \tag{1.7}$$

where Γ is the CO_2 partial pressure at the compensation point and r_i is the "internal" resistance to CO_2 uptake. As such r_i is not a true resistance to diffusion, but is used to represent the cellular response of CO_2 uptake to CO_2 partial pressure.

Combining Equations 1.6 and 1.7 yields

$$A/T = \frac{(r_s + r_b)}{(r_s' + r_b' + r_i)} \quad \frac{(p_a - \Gamma)}{(e_i - e_a)} \quad . \tag{1.8}$$

Given constant values of p_a, Γ, e_i and e_a, then A/T is proportional to the ratio $(r_s + r_b)/(r_s' + r_b' + r_i)$. Thus, increasing the gas-phase resistance, for example by increasing r_s, will result in a proportionally larger change in the numerator and A/T will increase. A reduction in r_i, i.e. an increase in photosynthetic capacity, will likewise reduce the denominator and A/T will again increase. In the sense that changes in either will lead to changes in A/T, the two components, $(1 - p_i/p_a)$ from equation 1.3 and $(r_s + r_b)/(r_s' + r_b' + r_i)$ from equation 1.8, are equivalent.

Many gas exchange studies, involving both inter- and intra-specific comparisons of C_3 species, have demonstrated correlations between water-use efficiency at the leaf level and either the resistance ratio or p_i/p_a. Rawson et al. (1977), Field et al. (1983) and Yoshie (1986) demonstrated the correlation when comparisons were made over a wide range of species. Khairi and Hall (1976) compared the leaf gas exchange of five citrus stocks, Planchon (1979) compared eight genotypes of a range of wheat species, while Frank and Barker (1976) compared the water use efficiencies of six range-grass species. All these studies showed positive correlations between water-use efficiency at the leaf level and either $(r_s + r_b)/(r_s' + r_b' + r_i)$ or $(1 - p_i/p_a)$, with genotypic variation in water-use efficiency being attributed to variation in stomatal resistance, assimilation capacity or both.

Many researchers have sought to increase the water-use efficiency of crop species by screening genotypes for stomatal characters. Jones (1987) has recently reviewed such attempts, pointing out several reasons for the lack of success. He suggests that the major reasons are the inherent variability and the strong phenotypic component of stomatal characters, whether one is measuring aspects of stomatal morphology, such as density or size, or measuring stomatal resistance or conductance. Dobrenz et al. (1969b) sought a correlation between stomatal density and water-use efficiency in clones of *Panicum antidotale*. They found none; the two most extreme clones for density had the same water-use efficiency. Others, however, have had more success. Miskin et al. (1972) found the expected inverse correlation between stomatal density and water-use efficiency in barley, while Nerkar et al. (1981), having identified genotypic differences in stomatal characters such as density and length, correlated these with resistance in genotypes of *Vicia faba* and found a positive correlation between resistance and water-use efficiency.

Although selection for improved water-use efficiency based on reduced stomatal conductance seems possible, the improvement in water-use efficiency that should result from such selection may not be as great as expected on the basis of the simple gas exchange theory presented in Equations 1.3 and 1.8. An important simplification in those equations is that the vapour pressure difference, $(e_i - e_a)$, is an independent variable. This is generally not the case.

As stomata close, leaf temperature will increase unless the boundary layer conductance to the diffusion of sensible heat is very large. An increase in leaf temperature will result in an increase in e_i, driving transpiration at a rate faster than would be expected if stomatal closure had no effect on leaf temperature at all. As well, if the leaf is, say, one of many in a canopy all behaving in a similar fashion, the air in the canopy around the leaves will become drier and hotter, decreasing e_a and further offsetting the expected decrease in transpiration (Jarvis and McNaughton, 1986).

The extent of this effect will depend on canopy characteristics, such as aerodynamic roughness, and the area over which the canopy extends. These features influence the degree of "coupling" between the canopy and the ambient atmosphere outside the boundary layer (Jarvis and McNaughton, 1986). For aerodynamically rough canopies, coupling will be relatively high, and changes in stomatal resistance will be largely reflected in changes in canopy transpiration. For poorly coupled canopies, changes in stomatal resistance may have little effect on canopy transpiration.

Cowan and Troughton (1971) and Jones (1976), on the basis of theoretical considerations, derive circumstances under which water-use efficiency may actually decrease as stomatal resistance increases (and vice versa). These circumstances are determined by the relative magnitudes of the stomatal resistance, boundary layer resistance and internal resistance.

Following upon the arguments presented by Cowan and Troughton (1971), Cowan (1977) presented theory from which he derived a term, "supraresistance to transfer of CO_2", which incorporated the relative contributions of the boundary layer and internal resistances. He defined this term mathematically for a single leaf in the following way:

$$\text{Supraresistance, } r_i^* = r_i - 1.6\, \varepsilon\, r_b^h - 0.2\, r_b \quad , \qquad (1.9)$$

where r_i is the internal resistance to CO_2, r_b and r_b^h the boundary layer resistances to water vapour and heat respectively, and ε is the rate of increase in the latent heat content of saturated air with increase in sensible heat content. This formulation moderates the relative significance of the internal resistance to CO_2 transfer by allowing for the extra "resistance" to water vapour transfer, in essence a "heat transfer resistance" (Farquhar et al., 1988).

The significance of this "supraresistance" in terms of instantaneous leaf water-use efficiency, is that, when r_i^* is positive, small increases in stomatal resistance (decreases in conductance) that reduce T and p_i/p_a also result in a higher leaf water-use efficiency. However, if r_i^* is negative, the instantaneous leaf water-use efficiency actually decreases for small increases in stomatal resistance.

If this theory is extended to incorporate the aerodynamic resistance, r_a, associated with a canopy, and fluxes and resistances are expressed in terms of ground area, then (Farquhar et al., 1988, Equation 14):

$$r_i^* = r_i - 0.2\, r_b - 1.6\, \varepsilon\, r_b^h - 0.6\, r_a - 1.6\, \varepsilon\, r_a \quad . \qquad (1.10)$$

Thus, r_i^* has a tendency to be smaller (i.e. closer to being negative) for canopies than for individual leaves. In other words, selection for improved water-use efficiency based on increased stomatal resistance may be even less effective when plants are grown as canopies.

It can be seen from equation 1.10 that "supraresistance" will tend to be least when canopy r_i is small, such as with high leaf area index of well-fertilized, well-watered C_3 vegetation at high light intensity, when ε is large (high temperatures) and when r_a and r_b are large, as with large leaves and small wind velocities.

The preceding analysis indicates that selection for improved water-use efficiency on the basis of changes in p_i/p_a resulting from variation in stomatal conductance may not be effective when plants are grown in field canopies. It must be recognised that selecting for improved water-use efficiency in this way is also likely to be at the expense of dry matter production (Jones, 1987). Reducing stomatal conductance means that the assimilation rate per unit leaf area will also be reduced.

On this basis, perhaps a better means of improving the water-use efficiency of crop plants may be to select for higher photosynthetic capacity. This should not only improve water-use efficiency, but should also lead to greater productivity in general. The greater water-use efficiency of C_4 species over C_3 species (Morison and Gifford, 1983) is largely attributed to the greater photosynthetic capacity of the former (Downes, 1969; Slatyer, 1973; Rawson et al., 1977), although C_4 species often have lower values of stomatal conductance as well (Downes, 1969; Rawson et al., 1977). For both C_3 and C_4 species, increasing the ambient CO_2 concentration, thereby reducing the relative influence of stomatal conductance, also enhances water-use efficiency (Morison and Gifford, 1984).

Evans (1976) points out that, for many C_3 species, selection for greater yield has not led to an increase in photosynthetic rate per unit area (i.e. photosynthetic capacity). He cites several studies where yield and leaf area, rather than yield and photosynthetic capacity, were positively correlated. In fact, selection for yield has often resulted in a negative correlation between leaf area and photosynthetic rate per unit area. In this regard wheat is a prime example. In studies comparing modern wheats with primitive relatives, Khan and Tsunoda (1970) and Evans and Dunstone (1970) found the primitive wheats to have higher photosynthetic rates per unit area but leaf area was smaller. On the basis of leaf nitrogen content, photosynthetic rates of the modern wheats are generally equivalent to that of the more primitive wheats, although Austin et al. (1984), expressing photosynthetic rate on a unit chlorophyll basis, found some *Triticum* diploids to have higher rates than more modern hexaploid and tetraploid wheats. The benefits to be gained by selecting for greater photosynthetic capacity must therefore be weighed against the disadvantages of the likely reduction in leaf area. As discussed earlier, in some environments and seasons, slow leaf area development may be advantageous to water-use efficiency at the crop level. In other environments this may well not be so.

Clearly, there are several problems associated with selection for improved water-use efficiency based on a reduction in the ratio p_i/p_a. These are largely associated with the gas-exchange and dry matter production of canopies growing under well-watered conditions. It may be that circumstances under which "supraresistance" will be negative are less likely to occur in water-limited environments, but the degree to which this is so will depend on the extent and timing of any such limitation.

In some environments, such as where the crop is grown largely on stored water, both leaf area index and stomatal conductance may be relatively low for much of the growing season. Under such circumstances, r_i^* will tend to be positive (Cowan, 1977), and the canopy more closely coupled to the the atmosphere (Jarvis and McNaughton, 1986). Assuming that genotypic variation in p_i/p_a persists under these conditions, selection for improved water-use efficiency based on variation in p_i/p_a may prove worthwhile.

In environments where current rainfall accounts for much of the crop water supply, crops may be relatively unstressed for much of the period of canopy growth. In such environments, selection for improved water-use efficiency may be ineffective if it is based on variation in p_i/p_a resulting from differences in stomatal conductance, except perhaps for those seasons when rainfall during the early part of the season is low and canopy development is restricted.

Thus, as for many proposed physiological traits (Turner, 1981), the value of selecting for improved water-use efficiency based on variation in p_i/p_a is likely to depend on the environment in which the crop is to be grown. The source of variation in p_i/p_a, i.e. stomatal conductance or photosynthetic capacity, is also likely to be important.

But how would one select for variation in p_i/p_a? Leaf gas exchange studies are laborious and would require careful duplication of conditions to detect true and consistent genotypic differences. As a screening technique they are unsuitable, suffering even more so from the problems that bedevil selection for stomatal characters. An alternative may lie in the use of carbon isotope discrimination. As outlined in the following section, carbon isotope discrimination may provide an indirect means of detecting genotypic differences in p_i/p_a and, by inference, genotypic differences in water-use efficiency.

1.2.7 Carbon Isotope Discrimination.

Atmospheric CO_2 contains the stable isotopes ^{12}C and ^{13}C in the approximate ratio of 89:1 (O'Leary, 1981). In plants there is proportionately less ^{13}C than in the air. This is a result of discrimination which takes place against $^{13}CO_2$ during carbon fixation (Troughton, 1979). Plants with the C_3 pathway of photosynthesis have a $^{13}C/^{12}C$ ratio about 22 per mil (‰) less than that of the atmosphere, while plants with the C_4 pathway have a ratio which is lower than the atmosphere by about 12‰. An intermediate ratio is found in plants exhibiting crassulacean acid metabolism (Troughton, 1979).

The isotopic composition ($\delta^{13}C$) of plant material is conventionally determined by comparing it to that of the historic standard Pee Dee belemnite (PDB), a carbonaceous rock. Hence, (O'Leary, 1981, Equation 2):

$$\delta^{13}C\ (‰) = (R/R_{PDB} - 1) \times 1000 \quad , \qquad (1.11)$$

where R is the molar abundance ratio, $^{13}C/^{12}C$, of the plant material and R_{PDB} is that of the standard PDB. On this basis, the composition of C_3 plant material is typically about -30‰ (i.e. the plant material is depleted in ^{13}C compared to PDB). Relative to PDB, the air has a composition of about -8‰ and thus, relative to air, the composition of C_3 plant material is -22‰.

Now, the discrimination against ^{13}C is determined using the following expression (O'Leary, 1981, Equation 3):

$$\text{Discrimination} = \frac{\delta^{13}C_{(source)} - \delta^{13}C_{(product)}}{1 + \delta^{13}C_{(source)}/1000} \quad . \qquad (1.12)$$

Since invariably, the $\delta^{13}C$ of plant material is more negative than that of the source, i.e. the air, the resulting value for discrimination is positive. Thus, while discrimination is positive, $\delta^{13}C$ values are negative. O'Leary (1981) points out that this can lead to considerable confusion, claiming that errors in the literature have often resulted from improper allocation of algebraic sign to discrimination values. Further, composition, $\delta^{13}C$, is sensitive to changes in the isotopic composition of the air even with a constant value for discrimination.

In an effort to overcome this confusion, Farquhar and Richards (1984) proposed a revision of the way in which the isotopic composition of plant material is expressed. They defined the overall isotope effect during carbon accumulation, α, as

$$\alpha = R_a/R_p \quad , \tag{1.13}$$

where a and p denote air and plant respectively (Farquhar and Richards, 1984, Equation A4). This is similar to the practice of chemists investigating isotope fractionations. Given a sufficiently large source reservoir that is not appreciably depleted by product formation, results of such studies are expressed in terms of the ratio $R_{(source)}/R_{(product)}$ (O'Leary, 1981).

The discrimination or fractionation, Δ, is then defined as the deviation from unity, i.e. (Farquhar and Richards, 1984, Equation A5)

$$\Delta = \alpha - 1 \quad . \tag{1.14}$$

Using this notation, C_3 plants have a positive discrimination (against ^{13}C) of about 22 x 10^{-3}. In terms of the isotopic composition, $\delta^{13}C$, relative to PDB,

$$\Delta = \frac{\delta^{13}C_{(a)} - \delta^{13}C_{(p)}}{1 + \delta^{13}C_{(p)}} \quad , \tag{1.15}$$

where a and p again denote air and plant, respectively (Hubick et al., 1986).

Although the isotopic discrimination measured in C_3 plant material is regarded as being typically 22 x 10^{-3}, considerable variation from this value has been observed. Troughton (1979) and O'Leary (1981) have reviewed the extent and sources of this variation which has a range, in terms of $\delta^{13}C$ values of -22 to -40‰, corresponding to a range in Δ of 14 x 10^{-3} to 32 x 10^{-3}.

1.2.7.1 The relationship between Δ and p_i/p_a

The basis of the discrimination against ^{13}C by C_3 plants lies mainly in two processes ; the physical process of diffusion of CO_2 to the site of carboxylation, and the biochemical process of CO_2 fixation (O'Leary, 1981). Diffusion of CO_2 through the leaf boundary layer and stomata and into the intercellular spaces within the leaf results in discrimination against ^{13}C due to the larger mass of the $^{13}CO_2$ molecule. The diffusivity of $^{13}CO_2$ in air is 4.4‰ less than that of $^{12}CO_2$ in air. Thus, the discrimination associated with diffusion in air is 4.4‰. Biochemical discrimination against ^{13}C occurs because of the lower reactivity of ^{13}C. In C_3 plants, the extent of this discrimination (approximately 27‰) is greater than that associated with diffusion. These two sources of discrimination are not additive however.

Farquhar et al. (1982b) developed theory which suggests that the discrimination measured in C_3 plants reflects the extent to which the CO_2 supply for carboxylation is limited by diffusion. This theory is encapsulated in the following equation (Farquhar and Richards, 1984, Equation 2):

$$\Delta = a \cdot \frac{p_a - p_i}{p_a} + b \cdot \frac{p_i}{p_a} , \qquad (1.16)$$

where Δ is the net discrimination against $^{13}CO_2$, p_a and p_i are the atmospheric and intercellular partial pressures of CO_2 respectively, a is the fractionation occurring due to diffusion in air (4.4‰) and b is the fractionation due to carboxylation (27‰). By rearranging Equation 1.16, carbon isotope discrimination can be related directly to the ratio p_i/p_a as:

$$\Delta = a + (b - a)\, p_i/p_a , \qquad (1.17)$$

which, on substituting the appropriate values for a and b becomes (Farquhar and Richards, 1984, Equation 3):

$$\Delta = (4.4 + 22.6\, p_i/p_a) \times 10^{-3} . \qquad (1.18)$$

This equation indicates that, if stomata place no limitation on diffusion of CO_2, such that p_i equals p_a, Δ would be 27×10^{-3}. As stomatal conductance declines and diffusion becomes more limiting, p_i will also decline and Δ will tend towards 4.4×10^{-3}.

Equation 1.18, which will be used as the "operating" equation in the bulk of this thesis, is an approximate expression however, since it neglects the contribution to net discrimination of an apparent host of other factors. These factors, discussed in some detail in Farquhar et al. (1982b), Farquhar and Richards (1984) and Evans et al. (1986) are associated with the movement of CO_2 to the site of carboxylation and with the biochemical processes of carboxylation and decarboxylation (respiration). They are incorporated in the following equation, which is an extension of Equation 1.16 (Farquhar et al., 1982b; Farquhar and Richards, 1984):

$$\Delta = a_b \frac{(p_a - p_s)}{p_a} + a \frac{(p_s - p_i)}{p_a} + (b_s + a_l) \frac{(p_i - p_c)}{p_a} + b \frac{p_c}{p_a} - \frac{eR_d/k + f\Gamma^*}{p_a} \quad .(1.19)$$

In this equation, p_s is the partial pressure of CO_2 at the leaf surface, p_c that at the site of carboxylation and p_a and p_i are as defined for Equation 1.16. a and b remain as previously defined also. a_b is the fractionation occurring during diffusion in the boundary layer (2.9‰), b_s is the fractionation occurring as CO_2 enters solution (1.1‰ at 25°C), a_l is the fractionation due to diffusion in water (0.7‰), e and f are fractionations with respect to average carbon composition associated with 'dark' respiration in the light and with photorespiration, respectively, k is the carboxylation efficiency and Γ^* is the CO_2 compensation point in the absence of R_d.

Equation 1.19 presents an array of factors that could all affect net discrimination independently of p_i/p_a. On the basis of available evidence, Farquhar and Richards (1984) consider most of them to be insignificant, but express some concern over variation associated with the diffusion of CO_2 from the intercellular spaces to the sites of carboxylation, and over the relative contributions of PEP carboxylase and RuBP carboxylase to the value of b.

Evans et al. (1986), using concurrent measurements of Δ and p_i/p_a, have shown that the fractionations associated with diffusion of CO_2 from the intercellular spaces to the sites of carboxylation may result in substantial deviations of measured values of net discrimination from those expected using equation 1.18. In other words, fractionations associated with a "liquid phase" resistance may be substantial. K.T. Hubick, G.D. Farquhar and S.C. Wong (unpublished), working with barley, observed a smaller effect than that found by Evans et al. (1986).

The value attributed to b, the net fractionation caused by carboxylation, is dominated by discrimination due to the primary carboxylating enzyme RuBP carboxylase. About 10% of carboxylations in C_3 plants are thought to involve PEP carboxylase. Farquhar and Richards (1984) derived a value of 27‰ for b based on the relative contributions of these two enzymes using the following equations:

$$b = (1 - \beta)b_3 + \beta b_4 = b_3 - \beta(b_3 - b_4) \quad , \qquad (1.20)$$

where β is the molar proportion of carbon fixed by PEP carboxylase (about 0.1), b_3 is the fractionation attributed to RuBP carboxylase (about 30‰), and b_4 is that attributed to PEP carboxylase (about -5.7‰). Variation in any of β, b_3 or b_4 will be reflected in variation in the true value of b. For example, the most recent estimate of b_3 is 29‰ with respect to dissolved CO_2 (Roeske and O'Leary, 1984). Earlier estimates varied considerably (O'Leary, 1981).

The possible extent of fractionations associated with respiratory processes must remain even more speculative, since estimates of them either vary widely (Park and Epstein, 1961; Hsu and Smith, 1972; Troughton et al., 1974) or have yet to be made.

Notwithstanding the large number of simplifying assumptions associated with Equation 1.18, in recent years a substantial body of evidence, both direct and indirect, has been accumulating which supports a relationship between discrimination and p_i/p_a. Indirect evidence has been obtained in several studies where variation in Δ was measured corresponding to experimentally or genotypically generated variation in p_i/p_a. In several studies where this correlation was observed, increasing salinity was used to generate variation in p_i/p_a (Farquhar et al., 1982a; Downton et al., 1985; Seemann and Critchley, 1985; Guy and Reid, 1986). Winter (1981) and Winter et al. (1982) subjected plants to, respectively, water stress and decreasing relative humidity, treatments known to reduce stomatal conductance. Both Δ and p_i/p_a were lowered by these treatments. In the *flacca* tomato mutant, which is known to lack abscisic acid and have a higher stomatal conductance compared to its isogenic parent, phenotypic reversion of both Δ and p_i/p_a occurred when the mutant was sprayed with abscisic acid during its growth (Bradford et al., 1983). Ehleringer et al. (1985) observed that mistletoes, which tend to have higher stomatal conductances than their hosts, show greater discrimination and higher p_i/p_a than their hosts. In all these cases Δ and p_i/p_a were positively correlated.

Direct evidence for a relationship between Δ and p_i/p_a comes from the work of Evans et al. (1986), who measured p_i/p_a in leaves of *Triticum aestivum* and *Xanthium strumarium* in a gas exchange chamber. They also measured Δ of the CO_2 in the air sampled both before and after passing over the leaf. By varying the experimental conditions they were able to generate variation in p_i/p_a and corresponding variation in the discrimination observed. Similar results have been obtained by K.T. Hubick, G.D. Farquhar and S.C. Wong (unpublished) working on barley and peanuts.

1.2.7.2 Carbon isotope discrimination and plant water-use efficiency

Recent studies have also demonstrated, at the whole plant level, a negative correlation between carbon isotope discrimination and water-use efficiency. Farquhar and Richards (1984) found variation in plant water-use efficiency among wheat genotypes subjected to a range of watering regimes in the glasshouse. This variation was negatively correlated with carbon isotope discrimination measured in plant material. For a range in water-use efficiency from 2.0 to 3.7 mmol C/mol H_2O, the corresponding range of discrimination was 22.5×10^{-3} to 19.4×10^{-3}. Similar studies on a diverse range of peanut genotypes (Hubick et al., 1986) and also on a range of barley genotypes (Hubick and Farquhar, 1989) have confirmed the negative correlation in a wider range of C_3 crop species. In the peanut study, water-use efficiency ranged from 0.8 to 1.7 mmol C/mol H_2O as Δ ranged from 19.6×10^{-3} down to 15.8×10^{-3}. In both the wheat and peanut studies the increase in water-use efficiency was approximately 18% for a decrease in Δ of 1×10^{-3}.

The studies with wheat, peanuts and barley have all demonstrated that genotypic variation in carbon isotope discrimination exists and that plant water-use efficiency and Δ are negatively correlated. This suggests that measurement of carbon isotope discrimination may provide an effective means of selecting for improved water-use efficiency in C_3

crops. The experimental work that follows was conducted to assess the usefulness of this new technique in selecting for improved water-use efficiency in wheat.

1.3 EXPERIMENTAL OBJECTIVES

The experimental program had several objectives. These objectives addressed both the physiological rationale for the use of carbon isotope discrimination as a selection criterion for improved water-use efficiency (Farquhar and Richards, 1984), and its reliability and use in breeding (Garrity et al., 1982).

In the Introduction (Section 1.1), a general framework was outlined for the assessment of physiological traits proposed for improving the yield performance of crops in water-limited environments. This framework is restated here.

1. Development of a hypothesis concerning the potential contribution of a particular trait.
2. Search for genotypic variability for the trait.
3. Development of a rapid and efficient screening method.
4. Elucidation of the mode of trait inheritance and selection of an appropriate breeding method to incorporate it into agronomically superior genotypes.
5. Documentation of a strong causal relationship between high levels of a trait and improved yield performance in water-limited environments.
6. Assessment of the likely impact of trait selection on yield potential under more favourable conditions.

This framework formed the basis for the experiments reported in this thesis. Not all stages were given similar priority, however. Work directed at stages 3 and 4 is presented as an appendix to the body of the thesis (Appendix 1).

The Literature Review (Sections 1.2.6.4 to 1.2.7.2) has examined the background theory and supporting experimental evidence indicating a negative correlation between carbon isotope discrimination and water-use efficiency. The review provides some encouragement that Δ may prove a useful means of screening for improved water-use efficiency, and hence increased yield, of crops growing in water-limited environments.

However, several shortcomings were also revealed which could limit the application of Δ in selecting for greater crop water-use efficiency. These shortcomings involve ;

1. Changes in the effectiveness of stomatal control of gas-exchange as the scale of measurement increases from single leaves to whole plants and then to crop canopies,
2. Possible unfavourable relationships between factors causing variation in Δ at the leaf level (specifically, stomatal conductance and photosynthetic capacity) and productivity at the crop level,
3. Several assumptions regarding the biochemistry of discrimination measured at the leaf level and the expression of discrimination in plant parts.

In order to determine the relationships between water-use efficiency and Δ and yield and Δ in field environments, and also to examine the significance of the possible shortcomings outlined above, greater genotypic variability in Δ needed to be sought and utilised, if available. It was concluded that genotypic variability would best be sought in material already "adapted" for growth in the Australian wheat belt, since field experiments would be conducted in that environment.

Material from a range of sources was evaluated for variation in Δ. This material included recently released cultivars and advanced lines from plant breeding programmes based in different parts of the wheat belt. Large numbers of genotypes were grown together in the field in replicated experiments at several sites (Chapter 2 and Appendix 1). Genotypic variation in Δ was found to be substantial. Genotype x environment interaction for Δ was generally nonsignificant (Chapter 2), except when more extreme environments were contrasted (Appendix 1). These results indicated that Δ is under relatively strong genetic control and that further experimentation as to the usefulness of Δ as a selection criterion was warranted.

Field experiments reported in Chapter 2 also provided an opportunity to determine the relationships between grain yield and Δ and biomass production and Δ under conditions where pre-anthesis water supply was largely non-limiting. These relationships were found to be positive. For a relatively small increase in Δ, there were substantial increases in both grain yield and biomass production. The slope of these relationships suggested that, under these growing conditions, relationships between crop water-use efficiency and Δ were probably not strong, and possibly positive as well.

To examine possible causes for this, several genotypes were selected spanning a range of Δ values. Experiments using these genotypes were conducted at the leaf, single plant and crop canopy levels.

Experiments reported in Chapter 3 were conducted to confirm that differences in Δ among wheat genotypes reflected measured differences in the ratio p_i/p_a among them. If this was found not to be the case, then genotypic differences in water-use efficiency would not necessarily follow, even at the leaf level. Gas-exchange techniques were used to estimate p_i/p_a in flag leaves of 14 genotypes. Discrimination measured in dry matter and p_i/p_a measured in flag leaves were positively correlated. Variation in p_i/p_a was attributed to variation among genotypes in conductance and in photosynthetic capacity.

In the gas-exchange studies, a considerable amount of variation in Δ was not explained by the observed variation in p_i/p_a. This result suggests that there may also be considerable variation in water-use efficiency that is not accounted for by variation in Δ, and vice versa. The relationship between plant water-use efficiency and Δ was tested in the glasshouse using plants of 16 genotypes grown under well-watered and terminally water-stressed conditions (Chapter 4). Dry matter production, transpirational water use and Δ in dry matter were measured in replicated experiments. There were negative correlations between water-use efficiency and Δ under both sets of growing conditions. Under well-watered conditions, the response of plant water-use efficiency to a change in Δ was large, but less than the expected response of leaf transpiration efficiency. This result suggests that, even at the single plant level, scale effects may have a significant impact on the relationship between water-use efficiency and Δ.

Field experiments were conducted over two seasons to determine the relationship between water-use efficiency and Δ for wheat genotypes grown under a mediterranean environment (Chapter 5). The experiments also aimed to determine the influence on water use and dry matter production of factors associated with genotypic variation in Δ, in particular stomatal conductance, photosynthetic capacity and canopy development. Dry matter production and soil water use were monitored for several genotypes grown in replicated plots. In one experiment, crop evapotranspiration was partitioned into transpiration and direct soil evaporation using a model based on the proportion of incident radiation reaching the soil surface. Under the growing conditions of high winter/spring rainfall followed by terminal water stress, significant genotypic variation in canopy water-use efficiency was observed but there was no relationship between water-use efficiency and Δ.

CHAPTER 2

GENOTYPIC VARIATION IN CARBON ISOTOPE DISCRIMINATION AND THE RELATIONSHIP BETWEEN YIELD AND CARBON ISOTOPE DISCRIMINATION UNDER WELL-WATERED CONDITIONS IN THE FIELD

2.1 INTRODUCTION

In container studies on wheat (Farquhar and Richards, 1984), peanut (Hubick et al., 1986) and barley (Hubick and Farquhar, 1989), plant water-use efficiency was shown to be negatively correlated with Δ. The study on wheat involved a small number of genotypes. There was relatively little genotypic variation in water-use efficiency and Δ under well-watered conditions, but variation in both increased with increasing water stress.

In this study, further genotypic variation in Δ was sought among a larger number of wheat genotypes. Material was obtained from a wide range of sources and grown together under well-watered conditions in the field. Genotype x environment interaction for Δ was examined by growing the majority of genotypes at two field sites and a subset of the genotypes in pots at another location.

The study also examined the relationship between yield and Δ under well-watered conditions. While increased water-use efficiency may be considered a desirable objective under water-limited conditions, this should preferably not be at the expense of yield potential in favourable seasons.

These trials also permitted an initial, if indirect, assessment of sources of variation in Δ in wheat genotypes. The ratio p_i/p_a, and hence the value of Δ, depends on the balance between photosynthetic capacity (the amount and activity of enzymes and other components associated with the carbon fixation system) and stomatal conductance. There may be variation among genotypes in p_i/p_a if either (1) photosynthetic capacity remains constant but stomatal conductance varies, or (2) stomatal conductance remains constant but photosynthetic capacity varies. If (1) holds, increasing stomatal conductance should raise p_i/p_a and Δ but lower leaf water-use efficiency (provided supraresistance, r_i^*, is positive). Since p_i/p_a (i.e. the supply of CO_2) is raised, assimilation rate per unit leaf area will increase. That is, the relationship between Δ and assimilation rate will be positive. If (2) holds, increasing photosynthetic capacity should lower p_i/p_a and Δ but raise leaf water-use efficiency and assimilation rate per unit leaf area. That is, the relationship between Δ and assimilation rate will be negative.

2.2 MATERIALS AND METHODS

2.2.1 Field Experiments.

A diverse range of wheats were sown at the two sites, Yanco (146°E,34°S) and Wagga Wagga (147°E,35°S), in southern New South Wales in 1984. The soil at Yanco was a red sandy clay loam, that at Wagga Wagga a grey silty clay loam. Both sites had been under pasture in 1982 and wheat in 1983 and were maintained as cultivated fallow from January 1984 until the experiments were sown. The experiment at Yanco was sown on 30 May and that at Wagga Wagga on the following day. Before sowing, fertilizer was applied at the rates of 27 $kg.ha^{-1}$ P and 14 $kg.ha^{-1}$ N. During the growing season, herbicides (Bromoxynil, Diclofop methyl) and a systemic fungicide (Triadimefon) were applied when required.

Twenty-four common wheats (*Triticum aestivum* L.), chosen for their diversity, comprising 13 cultivars grown commercially in Australia or elsewhere and 11 breeding lines, one durum wheat (*Triticum turgidum* L. *durum*) and two triticales (*Triticosecale* Wittmack) were grown (see Table 2.2). Twenty-one of the common wheats and the durum were represented in both trials. The genotypes were sown in a two-replicate randomized block design. The plots were 6.3 m x 8 rows with 0.17 m row spacing seeded at a density of 160 seeds m^{-2}. Mean anthesis dates at Yanco and Wagga Wagga were 11 October and 16 October, respectively. Both trials were harvested on 4 December.

At maturity, a 0.5 m^2 subsample was harvested at ground level from each plot to estimate harvest index (i.e. ratio of grain yield to above-ground dry matter). Above-ground biological yield per unit area was calculated from the quotient of grain yield and harvest index. Grain yield was obtained by machine harvesting the inside six rows in each plot after discarding 0.3 m from the ends.

Carbon isotope discrimination, Δ, was measured on peduncle material, i.e. stem material from above the flag leaf, from the subsample taken for harvest index. The material was dried at 80°C for 48 h and then ground to pass a 100 μm sieve. Δ was measured by ratio mass spectrometry as described below (Section 2.2.3). For each genotype all Δ values and harvest data are the means from the two replicate plots at each site.

Meteorological data from the two sites are summarised in Table 2.1. At both sites, rainfall was above average from July to October, i.e., the period preceding anthesis when the major part of crop growth occurs. Changes in soil water content were not followed in these experiments, but data were available from immediately adjacent experiments (Appendix 2.1). These data showed that depletion of the soil moisture store from just after sowing to anthesis was approximately 25% of that to maturity at both sites. This information, combined with that on seasonal rainfall given in Table 2.1, indicates that, at least to anthesis and possibly for some time beyond, soil water supply was not limiting crop growth.

2.2.2 Pot Experiment.

Ten genotypes (indicated in Table 2.2) from the field experiments, spanning the range of Δ values obtained, were subsequently grown in pots at Canberra (149°E, 35°S) in 1985. Six more genotypes which were to be grown in other field experiments were also grown (Table 2.3).

Pregerminated seed was sown into 3 L pots containing a loam-based potting mix on 10 June. Following emergence, plants were thinned to four per pot and the pots were placed in a three-replicate randomized block design on benches outdoors (to simulate the temperature and light regimes encountered in the field). The pots were watered every second day and kept well supplied with nutrients throughout. Δ for each genotype was measured on mainstem material harvested on 4 October when the plants were at or approaching ear emergence. The four mainstems from each pot, i.e. the whole of the stems minus the leaf blades, were bulked together and processed in the same manner as the samples from the field experiments. Δ values presented are the means of the three replicate pots per genotype.

Leaf conductance for each genotype was measured on recently expanded leaves 5 and 6 over a period of 3d of cloudless weather in early September, during stem elongation. For each genotype 10 measurements were made on both the adaxial and abaxial leaf surfaces using a Delta-T Mk3 diffusive resistance porometer. Since wheat leaves have a habit of twisting, the 10 measurements per leaf surface consisted of two groups of 5 measurements each, one group taken on surfaces facing the sun, the

Table 2.1. Monthly summary of meteorological data for the 1984 growing season at Wagga Wagga and Yanco. Average daily mean temperature (T), monthly rainfall (P), and average daily class A pan evaporation (E^*) are shown. Long-term average monthly rainfall is also shown, in parentheses, for each site.

	Yanco			Wagga Wagga		
	T (°C)	†P (mm)	E^* ($mm.d^{-1}$)	T (°C)	†P (mm)	E^* ($mm.d^{-1}$)
June	10.1	2.1(34)	2.04	9.3	5.1(50)	1.76
July	8.8	71.3(34)	1.53	7.8	89.7(47)	1.24
August	10.3	57.1(37)	2.51	10.0	94.9(49)	2.06
September	10.8	30.4(38)	3.78	10.2	38.2(44)	2.97
October	15.5	56.9(48)	5.49	15.1	35.6(54)	4.48
November	20.4	13.4(34)	8.56	19.6	19.2(41)	7.74
December	21.6	7.1(29)	10.24	20.8	5.1(42)	9.60

†P (January - May); 260 mm at Yanco, 320 mm at Wagga Wagga.

other on surfaces facing away from the sun. The mean conductance of each leaf surface was determined using all 10 measurements. A total leaf conductance, taken to represent the population of leaves of a given genotype, was determined by summing the mean conductances of the adaxial and abaxial surfaces.

2.2.3 Carbon isotope discrimination

To determine the stable carbon isotope composition of dry matter from field- and pot-grown plants, a subsample of finely ground material was combusted to CO_2 in a VG Isoprep 13 organic preparation system. The CO_2 was trapped in a liquid N_2-cooled glass tube supplied by the manufacturer. The CO_2 was then thawed and transferred directly to the inlet of the ratio mass spectrometer (VG Micromass 602D), where it was compared with a working standard of known carbon isotope composition (-35.08‰ relative to Pee Dee Belemnite). The value of Δ was then calculated according to Equation 1.15 using a value for $\delta^{13}C_{(air)}$ of -7.8‰ (Farquhar and Richards, 1984). Generally, single measurements only were taken on dry matter samples. Random replicate measurements were also performed as checks. The difference between these replicates was always very close to the 0.2‰ uncertainty of the combustion and analysis system.

2.3 RESULTS

2.3.1 Genotypic variation in Δ and its relationship with yield.

Δ varied significantly among genotypes at both Wagga Wagga and Yanco (Table 2.2). At Wagga Wagga, Δ had a range of 1.7×10^{-3}, at Yanco, 2.4×10^{-3}. The highest and lowest values of Δ at Yanco, 19.7×10^{-3} and 17.3×10^{-3}, correspond to p_i/p_a ratios of 0.68 and 0.57 (from Equation 1.18), near the range of 0.7 ± 0.1 said to be typical for C_3 plants at 2 kPa vapour pressure difference (Wong et al. 1979). At a p_a of 33.0 Pa these ratios represent p_i's of 22.4 Pa and 18.8 Pa respectively.

Genotype x site interaction for Δ was not significant (variance ratio = 1.09). This is also evident in the significant associations between Δ measured on genotypes at Wagga Wagga and at Yanco (Fig. 2.1a) and between Δ measured in pots at Canberra and mean Δ for genotypes at both field sites (Fig. 2.1b).

Table 2.2. Summary of cultivars, anthesis dates, grain yield (GY), biological yield (BY), and carbon isotope discrimination (Δ) of peduncle material for the two field experiments.

	Wagga Wagga				Yanco			
	Anthesis	Yield GY	Yield BY	$10^3 \times \Delta$	Anthesis	Yield GY	Yield BY	$10^3 \times \Delta$
	day	$t.ha^{-1}$			day	$t.ha^{-1}$		
Triticum aestivum L.								
Hartog*	293	5.01	12.86	18.86	-	-	-	-
Cleopatra 74*	286	4.51	10.03	18.66	276	4.24	10.13	19.73
Millewa*	296	4.43	12.13	18.27	285	3.88	10.79	18.69
Pitic 62	295	4.26	10.67	18.09	284	3.96	11.39	18.58
Takari	293	4.33	10.39	17.99	281	3.78	10.48	18.49
Spear	296	3.83	9.37	17.93	284	4.10	11.06	18.69
Kite	297	3.56	9.55	17.92	285	3.42	9.85	18.51
Sunstar*	294	3.53	10.79	17.77	282	3.78	10.63	18.24
Dagger	298	3.69	8.74	17.68	285	3.70	10.11	18.47
Blade*	296	4.33	10.12	17.51	285	4.06	11.07	18.19
Olympic	-	-	-	-	289	3.28	9.82	18.00
Cook*	295	4.03	8.96	17.36	284	4.20	9.85	17.79
Banks*	295	3.72	9.16	16.87	285	3.60	8.87	17.75
RAC 429	298	4.76	10.04	18.30	285	3.96	10.69	18.46
RAC 430*	296	4.72	11.30	18.02	286	4.06	11.76	18.86
RAC 460*	290	4.13	9.93	18.06	284	3.67	9.45	18.24
RAC 495	287	3.03	8.00	18.02	280	3.42	7.86	18.48
F79	-	-	-	-	283	4.22	11.06	18.44
CP1	292	3.74	10.60	17.67	284	3.51	11.08	18.48
KM1	294	3.13	9.93	17.41	283	3.05	9.76	18.06
CT2	289	3.60	9.54	17.30	283	3.64	11.09	17.99
CP	291	3.53	9.17	17.29	284	3.24	10.67	17.89
KM2*	293	2.82	9.55	17.18	287	2.99	9.42	17.74
CT1	289	3.67	9.70	17.18	282	3.35	9.52	17.36
Triticum turgidum L. *durum*								
Kamilaroi	296	3.54	8.05	17.51	284	3.29	9.34	17.82
Triticosecale Wittmack								
Dua	288	4.80	12.50	18.28	-	-	-	-
Ninghadu*	295	3.99	9.71	18.73	-	-	-	-
LSD ($P<0.05$)	1.2	0.57	1.39	0.35	1.3	0.45	1.34	0.57

* Genotypes also grown in pots in 1985 covering the range of Δ values measured in the field.

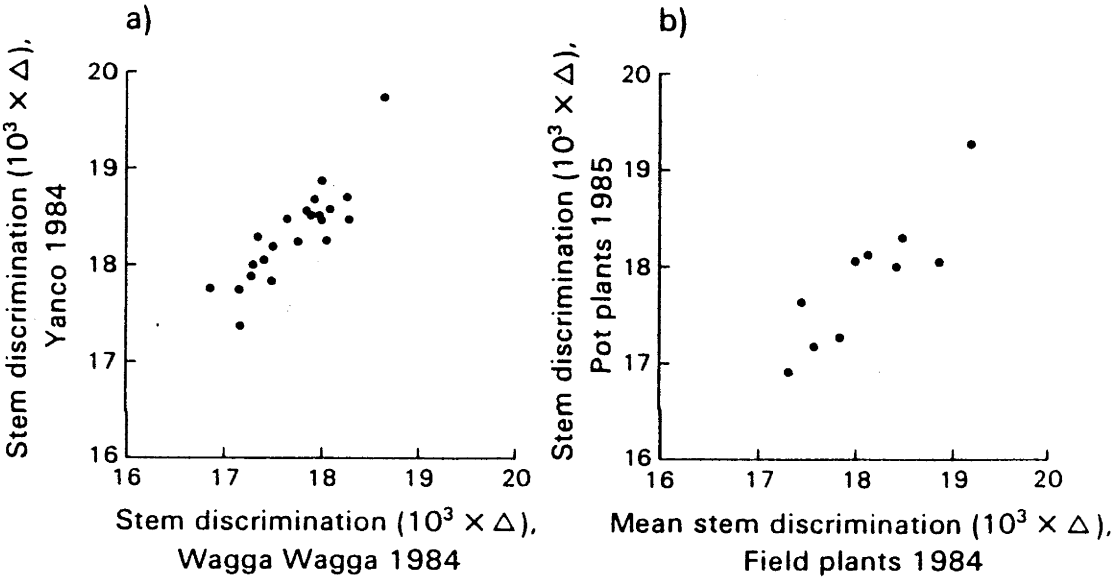


Fig. 2.1 (a) Relationship between carbon isotope discrimination, Δ, in stem material of 22 genotypes from the field trials at Wagga Wagga and Yanco in 1984. Correlation coefficient $r = 0.88^{**}$.

(b) Carbon isotope discrimination, Δ, in stems of 10 genotypes grown in pots in 1985 as a function of the mean Δ in stems of the same genotypes grown in the two field trials in 1984. Correlation coefficient $r = 0.88^{**}$. (** indicates statistical significance at the 0.01 level).

Significant positive correlations between grain yield and Δ and biological yield and Δ were obtained at both Wagga Wagga and Yanco (Fig. 2.2 and Fig. 2.3). For grain yield and biological yield both the slope and correlation coefficient were higher at Wagga Wagga than at Yanco.

2.3.2 The relationship between Δ and conductance.

In the pot experiment, the relationship between stem Δ and total leaf conductance was positive (Fig. 2.4a) but not highly significant ($P = 0.10$). When the conductance of each leaf surface was considered separately, no correlation was found between stem Δ and adaxial conductance (Fig. 2.4b) but a highly significant ($P < 0.01$) positive correlation was found between stem Δ and abaxial conductance (Fig. 2.4c). Abaxial conductance was less than adaxial conductance in all genotypes (Table 2.3).

2.4 DISCUSSION

The range in Δ values measured in these experiments indicates that significant variation in Δ exists among conventional, agronomically acceptable wheat genotypes. This result, as well as the low level of genotype x environment interaction for Δ found here, suggest that, if low Δ were to be shown to be a worthwhile trait, its incorporation into breeding programs may not be difficult. Genotypic ranking for Δ across field sites and also between field and pot-grown plants was consistent (Fig. 2.1a and 2.1b), indicating that Δ is under relatively strong genetic control. The low coefficient of variation in Δ, only 1.3% compared to 7.1% for biological yield, also indicates that Δ should be relatively easy to manipulate in breeding programs. However, the relationships found between yield and Δ in these experiments suggest that the use of Δ to screen for improved water-use efficiency in wheat will not be straightforward.

The positive correlations observed between yield and Δ also indicate a positive correlation between canopy water-use efficiency and Δ. This is in contrast to the conclusions of the container experiments on wheat (Farquhar and Richards, 1984), barley (Hubick and Farquhar, 1989) and *Arachis* genotypes (Hubick et al., 1986) summarised in the Literature Review. In all of these experiments, plant water-use efficiency and Δ were negatively correlated when water supply was limiting.

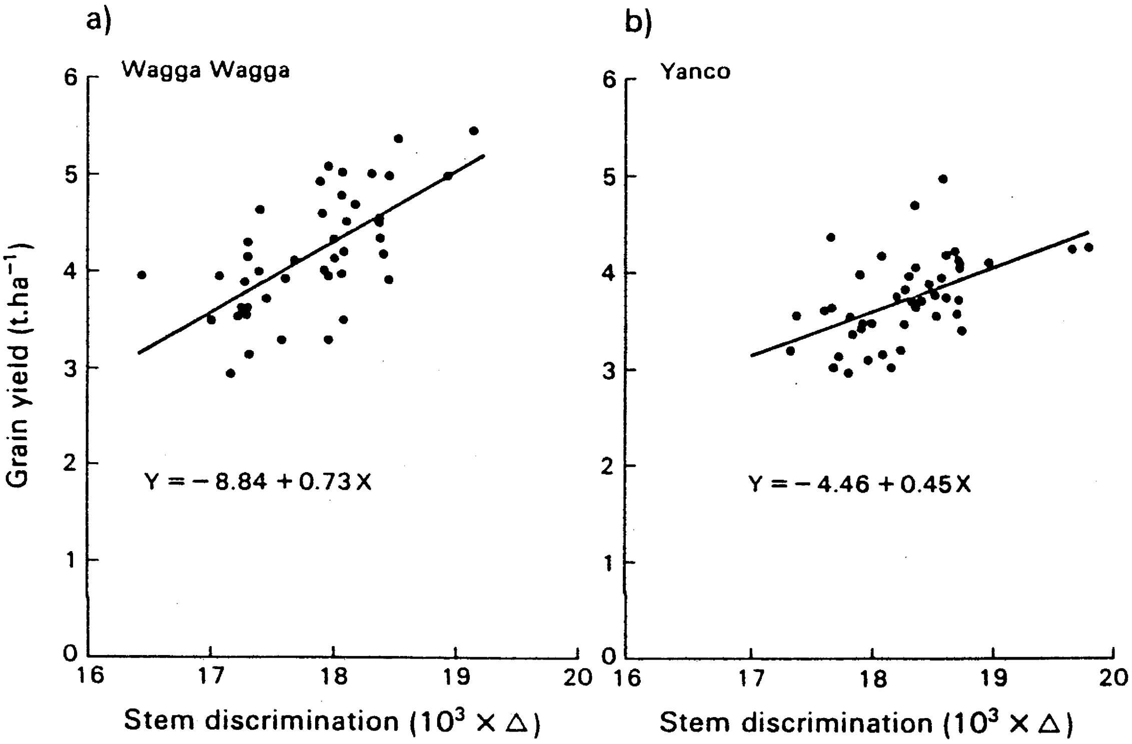


Fig. 2.2 Relationships between grain yield, (t.ha^{-1}), and carbon isotope discrimination, Δ, of stem material for the 1984 field trials at (a) Wagga Wagga and (b) Yanco. For (a) r = 0.65**. For (b) r = 0.51**.

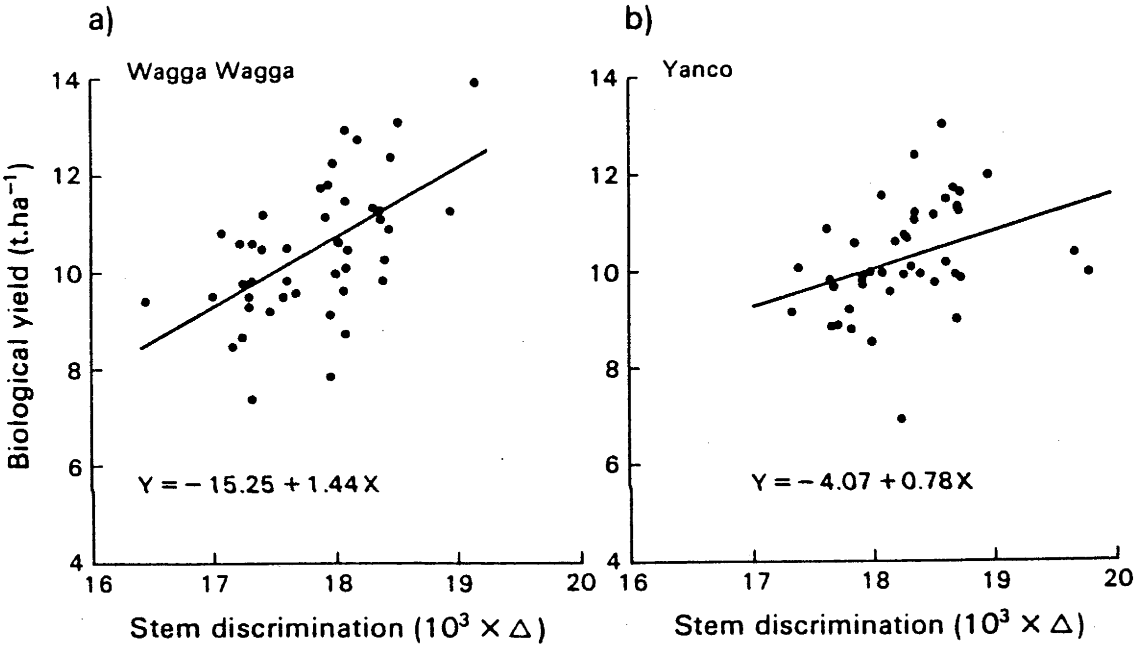


Fig. 2.3 Relationships between biological yield (t.ha^{-1}), and carbon isotope discrimination, Δ, in stem material for the 1984 field trials at (a) Wagga Wagga and (b) Yanco. For (a) r = 0.57**. For (b) r = 0.36**.

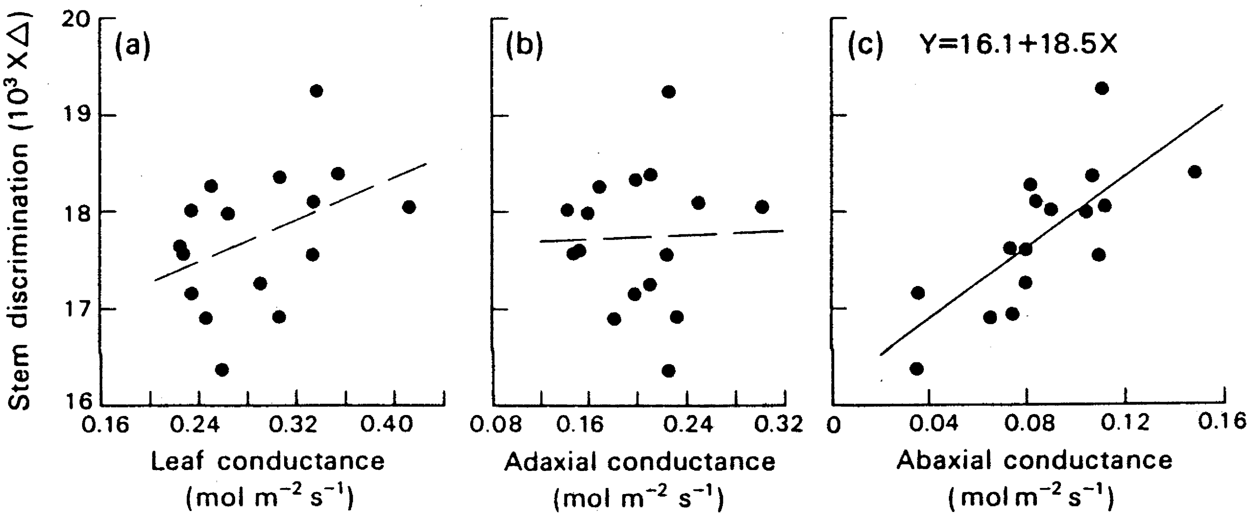


Fig. 2.4 Relationships between carbon isotope discrimination, Δ in stem material and leaf conductances, (mol m^{-2} s^{-1}), in pot-grown plants of 16 genotypes. For (a) total leaf conductance, r = 0.42 (P = 0.10). For (b) adaxial surface conductance, r = 0.03 (n.s.). For (c) abaxial surface conductance, r = 0.75**.

Table 2.3. Carbon isotope discrimination (Δ) of stem material, total leaf conductance (g_L), adaxial surface conductance (g_{AD}) and abaxial surface conductance (g_{AB}) of the cultivars grown in the 1985 Pot experiment.

Genotype	$10^3 \times \Delta$	g_L	g_{AD}	g_{AB}
			(mol m^{-2} s^{-1})	
Triticum aestivum L.				
Hartog	18.03	0.23	0.14	0.09
Cleopatra	19.27	0.34	0.23	0.11
Millewa	18.27	0.25	0.17	0.08
Sunstar	18.06	0.34	0.23	0.11
Blade	17.27	0.29	0.21	0.08
Cook	17.16	0.24	0.20	0.04
Banks	16.90	0.25	0.18	0.07
Yaqui 50E	18.36	0.31	0.20	0.11
Sundor	16.93	0.31	0.23	0.07
Quarrion	16.37	0.26	0.22	0.04
Gutha	18.40	0.36	0.21	0.15
RAC 430	17.99	0.26	0.16	0.11
RAC 460	18.11	0.33	0.25	0.08
KM2	17.62	0.23	0.15	0.08
Triticum turgidum L. *durum*				
Yallaroi	17.57	0.33	0.22	0.11
820357	17.59	0.23	0.15	0.08

Water use was not measured in this field study. Differences among genotypes in the extent of soil water use and in the amount of direct soil evaporation may partly account for this apparent contradiction. Reduced stomatal control of gas-exchange processes at the canopy level compared to that at the leaf level may have also contributed (Jarvis and McNaughton, 1986). Cowan (1977) and Farquhar et al. (1988), in discussing the implications of supraresistance to CO_2 transfer, r_i^*, suggest that, under some circumstances, small increases in stomatal resistance (decreases in conductance) may actually lead to decreased water-use efficiency. Such conditions (canopies of well-fertilized, well-watered C_3 vegetation with high leaf area index and at high light intensity) were duplicated reasonably well in these studies. If the genotypic variation in Δ found here was the result of genotypic variation in stomatal conductance then the growth conditions may well have contributed to the apparent positive correlation found between water-use efficiency and Δ.

The positive correlations found between biological yield and Δ indicate that, for this range of genotypes, biological yield was more strongly influenced by stomatal conductance than by variation in photosynthetic capacity. If variation in photosynthetic capacity had been the major influence, one may have expected the relationships between biological yield and Δ to be negative, as discussed in the Introduction. However, the relationship between Δ and total leaf conductance observed in the pot experiment was not strong either, indicating that the relationship between yield and stomatal conductance may not be as direct as suggested by the yield/Δ relationships.

A surprising feature of the relationships between biological yield and Δ is the extent of increase in yield obtained for a given increase in Δ. At Wagga Wagga biological yield increased 30% as Δ increased from 17×10^{-3} to 19×10^{-3} (i.e. as p_i/p_a increased from 0.56 to 0.65). At Yanco the increase was 17%. These figures represent substantial increases for what appears to be a relatively small change in p_i/p_a. Studies where wheat canopies have been grown under elevated CO_2 levels (e.g. Gifford, 1977) suggest that the increases in p_i implicit in the range in Δ measured here should result in increases in biological yield less than half those found. This also suggests that variation in stomatal conductance alone was insufficient to account for the observed yield increases.

Another hypothesis is that photosynthetic capacity may have been lower in lines with high Δ. Dry matter production is a function of radiation interception, determined by the size and number of leaves, and the efficiency with which intercepted radiation is converted to dry matter. In wheat, leaf size and photosynthetic rate per unit leaf area are negatively correlated (Evans and Dunstone, 1970; Rawson et al., 1983). It may be that high yielding lines have a lower photosynthetic rate per unit area, i.e. a lower photosynthetic capacity, but a relatively faster rate of leaf area development. This would result in a positive correlation between yield and Δ, as observed here, since p_i/p_a would be higher in lines with lower photosynthetic capacity. It is therefore possible that both higher stomatal conductance and lower photosynthetic capacity were contributing to the positive relationships observed between biological yield and Δ.

The strong positive correlation between Δ and abaxial conductance (and, by inference, yield and abaxial conductance) was unexpected. Rawson et al. (1976) monitored photosynthetic rate and stomatal conductance on both sides of wheat flag leaves in experiments in which they altered the requirement for assimilate in the ear. They observed an increase in leaf photosynthetic rate with increasing demand for assimilate in the ear which was entirely due to changes on the abaxial surface, i.e. the surface contributing least to whole leaf photosynthesis. Both abaxial conductance and abaxial photosynthetic rate increased with increasing demand. The relationship inferred here between yield and abaxial conductance may be indicative of a similar response, high yielding lines having a greater demand for assimilate from their leaves. Shimshi and Ephrat (1975) and Fischer et al. (1981) observed positive correlations betweeen yield and leaf permeability measured by viscous-flow porometry. The output from such porometers is dominated by the surface having the lowest conductance i.e. the abaxial surface. Hence, a positive correlation between yield and abaxial conductance may also be inferred from their results.

The extent of variation about the regression lines relating yield to Δ is not surprising. Δ may vary as a result of variation in stomatal conductance, photosynthetic capacity, or possibly both as discussed above. Yield may also vary because of genotypic differences in allocation of assimilate to the roots or respiration. Another cause of the variation observed may have been the organ chosen for the measurement of Δ. The peduncle was chosen since it is an organ that grows near the time when yield potential is being established. It was also easy to sample uniformly from the dry matter subsample taken for harvest index. The close correlation observed between Δ measured on whole stems (minus leaf blades) from the pot experiment and Δ measured on the peduncle only from the field experiments indicates that the peduncle gives an adequate ranking for Δ, given the ease with which it can be sampled when plants are harvested at maturity. Data from subsequent experiments (Appendix 1), indicate that, under less favourable conditions than in these experiments, interactions may develop between Δ and seasonal water use. This suggests that genotypic ranking for Δ may be better estimated if organs are sampled at an earlier stage of growth.

2.5 CONCLUSIONS

The field studies presented here demonstrate that substantial genotypic variation in Δ exists in wheat and that, by virtue of the consistent ranking for Δ across environments, Δ appears to be under relatively strong genetic control.

It is apparent, however, that the use of Δ to screen for improved water-use efficiency must be weighed against the potentially large yield penalty associated with selecting lines with low values of Δ.

The slopes of the relationships between biological yield and Δ indicate that the relationships between water-use efficiency and Δ were probably poor, and possibly positive also. This may have been due to either a poor relationship between Δ and p_i/p_a, and hence leaf water-use efficiency, or to reduced stomatal control of gas exchange in field canopies.

The effects of "scale" are likely to be more important if variation in Δ is mainly due to variation in stomatal conductance. The results of this study indicate that there may also be a substantial component of genotypic variation in Δ that is due to variation in photosynthetic capacity.

In the next chapter, results of experiments which examine the relationship between Δ measured in dry matter and p_i/p_a measured in leaves are presented. The contributions of variation in conductance and variation in capacity to genotypic variation in Δ will also be examined.

CHAPTER 3

THE RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION AND THE RATIO OF INTERCELLULAR TO ATMOSPHERIC PARTIAL PRESSURES OF CARBON DIOXIDE

3.1 INTRODUCTION

The theoretical relationship between water-use efficiency and Δ arises indirectly from two independent relationships: that between Δ and the ratio of the intercellular to atmospheric partial pressures of CO_2, p_i/p_a; and that between water-use efficiency and p_i/p_a (Farquhar et al., 1982b; Farquhar and Richards, 1984).

The relationship between Δ and p_i/p_a is summarised in the following simplified expression (Equation 1.18), in which Δ is described as being linearly dependent on p_i/p_a.

$$\Delta = (4.4 + 22.6 p_i/p_a) \times 10^{-3} \quad , \qquad (3.1)$$

where the constants 4.4 and 22.6 (= 27 - 4.4) are associated with the kinetic isotope effects for discrimination during diffusion of CO_2 into the intercellular airspaces of the leaf (4.4) and during the carboxylation step itself (27). The value of p_i/p_a is determined by the balance between stomatal conductance and the photosynthetic capacity of leaves.

Correlations between Δ and p_i/p_a have been demonstrated in several studies in which p_i/p_a was caused to vary widely by imposing treatments which altered the gas-exchange characteristics of the species being examined, such as salt and water stress and variation in irradiance and vapour pressure deficit (Farquhar et al., 1982a; Bradford et al., 1983; Downton et al., 1985; Evans et al., 1986; Guy and Reid, 1986; Brugnoli et al., 1988).

As well as the processes summarised in Equation 3.1, there are numerous other potential sources of variation in Δ measured in plant dry matter. Variation in Δ of dry matter may potentially result from variation in the extent of fractionations occuring before, after, or in parallel with those associated with CO_2 fixation by the principal carboxylating enzyme, RuP_2 carboxylase. There may be variation in the partial pressure drop across the liquid phase resistance prior to CO_2 fixation (Evans et al., 1986) and variation in the proportion of carboxylations via PEP carboxylase (Farquhar and Richards, 1984). Fractionations associated with respiratory processes could alter measured Δ either by altering the isotopic composition of CO_2 outside (or inside) the leaf prior to its fixation (or refixation) (Medina and Minchin, 1980) or by changing the isotopic composition of carbon after its incorporation into dry matter. Fractionations may occur during the formation of photosynthetic products such as starch and soluble sugars (Brugnoli et al., 1988). Further fractionations may occur during the translocation of these products and the synthesis of dry matter constituents from them (O'Leary, 1981; Francey et al., 1985).

Any or all of these potential sources of variation in Δ measured in dry matter could be subject to intraspecific variation that may not be revealed by imposing treatments on single representatives of species.

This study was conducted to confirm that genotypic differences in Δ reflected genotypic differences in p_i/p_a. If this was found not to be the case, then genotypic differences in water-use efficiency would not necessarily follow from differences in Δ, even at the leaf level. The study also sought to examine the extent to which variation in stomatal conductance and/or photosynthetic capacity were contributing to any differences observed in p_i/p_a among the wheat genotypes tested.

3.2 MATERIALS AND METHODS

3.2.1 Plant material

The fourteen wheat genotypes used in these studies are listed in Table 3.1. They were selected on the basis of variation in Δ from a large range of wheat genotypes grown in the field at several locations (Chapter 2; Appendix 1), and included 12 hexaploid wheats (*Triticum aestivum* L.) and 2 tetraploid durum wheats (*T. turgidum* L. *durum*). These 14 genotypes made up the majority of 20 genotypes used in experiments described in Chapters 4 and 5, which examined the relationship between water-use efficiency and Δ for single plants and for field canopies.

Two sets of plants were used for these leaf gas-exchange studies. For the first experiment (Experiment I), plants were sown on 29 May in 3L pots containing a high-fertility, loam-based potting mix. Following emergence, plants were thinned to 3 per pot with 3 pots per genotype. All plants were well watered, twice-weekly initially and finally twice daily. Supplemental nutrients were applied during the stem-elongation phase.

For the second experiment (Experiment II), well-watered plants from a water-use efficiency study reported in Chapter 4 were used. Growth conditions for these plants are described briefly here and more fully in Chapter 4. Plants were sown on September 5 in 0.1 x 0.5m PVC tubes sealed at the base and containing a sandy river loam with supplemental nitrogenous fertilizer (1.5g of ammonium nitrate). The soil in the tubes was watered-up to 25% gravimetric soil water content before sowing. Plants were watered daily and transpired water was replaced so that soil water content never fell below 75% of the maximum. After emergence, plants were thinned to one per pot. Plants from 3 replicate blocks harvested at maturity (December 15) were used for the gas-exchange and Δ measurements reported here.

For both experiments, seeds of all genotypes were germinated on moist filter paper and then vernalised in the dark at 1-2°C for 40d to satisfy the vernalisation requirements of Quarrion and M3844. Plants were grown at Canberra in a well-ventilated glasshouse maintained at 20±2°C during the day and 8±2°C at night. Humidity was not controlled in the glasshouse. The daytime vapour pressure deficit was typically 1.2±0.2 kPa but nearer 0.8 kPa during wet weather.

3.2.2 Gas-exchange studies.

In Experiment I, gas-exchange measurements were made on 3 flag leaves of each genotype, one from each pot. All leaves were measured between 6 and 8d after ligule emergence, corresponding to the period when CO_2-assimilation rate should have been near maximal (Rawson et al., 1983). Flag leaves measured were either on mainstems or equally tall major tillers. All genotypes were measured within a 7d period in late August and early September, two genotypes being measured each day by using two chambers running concurrently.

Measurements were made using the open gas-exchange system described by Evans (1983). Briefly, the central part of the flag leaf was enclosed, adaxial surface up, in a double-sided leaf chamber (2.25 cm^2) which measured gas-exchange from each side of the leaf separately. CO_2-assimilation rate, A, leaf (stomatal plus cuticular) conductance, g_L, and p_i/p_a were measured first at an ambient CO_2 partial pressure, p_a, of 35 Pa and at 1000μmol quanta PAR m^{-2} s^{-1}, with air temperature 20°C and leaf-to-air vapour pressure difference 1.2 kPa. These conditions were chosen to duplicate those under which the plants were growing in the glasshouse. After obtaining several stable readings under these conditions (generally after approximately 1h), A was determined as a function of p_i, by first lowering p_a to 5 Pa and then raising it through 15, 25, 35, 45 and 70 Pa at a constant irradiance of 1000µmol quanta PAR m^{-2} s^{-1} (Appendix 3). As a measure of photosynthetic capacity (Caemmerer and Farquhar, 1984), the initial slope of the A:p_i curve for each leaf was obtained by linear regression of the data obtained at p_a levels of 5 and 15 Pa.

In Experiment II, gas-exchange of flag leaves was measured after ear emergence. All genotypes were measured on the one day (October 21) during a period of clear skies. Measurements were taken on two flag leaves from each plant using a portable gas-exchange system (Model LA-2, Analytical Development Corp., Hoddesdon, U.K.). Air temperature in the assimilation chamber was 26±2°C, vapour pressure deficit 1.5±0.3 kPa, and incident PAR 1700±200 µmol quanta m^{-2} s^{-1}. Leaf temperature (and hence conductance and p_i/p_a) was estimated using an energy budget subroutine.

3.2.3 Carbon isotope discrimination

For Experiment I, carbon isotope analysis was done on growing ears which had been enclosed in the flag leaf sheath during the gas-exchange measurements. Ears were removed from tillers 7d after gas-exchange measurements on the corresponding flag leaf, before they had emerged from the leaf sheath. For Experiment II, 3 large tillers from each plant, including the ears, were subsampled at maturity for Δ analysis. All plant samples were oven-dried at 80°C for 48h and finely ground. During grinding, the various dry matter components were mixed thoroughly to minimise variation in Δ due to further subsampling.

The genotypic ranking for Δ of glasshouse-grown plants was verified using dry matter sampled from an experiment in which all genotypes were grown together under well-watered conditions in the field (described fully in Chapter 5). For field-grown material, discrimination was measured in the whole of the tops of plants sampled at the 5-leaf stage and in flag leaves sampled at ear emergence. The field data presented are the means of data from these two harvests.

The stable carbon isotope composition of all dry matter samples was determined using the techniques and equipment described in Section 2.2.3.

3.3 RESULTS

3.3.1 Genotypic variation in Δ and p_i/p_a.

The 14 genotypes are listed in Table 3.1, from high to low Δ, on the basis of Δ values measured under well-watered conditions in the field sowing. The ranking of these values was similar to that anticipated on the basis of earlier measurements of field-grown material. The mean value of Δ in the field was 20.1×10^{-3} with a range of 1.8×10^{-3}. In both glasshouse studies, the range (1.8×10^{-3}) and mean values of Δ (20.0×10^{-3}) were similar to those in the field.

Values of Δ measured in the two glasshouse experiments were strongly correlated ($r = 0.81$, $P<0.01$; Fig. 3.1a). In general, values of Δ measured in the glasshouse corresponded well to those measured in the field (Fig. 3.1b), although the values of Δ measured in cv. Gutha grown in the glasshouse were substantially lower than had been expected on the basis of these (and earlier) field measurements. This was probably due to a failure to maintain the large Gutha plants adequately watered in the glasshouse. Symptoms of water stress, including wilting and senescence of lower leaves, were observed in the Gutha plants grown in the glasshouse experiments.

In both glasshouse experiments, there was significant variation among genotypes in p_i/p_a (Table 3.1). The range of p_i/p_a was similar in both experiments but was relatively small (0.07 in Experiment I, 0.08 in Experiment II). The mean values of p_i/p_a from each experiment were significantly different ($P<0.01$); 0.69 when using the laboratory-based system in Experiment I, 0.77 when measured using the portable system in Experiment II. There was no correlation between values of p_i/p_a measured in the two experiments.

Relationships between genotype means of Δ measured in dry matter and p_i/p_a measured in flag leaves are shown in Figure 3.2. The relationship from Experiment II was highly significant ($r = 0.78$, $P<0.01$; Fig. 3.2b), whereas that from Experiment I was significant only when the data point for cv. Gutha was excluded (Fig. 3.2a). Relationships between Δ and p_i/p_a for *individual* tiller data from Experiment I were highly significant ($r = 0.52$, $P<0.01$, $n = 42$, with tillers of Gutha included; $r = 0.64$, $P<0.01$, $n = 39$, with tillers of Gutha excluded).

Table 3.1. Summary of variation among 14 wheat genotypes in carbon isotope discrimination, Δ, measured in dry matter of plants grown in the field and in two glasshouse experiments. Flag leaf gas-exchange characteristics of glasshouse-grown plants are also shown. These include the ratio of intercellular to atmospheric partial pressures of CO_2, p_i/p_a, leaf conductance, g_L, and the initial slope of the $A:p_i$ curve, b. The genotypes are listed in an approximate ranking for Δ, from high to low, on the basis of Δ values measured before anthesis in field-grown material.

Genotype†	Field	Glasshouse Experiment I				Glasshouse Experiment II		
	$10^3 \times \Delta$ at anthesis	$10^3 \times \Delta$	p_i/p_a	g_L (mol m^{-2} s^{-1})	b (µmol m^{-2} s^{-1} Pa^{-1})	$10^3 \times \Delta$	p_i/p_a	g_L (mol m^{-2} s^{-1})
1. Yallaroi	21.0	20.8	0.70	0.43	1.37	20.7	0.78	1.04
2. Gutha	21.0	19.6	0.72	0.51	1.53	19.4	0.71	0.71
3. Cranbrook	20.5	20.3	0.69	0.48	1.81	20.2	0.80	1.11
4. Blade	20.4	20.1	0.67	0.51	1.86	20.5	0.79	1.11
5. Comet	20.3	19.8	0.70	0.53	1.60	20.4	0.75	0.75
6. 820357	20.2	19.8	0.67	0.39	1.39	20.1	0.80	0.80
7. Veery 3	20.1	21.0	0.72	0.55	1.66	20.9	0.80	1.09
8. K1056	20.0	19.8	0.69	0.46	1.60	19.8	0.75	0.86
9. Sunstar	19.9	20.4	0.68	0.56	2.06	20.5	0.78	1.13
12. RAC 430	19.8	19.9	0.71	0.50	1.70	20.3	0.79	1.26
13. Hartog	19.8	20.2	0.71	0.49	1.56	20.0	0.75	0.75
14. M3844	19.7	19.3	0.65	0.43	1.79	19.6	0.77	1.00
17. Sundor	19.5	19.7	0.67	0.46	1.79	19.3	0.73	0.73
20. Quarrion	19.2	19.5	0.68	0.48	1.68	19.1	0.72	0.59
$\bar{x}$	20.1	20.0	0.69	0.48	1.67	20.0	0.77	0.92
LSD (0.05)	0.5	0.8	0.03	0.07	0.33	0.6	0.05	0.35
CV (%)	1.8	2.4	2.8	8.6	12.0	1.9	9.5	55.3

†Yallaroi and 820357 are durum wheats (*Triticum turgidum* L. *durum*), the remaining 12 genotypes are bread wheats (*T. aestivum* L.). Genotype code corresponds to ranking of all 20 genotypes grown in the field experiment as given in Table 5.3.

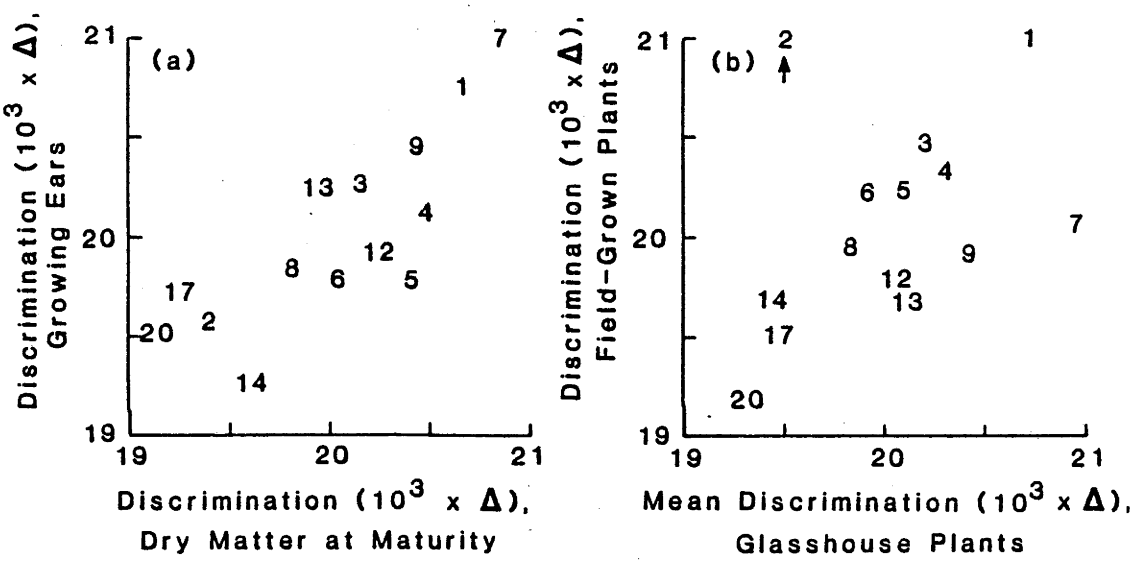


Fig. 3.1. (a) Relationship among 14 wheat genotypes between carbon isotope discrimination, Δ, measured in the growing ear in Experiment I and in total above-ground dry matter at maturity in Experiment II. $r = 0.81$, $P<0.01$.

(b) Carbon isotope discrimination, Δ, in dry matter of 14 wheat genotypes grown in the field in 1986 as a function of the mean Δ in dry matter of the same genotypes grown in two glasshouse experiments. $r = 0.42$, n.s. When arrowed data point for cv. Gutha excluded, $r = 0.71$, $P<0.01$.

Genotype code as in Table 3.1.

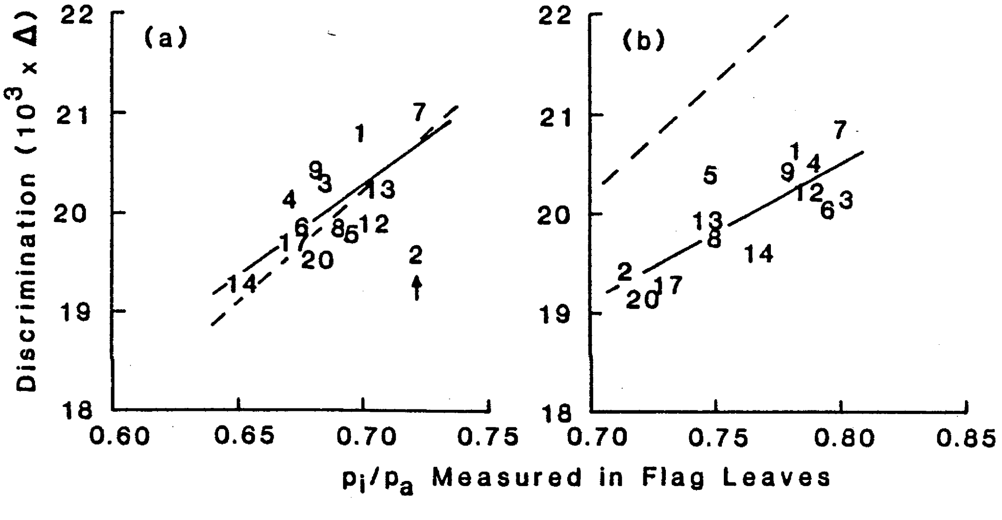


Fig. 3.2. (a) Relationship between Δ measured in growing ears and p_i/p_a measured in flag leaves of 14 wheat genotypes in Experiment I. Solid line, fitted regression excluding data point for cv. Gutha (arrowed). $Y = 18.61X + 7.26$; $r = 0.70$, $P<0.01$. Dashed line, Equation 3.1. Genotype code as in Table 3.1.

(b) Relationship between carbon isotope discrimination, Δ, measured in above-ground dry matter and the ratio of intercellular to atmospheric partial pressures of CO_2, p_i/p_a, measured in the flag leaves of 14 wheat genotypes in Experiment II. Solid line, fitted regression. $Y = 14.1X + 9.24$; $r = 0.78$, $P<0.01$. Dashed line, Equation 3.1. Genotype code as in Table 3.1.

3.3.2 Sources of variation in p_i/p_a.

The ratio p_i/p_a depends on the balance between leaf conductance, g_L, and photosynthetic capacity, here estimated as the initial slope, b, of the A:p_i relationship. There was substantial genotypic variation in g_L and b in Experiment I and in g_L in Experiment II (Table 3.1). The correlation between p_i/p_a and g_L was strong in Experiment II ($r = 0.90$, $P<0.01$; Fig 3.3b), but was non-significant in Experiment I ($r = 0.48$, $P<0.10$; Fig. 3.3a). Genotype means of g_L measured in the two experiments were not correlated. The relationship between genotype means of p_i/p_a and b from Experiment I was negative but nonsignificant ($r = -0.39$). The relationships between p_i/p_a and g_L and p_i/p_a and b for *individual* leaf data from Experiment I, rather than for genotype means, were both significant at the 1% level, with $r = 0.56$ and $r = -0.46$ respectively.

3.4 DISCUSSION

In these experiments, genotypic variation in Δ measured in dry matter was correlated with instantaneous measurements of p_i/p_a in leaves. Similar results have been obtained in experiments in which variation in p_i/p_a was due largely to physiological treatments (Winter, 1981; Farquhar et al., 1982a; Seemann and Critchley, 1985; Downton et al., 1985; Guy and Reid, 1986). The relationships observed here extend these observations to variation in p_i/p_a attributable to genotypic effects.

The relationships between Δ and p_i/p_a were positive but there was considerable variation about them. From the fitted regressions (Figs. 3.2a and 3.2b), variation in p_i/p_a accounted for, on average, 55% of the observed variation in Δ. Some of the variation about these relationships probably resulted from the experimental approach used, while some variation may have also been due to limitations of the simplified model represented by Equation 3.1.

One limitation of the experimental approach lay in seeking relationships between the short-term (Experiment I) or instantaneous (Experiment II) measurement of p_i/p_a in leaves and the value of Δ measured in plant dry matter harvested at a later date.

In Experiment II, there was a large difference in time-scale between the measurement of p_i/p_a in the flag leaf after ear emergence and the measurement of Δ in the whole of the above-ground dry matter at maturity. Further, the flag leaf is only one of many sources of carbon accumulated in the plant.

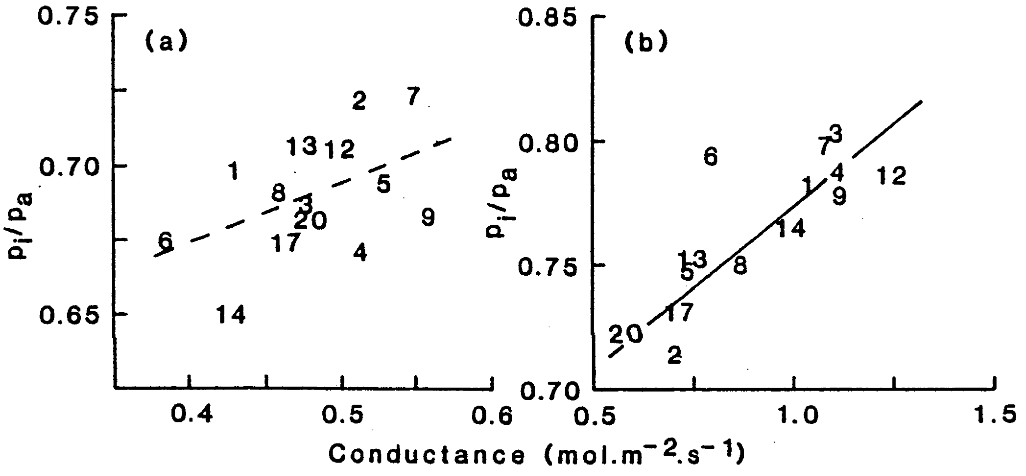


Fig. 3.3. Relationships between the ratio of intercellular to atmospheric partial pressures of CO_2, p_i/p_a, and conductance, for 14 wheat genotypes in (a) Experiment I ($r = 0.48$, $P<0.10$) and (b) Experiment II ($r = 0.90$, $P<0.01$). Genotype code as in Table 3.1.

Experiment I was aimed at linking the measurements of Δ in dry matter and p_i/p_a in leaves more directly. The growing ear/flag leaf system was selected for this purpose since the flag leaf has been shown to be the dominant source of carbon for the growing ear (Rawson and Hofstra, 1969). It was assumed that, being enclosed in the leaf sheath, the ear would have limited opportunity to fix carbon itself.

For the short-term (less than 1h) measurement of p_i/p_a in Experiment I, flag leaves were exposed to conditions approximating those to which they were exposed in the glasshouse. However, ear development was not coincident among the genotypes. Those genotypes developing earliest had p_i/p_a measured earliest and their ears harvested earliest. Temporal variation in environmental conditions over the period of ear growth, such as variation in incident radiation and vapour pressure deficit, would be expected to lead to temporal variation in p_i/p_a (Caemmerer and Farquhar, 1984; Morison and Gifford, 1983). For example, although bright sunshine prevailed for most of the experimental period (giving, on average, 18.7 MJ m^{-2} d^{-1} of solar radiation), the radiation receipt on one particular day was only 6.1 MJ m^{-2} d^{-1}.

It was anticipated that by allowing the ears to grow for several days, differences among genotypes in the effects of temporal environmental variation would be minimised. In the case of cv. Gutha the reverse may have been true. In the field under well-watered conditions, Δ measured in Gutha was similar to that in Yallaroi and Cranbrook (Table 3.1). The value of Δ measured in the ears of glasshouse-grown plants of Gutha was considerably lower than that expected (Fig. 3.1b), most probably because, in the glasshouse, the Gutha plants were subject to water deficit during ear growth due to inadequate watering. Reduced Δ in response to water stress is a common observation (Farquhar and Richards, 1984; Hubick and Farquhar, 1986; Hubick and Farquhar, 1989) and is associated with stomatal closure. Stomata of well-watered plants tend to be more sensitive to water deficits than plants that have previously encountered water stress (McCree, 1974). This is particularly so in glasshouse-grown plants (Davies, 1977).

It is difficult to assess the contribution of further discrimination (beyond that associated with photosynthesis) to the variation observed about the relationships between Δ and p_i/p_a. The extent of variation about the relationships between Δ and p_i/p_a observed here was relatively small compared to that observed in more direct experiments, which have considered discrimination associated with photosynthesis alone (Evans et al., 1986; Farquhar et al., 1988) or discrimination associated with the short-term formation of photosynthetic products such as starch and soluble sugars (Brugnoli et al., 1988). In those studies, physiological treatments were used to generate a considerably greater range in p_i/p_a and Δ than the range observed in the present study on genotypic variation.

The data obtained here differ in another important feature from those obtained in other studies. In the study by Evans et al. (1986) and that by Brugnoli et al. (1988), Δ measured at a given value of p_i/p_a was up to 4×10^{-3} lower than that expected on the basis of the simplified theory summarised by Equation 3.1. In Experiment I, the values of Δ measured in the growing ears fell very close to the relationship predicted from Equation 3.1 (Fig. 3.2a). Over the range of p_i/p_a observed in Experiment II there was an offset of about 1×10^{-3} (Fig. 3.2b). This was probably due to underestimation of leaf temperature, and hence overestimation of leaf conductance and p_i/p_a, by the portable gas-exchange system used. The model represented by Equation 3.1 was proposed partly because it fitted observations of Δ measured in dry matter (Farquhar et al., 1982b). These observations are supported by those from the present study.

Positive relationships between yield (as both total above-ground dry matter and grain yield) and Δ measured in stem material were observed under well-watered conditions in the field experiments reported in Chapter 2. It was proposed that such relationships could result from a positive association between Δ and stomatal conductance (and hence photosynthetic rate per unit leaf area) and/or a negative association between Δ and photosynthetic capacity (with high Δ lines having a relatively faster rate of leaf area development).

The present study revealed considerable variation among genotypes in both conductance and photosynthetic capacity. In Experiment II, g_L varied by more than 100% over the range of genotype means observed (Table 3.1). In Experiment I, the relative variation in g_L over the range of genotype means was 43%. The relative variation in b was 50% (Table 3.1). The extent of variation in both these parameters was similar to that found in other studies on genotypic variation in conductance (Nerkar et al., 1981; Shimshi and Ephrat, 1975) and photosynthetic capacity (Dunstone et al., 1973; Evans and Seemann, 1984).

The relative variation in p_i/p_a among genotypes (11% in both experiments) was considerably less than that in either b or g_L. There was a tendency for p_i/p_a to be higher when conductance was higher or when capacity was lower. However, the relative influence on p_i/p_a of conductance and capacity was confounded by the positive relationship between the two ($r = 0.54$, $P<0.05$, Fig. 3.4). Thus, in Experiment I, the relationship between genotype means of p_i/p_a with either g_L or b was not significant. Partial correlation coefficients for the relationships between p_i/p_a and g_L with b held constant ($r = 0.89$) and between p_i/p_a and b with g_L held constant ($r = -0.88$) were both highly significant, but opposite in sign. These results, as well as the similar extent of variation in g_L and b, indicate that variation in p_i/p_a among this set of genotypes was associated approximately equally with variation in conductance and with variation in photosynthetic capacity. A multiple regression analysis between p_i/p_a, g_L and b for all data collected in Experiment I yielded the following equation;

$$p_i/p_a = 0.651 + 0.308g_L - 0.066b \quad . \qquad (3.2)$$

Equation 3.2 accounted for 70% of the observed variation in p_i/p_a.

A positive association between g_L and b has been observed previously among different species (Körner et al., 1979; Werk et al., 1983) and among different physiological treatments within species (Wong et al., 1979). This association prompted the latter authors to suggest that stomata respond to variations in photosynthetic capacity in such a way as to maintain p_i approximately constant (at constant p_a). Certainly, and as has been observed frequently (Morison, 1987), p_i/p_a was a much more conservative parameter than either stomatal conductance or photosynthetic capacity.

While the variation in p_i/p_a appears relatively small, the relationships in Figure 3.1 indicate that these small differences are conserved over the time-scale in which carbon is laid down in dry matter. It seems likely, given the poor relationship between p_i/p_a measured in the two experiments, that short-term measurements of p_i/p_a by gas-exchange are more variable, and therefore less useful for applications that require long-term means.

In terms of plant water-use efficiency, it is more appropriate to consider variation in $(1 - p_i/p_a)$ than variation in p_i/p_a (Tanner and Sinclair, 1983; Farquhar and Richards, 1984). Relative variation in

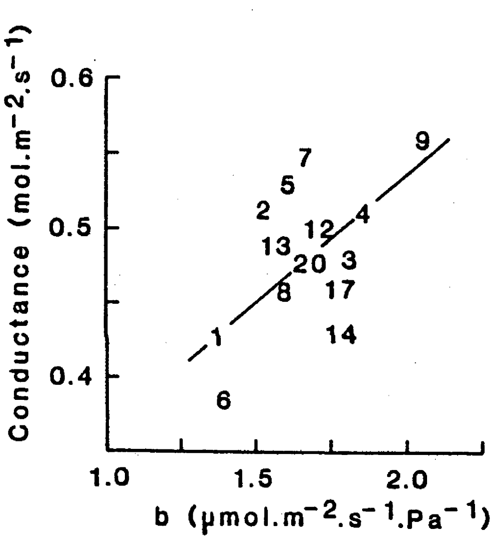


Fig. 3.4. Relationship between conductance and the initial slope, b, of the A:p_i curve for 14 wheat genotypes in Experiment I. ($r = 0.54$, $P<0.05$). Genotype code as in Table 3.1.

$(1 - p_i/p_a)$ was considerably greater than that in p_i/p_a (45% in Experiment I, 25% in Experiment II). At constant vapour pressure difference, variation in leaf transpiration efficiency would be expected to be similar to variation in $(1 - p_i/p_a)$, and this was observed. In Experiment II, in which the leaf-to-air vapour pressure difference was maintained constant at 1.2 kPa, leaf transpiration efficiency varied from 5.3 mmolCO_2/molH_2O to 6.7 mmolCO_2/molH_2O among these genotypes.

3.5 CONCLUSIONS

In these experiments, positive relationships between Δ and p_i/p_a were observed among genotypes selected on the basis of variation in Δ. This result indicates that genotypic variation in Δ may provide a reasonable estimate of genotypic variation in long-term mean p_i/p_a. Among the genotypes used here, variation in p_i/p_a was attributed, approximately equally, to variation in stomatal conductance and to variation in photosynthetic capacity.

While the relationships between Δ and p_i/p_a were positive, considerable variation in Δ (approximately 45%) was not explained by variation in p_i/p_a. This may have been due to short-term variation in p_i/p_a or to fractionations which contribute to variation in Δ measured in dry matter, but which may not be directly related to discrimination during photosynthesis.

To the extent that variation in Δ does not correspond to variation in p_i/p_a, there may also be considerable variation in Δ that does not correspond to variation in plant water-use efficiency, and vice versa.

The following chapter presents the results of experiments which examine the relationships between water-use efficiency and Δ in well-watered and water-stressed plants of these genotypes selected on the basis of variation in Δ.

CHAPTER 4

THE RELATIONSHIP BETWEEN CARBON ISOTOPE DISCRIMINATION AND WATER-USE EFFICIENCY AT THE SINGLE PLANT LEVEL

4.1 INTRODUCTION

The gas-exchange studies reported in Chapter 3 confirmed a positive relationship between Δ and p_i/p_a among wheat genotypes selected on the basis of variation in Δ. In the experiments on whole plants reported by Farquhar and Richards (1984), the major source of variation in water-use efficiency and Δ was water stress and differences in cultivar responses to it.

The aim of the experiments presented in this chapter was to determine the relationship between plant water-use efficiency and Δ using genotypes as the sole source of variation in Δ. The relationship was studied in both well-watered and terminally water-stressed plants of 16 wheat genotypes selected on the basis of variation in Δ. Emphasis was placed on well-watered plants since Δ measured under these conditions should largely represent constitutive genotypic variation in this trait.

4.2 MATERIALS AND METHODS

Sixteen genotypes, including 14 hexaploid wheats (*Triticum aestivum* L.) and 2 tetraploid durum wheats (*T. turgidum* L. *durum*), were used in these experiments. The genotypes used included the 14 used in the gas-exchange experiments described in Chapter 3, plus the genotypes Millewa and M3458. Cultural conditions for this study, described in part in Chapter 3, are presented more fully here.

Seed of all genotypes was germinated and vernalised as described in Section 3.2.1. Plants were sown on September 5 and were thinned to one plant per pot after emergence. The plants were grown at Canberra in a well-ventilated glasshouse maintained at 20±2°C during the day and 8±2°C at night. Humidity was not controlled in the glasshouse. The daytime vapour pressure deficit was typically 1.2±0.2 kPa but nearer 0.8 kPa during wet weather. There were six well-watered plants and four water-stressed plants of each genotype except 820357. Poor germination resulted in only three well-watered plants of this genotype.

For the study under well-watered conditions, plants were grown in PVC tubes 0.1 x 0.5 m, sealed at the base and containing a sandy river loam. The tubes were watered-up to 25% gravimetric soil water content prior to sowing, 1.5 g of ammonium nitrate (giving 200 kg $N.ha^{-1}$ on an area basis) being applied during watering up. For the study on water-stressed plants, sealed tubes 0.1 x 1.0 m were used. These tubes also contained the sandy river loam and were watered up to hold 2L of water. 0.75 g of ammonium nitrate was supplied to these tubes during watering up. All tubes were sealed at the soil surface with a layer of black polythene to prevent direct evaporation. A 1 cm layer of perlite was placed on top of the polythene in the long tubes.

All tubes were brought to starting weight after thinning. No further water was added to the long tubes, the plants being subjected to a gradually increasing terminal stress. The well-watered tubes were weighed regularly, initially every 4 d and then every 2 d after the start of stem elongation. Transpired water from these tubes was replaced so that soil water content was maintained above 75% of the maximum at all times. From the start of stem elongation, pots were watered daily (for some genotypes more frequently). For the well-watered plants, 4 tubes without plants were used to estimate direct evaporative losses. Two such dummy tubes were used for the water-stressed plants. Plants in the 0.5 m tubes were grown as spaced plants and were arranged in 6 replicate blocks, tubes in each block being rerandomised when weighed. The 1.0 m tubes were left in the same positions throughout the experiment and were arranged in a 4-replicate randomised block design.

Plants were harvested at two times during the experiment; at ear emergence and at maturity. Ear emergence was defined for the well-watered plants as when 3 ears were totally emerged and for the water-stressed plants, which had greatly reduced tiller number, as when the mainstem ear had fully emerged. Three replicates of the well-watered plants and two of the water-stressed plants were harvested at each time. The harvest at ear emergence took place over 7 d from November 4 to November 11, all replicate plants of a given genotype being harvested on the same day. Well-watered plants of 820357 were not harvested at ear emergence. The three replicate plants of this genotype were held over until the final harvest. At each harvest, plant tops were partitioned into dry matter components which were oven-dried at 80°C for 48 h prior

Corrigendum

Page 66.
The following sentence should be included at the end of paragraph 2.

"Statistical analyses (ANOVAR) showed no significant effect of harvest time or genotype x harvest interaction for either W* or Δ in water-stressed plants."

to weighing. Root weights were only determined on the well-watered plants harvested at ear emergence.

Plant water-use efficiency (W when calculated on a whole-plant basis, W^* when calculated for plant tops only) was determined from the weight of plant material harvested and the water use to the time of harvest. Plant water use to each harvest was corrected for direct evaporative losses by subtracting the average amount of water lost to that time from the tubes without plants. For the water-stressed plants, water use to the harvest at ear emergence was greater than 90% of the final water use for all genotypes (mean 95%). Since the difference in W^* and Δ measured at the two harvests was negligible, the data from both harvests were combined to determine genotype means.

Carbon isotope discrimination (Δ) was measured using the whole of the above-ground material from the water-stressed plants. For the well-watered plants, 3 large tillers, taken to be representative of the whole plant, were sampled from each plant at each harvest. All plant material was oven-dried at 80°C for 48 h and then finely ground. Carbon isotope composition was determined using the techniques and equipment described in Section 2.2.3.

4.3 RESULTS

4.3.1 Water-use efficiency and Δ.

There was substantial genotypic variation in water-use efficiency and Δ in both the well-watered and the water-stressed treatments (Table 4.1; Appendix 4).

In well-watered plants, the range and the mean value of Δ were lower at ear emergence than at maturity. In terminally water-stressed plants, the mean value of Δ was considerably lower than the average value measured in well-watered plants.

Genotypic variation in Δ of stressed plants was strongly correlated with Δ measured in well-watered plants at maturity ($r = 0.79$, $P<0.01$). However, correlations were poor between Δ measured in well-watered plants at ear emergence and Δ measured in either well-watered or water-stressed plants at maturity.

In each treatment and at each harvest, water-use efficiency varied by approximately 28% around the respective means (Table 4.1). In water-

Table 4.1. Summary of variation in carbon isotope discrimination (Δ) and plant water-use efficiency, on a whole-plant basis (W) and an above-ground dry matter basis (W^*), among wheat genotypes grown under well-watered and water-stressed conditions in the glasshouse.

	Well-watered Plants					Water-stressed Plants	
	Ear Emergence			Maturity			
	$10^3 \times \Delta$	W ($g.kg^{-1}$)	W^* ($g.kg^{-1}$)	$10^3 \times \Delta$	W^* ($g.kg^{-1}$)	$10^3 \times \Delta$	W^* ($g.kg^{-1}$)
[1] $\bar{x}$	19.53	5.25	4.41	20.01	4.65	16.11	5.55
LSD($P<0.05$)	0.85	0.52	0.35	0.64	0.33	0.70	0.38
Maximum	20.28	6.18	5.07	20.86	5.27	16.94	5.95
Minimum	18.92	4.86	3.99	19.13	4.06	15.27	4.69

[1] Genotype means of these data are given in Appendix 4, Tables A4.1, A4.2 and A4.3.

stressed plants, the mean value of W^* was approximately 23% greater than the average value of W^* in well-watered plants.

In well-watered plants, genotypic variation in water-use efficiency was negatively correlated with variation in Δ measured in dry matter, at both ear emergence and at maturity. This was so whether water-use efficiency was expressed on a whole-plant basis (measured at ear emergence only, with $r = -0.54$, $P<0.05$) or on an above-ground dry matter basis (measured at ear emergence (Fig. 4.1a; $r = -0.57$, $P<0.05$), and at maturity (Fig. 4.1b; $r = -0.74$, $P<0.01$)).

Water-use efficiency and Δ were also negatively correlated in plants subjected to terminal water stress. This was so whether W^* was plotted as a function of Δ measured in dry matter of water-stressed plants ($r = -0.75$, $P<0.01$; Fig. 4.2a) or in dry matter of well-watered plants ($r = -0.74$, $P<0.01$; Fig. 4.2b). Indeed, W^* under water stress was positively correlated with W^* under well-watered conditions ($r = 0.64$, $P<0.01$).

4.3.2 Water use and dry matter production.

At both harvests of the well-watered plants, there was significant genotypic variation in water use and dry matter production (Table 4.2). At maturity, water use to that time was positively correlated with Δ ($r = 0.54$, $P<0.05$; Fig. 4.3a). There was no relationship between above-ground dry matter and Δ or between grain yield and Δ. At ear emergence, plants were harvested on different dates. In these plants, there was no relationship between either water use, total dry matter or above-ground dry matter and Δ. Water use and above-ground dry matter production at ear emergence averaged, respectively, 44% and 42% of that at maturity.

In water-stressed plants, water use was constrained to be approximately equal for all genotypes (Table 4.2). Under these conditions, above-ground dry matter production by water-stressed plants was negatively correlated with Δ measured in dry matter of stressed plants ($r = -0.56$, $P<0.05$; Fig. 4.3b). The relationship with Δ measured in dry matter of well-watered plants ($r = -0.46$) was significant at the 10% level only. There was no relationship between grain yield or harvest index of stressed plants and Δ of either stressed or well-watered plants.

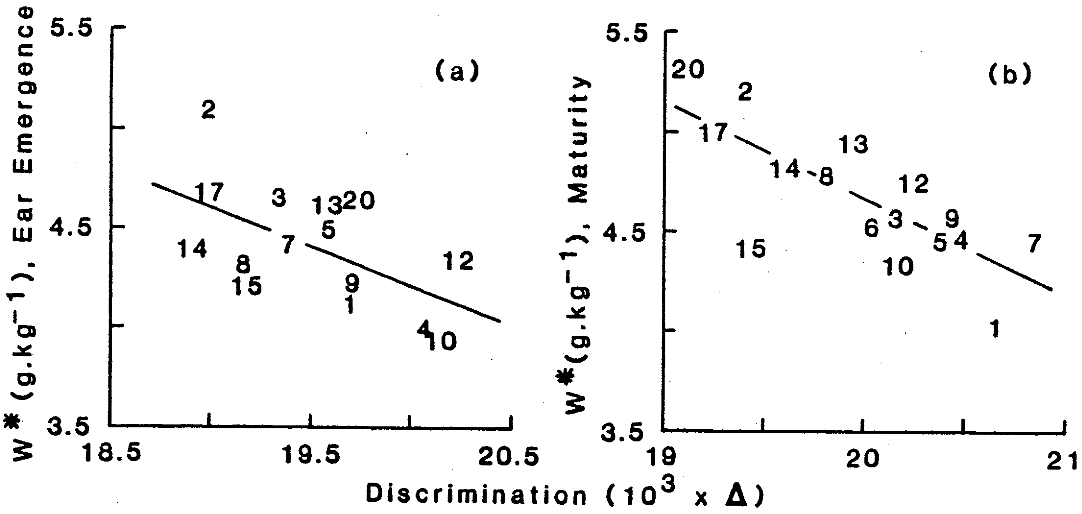


Figure 4.1. (a) The relationship at ear emergence between plant water-use efficiency measured on an above-ground dry matter basis, W^*, and carbon isotope discrimination, Δ, measured in dry matter of well-watered plants of 15 wheat genotypes. Solid line, fitted regression; $Y = -0.38X + 11.80$. ($r = -0.57$, $P<0.05$).

(b) The relationship at maturity between plant water-use efficiency measured on an above-ground dry matter basis, W^*, and carbon isotope discrimination, Δ, measured in dry matter of well-watered plants of 16 wheat genotypes. Solid line, fitted regression; $Y = -0.46X + 13.76$. ($r = -0.74$, $P<0.01$).

Genotype code : 1. Yallaroi; 2. Gutha; 3. Cranbrook; 4. Blade; 5. Comet; 6. 820357; 7. Veery 3; 8. K1056; 9. Sunstar; 10. M3458; 12. RAC 430; 13. Hartog; 14. M3844; 15. Millewa; 17. Sundor; 20. Quarrion.

(This code corresponds to that used for the same genotypes in Chapter 3 and Chapter 5.)

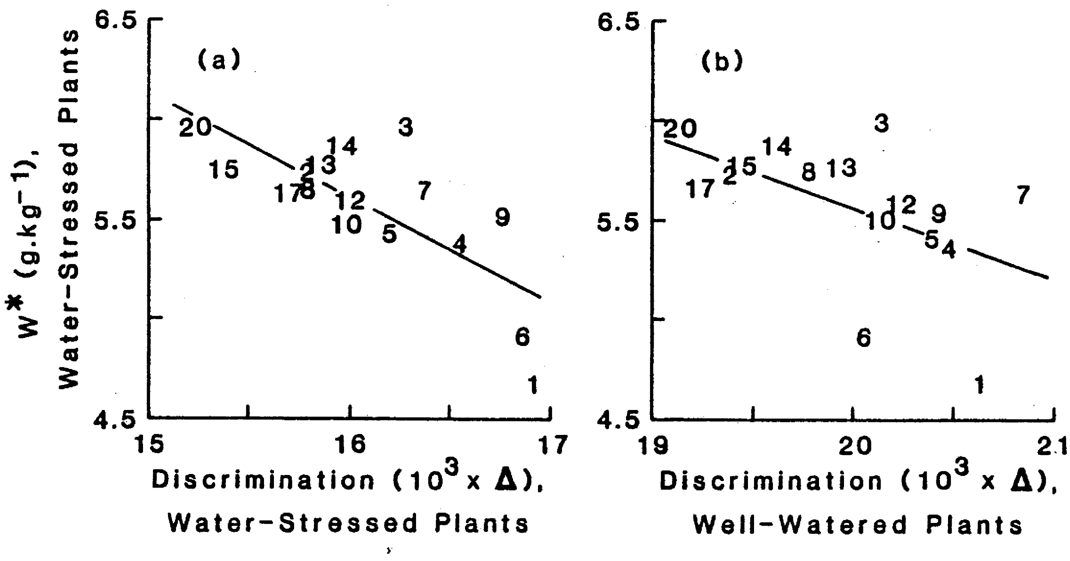


Figure 4.2. (a) The relationship between plant water-use efficiency measured on an above-ground dry matter basis, W^*, in water-stressed plants and carbon isotope discrimination, Δ, measured in dry matter of water-stressed plants of 16 wheat genotypes. Solid line, fitted regression; $Y = -0.51X + 13.76$. ($r = -0.75$, $P<0.01$). Genotype code as in Figure 4.1.

(b) The relationship between plant water-use efficiency measured on an above-ground dry matter basis, W^*, in water-stressed plants and carbon isotope discrimination, Δ, measured in dry matter of well-watered plants of 16 wheat genotypes. Solid line, fitted regression; $Y = -0.34X + 12.27$. ($r = -0.52$, $P<0.05$). Genotype code as in Figure 4.1.

Table 4.2. Summary of variation in water use (T) and dry matter production, on a whole-plant basis (TDM) and an above-ground dry matter basis (AGDM), among wheat genotypes grown under well-watered and water-stressed conditions in the glasshouse.

	Well-watered Plants					Water-stressed Plants	
	Ear Emergence			Maturity			
	T (kg)	TDM (g)	AGDM (g)	T (kg)	AGDM (g)	T (kg)	AGDM (g)
[1] $\bar{x}$	4.61	24.3	20.4	10.48	48.7	1.18	6.56
LSD(P<0.05)	0.58	4.5	3.2	0.78	5.4	0.11	0.69
Maximum	5.22	32.3	26.5	11.62	57.9	1.28	7.15
Minimum	3.88	20.1	16.8	8.97	42.8	1.07	5.57

---

[1] Genotype means of these data are given in Appendix 4, Tables A4.1, A4.2 and A4.3.

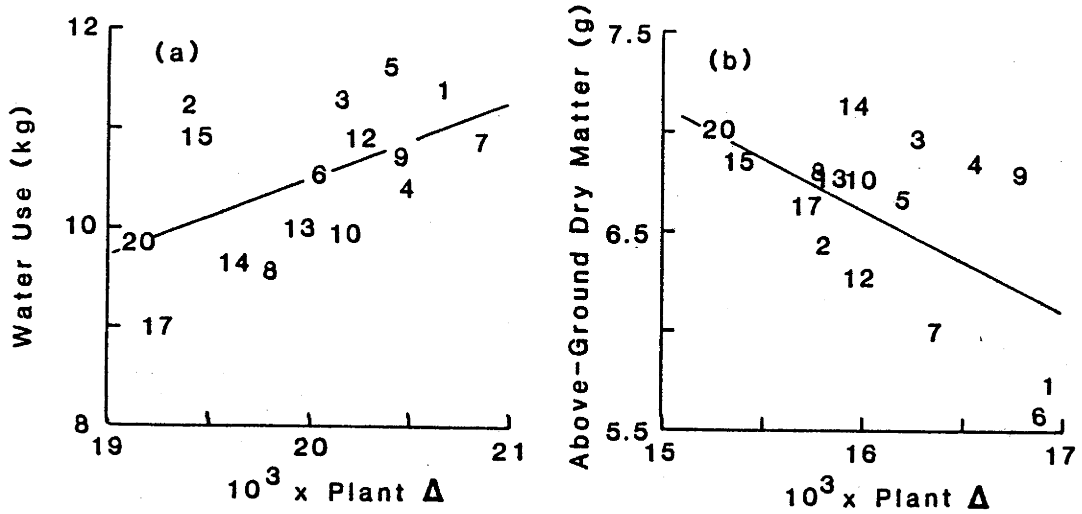


Figure 4.3. (a) The relationship at maturity between plant water use and carbon isotope discrimination, Δ, measured in dry matter of well-watered plants of 16 wheat genotypes. Solid line, fitted regression; $Y = 0.78X - 5.09$. ($r = 0.54$, $P<0.05$). Genotype code as in Figure 4.1.

(b) The relationship between above-ground dry matter production and carbon isotope discrimination, Δ, measured in dry matter of water-stressed plants of 16 wheat genotypes. Solid line, fitted regression; $Y = -0.52X + 14.97$. ($r = -0.56$, $P<0.05$). Genotype code as in Figure 4.1.

4.4 DISCUSSION

This study has demonstrated substantial variation in water-use efficiency in both well-watered and water-stressed plants of a range of wheat genotypes. Genotypic variation in water-use efficiency of wheat has been reported previously in experiments designed to examine the relationship between morphophysiological traits and yield under water stress (Passioura, 1977; Richards and Townley-Smith, 1987). Dry matter from the latter study was assessed for variation in Δ (Farquhar and Richards, 1984). The measurements confirmed the negative correlation between water-use efficiency and Δ predicted by theory (Farquhar et al., 1982b).

In this study, the genotypes used were selected on the basis of variation in Δ. Water-use efficiency and Δ were again negatively correlated, indicating that assessment of genotypic variation in Δ may provide an effective, indirect means of assessing genotypic variation in water-use efficiency at the single plant level. Moreover, the relationship obtained between W^* under terminal stress and Δ measured in dry matter of well-watered plants, indicates that selection for improved water-use efficiency under severe water limitation should be possible based on Δ measured in well-watered plants. This offers considerable advantages over other physiological criteria which have been proposed for enhancing performance in water-limited environments, such as osmotic adjustment (Morgan, 1983), stomatal sensitivity (Jones, 1983), leaf rolling (O'Toole, 1982), or glycine-betaine accumulation (Hanson and Hitz, 1982), but which require the imposition of water stress for their expression or measurement. Hubick and Farquhar (1989) observed that barley genotypes also maintained their ranking for W and Δ when tested over a range of environments.

The regressions fitted to the relationships between water-use efficiency and Δ indicate that variation in Δ accounted for between approximately 30% and 55% of the observed variation in water-use efficiency. These values are not high, but are comparable to values for many other traits used in screening for other purposes such as improved quality (Orth et al., 1972).

A considerable amount of the unexplained variation in water-use efficiency could be due to variation about the relationship between Δ and p_i/p_a examined in Chapter 3. In those experiments, variation in p_i/p_a accounted for, on average, 55% of the observed variation in Δ.

Plant water-use efficiency may also vary among genotypes or treatments because of variation in factors other than those leading to changes in p_i/p_a. There may be variation in W^* because of differences in the proportion of carbon allocated to the roots. Leaf temperature (and hence leaf-to-air vapour pressure difference) may vary directly as a result of variation among genotypes in, for instance, the presence or absence of a reflective waxy coating on leaves (Richards et al., 1986) or, indirectly, via effects on sensible heat exchange, through variation in stomatal conductance (Jones, 1983). There may also be variation in the proportion of carbon that is fixed by the leaves but lost through respiratory processes in the leaves (Wilson, 1984) or the rest of the plant (Stahl and McCree, 1988).

Increased root/shoot ratio and higher leaf temperature are probably more important in explaining the relatively low values of W^* of the water-stressed plants in this study. At the mean value of Δ for water-stressed plants (16.1×10^{-3}), the corresponding value of W^* calculated from the regression fitted to the relationship between W^* and Δ in stressed plants is 5.56 $g.kg^{-1}$ (Fig. 4.2a). This value is well below that expected from extrapolation of the regression fitted to the data on W^* and Δ for well-watered plants at maturity, 6.43 $g.kg^{-1}$ (Fig. 4.1b).

Gas-exchange studies on plants of 14 of the 16 genotypes used here (Chapter 3) indicated that variation in both stomatal conductance and in photosynthetic capacity contributed to genotypic variation in Δ. The extent to which variation in conductance (and to a lesser extent, capacity) will be reflected in variation in water-use efficiency is dependent on the scale of interest (Cowan and Troughton, 1971; Jones, 1983; Jarvis and McNaughton, 1986; Cowan, 1988). Jarvis and McNaughton (1986) have proposed a "decoupling factor", Ω, to account for the degree to which evaporation from plant canopies is decoupled from changes in leaf conductance as a result of changes in the boundary layer conductance. For wheat crops growing under certain conditions, they estimate a value for Ω of approximately 0.6. For spaced plants growing in a well-ventilated glasshouse, the response of water-use efficiency to a change in stomatal conductance will also be reduced, but by a factor somewhat less than 0.6. In these experiments, the regressions fitted to the relationships between W (and W^*) and Δ in well-watered plants indicate that for a decrease in Δ from, say, 21×10^{-3} to 19×10^{-3}, water-use efficiency increased by $21 \pm 1\%$. The theory relating Δ to transpiration efficiency of single leaves in well-stirred, rapidly flushed gas-exchange cuvettes (i.e. with negligible boundary layer resistance and constant leaf-to-air vapour pressure difference) indicates that, for the same change in Δ, transpiration efficiency should increase by 33%. This suggests an "effective" value for Ω of about 0.35 under these growing conditions. This value may underestimate boundary layer effects on transpiration, since the calculation also incorporates the effects on water-use efficiency of variation in photosynthetic capacity, which was not constant among these genotypes (Table 3.1).

As Farquhar and Richards (1984) point out, assessment of variation in water-use efficiency using Δ provides no information on the extent of variation in either component of the ratio that makes up water-use efficiency, i.e. dry matter production or water use. In this study, there was no relationship observed between dry matter production and Δ under well-watered conditions. This result differs from the positive relationships observed in Chapter 2 between dry matter production and Δ in wheat grown under well-watered conditions in the field. Under the high fertility conditions of this glasshouse study, a large leaf area was maintained for several weeks either side of ear emergence. At ear emergence, leaf area averaged 0.19 m^2 per plant. This corresponded to a leaf area index of 24 on a pot area basis and approximately 7 in terms of the bench area occupied by each pot. Under these conditions, both self-shading and mutual shading may have largely eliminated any potential dry matter advantage of genotypes with high values of Δ associated with greater stomatal conductance or more rapid leaf area development (Austin, 1982; Gifford and Jenkins, 1982).

The very large leaf area per plant may have also resulted in inadequate watering of some genotypes during the period around ear emergence, when leaf area was maximal. The mean Δ value and the range of Δ values measured at ear emergence were lower than at maturity. This was largely due to lower than expected values of Δ in those genotypes with typically high Δ values (Fig. 4.1; Appendix 4, Table A4.1). As well as causing a relatively lower Δ value in these lines, transient water deficit around ear emergence may have reduced actual dry matter production during this phase of growth. Potential dry matter production during the period after anthesis, when watering appears to have been sufficient to restore genotypic ranking for Δ, may have also been reduced, via effects on both source and sink size (Fischer, 1980).

The effect of transient water deficit was particularly marked in cv Gutha, which, at ear emergence, had used up to 40% more water at the time the first set of genotypes were harvested (November 1) than several of the other genotypes harvested later (Table 4.2; Appendix 4, Table A4.1). In Gutha, measured values of Δ were substantially lower than expected at both ear emergence and at maturity (Fig. 4.1). Gutha also ranked substantially lower than expected in the water-stress treatment (Fig. 4.2). Since all genotypes grew on a similar, limited water supply in the water-stress treatment, this latter observation may indicate relatively greater stomatal sensitivity to reduced soil water availability in Gutha compared to the other genotypes used in this study. Under some circumstances, greater stomatal sensitivity to soil water deficit may be a more desirable trait than either stomatal insensitivity or a constitutively low stomatal conductance (Ludlow, 1980; Jones, 1983; McCree and Richardson, 1987). Certainly, water-use efficiency was substantially higher in Gutha in both the well-watered and terminal water stress treatments than would have been expected on the basis of Δ measured in the field. Changes in genotypic ranking for Δ under contrasting watering regimes may be an effective means of detecting variation among genotypes in stomatal sensitivity.

4.5 CONCLUSIONS

This study has demonstrated substantial genotypic variation in plant water-use efficiency among a range of conventional Australian wheat genotypes selected on the basis of variation in Δ.

Genotypic variation in water-use efficiency was negatively correlated with Δ under both well-watered and water-stressed conditions. Water-use efficiency under water-stressed conditions was also negatively correlated with Δ measured in well-watered plants.

Relationships between water-use efficiency and Δ were reasonably strong, variation in Δ accounting for between approximately 30% and 55% of the observed variation in water-use efficiency. These values are comparable to those of other traits used in screening for other purposes in breeding programmes.

These results suggest that selection for low Δ may prove useful for improving plant water-use efficiency in wheat under both well-watered and water-stressed conditions.

For such selection to prove worthwhile, it must be shown to lead to improved water-use efficiency in the field. Even in these experiments, the response of plant water-use efficiency to a change in Δ, though relatively large, was approximately 35% less than the change in leaf transpiration efficiency that would be expected under conditions of negligible boundary layer resistance and constant leaf-to-air vapour pressure difference.

This effect, attributed largely to the increased influence of boundary layer resistance on transpirational water loss, is likely to be greater in the field. In the next chapter, results of experiments which examine the relationship between water-use efficiency and Δ for a range of wheat genotypes grown in field canopies, are presented.

CHAPTER 5

CARBON ISOTOPE DISCRIMINATION AND WATER-USE EFFICIENCY IN FIELD CANOPIES.

5.1 INTRODUCTION

If carbon isotope discrimination is to be used as a selection criterion for improved water-use efficiency, it must be shown that such selection leads to enhanced water-use efficiency in the field.

While the experiments reported in Chapter 2 demonstrated substantial and encouraging variation in Δ among conventional wheat genotypes, under the well-watered conditions in which the plants were grown, strong positive relationships were observed between above-ground dry matter production and Δ. This result indicated that under the well-watered conditions of those studies, relationships between crop water-use efficiency and Δ were probably weak (and possibly positive also).

A poor relationship between water-use efficiency and Δ may result from either a poor relationship between Δ and the ratio p_i/p_a (and hence leaf transpiration efficiency) or a poor relationship between leaf transpiration efficiency and crop water-use efficiency.

Discrimination and p_i/p_a were positively correlated in experiments reported in Chapter 3 and strong negative correlations between water-use efficiency and Δ were confirmed at the single plant level in experiments on both well-watered and water-stressed plants reported in Chapter 4.

However, it was noted that even in "scaling up" from leaf gas-exchange to water-use efficiency of single plants, the change in water-use efficiency per unit change in Δ was approximately 35% less than the change in leaf transpiration efficiency that would be expected under conditions of constant leaf-to-air vapour pressure difference. This result indicates that the problem of "scale" (Jarvis and McNaughton, 1986; Cowan, 1988) may become even more important at the crop canopy level.

In this chapter we return to the field to investigate the relationship between water-use efficiency and Δ using genotypes studied in Chapters 3 and 4, i.e. genotypes for which we have confirmed negative relationships between water-use efficiency and Δ at both the leaf and plant levels.

Experiments were conducted over two seasons. In 1985, eight genotypes spanning a range in Δ of approximately 2×10^{-3} were studied in a detailed investigation of seasonal patterns of water use and dry matter production. In 1986, twenty genotypes were grown, including those grown the previous season. Dry matter production was studied in all genotypes. Water use was monitored in four genotypes spanning the range of Δ.

The major aim of these experiments was to determine the relationship between water-use efficiency and Δ for wheat genotypes selected on the basis of variation in Δ and grown in field plots under a mediterranean environment. The experiments also aimed to determine the influence on water use and dry matter production of factors associated with genotypic variation in Δ, in particular stomatal conductance, photosynthetic capacity and canopy development. Each of these factors had been proposed as contributing to the positive relationships between dry matter production and Δ observed in 1984.

5.2 MATERIALS AND METHODS

5.2.1 Plant material

The genotypes grown in 1985 and 1986 are listed in Tables 5.2 and 5.3 respectively. The 8 genotypes grown in 1985 were a subset of those grown in 1986. Of the 20 genotypes grown in 1986, 16 were grown in the glasshouse experiments described in Chapter 4. All 20 genotypes were either recently released Australian cultivars or advanced lines from Australian breeding programmes. Details of their pedigrees and background are given in Appendix 5.1. Two of the genotypes, Yallaroi and 820357, are durum wheats (*Triticum. turgidum* L. *durum*. The remaining 18 are bread wheats (*T. aestivum* L.). All of the genotypes except Gutha would be classed as medium height, semi-dwarf wheats in eastern Australia. Gutha is a tall wheat.

5.2.2 Cultural conditions

The experiments were conducted at Moombooldool (146°35′E, 34°18′S), 300 km west of Canberra in south-west New South Wales, in the western half of the wheat belt (long-term April to November precipitation approximately 305 mm). Both experiments were conducted in 1 km square paddocks, which were totally sown to wheat. The sites were flat and the soil-type was similar in both years; a solonized, brown mallee soil (Stephens, 1962) with no sharp texture changes. The site used in 1985 had been under legume pasture for the previous four years until October

1984, at which time it was cultivated and left fallow. For the 1986 study, the site had been under mixed grass/legume pasture for the previous two years and was cultivated in December 1985 and left fallow until sowing.

The experiments were sown as 4-replicate, randomised block designs on 22 May 1985 and 26 May 1986. In both years, plots were 15 m x 8 rows with a 0.17 m row spacing and were sown in an east-west direction. Seeding rate averaged 140 seeds.m^{-2} for all genotypes. Phosphorus in 1985 (at 1.4 g.m^{-2} P) and phosphorus and nitrogen in 1986 (at 2.0 g.m^{-2} P, 1.1 g.m^{-2} N), were applied at sowing. Emergence was approximately 10 d after sowing in both years. Herbicides applied in July 1985 (Hoegrass® and Brominil®) and in August 1986 (Hoegrass® and Homix®), gave effective weed control. Mean anthesis date was 6 October in both years (138 d and 134 d after sowing in 1985 and 1986, respectively). Plots were machine harvested on 12 December 1985 and 26 November 1986.

5.2.3 Meteorological data

An automatic weather station was located adjacent to the plots in both years. Daily wind run, maximum and minimum temperatures, maximum and minimum relative humidity, solar radiation, precipitation, and Class A pan evaporation were monitored. Data from both seasons are summarised in Table 5.1.

5.2.4 Crop growth measurements

In 1985, at approximately 21 d intervals commencing on 10 July (by which time the third leaf was fully emerged), quadrats were harvested from each plot at the soil surface to estimate above-ground dry matter production, green leaf area and the manner in which the dry matter was partitioned. The quadrats were 0.30 m x 4 rows, the inside 4 rows only being sampled. There were eight hand harvests through the season. At the final machine harvest, a quadrat the same size as those taken earlier was sampled for estimation of harvest index. The machine harvested area consisted of 6 rows x 6 m of uninterrupted plot length, the outside rows having been removed prior to harvesting. The machine harvest yields were corrected for grain loss from shattering using the mean grain number per square metre calculated from the harvest index sample and the quadrat taken at physiological maturity on 21 November.

Table 5.1. Summary of meteorological data collected for the 1985 and 1986 growing seasons at Moombooldool.

		June	July	Aug	Sep	Oct	Nov	Dec
[1] Precipitation (mm)	1985	28.2	8.7	57.3	32.9	39.2	93.6	50.2
	1986	12.0	83.1	84.8	28.3	42.8	22.3	-
	[2] Long-term mean	37.0	34.5	39.5	33.5	46.5	35.0	32.5
	Long-term median	33.5	32.5	38.0	26.5	36.5	26.5	25.0
Number of Rain days	1985	7	4	10	6	8	13	5
	1986	4	11	8	5	6	6	-
	Long-term mean	8	9	9	7	8	6	5
[3] Maximum Temperature	1985	-	14.6	15.0	18.1	23.4	27.5	29.5
	1986	-	12.9	15.4	18.0	20.4	27.0	-
	Long-term mean	15.5	14.8	16.4	19.4	23.6	26.9	30.7
[3] Minimum Temperature	1985	-	2.5	3.8	3.0	8.4	12.1	14.6
	1986	-	3.2	3.4	5.7	5.7	10.2	-
	Long-term mean	3.5	2.8	4.0	6.0	9.5	11.5	14.5
[3] Pan evaporation ($mm.d^{-1}$)	1985	-	1.2	2.2	2.8	4.4	7.1	-
	1986	-	1.5	2.1	3.0	3.7	7.0	-
[3] Solar radiation ($MJm^{-2}.d^{-1}$)	1985	-	10.5	11.2	17.4	20.5	24.1	23.5
	1986	-	8.0	-	-	19.1	25.2	-

---

[1] May precipitation 39.3 mm in 1985 and 48.8 mm in 1986. May to October totals, 201 mm in 1985 and 300 mm in 1986.

[2] Long-term means are averages of data from Ardlethan, 22km east, and Yenda, 40 km west of site.

[3] Monthly average

Dry matter cuts were taken on four dates during 1986 : during tillering (August 13), at ear emergence (September 23), after anthesis (October 16) and at maturity (November 21). The sample taken at maturity was used to estimate harvest index. Two 0.30 m x 4 row quadrats were taken from each plot at each harvest. On July 31, ten plants were pulled from all plots in two of the four replicate blocks. These plants were divided into above- and below-ground parts (the latter not including roots). The machine harvested area was the same in 1986 as in 1985.

The weights of all dry matter components from hand harvests in both years were measured after oven drying at 80°C for 48 h. For both studies, dry matter at anthesis was calculated from linear interpolation of data obtained at harvests either side.

5.2.5 Radiation interception

At each sampling date in 1985, interception of radiant energy by the crop canopies was estimated for each plot by placing a 1 m line quantum sensor (Licor Model Li-191SB) horizontally on the ground beneath the canopy perpendicular to the row direction. Two measurements were taken from each plot at each sampling time. Estimates of incident radiation were obtained at regular intervals during the measurement of canopy interception by placing the line sensor on bare ground adjacent to the plots. All measurements were taken in bright sunshine between 1000 h and 1400 h. From the bare soil and crop canopy measurements, the proportion of incident radiation intercepted by the canopy, α, was determined for each plot at each sampling date.

In 1986, measurements of α were obtained on July 31, August 13 and October 2 using the same technique as in 1985. Persistent broken cloud disrupted measurements on other dates.

5.2.6 Soil water measurements

Water use during the season was monitored using the neutron attenuation technique. In 1985, two neutron moisture meter access tubes were installed in each plot during June. One of the tubes extended beyond 2 m below the soil surface, the other to 1.3 m. Soil water readings were taken at several dates through the season corresponding to the dates of dry matter harvests starting on July 10, 48 d after sowing. Readings of 1 min duration were taken at depths of 0.15, 0.25 and 0.35 m and then every 0.2 m to a depth of 1.95 m in the long tubes and 1.15 m in the short tubes. The neutron moisture meters (Troxler Model 3222) were field-calibrated using holes augered at the start and end of the season. Volumetric soil moisture content, θ_v, was determined for each depth using a single calibration curve for all depths. Moisture in the top 0.10 m was determined from soil samples, two per plot, taken using volumetric samplers each time soil moisture content was measured. The soil samples were oven dried at 105°C for 48 h for moisture content determination. The soil water content to 2.0 m was calculated by multiplying θ_v determined at each sampling depth by the appropriate sampling interval, to give soil moisture content in mm of water per sampling interval, and then summing over the profile. Changes in the soil water content at each depth over the course of the season are summarised for each genotype in Appendix 5.2.

In 1986, crop water use was measured on four genotypes spanning the range of Δ anticipated : Cranbrook, Sunstar, Millewa and Quarrion. There was only one access tube per plot in 1986 and tubes were read to a depth of 1.35 m only. Soil water measurements were first taken on August 13 and then at each subsequent harvest.

5.2.7 Crop water use

Crop evapotranspiration, ET, for the periods between soil water measurements, was calculated using the moisture budget equation :

$$ET = P - S - R - D \qquad (5.1),$$

where P is the precipitation during a given period, S is the change in total moisture in the profile, R is surface runoff and D is drainage beyond the depth of measurement. There was no evidence that either runoff or drainage occurred in 1985 and these were taken to be zero in that year. There was some evidence of possible water extraction beyond the 2.0 m depth of sampling (Appendix 5.2). It was considered that any such extraction would have been negligible since the initial water content of the soil beyond 2.0 m was extremely low. In 1986 there appeared to be some drainage beyond 1.35 m, the depth of measurement in most plots, and also beyond 2.0 m in some of the remaining plots in which longer tubes had been located. The data obtained in 1986 are unlikely to give an accurate measure of crop water use.

Evaporation from uncropped bare soil, E_s, was also measured during the 1985 study using the same technique used for ET measurements. Four access tubes were located to a depth of 1.5 m in bare ground immediately adjacent to the border plots surrounding the experiment. E_s was also computed according to Equation 5.1.

In 1985, soil water content was first measured for all plots 48 d after sowing, when the third leaf was fully emerged. ET was estimated for the intervening period as being 31 mm, and was assumed to be the same for all genotypes and the bare soil. This amount is equivalent to ET accumulated at a rate of 0.65 $mm.d^{-1}$, which was the mean rate of water loss from bare soil during the period in mid-July from the first to second soil water readings.

For the 1985 study, crop ET was partitioned into components of soil evaporation, E, and transpiration, T, using a model based on radiation penetration to the soil surface under crop canopies and evaporation from bare soil (Cooper et al., 1983). The model and the procedure used to partition ET are described more fully in Appendix 5.3. This procedure was not applied to the water use data obtained in 1986. Soil water and radiation interception measurements were curtailed by persistent rainfall in the early part of that year.

5.2.8 Water-use efficiency

Crop water-use efficiency for above-ground dry matter production was calculated for the 1985 study based on ET, W^*_{ET}, and on T, W^*_T. Water-use efficiency was calculated as the ratio of dry matter produced to water used over periods from sowing to maturity, sowing to anthesis, and anthesis to maturity. For the 1986 study, estimates of W^*_{ET} were calculated for the period from August 13 to maturity.

5.2.9 Stomatal conductance measurements

On several dates during 1985, stomatal conductance data were obtained on all eight genotypes using a Delta-T Mk III diffusive resistance porometer. Data were obtained on uppermost fully expanded leaves only, between 1000 h and 1500 h (depending on cloud cover), on days with extended periods of bright sunshine and clear sky or (on one occasion) of uniform overcast. Measurements were taken on both the adaxial and abaxial surface for all genotypes but only on surfaces facing upwards. For each genotype, the mean conductance for each surface was calculated and these were summed to give a leaf conductance, g_L. Genotypic variation is summarised below using the mean values of g_L from the sampling dates before anthesis. Genotypic and seasonal variation in conductance during 1985 is considered in more detail in Appendix 1.

In 1986, variation in canopy temperature was used as an index of variation in conductance. Measurements were taken on all plots using an infrared thermometer (Everest Interscience, Model 210) on three dates after canopy closure (September 23, October 2 and October 16).

5.2.10 Carbon isotope discrimination

During the 1985 season, several plant organs were separated for measurement of carbon isotope discrimination, Δ. For each of the harvests before anthesis, a subsample of the last fully expanded leaves of each genotype was taken. These corresponded to, approximately, leaves 3.5, 5, 6.5, 8 and 9 (the flag leaf). Stem material from the third harvest was also measured. From the final hand harvest, the peduncle (i.e. stem material from above the flag leaf node), grain and rachis were measured. All plant organs were measured for Δ using the techniques and equipment described in Section 2.2.3. Single measurements only were made on each sample. Data presented are the means of the four replicate plots of each genotype. These measurements are presented comprehensively in Appendix 1. They are summarised below by calculating values for mean leaf Δ, plant Δ at anthesis and plant Δ at maturity.

Mean leaf Δ was calculated as the arithmetic mean of Δ values measured in the leaves sampled from each plot. Plant Δ at anthesis was calculated as the weighted average of the mean leaf Δ value and a stem Δ value (the average of the August 13 stem and maturity rachis measurements). The leaf and stem Δ values were weighted using the data on dry matter partitioning obtained on September 25. Plant Δ at maturity was obtained using weighted averages for leaf, stem and grain Δ. Values were weighted according to the data on dry matter partitioning obtained on November 21.

For the 1986 study, Δ was measured on the whole of the tops of plants from two replicate blocks sampled on July 31, on flag leaves subsampled from all replicates of the harvest on September 23, and on grain subsampled from all replicates of the harvest on November 21. A value of pre-anthesis plant Δ was calculated as the mean of the Δ values measured on the plant tops and flag leaves. A value of plant Δ at maturity was calculated as the mean of the calculated pre-anthesis Δ and the measured grain Δ.

5.3 RESULTS

5.3.1 Variation in Δ.

There was significant and substantial genotypic variation in Δ in both 1985 (Table 5.2) and 1986 (Table 5.3). Ranking for Δ among the eight genotypes common to the two experiments was similar in both seasons, at anthesis ($r = 0.89$, $P<0.01$) and at maturity ($r = 0.97$, $P<0.01$). The range of Δ values was also similar in both seasons (approximately 1.8×10^{-3} at anthesis and 1.5×10^{-3} at maturity) but mean Δ values were lower in 1985.

The lower Δ values in 1985 reflected the lower seasonal precipitation in 1985 compared to 1986 (Table 5.1). In 1985, the May-October total was 201 mm, and in 1986, 300 mm. The mean Δ value measured in peduncle material in 1985 (16.7×10^{-3}) was also substantially lower than that measured in 1984 at both Wagga Wagga (17.8×10^{-3}) and Yanco (18.3×10^{-3}). Rainfall at the 1984 sites was similar to that at Moombooldool in 1986 (Table 2.1).

Interactions between Δ and water supply on a seasonal basis are considered in greater detail for the 1985 season in Appendix 1.

5.3.2 Sources of genotypic variation in Δ.

In the 1985 experiment, with the exception of cv Hartog, sources of genotypic variation in Δ appeared to closely reflect those observed in the gas-exchange studies reported in Chapter 3 (Table 3.1). The data summarised in Figure 5.1 indicate that, among the semi-dwarf bread wheats, variation in Δ was mainly due to variation in stomatal conductance ($r = 0.89$, $P<0.05$). For all eight genotypes (i.e. including the tall wheat, Gutha, and the two durum wheats), the relationship between Δ and g_L was nonsignificant.

For a given value of conductance, Δ of the two durums and cv Gutha was substantially greater than in the semi-dwarf bread wheats, indicating lower photosynthetic capacity in these three lines, as found in the gas-exchange study.

Table 5.2. Variation in carbon isotope discrimination (Δ) and biological yield (BY) at maturity and at anthesis, and variation in grain yield (GY) and harvest index (HI), among the eight genotypes grown in 1985.

	Maturity				Anthesis	
†Genotype	$10^3 \times \Delta$	BY ($t.ha^{-1}$)	GY ($t.ha^{-1}$)	HI	$10^3 \times \Delta$	BY ($t.ha^{-1}$)
1. Yallaroi	17.63	10.92	4.86	0.45	19.36	7.99
2. Gutha	17.35	9.57	3.79	0.40	18.99	8.35
4. Blade	17.08	8.85	3.69	0.42	18.47	7.14
6. 820357	17.12	9.69	4.33	0.45	18.86	7.67
12. RAC 430	17.28	10.30	4.44	0.43	18.68	7.00
13. Hartog	17.01	8.98	3.71	0.41	18.40	6.49
17. Sundor	16.57	9.49	3.99	0.42	17.96	6.99
20. Quarrion	16.09	9.56	3.93	0.41	17.50	6.65
$\bar{x}$	17.02	9.67	4.09	0.42	18.53	7.29
LSD(P<0.05)	0.43	1.20	0.48	0.02	0.41	0.67

† Number code corresponds to that used to distinguish the 20 genotypes grown in 1986 as shown in Table 5.3.

Table 5.3. Variation in carbon isotope discrimination (Δ) and biological yield (BY) at maturity and at anthesis, and variation in grain yield (GY) and harvest index (HI), among the twenty genotypes grown in 1986.

	Maturity				Anthesis	
†Genotype	$10^3 \times \Delta$	BY (t.ha^{-1})	GY (t.ha^{-1})	HI	$10^3 \times \Delta$	BY (t.ha^{-1})
1. Yallaroi	18.58	10.54	4.35	0.41	21.03	7.78
2. Gutha	18.42	12.54	4.57	0.36	21.00	9.25
3. Cranbrook*	18.50	11.85	5.23	0.44	20.48	8.88
4. Blade	18.10	11.14	4.46	0.40	20.35	7.80
5. Comet	17.96	13.15	5.04	0.38	20.26	8.72
6. 820357	17.98	10.18	4.37	0.43	20.22	7.85
7. Veery 3	18.31	11.48	4.69	0.41	20.08	8.79
8. K1056	17.81	11.55	4.80	0.42	19.96	8.03
9. Sunstar*	17.97	11.40	4.83	0.42	19.92	7.68
10. M3458	17.65	10.82	4.76	0.44	19.82	8.31
11. Vulcan	17.82	12.09	5.32	0.44	19.81	8.78
12. RAC 430	17.96	11.97	4.79	0.40	19.76	8.94
13. Hartog	17.87	12.31	4.98	0.40	19.75	8.36
14. M3844	17.54	11.97	4.49	0.38	19.68	7.89
15. Millewa*	17.66	11.04	4.55	0.41	19.65	8.34
16. Dollarbird	17.60	11.56	4.77	0.41	19.64	8.64
17. Sundor	17.48	10.73	4.51	0.42	19.51	7.35
18. Skua	17.51	10.36	4.28	0.41	19.44	7.36
19. Sunbird	17.39	10.11	4.25	0.42	19.41	7.42
20. Quarrion*	17.08	11.37	4.11	0.36	19.19	7.23
$\bar{x}$	17.86	11.41	4.66	0.41	19.86	8.17
LSD($P<0.05$)	0.41	1.60	0.70	0.03	0.48	1.35

† Genotypes listed in order of Δ values at anthesis.

* Genotypes monitored for soil water use.

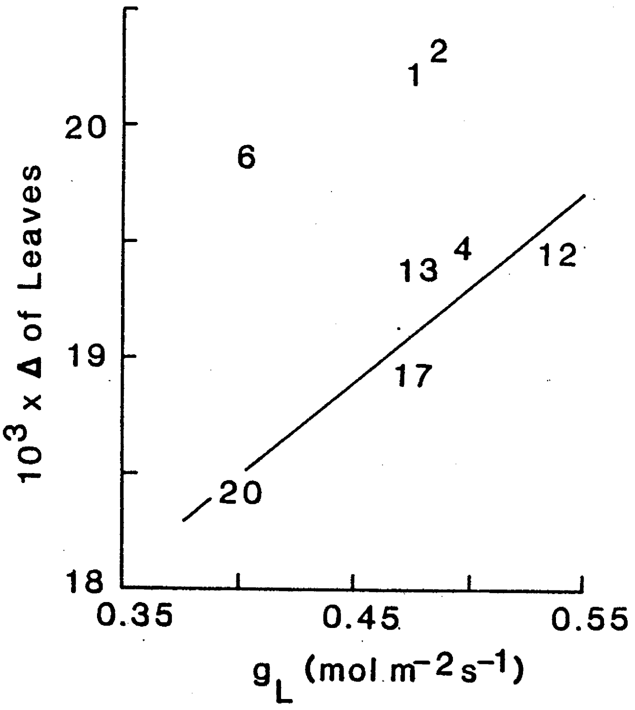


Figure 5.1. The relationship between the mean value of leaf conductance, g_L, measured during the pre-anthesis period and the mean value of carbon isotope discrimination, Δ, measured in leaves, for the eight genotypes grown in 1985. The solid line is a fitted regression to the data points for semi-dwarf bread wheats (genotypes 4,12,13,17 and 20) and is described by; $Y = 8.23X + 15.20$ ($r = 0.89$, $P<0.05$). The relationship for all eight genotypes was not significant. Genotype code as in Table 5.2.

In 1986, variation in canopy temperature under well-watered conditions was used as an index of variation in stomatal conductance among the twenty genotypes grown. There was significant genotypic variation in canopy temperature observed under well-watered conditions before anthesis, but the relationship with Δ was nonsignificant.

5.3.3 Above-ground dry matter production and grain yield.

Differences between the 1985 and 1986 seasons in precipitation and in Δ were reflected in differences in average dry matter production and grain yield (Tables 5.2 and 5.3). For the eight genotypes common to the two experiments, above-ground dry matter production at maturity averaged 17% less and grain yield 11% less, in 1985, than in 1986.

In 1986, there were significant, positive relationships between above-ground dry matter production and Δ at maturity ($r = 0.50$, $P<0.05$) and between grain yield and Δ at maturity ($r = 0.58$, $P<0.05$). Among the eight genotypes grown in 1985, these relationships were not significant.

In both seasons, and particularly in 1985 (Appendix 1), post-anthesis dry matter production by some genotypes appeared to be limited by soil water availability. There was no relationship, in either season, between post-anthesis dry matter production and Δ.

The relationships between above-ground dry matter production and Δ for the period to anthesis, when water supply was less limiting, were strongly positive in both 1985 ($r = 0.77$, $P<0.05$; Figure 5.2a) and 1986 ($r = 0.67$, $P<0.01$).

5.3.4 Canopy radiation interception

Genotypic variation in the proportion of radiation intercepted by the canopy (α) was significant in both seasons only for sampling dates in July and August.

In 1985, relationships between α and mean leaf Δ were positive at $P < 0.10$ for measurements taken on July 9 (mean α, 0.09; $r = 0.62$) and July 24 (mean α, 0.20; $r = 0.64$) and at $P < 0.01$ for measurements on August 13 (mean α, 0.50; $r = 0.84$). In 1986, there was little change in α between July 31 (mean α, 0.57) and August 13 (mean α, 0.60). The relationship between the average values of α from these two dates and Δ at anthesis was significant at $P < 0.05$ ($r = 0.55$).

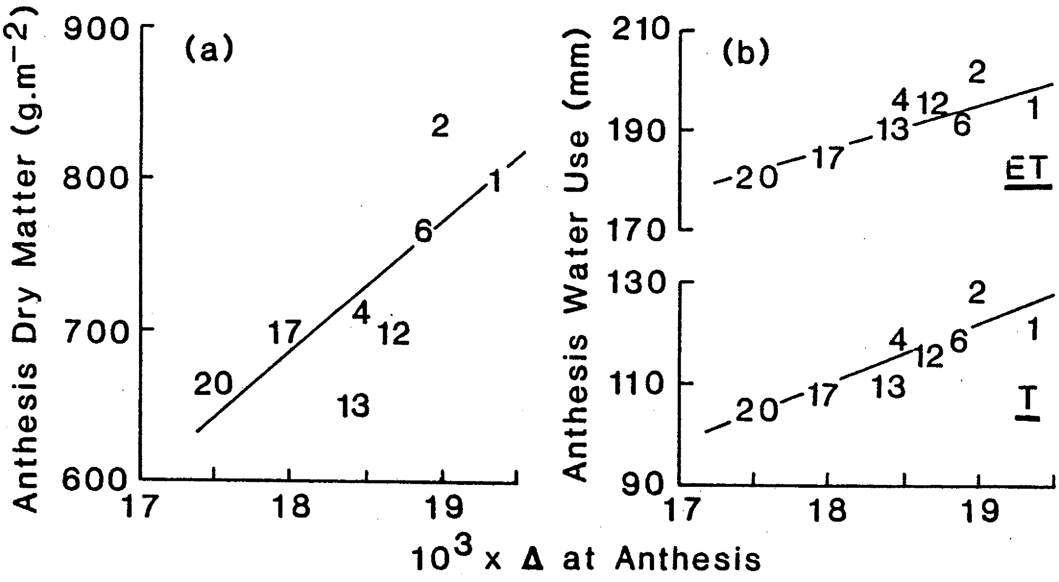


Figure 5.2. Relationships for the period from sowing to anthesis in 1985 between carbon isotope discrimination, Δ, measured in plant material and (a) above-ground dry matter and (b) water use as evapotranspiration, ET, and transpiration, T. The solid lines are fitted regressions. For dry matter, $Y = 86X - 862$ ($r = 0.77$, $P<0.05$). For ET, $Y = 8.91X + 26.21$ ($r = 0.77$, $P<0.05$). For T, $Y = 11.62X - 99.24$ ($r = 0.85$, $P<0.01$). Genotype code as in Table 5.2.

5.3.5 Water use

Total water use (ET) varied significantly among genotypes in 1985 but the range was relatively small, from 265 mm for cv 820357 to 285 mm for cv Gutha (Table 5.4). Genotypic differences in T and E for the whole season were not significant and there was no relationship between T and Δ or ET and Δ at maturity.

There was significant genotypic variation in T and in ET for the period to anthesis. Both T and ET were positively correlated with Δ at anthesis (Figure 5.2b), ($r = 0.85$, $P<0.01$ for T and Δ; $r = 0.77$, $P<0.05$ for ET and Δ).

In 1986, no significant differences in ET were observed among the four genotypes monitored for soil water use. Measurements of canopy radiation interception were too infrequent to partition ET into its components.

5.3.6 Variation in water-use efficiency

For the 1985 study, there was significant genotypic variation in seasonal water-use efficiency for above-ground dry matter production, based on both ET (W^*_{ET}) and T (W^*_T), (Table 5.4). Genotypic variation in water-use efficiency for grain yield, based on both ET and T, was also significant (data not shown).

Because of the small range in water use (as either ET or T), variation in both W^*_{ET} and W^*_T largely reflected genotypic variation in dry matter production (Fig. 5.3a). Relationships between W^*_T and T and between W^*_{ET} and ET were negative but nonsignificant.

There was no relationship between genotypic variation in seasonal water-use efficiency (as either W^*_T or W^*_{ET}) and genotypic variation in Δ (Fig. 5.3b).

At anthesis, the regressions fitted to the relationships between above-ground dry matter production and Δ and water use (as either ET or T) and Δ indicated that, per unit change in Δ, there was a relatively greater change in dry matter production than in either ET or T. However, there were no significant differences in W^*_{ET} or W^*_T at anthesis and, while the relationships at anthesis between W^*_{ET} and Δ and W^*_T and Δ were both positive, neither relationship was significant.

In 1986, there were no significant differences in W^*_{ET} among the four genotypes monitored for water use, either at maturity or at anthesis.

Table 5.4. Variation in seasonal evapotranspiration (ET), estimated totals of direct evaporation (E) and transpiration (T), and water-use efficiency for above-ground dry matter production based on evapotranspiration (W^*_{ET}) and transpiration (W^*_T), among the eight genotypes grown in 1985.

Genotype	[1]ET (mm)	E (mm)	T (mm)	[2]W^*_{ET} ($g.m^{-2}.mm^{-1}$)	W^*_T ($g.m^{-2}.mm^{-1}$)
Yallaroi	271	91	180	4.04	6.07
Gutha	285	92	193	3.36	4.96
RAC 430	282	99	183	3.66	5.63
820357	265	89	176	3.55	5.51
Blade	284	98	186	3.12	4.76
Hartog	277	97	180	3.25	4.99
Sundor	270	93	177	3.51	5.36
Quarrion	267	93	174	3.58	5.49
$\bar{x}$	275	94	181	3.51	5.35
LSD($P<0.05$)	12	n.s.	n.s.	0.45	0.70

---

[1] Measured ET plus 31.5 mm for the 48 d period from sowing to the first soil water readings. This figure equivalent to the mean rate of evaporation of $0.65\ mm.d^{-1}$ measured for bare soil in early July.

[2] Water-use efficiency is given in units of $g.m^{-2}.mm^{-1}$ which is equivalent to $g.kg^{-1}$ as used in the glasshouse studies.

$1\ g.m^{-2}.mm^{-1} = 1 \times 10^{-2}\ t.ha^{-1}.mm^{-1}$

$= 10\ kg.ha^{-1}.mm^{-1}$

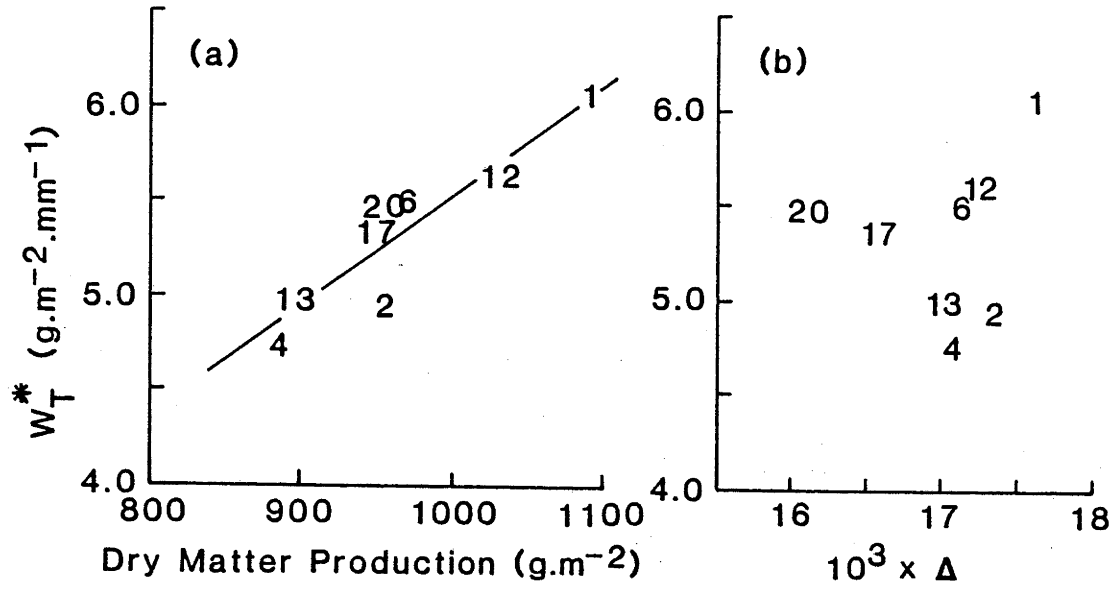


Figure 5.3. (a) Relationship between water-use efficiency based on transpiration, W_T^*, at maturity and final above-ground dry matter for the eight genotypes grown in 1985. The solid line is a fitted regression described by; $Y = 0.0058X - 0.26$ ($r = 0.91$, $P<0.01$).

(b) Relationship between water-use efficiency based on transpiration, W_T^*, at maturity and carbon isotope discrimination, Δ, at maturity for the eight genotypes grown in 1985.

Genotype code as in Table 5.2.

5.4 DISCUSSION

The hypothesis forming the basis of this investigation is that, providing supraresistance (r_i^*) is positive, water-use efficiency should be negatively correlated with carbon isotope discrimination. In these experiments, wheat genotypes selected on the basis of variation in Δ were grown in a field environment with relatively high winter/spring rainfall. Under these growing conditions, significant genotypic variation in canopy water-use efficiency was observed but there was no relationship between water-use efficiency and Δ. This was despite the strong negative correlations observed between leaf transpiration efficiency (at constant leaf-to-air vapour pressure difference) and Δ and between whole-plant water-use efficiency and Δ under well-watered and water-stressed conditions in the glasshouse. These earlier studies involved most of the genotypes grown in these field experiments.

The discrepancy between the field and glasshouse experiments may be attributable to several factors. As well as the expected effects of the increased scale of measurement on the sensitivity of water-use efficiency to a change in stomatal conductance (Jones, 1983; Jarvis and McNaughton, 1986; Cowan, 1988), these may have also included factors associated with the positive relationship between dry matter production and Δ under well-watered conditions in the field, and genotypic variation in the effects of increasing soil water deficit on dry matter accumulation after anthesis.

5.4.1 Water-use efficiency under well-watered conditions.

The results of the gas-exchange studies reported in Chapter 3 and the data summarised in Figure 5.1 for these studies, indicate that variation in Δ among the genotypes used here could be attributed to variation in both stomatal conductance and photosynthetic capacity.

For the 1985 study, water supply was largely non-limiting for the period to anthesis. The relative effects of variation in stomatal conductance and variation in photosynthetic capacity on W_T^* during this period are summarised in Table 5.5, in which W_T^* is compared in sets of genotypes that differed mainly in conductance and in sets of genotypes that differed mainly in photosynthetic capacity.

The effect of a change in stomatal conductance is assessed by comparing two bread wheat genotypes with low conductance, cvs. Quarrion

Table 5.5. The effects of genotypic variation in stomatal conductance (g_L) and genotypic variation in photosynthetic capacity (b) on canopy water-use efficiency (W_T^*), cumulative interception of photosynthetically active radiation (J), and the conversion efficiency of radiation into above-ground dry matter (ε) during the pre-anthesis period in 1985. Average values of carbon isotope discrimination (Δ) at anthesis and the corresponding values of (1 - p_i/p_a) are also shown for each set of genotypes.

	Conductance			Capacity		
	Low g_L		High g_L	High b		Low b
[1]Genotypes	18,20		5,9	5,9,18,20		1,3,6
[2]Mean g_L ($mol.m^{-2}.s^{-1}$)	0.53	(.05)	0.74	0.63	(n.s)	0.58
[3]Mean b ($\mu mol.m^{-2}.s^{-1}.Pa^{-1}$)	1.74	(n.s)	1.78	1.76	(.01)	1.43
$10^3 \times \Delta$	17.73	(.10)	18.58	18.15	(.05)	19.07
$(1 - p_i/p_a)$	0.41		0.37	0.39		0.35
W_T^* ($g.m^{-2}.mm^{-1}$)	6.40	(.05)	6.02	6.21	(.10)	6.50
[4]J ($MJ.m^{-2}$)	625	(n.s)	605	615	(.01)	674
[5]ε ($g.MJ^{-1}$)	1.04	(.05)	1.11	1.08	(n.s)	1.14

---

[1] Genotypes listed according to numerical code in Table 5.2.

[2] Mean of values to anthesis shown in Figure 5.1 and values from glasshouse experiments I and II given in Table 3.1.

[3] Mean of values given in Table 3.1 for glasshouse experiment II.

[4] J was estimated for the period from d.64 (when measurements of incident radiation commenced) to d. 139 (mean anthesis date) by summing the products of the daily measurements of incident PAR and daily estimates of radiation interception derived from the curves fitted to the data obtained on radiation penetration through the canopy (Appendix 5.3).

[5] ε was calculated for the period from d.64 to d.139, as the increment in above-ground dry matter from the harvest at d.64 to anthesis, divided by the estimated value of J for the same period.

[6] Figures in brackets indicate the level of significance for differences between groups within a comparison.

and Sundor, with two bread wheat genotypes with high conductance, cvs. Blade and RAC 430. On average, W_T^* at anthesis was 6% greater in the low g_L pair. From the mean Δ values at anthesis measured in these two sets of genotypes, an 11% difference in leaf transpiration efficiency would be expected in a well-stirred gas-exchange cuvette at constant leaf-to-air vapour pressure deficit, i.e. approximately twice the observed increase at the canopy level. This reduction in the sensitivity of W_T^* at the canopy level to a change in stomatal conductance is close to that calculated by Cowan (1988) for wheat plots of this size growing under similar conditions. With increasing plot size, the canopy boundary layer conductance will become relatively smaller still, and canopy transpiration even more insensitive to changes in stomatal conductance (Jarvis and McNaughton, 1986; Cowan, 1988). Over a fetch of several hundred metres and under well-watered conditions such as experienced in this phase of crop growth, W_T may, in fact, be expected to be greater for lines with high conductance than for lines with low conductance.

The effect on W_T^* of variation in photosynthetic capacity differed from that expected. Cowan (1988) suggests that scale effects should have little influence on relative differences in water-use efficiency when these differences are due to changes in photosynthetic capacity alone. When the average W_T^* to anthesis of sets of genotypes differing in photosynthetic capacity were compared (Table 5.5), W_T^* in those genotypes with relatively low capacity (cvs. Gutha, Yallaroi and 820357) was similar to (6% greater at $P < 0.10$) the average W_T^* of genotypes with higher capacity (cvs. Quarrion, Sundor, Blade and RAC 430). On the basis of mean Δ values, W_T^* would have been expected to average 11% less in the low capacity genotypes than in the set of lines with higher capacity.

That water-use efficiency to anthesis was not less for genotypes with relatively low photosynthetic capacity may be due to several factors associated with their substantially greater anthesis biomass.

Dry matter production may be seen as a function of radiation interception and the efficiency with which intercepted radiation is converted into dry matter (Monteith, 1977). During the early part of the season in both years of this study, estimates of the proportion of radiation intercepted by the canopy, α, were positively correlated with Δ. In 1985, this was largely because of the relatively high values of α of genotypes with low photosynthetic capacity. A negative relationship between canopy leaf area development and photosynthetic capacity is a common observation among crop species, and has been cited as a major reason for the generally poor relationship between photosynthetic rate per unit leaf area and crop yield (Elmore, 1980; Bhagsari and Brown, 1986; Poskuta and Nelson, 1986).

The high values of α for those genotypes in the 1985 experiment with relatively low photosynthetic capacity are reflected in estimates of cumulative PAR interception (J) by each genotype calculated for the period from d.64 (when measurements of incident PAR started) to d.139 (the mean anthesis date) and summarised in Table 5.5. Average values of J for the "low" and "high" capacity groups were 674 ± 3 and 615 ± 6 $MJ.m^{-2}$ respectively. These values probably underestimate J by approximately 10-20% (Hipps et al., 1983; Muchow, 1985) because radiation penetration through the canopy is greatest during the middle of the day, when measurements were taken in this study. Nevertheless, they indicate that radiation interception was considerably greater for the "low" capacity lines than for lines with higher capacity.

While greater radiation interception is associated with greater dry matter production (Brougham, 1956; Hesketh and Baker, 1967; Gifford and Evans, 1981), it should also reflect greater transpirational water use (Squire and Black, 1981), as was observed in this study. However, the sensitivity of canopy transpirational losses to increased leaf area may not be as great as expected, since increased transpirational water loss into the canopy boundary layer will increase the vapour pressure there, lowering the evaporative demand somewhat (Jarvis and McNaughton, 1986). Although similar in principle, the effect on W_T will be less than that discussed above in relation to changes in stomatal conductance, because there should be a smaller effect on canopy temperature.

Other differences in the pattern of dry matter accumulation before anthesis, acting via effects on the efficiency of conversion of intercepted radiation into above-ground dry matter (ε), may have also countered the expected effect of lower photosynthetic capacity on W_T^*. Estimates of ε for the period from d.64 to anthesis are also given in Table 5.5. These estimates are not corrected for possible underestimation of J discussed above, since the magnitude of any correction factor cannot be estimated precisely.

Conversion efficiency (as estimated) was greater in lines with relatively high stomatal conductance than in lines with low conductance. This result was expected since photosynthetic rate per unit leaf area should be lower in genotypes with lower stomatal conductance (Muchow et al., 1986).

Lower photosynthetic capacity may also be expected to decrease conversion efficiency, but there was no significant difference in ε between genotypes with "low" and "high" capacity. This may have been due to a greater allocation of carbon to above-ground parts by genotypes with relatively "low" capacity or to the nature of the dry matter being produced these lines (Teare et al., 1973).

In both 1985 and 1986, the proportion of above-ground dry matter found in the leaves at anthesis was negatively correlated with Δ at anthesis. In 1985, this proportion ranged from 0.20 in Gutha, Yallaroi and 820357 to 0.25 in Quarrion and Sundor and in 1986, from 0.19 in Gutha, Cranbrook and Veery 3 to 0.27 in Quarrion and M3844. One effect of greater carbon allocation to stems and sheaths may be a decrease in ϕ (i.e. the proportion of carbon fixed during photosynthesis that is lost in respiratory processes) (McCree, 1988) resulting from both an increase in the efficiency of new biomass synthesis and a decrease in the maintenance requirement of existing biomass (Stahl and McCree, 1988).

In these field experiments, LAI reached a maximum of approximately 3.5 in 1985 and 4.0 in 1986 and then fell rapidly in both seasons. At higher values of LAI, above full light interception, further increases in leaf area would lead to reduced carbon use efficiency (Gifford and Jenkins, 1982). If such higher LAI values were reached earlier by high Δ lines, any putative respiratory advantage of subsequent changes in carbon allocation may be eliminated. This may partly explain the lack of dry matter advantage of high Δ lines in the glasshouse experiment, where LAI values were considerably greater than in the field and were maintained over several weeks.

Differences in dry matter allocation between below- and above-ground dry matter may have also enhanced W_T^* (and the apparent efficiency of radiation conversion into above-ground dry matter) in "low" capacity, high Δ lines. For the July 31 harvest of the 1986 study, at which plants were separated into above-ground and below-ground dry matter (excluding roots), there was a greater proportion of dry matter in below ground parts in low Δ lines ($r = -0.54$, $P<0.05$). This early difference in dry matter partitioning may have also been associated with the slower canopy development of low Δ lines and could result in a substantial effect on above-ground dry matter production by anthesis.

High Δ lines may have also been partitioning less dry matter to their roots. In a recent series of experiments on the effects of soil strength on growth and water-use efficiency of wheat seedlings (Masle and Passioura, 1987; Masle and Farquhar, 1988), root/shoot ratio and Δ were found to be negatively correlated. It is not known whether a similar correlation exists when Δ varies genotypically. In the glasshouse experiment (Chapter 4), there were no consistent differences in root/shoot ratio at ear emergence between high and low Δ lines (Table A4.1). However, measurements made at ear emergence (when root dry matter is near its maximum) on roots growing in a restricted volume may not detect genotypic differences present earlier in ontogeny.

5.4.2 Water-use efficiency under post-anthesis water deficit.

In these studies, soil water deficits were largely confined to the post-anthesis period. In 1985, variation in W_T^* during this phase of crop growth was independent of variation in Δ but was strongly correlated with post-anthesis dry matter production ($r = 0.96$, $P<0.01$; Fig. 5.4).

In this field environment, variation in dry matter production during post-anthesis water stress may be seen as being a function of the extent of soil water depletion to anthesis, which largely reflects dry matter production to that time (Fischer and Kohn, 1966a; Doyle and Fischer, 1979; Figure 5.2, this study), and genotypic variation in the sensitivity of dry matter production to soil water deficits.

Conservation of soil water by low Δ genotypes during the pre-anthesis phase may favour greater dry matter production by these lines after anthesis. However, any such water conserved for use after anthesis will be used relatively inefficiently since the evaporative demand generally increases rapidly during the post-anthesis period (Nix, 1975). As well, for all genotypes, senescence of the canopy after anthesis will restrict the capacity for CO_2 fixation during this phase (Gifford and Jenkins, 1982).

The effects of senescence on CO_2 fixation and carbon balance during post-anthesis stress may vary with genotypic variation in stomatal sensitivity to soil water depletion. Jarvis and McNaughton (1986) suggest that the sensitivity of canopy transpiration to a change in stomatal conductance should be relatively large under water stress, since canopy conductance will again be small relative to the boundary layer conductance. Cowan (1989) extends this analysis, arguing that the

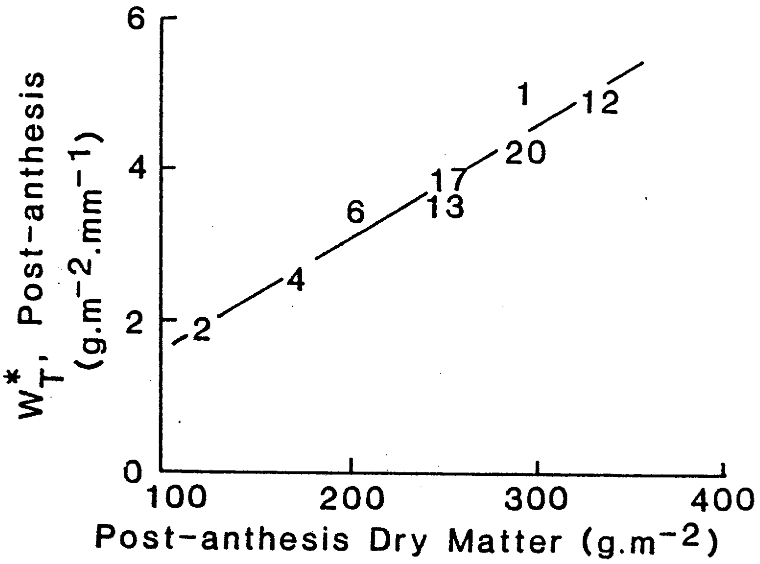


Figure 5.4. Relationship between water-use efficiency based on transpiration, W_T^*, for the post-anthesis period in 1985 and above-ground dry matter production over that period. The solid line is a fitted regression described by; $Y = 0.0151X + 0.09$ ($r = 0.96$, $P<0.01$). Genotype code as in Table 5.2.

sensitivity of canopy water-use efficiency to a change in stomatal conductance should also be relatively large under water stress. This may be so for the ratio of gross photosynthetic carbon input to transpiration. However, in several studies, crop water-use efficiency has been observed to decline as a result of imposition of water stress on previously well-watered, dense canopies (Sinclair et al., 1975; Zur and Jones, 1984; Baldocchi et al., 1985). This effect has been attributed to increased canopy temperature due to stomatal closure, and also to the effects of water stress on canopy photosynthetic capacity and to an increase in the proportion of fixed carbon lost through respiratory processes (Legg et al., 1979; Muchow et al., 1986). For well-developed canopies experiencing terminal water stress, relative stomatal insensitivity to declining soil water availability may be advantageous in maintaining a more positive carbon balance and greater water-use efficiency.

5.5 CONCLUSIONS

In these field experiments, variation in crop water-use efficiency was studied among wheat genotypes selected on the basis of variation in Δ. Under the prevailing conditions of high winter/spring rainfall, no relationship was observed between W_T^* and Δ measured in dry matter.

Genotypic variation in Δ was attributed to variation in both stomatal conductance and photosynthetic capacity. Under the predominantly well-watered conditions up to anthesis, variation in W_T^* resulting from a change in stomatal conductance was less than expected from leaf gas-exchange theory. This was probably due to the relatively greater influence of boundary layer conductance on transpirational water loss from field canopies. The sensitivity of crop water-use efficiency to a change in stomatal conductance is likely to be further reduced as the area increases over which such a change in conductance is applied.

There was no effect on W_T^* of reduced photosynthetic capacity. This may have been partly due to the relatively greater influence of boundary layer conductance on transpirational water loss, and partly due to possible effects of genotypic variation in dry matter distribution on the ratio of above-ground to total dry matter and on the relative extent of respiratory carbon losses.

Water deficits were largely confined to the post-anthesis period. Water-use efficiency during this phase of crop growth was also independent of variation in Δ but was strongly correlated with post-anthesis dry matter production. Greater dry matter production during this phase was favoured in low Δ genotypes that had conserved soil water in the period up to anthesis. However there was also substantial variation in post-anthesis dry matter production among high Δ lines that had used considerably more water at anthesis.

The cause of this latter variation among high Δ lines is unknown, but may involve genotypic variation in stomatal sensitivity to soil water deficits.

CHAPTER 6

GENERAL DISCUSSION

6.1 INTRODUCTION

Crop production in many parts of the world is limited by an inadequate water supply. In the Australian wheatbelt, the extent of this limitation varies as one moves from the typically moister areas nearest the coast, to the drier margins towards the continent's interior. Water supply also varies from year to year, at a given location, according to the incidence of rainfall. The extent and timing of this rainfall limitation overlays further climatic variation as one moves from the western and southern parts of the wheat belt, where the crop is grown predominantly on current rainfall, to the northern part, where the major part of the water used by the crop is moisture stored in the soil profile from previous summer rains.

Increasingly, crop breeders are seeking alternative selection procedures to improve yield stability and to continue the yield gains they have made using conventional, empirical breeding techniques. The search for appropriate physiological selection criteria for water-limited environments has followed either a "black box" approach or an "ideotype" approach. Passioura (1977), in espousing the latter, suggested that improving the water-use efficiency of dry matter production, i.e. the dry matter produced per unit water transpired by the crop, may lead to improved grain yields in water-limited environments.

Tanner and Sinclair (1983) and Farquhar et al., (1982b), among others, proposed that one means of achieving greater crop water-use efficiency may be to seek and exploit genotypic variation in the ratio of the intercellular to atmospheric partial pressures of CO_2, p_i/p_a. At the leaf level, under conditions of constant leaf-to-air vapour pressure difference and negligible boundary layer resistance, p_i/p_a is negatively related to transpiration efficiency (the ratio of the instantaneous rates of CO_2-assimilation and transpiration).

The routine measurement of p_i/p_a presents practical difficulties in breeding programmes. Farquhar et al., (1982b) developed theory which proposed that, for C_3 species, carbon isotope discrimination (Δ) should be positively related to p_i/p_a. They suggested that, as an alternative to the measurement of p_i/p_a itself, Δ may prove useful as a selection criterion for improved crop water-use efficiency. This suggestion was given experimental support by the results of the study by Farquhar and Richards (1984), which demonstrated a negative correlation between plant water-use efficiency and Δ for a group of wheat genotypes subjected to a range of watering regimes.

The broad aim of the work presented in this thesis, then, was to assess the usefulness of carbon isotope discrimination in selecting for improved water-use efficiency in wheat. The ultimate objective of such selection would be to improve the grain yield of wheat growing in water-limited environments.

6.2 EXPERIMENTAL OBJECTIVES

The experimental program was based on a framework suggested by Garrity et al. (1982) for the assessment of physiological traits proposed for improving the yields of crops growing in water-limited environments. This framework consisted of six stages.

1. Development of a hypothesis concerning the potential contribution of a particular trait.
2. Search for genotypic variability for the trait.
3. Development of a rapid and efficient screening method.
4. Elucidation of the mode of trait inheritance and selection of an appropriate breeding method to incorporate it into agronomically superior genotypes.
5. Documentation of a strong causal relationship between high levels of a trait and improved yield performance in water-limited environments.
6. Assessment of the likely impact of trait selection on yield potential under more favourable conditions.

As summarised above, the Literature Review provided some encouragement that improved crop water-use efficiency may be one means of improving the yields of crops growing in water-limited environments, and that Δ may prove useful in selecting for improved water-use efficiency. However, the Literature Review also revealed several possible shortcomings which could limit the application of Δ for this purpose.

Firstly, there are several potential sources of variation in carbon isotope composition of plant dry matter which are largely independent of variation in p_i/p_a. Hence, genotypic variation in Δ may not adequately represent genotypic variation in p_i/p_a. Secondly, there may be substantial changes in the effectiveness of stomatal control of gas-exchange as the scale of measurement increases from single leaves to crop canopies. These scale effects may alter considerably the extent to which variation in leaf p_i/p_a and, by inference, transpiration efficiency at the single leaf level and plant Δ, are reflected in variation in crop water-use efficiency. Thirdly, crop productivity under more favourable conditions may be restricted, either directly or indirectly, by selecting for characteristics which favour improved leaf transpiration efficiency, i.e. low stomatal conductance and high photosynthetic capacity.

The experimental framework outlined above was modified somewhat to determine the significance of these possible shortcomings. The experimental programme addressed not only the reliability and use of Δ in breeding, but also the physiological rationale for the use of Δ as a selection criterion for improved crop water-use efficiency.

6.3 RESULTS

An essential prerequisite for the use of any trait in breeding programmes is that there be genotypic variation in the trait. Genotypic variation in Δ was sought in material obtained from a range of sources and grown together in several field experiments (Chapter 2 and Appendix 1). These experiments revealed substantial variation in Δ among wheat genotypes. The majority of the genotypes grown were either recently released Australian cultivars or advanced lines from breeding programmes based in different parts of the Australian wheat belt. Among these genotypes, the range of variation in Δ was up to 2.5×10^{-3}.

These results indicate that, if selection based on Δ was shown to be worthwhile, there is already considerable variation in Δ present among conventional, agronomically acceptable wheat genotypes, that could be utilised to improve crop water-use efficiency.

The adoption of many putative physiological traits has been impeded by the lack of rapid and efficient screening techniques (Wilson, 1984). The measurement of Δ is certainly rapid. Techniques currently available enable many thousands of samples to be processed annually (Farquhar et al., 1988). The efficiency of screening for Δ would be enhanced by minimising both non-genotypic variation in Δ and genotype x environment interaction for Δ. The extent of non-genotypic variation in Δ, as well as some likely sources of this variation, were examined in experiments reported in Appendix 1. Genotype x environment interaction for Δ was considered in experiments presented in Chapter 2 and Appendix 1.

There was substantial genotypic variation in Δ at each field site used and also substantial variation in site-mean Δ. When genotypic ranking for Δ was compared across sites, genotype x environment interaction for Δ was generally non-significant, except when more extreme environments were contrasted. Estimates of broad-sense heritability for Δ in these experiments ranged from 40% to 80%, and averaged 63%. These estimates, as well as the generally non-significant genotype x environment interaction for Δ, indicate that Δ is under reasonably strong genetic control.

Results of field experiments (Appendix 1) and glasshouse experiments (Chapter 4) indicate that some variation in genotypic ranking for Δ may arise as a result of variation among genotypes in the extent of soil water use. This indicates that assessment of genotypic variation in Δ should be most efficient if Δ is measured early in plant development on plants grown under well-watered conditions.

The mode of inheritance of Δ was not studied as part of this experimental program.

Several of the experiments used in the assessment of genotypic variation in Δ and genotype x environment effects on Δ, were conducted under relatively favourable moisture conditions. Above-average winter/spring rainfall meant that water supply was largely non-limiting for the pre-anthesis period and, in some experiments, beyond anthesis.

In all of these studies, relationships between grain yield and Δ and between above-ground biomass and Δ were positive, frequently strongly so. For relatively small increases in Δ, there were substantial increases in both grain yield and biomass production. These results confirm that selection for low Δ (i.e. improved transpiration efficiency at the leaf level) could have a substantial, deleterious impact on yield potential under favourable conditions.

It was concluded that the positive relationships between biomass production and Δ were associated with genotypic variation in stomatal conductance and in photosynthetic capacity, both of which varied significantly among genotypes grown in these studies. Gas-exchange studies on a set of 14 genotypes (Chapter 3) indicated that genotypic variation in conductance and capacity contributed approximately equally to the genotypic variation in Δ observed. From observations made in the field (Chapter 5), it was considered that low stomatal conductance decreased dry matter production directly, as a result of a decreased rate of CO_2-assimilation per unit leaf area. The influence of variation in photosynthetic capacity was thought to be indirect, lower capacity being associated with more rapid canopy development and greater radiation interception and hence, greater dry matter production. There was no evidence that lower photosynthetic capacity resulted in a lower efficiency of conversion of PAR into above-ground biomass, as might have been expected. This may have been due to a reduced allocation of carbon to below-ground biomass, or to a reduction in the proportion of carbon fixed by the leaves in photosynthesis but lost through plant respiratory processes, among lines with relatively low photosynthetic capacity.

While all these possible causes may explain the positive relationships between biomass and Δ, the slopes of these relationships indicate that, under these growing conditions of relatively high winter/spring rainfall, the relationships in the field between crop water-use efficiency and Δ were probably poor, and possibly positive also.

A poor relationship between crop water-use efficiency and Δ may be the result of a poor relationship between Δ and p_i/p_a, a poor relationship between leaf p_i/p_a and crop water-use efficiency, or both. These relationships were examined using a set of genotypes selected to span a range of Δ values. Using these genotypes as a source of variation in Δ, the relationships between Δ and water-use efficiency at the leaf, single plant and crop canopy levels were determined.

Experiments reported in Chapter 3 were conducted to confirm that differences in Δ among wheat genotypes reflected measured differences among them in p_i/p_a. Discrimination measured in dry matter was positively correlated with p_i/p_a measured in flag leaves of well-watered plants of 14 genotypes. The fitted regressions fell close to the simplified, theoretical relationship between Δ and p_i/p_a proposed by Farquhar and Richards (1984).

These relationships indicate that Δ provides a reasonable estimate of genotypic variation in p_i/p_a. However, approximately 45% of the observed variation in Δ was not accounted for by variation in p_i/p_a. This result indicates that there may also be considerable variation in water-use efficiency that is not accounted for by variation in Δ, and vice versa.

The relationship between plant water-use efficiency and Δ was determined using plants of 16 genotypes grown under well-watered and water-stressed conditions in the glasshouse. Substantial genotypic variation in plant water-use efficiency was demonstrated under both sets of conditions among this set of conventional Australian wheat genotypes. For both well-watered plants and for plants subjected to terminal water stress, plant water-use efficiency and Δ were negatively correlated. Water-use efficiency under terminal water stress was also negatively correlated with Δ measured under well-watered conditions.

The relationships between water-use efficiency and Δ were reasonably strong, variation in Δ accounting for between approximately 30% and 55% of the observed variation in plant water-use efficiency. These results indicate that low Δ may prove useful in selecting for improved plant water-use efficiency in wheat.

In these experiments, under well-watered conditions, the response of plant water-use efficiency to a change in Δ, though relatively large, was approximately 35% less than the change in leaf transpiration efficiency that would be expected, for a similar change in Δ, under conditions of negligible boundary layer resistance and constant leaf-to-air vapour pressure difference. This was attributed largely to the increased influence of boundary layer resistance on transpirational water loss. This boundary layer effect may, in fact, have been underestimated, since variation in Δ was not solely attributed to variation in stomatal conductance, but also to variation in photosynthetic capacity.

Field experiments were conducted over two seasons to determine the relationship between water-use efficiency and Δ in field plots (Chapter 5). A range of wheat genotypes was grown under a mediterranean-type environment of reasonably high winter/spring rainfall, followed by a period of gradually increasing terminal stress during the post-anthesis phase.

Significant genotypic variation in crop water-use efficiency was observed, but there was no relationship between crop water-use efficiency and Δ.

Under the predominantly well-watered conditions up to anthesis, variation in water-use efficiency resulting from a change in stomatal conductance was less than expected from leaf gas-exchange theory. This was probably due to the relatively greater influence of boundary layer conductance on transpirational water loss from field canopies.

There was no effect on crop water-use efficiency of reduced photosynthetic capacity. This may have been partly due to the increased influence of boundary layer conductance on canopy transpiration, transpiration during the pre-anthesis phase being greater from the faster developing canopies of genotypes with relatively low photosynthetic capacity, and partly due to possible effects of genotypic variation in dry matter distribution on the ratio of above-ground to total dry matter and on the relative extent of respiratory carbon losses.

Water deficits were largely confined to the post-anthesis period. Water-use efficiency during this phase of crop growth was also independent of variation in Δ but was strongly related to post-anthesis dry matter production. Greater post-anthesis dry matter production during this phase was favoured in low Δ genotypes that had conserved soil water in the period up to anthesis. However, there was also substantial post-anthesis dry matter production in some high Δ lines that had used considerably more water at anthesis.

6.4 DISCUSSION AND CONCLUSIONS

The major results of the experimental programme may be summarised as follows :

(1) There is substantial variation in Δ among conventional wheat genotypes.
(2) Variation in Δ appears to be under relatively strong genetic control.
(3) Genotypic variation in Δ provides a reasonable measure of genotypic variation in leaf p_i/p_a.

In the glasshouse,

(4) Δ is negatively correlated with plant water-use efficiency under both well-watered and terminally water-stressed conditions.
(5) Δ is considerably faster and easier to measure than plant water-use efficiency.

In the field, for wheat grown in a mediterranean-type environment,

(6) Δ is poorly correlated with crop water-use efficiency.
(7) Δ is positively related to above-ground dry matter production and grain yield when there is little pre-anthesis water limitation.

Many of these results have now been observed in other C_3 species. Substantial variation in Δ has been observed among conventional genotypes of peanut (Hubick et al., 1986), barley (Hubick and Farquhar, 1989; Crauford et al., 1988) and common bean (White et al., 1988). Among genotypes of barley (Crauford et al., 1988) and common bean (White et al., 1988), Δ values were strongly correlated when genotypes were grown in similar rainfed environments, but not when more contrasting environments were compared. Genotype x environment interaction for Δ was found to be non-significant for a range of peanut genotypes grown at several rainfed sites (Hubick et al., 1989). In the multi-site comparison of peanut genotypes, broad-sense heritability for Δ was estimated to be approximately 80%.

Positive relationships between Δ and p_i/p_a have been demonstrated for peanut (Hubick et al., 1989) and barley (K.T. Hubick and G.D. Farquhar, unpublished data), using on-line gas-exchange techniques.

Negative relationships between plant water-use efficiency and Δ have been observed for container-grown plants of peanut (Hubick et al., 1986; Hubick et al., 1989), barley (Hubick and Farquhar, 1989) and tomato (Martin and Thorstenson, 1988).

Relationships in the field between yield and Δ and water-use efficiency and Δ have been variable. Relationships between yield and Δ were determined for 10 bean cultivars grown under rainfed and irrigated conditions in Colombia (White et al., 1988). Under irrigation, there was no relationship between yield and Δ. Under relatively favourable rainfed conditions, yield (as both grain and biomass) was positively correlated with Δ. Under less favourable conditions, yield was greatest at intermediate values of Δ.

In a study on barley (Crauford et al., 1988), thirty-six 2-row genotypes were grown in field trials at three sites varying in annual rainfall in Syria (which has a mediterranean-type rainfall distribution) and under irrigation and terminal drought (using a raincover) at Cambridge, in the U.K.. For all but the irrigated treatment at Cambridge, relationships between yield (as grain and biomass) and Δ were positive. The authors suggest that there may have been some interaction between Δ and maturity, since higher yielding lines were also earlier to head.

Negative relationships between biomass and Δ have also been observed in some field studies. Above-ground biomass and Δ were negatively correlated among 16 peanut genotypes grown under rainfed conditions in southern Queensland (Hubick et al., 1989). Relationships between grain yield and Δ and harvest index and Δ were positive. For barley genotypes grown under a terminal stress imposed using a raincover, above-ground biomass and Δ were negatively correlated (B. Read, P.J. Cornish, K.T. Hubick and G.D. Farquhar, unpublished data). Finally, when 12 of the wheat genotypes used in the field studies presented in Chapter 5 were grown in the field in 1987 under conditions of low pre-anthesis rainfall and a greater dependence on stored soil moisture, the relationship between above-ground biomass and Δ was also negative (R.A. Richards, personal communication).

In most of the above studies, water use was not measured. For the raincover study on barley by Read et al., water use was measured but no attempt was made to partition direct soil evaporation from crop transpiration. In that study, water-use efficiency, based on evapotranspiration, was negatively correlated with Δ measured in plant dry matter. Water-use efficiency (on a transpirational basis) may also have been negatively correlated with Δ in this study on barley, and in the other studies cited above in which final biomass and Δ were negatively correlated. A negative correlation between crop water-use efficiency and Δ seems unlikely for those studies in which the relationships between final biomass and Δ were positive.

The relationship between crop water-use efficiency and Δ may vary, therefore, depending on the extent and timing of any water limitation. Under well-watered conditions, the relationship may also vary depending on the relationship between photosynthetic capacity and biomass production. In wheat, this relationship may be negative. In some other species, such as peanut, the relationship may be positive (Gifford et al., 1984).

Jones (1987) has reviewed attempts by breeders to develop varieties of crop plants having particular stomatal characteristics. He points out, particularly in relation to improved crop yields in water-limited situations, the problem of achieving a balance between the need for water conservation and survival and the need to retain productivity. Turner (1981) addressed the same problem in discussing other physiological criteria. He suggested that progress in developing suitable criteria had been slow because often, proposed traits were more applicable to survival than productivity. The results of the present study indicate that, for wheat, carbon isotope discrimination may fall into a similar category.

The water use data obtained in the field experiments reported in Chapter 5 indicate that the greater above-ground dry matter production to anthesis of high Δ lines was achieved at the expense of greater soil water use. Much of the dry matter advantage of high Δ lines at anthesis had been eliminated by maturity, especially in the drier 1985 season. However, despite greater post-anthesis dry matter production, grain yield still tended to be lower in low Δ genotypes. Thus, even though low Δ lines may conserve water that is effectively used for dry matter production after anthesis, the water conserved may not be used effectively in enhancing grain yield under these growing conditions. Even for dry matter production, in this environment, water conserved for use after anthesis may not be used efficiently, since evaporative demand increases steeply after anthesis. In the absence of any genotypic differences in water-use efficiency, such water would be more efficiently used before anthesis, when the vapour pressure deficit is lower.

The results of the studies performed here indicate that, in this field environment, which is fairly typical of much of the southern Wheat Belt, selection for improved crop water-use efficiency in wheat based on low Δ would be inappropriate. For much of the pre-anthesis period, the crop is often largely unstressed. Under these circumstances, scale effects, associated with the relative increase in boundary layer resistance in the field, may eliminate or even reverse any water-use efficiency advantage at the leaf or plant level associated with low stomatal conductance. Direct soil evaporation is also likely to be greater from under the slower developing canopies of low Δ lines. Indeed, given the consistent positive relationships observed between grain yield and Δ, selection for high Δ could be much more useful in this environment.

As noted above, however, soil water use to anthesis was also positively correlated with Δ in these field experiments. Selection based on high Δ would therefore risk excessive soil water use even before anthesis in more marginal areas of the southern Wheat Belt and throughout the whole of the southern Wheat Belt in drier seasons. Additional selection criteria would also be necessary to impart greater yield stability. Such criteria could include osmotic adjustment, which has been associated with the maintenance of leaf turgor and relative water content at low water potentials in wheat (Morgan, 1983), and which has been shown to give a yield advantage in a range of field environments in eastern Australia (Morgan et al., 1986). The somewhat simpler characteristic of high leaf relative water content under stress has also been positively associated with higher grain yield under water-limited conditions (Schonfeld et al., 1988). At present, selection for high osmotic adjustment is not rapid (Morgan et al., 1986) and selection for high leaf relative water content under stress is poorly characterised. Nevertheless, initial selection for high Δ, to reduce the size of the population to be screened, followed by selection for either high osmotic adjustment or high leaf relative water content under water stess, may be an effective compromise between the requirement for a reasonable yield potential (Fischer, 1979) and the ability to capitalise on that potential (Passioura, 1986).

While the usefulness of low Δ appears to be limited in the southern Wheat Belt, low Δ may be a considerably more useful trait for crops grown in the northern Wheat Belt. In that type of environment, where current rainfall represents a smaller proportion of seasonal water supply, direct soil evaporation is less of a concern, and the need to conserve water for use both before and after anthesis is greater (Loomis, 1983; Richards and Townley-Smith, 1987). Water conservation should be aided by the slower rate of canopy development and lower stomatal conductance associated with low Δ. Some degree of incipient stress may also be likely relatively early in the season. Under such circumstances, stomatal conductance should be reduced, and the relative influence of canopy boundary layer resistance on transpirational water loss also reduced (Jarvis and McNaughton, 1986). Under these conditions, the relationship between crop water-use efficiency and Δ could, in fact, be negative (Cowan, 1988).

Clearly, much more work is necessary to confirm the applicability of using Δ as a selection criterion in either of these wheat-growing environments.

The relationships between grain yield and Δ and crop water-use efficiency and Δ need to be determined under conditions typical of the northern Wheat Belt and also under less favourable conditions in the southern Wheat Belt. For this work, it would be useful to make a clearer distinction between sources of variation in Δ, i.e. stomatal conductance and photosynthetic capacity. Simpler techniques to distinguish between these sources of variation, which may incorporate the measurement of Δ itself, could be developed.

Where variation in Δ is due to variation in stomatal conductance, the influence of scale effects should be determined in a range of field environments. Where Δ varies as a result of variation in photosynthetic capacity, more work is needed to determine the nature and influence of other characteristics that may be associated with low photosynthetic capacity in wheat. These include patterns of dry matter partitioning both above and below the ground and the proportion of carbon lost by respiratory processes.

Experiments in this thesis were done using diverse genotypes spanning a range of Δ values. It would be advantageous to develop populations which differ distinctly in either Δ, conductance or capacity, but which are more homogeneous for other traits. Given the seemingly complex nature of the processes responsible for and associated with variation in Δ, it seems unlikely that such populations would be "isogenic", but they should allow clearer conclusions to be drawn as to the usefulness of Δ as a selection criterion for wheat grown in different environments.

BIBLIOGRAPHY

Al-Khafaf, S., P.J. Wierenga and B.C. Williams. 1978. Evaporative flux from irrigated cotton as related to leaf area index, soil water, and evaporative demand. *Agron. J.*, **70**: 912-917.

Anderson, C.D. and D.W.L. Read. 1966. Water-use efficiency of some varieties of wheat, oats, barley and flax grown in the greenhouse. *Can. J. Plant Sci.*, **46**: 375-378.

Angus, J.F., H.A. Nix, J.S. Russell and J.E. Kruizinga. 1980. Water use, growth and yield of wheat in a subtropical environment. *Aust. J. Agric. Res.*, **31**: 873-886.

Antony, G. and J.P. Brennan. 1988. Improvements in yield potential and bread-making characteristics of wheat in New South Wales: 1925-26 to 1983-84. *NSW Dept. of Agric. Misc. Bull. No. 55.*

Austin, R.B. 1982. Crop characteristics and the potential yield of wheat. *J. Agric. Sci.*, **98**: 447-453.

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

Austin, R.B., M.A. Ford, C.L. Morgan, A. Kaminski and T.E. Miller. 1984. Genetic constraints on photosynthesis and yield in wheat. *Adv. Photos. Res. IV*, **2**: 103-110.

Baldocchi, D.D., S.B. Verma and N.J. Rosenberg. 1985. Water use efficiency in a soybean field : influence of plant water stress. *Agric. and For. Meteorol.*, **34**: 53-65.

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

Bidinger, F., R.B. Musgrave and R.A. Fischer. 1977. Contribution of stored pre-anthesis assimilate to grain yield in wheat and barley. *Nature*, **270**: 431-433.

Bierhuizen, J.F. and R.O. Slatyer. 1965. Effect of atmospheric concentration of water vapour and CO_2 in determining transpiration-photosynthesis relationships of cotton leaves. *Agric. Meteorol.*, **2**: 259-270.

Bleak, A.T. and W. Keller. 1973. Water requirement, yield and tolerance to clipping of some cool-season, semi-arid range grasses. *Crop Sci.*, **13**: 367-371.

Blum, A. 1979. Principles and methodology of selecting for drought resistance in sorghum. *Genet. Agrar.*, **4**: 205-215.

Bradford, K.J., T.D. Sharkey and G.D. Farquhar. 1983. Gas exchange, stomatal behaviour, and $\delta^{13}C$ values of the *flacca* tomato mutant in relation to abscisic acid. *Plant Physiol.*, **72**: 245-250.

Briggs, L.J. and H.L. Shantz. 1913. The water requirements of plants. I. Investigations in the Great Plains in 1910 and 1911. *USDA Bur. Plant Ind. Bull. No. 284.*

Brougham, R.W. 1956. Effect of intensity of defoliation on regrowth of pasture. *Aust. J. Agric. Res.* **7**: 377-387.

Brugnoli, E., K.T. Hubick, S. von Caemmerer, S.C. Wong and G.D. Farquhar. 1988. Correlation between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio of intercellular and atmospheric partial pressures of carbon dioxide. *Plant Physiol.* In press.

Caemmerer, S. von and G.D. Farquhar. 1984. Effects of partial defoliation, changes of irradiance during growth, short-term water stress and growth at enhanced $p(CO_2)$ on the photosynthetic capacity of leaves of *Phaseolus vulgaris* L. *Planta* **160**: 320-329.

Clarke, J.M. and T.N. McCaig. 1982. Excised-leaf water retention capability as an indicator of drought resistance of *Triticum* genotypes. *Can. J. Plant Sci.* **62**: 571-578.

Clarke, J.M. and T.F. Townley-Smith. 1986. Heritability and relationship to yield of excised-leaf water retention in durum wheat. *Crop Sci.*, **26**: 289-292.

Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. *Ann. Rev. Plant Physiol.* **36**: 77-115.

Clawson, K.L., J.E. Specht, B.L. Blad and A.F. Garay. 1986. Water use efficiency in soybean pubescence density isolines - a calculation procedure for estimating daily values. *Agron. J.*, **78**: 483-487.

Colwell, J.D. 1963. The effect of fertilizers and season on the yield and composition of wheat in southern New South Wales. *Aust. J. Exp. Agric. Anim. Husb.*, **3**: 51-61.

Cooper, P.J.M., J.D.H. Keatinge and G. Hughes. 1983. Crop evapotranspiration - a technique for calculation of its components by field measurements. *Field Crops Res.*, **7**: 299-312.

Cornish, E.A. 1950. The influence of rainfall on the yield of wheat in South Australia. *Aust. J. Sci. Res.* **B3**: 178-218.

Cowan, I.R. 1977. Stomatal behaviour and environment. *Adv. Bot. Res.*, **4**: 117-228.

Cowan, I.R. 1988. Stomatal physiology and gas exchange in the field. *In*: Flow and Transport in the Natural Environment: Advances and Applications. pp.160-172. Steffen, W.L. and O.T. Denmead (eds.). Springer-Verlag, Berlin.

Cowan, I.R. and J.H. Troughton. 1971. The relative role of stomata in transpiration and assimilation. *Planta*, **106**: 185-189.

Crauford, P.Q., N.J. Clipson, R.B. Austin and E. Acevedo. 1988. An approach to defining an ideotype for barley in low-rainfall Mediterranean environments. *In*: Proc. Int. Symp. on Improving Winter Cereals Under Temperature and Soil Salinity Stresses. Cordoba, Spain, 1987. ICARDA. In press.

Davies, W.J. 1977. Stomatal responses to water stress and light in plants grown in controlled environments and in the field. *Crop Sci.* **17**: 735-740.

Dobrenz, A.K., L.N. Wright, M.A. Massengale and W.R. Kneebone. 1969a. Water use efficiency and its association with several characteristics of blue panic grass (*Panicum antidotale* Retz.). *Crop Sci.,* **9**: 213-216.

Dobrenz, A.K., L.N. Wright, A.B. Humphrey, M.A. Massengale and W.R. Kneebone. 1969b. Stomate density and its relationship to water-use efficiency in blue panic grass (*Panicum antidotale* Retz.). *Crop Sci.,* **9**: 354-357.

Dobrenz, A.K., D.F. Cole and M.A. Massengale. 1971. Yield components and leaf characteristics associated with the water requirement of alfalfa. *Crop Sci.,* **11**: 124-125.

Donald, C.M. 1962. In search of yield. *J. Aust. Inst. Agric. Sci.,* **28**: 171-178.

Downes, R.W. 1969. Differences in transpiration rates between tropical and temperate grasses under controlled conditions. *Planta,* **88**: 261-273.

Downton, W.J.S., J.R. Grant and S.P. Robinson. 1985. Photosynthetic and stomatal response of spinach leaves to salt stress. *Plant Physiol.* **77**: 85-88.

Doyle, A.D. and H. Marcellos. 1974. Time of sowing and wheat yield in northern New South Wales. *Aust. J. Exp. Agric. Anim. Husb.,* **14**: 93-102.

Doyle, A.D. and R.A. Fischer. 1979. Dry matter accumulation and water use relationships in wheat crops. *Aust. J. Agric. Res.* **30**: 815-829.

Dudley, J.W. and R.H. Moll. 1969. Interpretation and use of estimates of heritability and genetic variances in plant breeding. *Crop Sci.* **9**: 257-262.

Dunstone, R.L., R.M. Gifford and L.T. Evans. 1973. Photosynthetic characteristics of modern and primitive wheat species in relation to ontogeny and adaptation to light. *Aust. J. Biol. Sci.* **26**: 295-307.

Ehleringer, J.R., E.-D. Schulze, H. Zeigler, O.L. Lange, G.D. Farquhar and I.R. Cowan. 1985. Xylem-tapping mistletoes : water or nutrients parasites? *Science,* **227**: 1479-1481.

Ellison, F.W., B.D.H. Latter and T. Anttonen. 1985. Optimal regimes of selection for grain yield and harvest index in spring wheat. *Euphytica,* **34**: 625-640.

Elmore, C.D. 1980. The paradox of no correlation between leaf photosynthesis rates and crop yields. *In*: Predicting Photosynthesis for Ecosystems. Vol 2. pp.155-167. Hesketh, J.D. and J.W. Jones (eds.). CRC Press, Boca Raton, Florida.

Evans, J.R. 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiol.,* **72**: 297-302.

Evans, J.R. and J.R. Seemann. 1984. Differences between wheat genotypes in specific activity of ribulose-1,5-bisphosphate carboxylase and the relationship to photosynthesis. *Plant Physiol.* **74**: 759-765.

Evans, J.R., T.D. Sharkey, J.A. Berry and G.D. Farquhar. 1986. Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. *Aust. J. Plant Physiol.,* **13**: 281-292.

Evans, L.T. 1976. Physiological adaptation to performance as crop plants. *Philos. Trans. R. Soc. Lond., Ser. B,* **275**: 71-83.

Evans, L.T. and R.L. Dunstone. 1970. Some physiological aspects of evolution in wheat. *Aust. J. Biol. Sci.,* **23**: 725-741.

Evans, L.T., J. Bingham, P. Jackson and J. Sutherland. 1972. Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears. *Ann. Appl. Biol.,* **70**: 67-76.

Farquhar, G.D., M.C. Ball, S. von Caemmerer and Z. Roksandic. 1982a. Effect of salinity and humidity on $\delta^{13}C$ value of halophytes - evidence for diffusional isotope fractionation determined by the ratio of intercellular/atmospheric partial pressures of CO_2 under different environmental conditions. *Oecologia,* **52**: 121-124.

Farquhar, G.D., M.H. O'Leary and J.A. Berry. 1982b. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust. J. Plant Physiol.,* **9**: 121-137.

Farquhar, G.D. and R.A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Aust. J. Plant Physiol.,* **11**: 539-552.

Farquhar, G.D., K.T. Hubick, A.G. Condon and R.A. Richards. 1988. Carbon isotope fractionation and plant water-use efficiency. *In*: Applications of Stable Isotope Ratios to Ecological Research (in press). Rundel, P.W., J.R. Ehleringer and K. Nagy (eds.). Springer Verlag, New York.

Field, C., J. Merino and H.A. Mooney. 1983. Compromises between water-use efficiency and nitrogen-use efficiency in five species of California evergreens. *Oecologia*, **60**: 384-389.

Fischer, R.A. 1979. Growth and water limitation to dryland wheat yield in Australia : a physiological framework. *J. Aust. Inst. Agric. Sci.*, **45**: 83-94.

Fischer, R.A. 1980. Influence of water stress on crop yield in semiarid regions. *In*: Adaptation of Plants to Water and High Temperature Stress. pp.323-339. Turner, N.C. and P.J. Kramer (eds.). John Wiley and Sons, New York.

Fischer, R.A. 1981. Optimizing the use of water and nitrogen through breeding of crops. *Plant and Soil*, **58**: 249-278.

Fischer, R.A. and G.D. Kohn. 1966a. The relationship between evapotranspiration and growth in the wheat crop. *Aust. J. Agric. Res.*, **17**: 255-267.

Fischer, R.A. and G.D. Kohn. 1966b. The relationship of grain yield to vegetative growth and post-flowering leaf area in the wheat crop under conditions of limited soil moisture. *Aust. J. Agric. Res.*, **17**: 281-295.

Fischer, R.A. and N.C. Turner. 1978. Plant productivity in the arid and semi-arid zones. *Ann. Rev. Plant Physiol.*, **29**: 277-317.

Fischer, R.A. and M. Sanchez. 1979. Drought resistance in spring wheat cultivars. II. Effects on plant water relations. *Aust. J. Agric.* Res., **30**: 801-814.

Fischer, R.A. and J.T. Wood. 1979. Drought resistance in spring wheat cultivars. III. Yield associations with morpho-physiological traits. *Aust. J. Agric. Res.*, **30**: 1001-1020.

Fischer, R.A., F. Bidinger, J.R. Syme and P.C. Wall. 1981. Leaf photosynthesis, leaf permeability, crop growth, and yield of short spring wheat genotypes under irrigation. *Crop Sci.*, **21**: 367-373.

Fletcher, R.J. 1984. Breeding for frost resistance in early flowering wheat. *In*: Proc. 6th Int. Wheat Genetics Symposium, Kyoto, Japan, 1983. pp.965-969.

Francey, R.J., R.M. Gifford, T.D. Sharkey and B. Weir. 1985. Physiological influences on carbon isotope discrimination in huon pine (*Lagarostrobos franklinii*). *Oecologia*, **66**: 211-218.

Frank, A.B. and R.E. Barker. 1976. Rates of photosynthesis and transpiration and diffusive resistance of six grasses grown under controlled conditions. *Agron. J.*, **68**: 487-490.

French, R.J. and J.E. Schultz. 1984. Water use efficiency of wheat in a mediterranean-type environment. I. The relation between yield, water use and climate. *Aust. J. Agric. Res.*, **35**: 743-764.

Garrity, D.P., C.Y. Sullivan and W.M. Ross. 1982. Alternative approaches to improving grain sorghum productivity under drought stress. *In*: Drought Resistance in Crops with Emphasis on Rice. pp.339-356. International Rice Research Institute, Los Banos, Philippines.

Gifford, R.M. 1977. Growth pattern, carbon dioxide exchange and dry weight distribution in wheat growing under differing photosynthetic environments. *Aust. J. Plant Physiol.,* **4**: 99-110.

Gifford, R.M. and L.T. Evans. 1981. Photosynthesis, carbon partitioning and yield. *Ann. Rev. Plant physiol.,* **32**: 485-509.

Gifford, R.M. and C.L.D. Jenkins. 1982. Prospects for applying knowledge of photosynthesis toward improving crop production. *In*: Photosynthesis. Vol II. Development, Carbon Metabolism, and Plant Productivity. pp.419-457. Govindjee (ed.). Academic Press, New York.

Gifford, R.M., J.H. Thorne, W.D. Hitz and R.T. Giaquinta. 1984. Crop productivity and photoassimilate partitioning. *Science,* **225**: 801-808.

Guy, R.D., D.M. Reid and H.R. Krouse. 1980. Shifts in carbon isotope ratios of two C_3 halophytes under natural and artificial conditions. *Oecologia,* **44**: 241-247.

Guy, R.D. and D.M. Reid. 1986. Photosynthesis and the influence of CO_2-enrichment on $\delta^{13}C$ values in a C_3 halophyte. *Plant, Cell and Environ.,* **9**: 65-72.

Hamblin, A.P., D. Tennant and H. Cochrane. 1982. Tillage and the growth of a wheat crop in a loamy sand. *Aust. J. Agric. Res.,* **33**: 887-897.

Hanson, A.D. and W.D. Hitz. 1982. Metabolic responses of mesophytes to plant water deficits. *Ann. Rev. Plant Physiol.,* **33**: 163-203.

Heichel, G.H. 1971. Confirming measurements of respiration and photosynthesis with dry matter accumulation. *Photosynthetica,* **5**: 93-98.

Hesketh, J. and D. Baker. 1967. Light and carbon assimilation in plant communities. *Crop Sci.,* **7**: 285-293.

Hipps, L.E., G. Asrar and E.T. Kanemasu. 1983. Assessing the interception of photosynthetically active radiation in winter wheat. *Agric. Meteorol.,* **28**: 253-259.

Hsu, J.C. and B.N. Smith. 1972. $^{13}C/^{12}C$ ratios of carbon dioxide from peanut and sunflower seedlings and tobacco leaves in light and darkness. *Plant and Cell Physiol.,* **13**: 689-694.

Hubick, K.T., G.D. Farquhar and R. Shorter. 1986. Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Aust. J. Plant Physiol.,* **13**: 803-816.

Hubick, K.T. and G.D. Farquhar. 1989. Genetic variation in carbon isotope discrimination and the ratio of carbon gained to water lost in barley. Submitted to *Plant, Cell and Environ.*.

Hubick, K.T., R. Shorter and G.D. Farquhar. 1989. Heritability and genotype x environment interactions of carbon isotope discrimination and transpiration efficiency of single plants of peanut (*Arachis hypogoea* L.). Submitted to *Aust. J. Plant Physiol.*.

Hunt, O.J. 1962. Water requirement of selected genotypes of *Elymus junceus* Fisch. and *Agropyron intermedium* and their parent-progeny relationships. *Crop Sci.*, **2**: 97-99.

Hunt, R. 1982. "Plant Growth Curves". Edward Arnold, London.

Hurd, E.A. 1976. Can we breed for drought resistance? *In*: Water Deficits and Plant Growth. Vol. IV. pp.317-353. Kozlowski, T.T. (ed.). Academic Press, New York.

Innes, P. and R.D. Blackwell. 1983. Some effects of leaf posture on the yield and water economy of winter wheat. *J. Agric. Sci.*, **101**: 367-375.

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

Jarvis, P.G. and K.G. McNaughton. 1986. Stomatal control of transpiration : scaling up from leaf to region. *Adv. Ecol. Res.*, **15**: 1-49.

Johnson, D.A., R.A. Richards and N.C. Turner. 1983. Yield, water relations, gas exchange, and surface reflectances of near-isogenic wheat lines differing in glaucousness. *Crop Sci.*, **23**: 318-325.

Jones, H.G. 1976. Crop characteristics and the ratio between assimilation and transpiration. *J. Appl. Ecol.*, **13**: 605-622.

Jones, H.G. 1977. Aspects of water relations of spring wheat (*Triticum aestivum* L.) in response to induced drought. *J. Agric. Sci.*, **88**: 267-282.

Jones, H.G. 1983. "Plants and Microclimate". Cambridge University Press, Cambridge, England.

Jones, H.G. 1987. Breeding for stomatal characters. *In*: Stomatal Function. pp.431-443. Zeiger, E.F., G.D. Farquhar and I.R. Cowan (eds.). Stanford University Press, Stanford, California.

Jones, R.J. and C.J. Nelson. 1979. Respiration and concentration of water soluble carbohydrates in plant parts of contrasting tall fescue genotypes. *Crop Sci.*, **19**: 367-372.

Jordan, W.R. and F.R. Miller. 1980. Genetic variability in sorghum root systems : implications for drought tolerance. *In*: Adaptation of Plants to Water and High Temperature Stress. pp.383-399. Turner, N.C. and P.J. Kramer (eds.). John Wiley and Sons, New York.

Kanemasu, E.T., L.R. Stone and W.L. Powers. 1976. Evapotranspiration model tested for soybean and sorghum. *Agron. J.*, **68**: 569-572.

Kaul, R. 1969. Relations between water status and yield of some wheat varieties. *Z. Pflanzenzuchtg*, **62**: 145-154.

Kaul, R. and W.L. Crowle. 1971. Relations between water status, leaf temperature, stomatal aperture, and productivity of some wheat varieties. *Z. Pflanzenzuchtg*, **65**: 233-243.

Keatinge, J.D.H. and P.J.M. Cooper. 1983. Kabuli chickpea as a winter-sown crop in northern Syria : moisture relations and crop productivity. *J. Agric. Sci.*, **100**: 667-680.

Keim, D.L. and W.E. Kronstad. 1981. Drought response of winter sheat cultivars under field stress conditions. *Crop Sci.*, **21**: 11-15.

Khairi, M.M.A. and A.E. Hall. 1976. Comparative studies of net photosynthesis and transpiration of some citrus species and relatives. *Physiol. Plant.*, **36**: 35-39.

Khan, M.A. and S. Tsunoda. 1970. Evolutionary trends in leaf photosynthesis and related leaf characters among cultivated wheat species and its wild relatives. *Japan J. Breeding*, **20**: 133-140.

Kirkham, M.B., E.L. Smith, C. Dhanasobbon and T.I. Drake. 1980. Resistance to water loss of winter wheat flag leaves. *Cer. Res. Comm.*, **8**: 393-399.

Kohn, G.D. and R.R. Storrier. 1970. Time of sowing and wheat production in southern New South Wales. *Aust. J. Exp. Agric. Anim. Husb.*, **10**: 604-609.

Körner, C., J.A. Scheel and H. Bauer. 1979. Maximum leaf diffusive conductance in vascular plants. *Photosynthetica*, **13**: 45-82.

Kramer, P.J. 1969. "Plant and Soil Water Relationships : A Modern Synthesis". McGraw-Hill, New York.

Legg, B.J., W. Day, D.W. Lawlor and K.J. Parkinson. 1979. The effects of drought on barley growth : models and measurements showing the relative importance of leaf area and photosynthetic rates. *J. Agric. Sci.*, **92**: 703-716.

Loomis, R.S. 1983. Crop manipulations for efficient use of water : an overview. *In*: Limitations to Efficient Water Use in Crop Production. pp.345-374. Taylor, H.M., W.R. Jordan and T.R. Sinclair (eds.). ASA-CSSA-SSSA, Madison, USA.

Ludlow, M.M. 1980. Adaptive significance of stomatal responses to water stress. *In*: Adaptation of Plants to Water and High Temperature Stress. pp.123-138. Turner, N.C. and P.J. Kramer (eds.). John Wiley and Sons, New York.

McCree, K.J. 1974. Changes in stomatal response characteristics of grain sorghum produced by water stress during growth. *Crop Sci.,* **14**: 273-278.

McCree, K.J. 1988. Sensitivity of sorghum grain yield to ontogenetic changes in respiration coefficients. *Crop Sci.,* **28**: 114-120.

McCree, K.J. and S.G. Richardson. 1987. Stomatal closure vs. osmotic adjustment : a comparison of stress responses. *Crop Sci.,* **27**: 539-543.

McWilliam, J.R. 1986. The national and international importance of drought and salinity effects on agricultural production. *Aust. J. Plant Physiol.,* **13**: 1-13.

Marshall, D.R. 1987. Australian plant breeding strategies for rainfed areas. *In*: Drought Tolerance in Winter Cereals. pp.89-99. Srivastava, J.P., E. Porceddu, E. Acevedo and S. Varma (eds.). John Wiley and Sons, New York.

Martin, B. and Y.R. Thorstenson. 1988. Stable carbon isotope composition ($\delta^{13}C$), water use efficiency and biomass productivity of *Lycopersicon esculentum, Lycopersicon pennellii,* and the F_1 hybrid. *Plant Physiol.,* **88**: 213-217.

Masle, J. and G.D. Farquhar. 1988. Effects of soil strength on the relation of water-use efficiency and growth to carbon isotope discrimination in wheat seedlings. *Plant Physiol.,* **86**: 32-38.

Masle, J. and J.B. Passioura. 1987. Effects of soil strength on the growth of wheat seedlings. *Aust. J. Plant Physiol.,* **14**: 643-656.

Medina, E. and P. Minchin. 1980. Stratification of $\delta^{13}C$ values of leaves in Amazonian rainforests. *Oecologia,* **45**: 377-378.

Miskin, K.E., D.C. Rasmussen and D.N. Moss. 1972. Inheritance and physiological effects of stomatal frequency in barley. *Crop Sci.,* **12**: 780-783.

Monteith, J.L. 1977. Climate and the efficiency of crop production in Britain. *Philos. Trans. R. Soc. Lond., Ser. B,* **281**: 277-294.

Morgan, J.M. 1980. Possible role of abscisic acid in reducing seed set in water stressed wheat plants. *Nature,* **285**: 607-614.

Morgan, J.M. 1983. Osmoregulation as a selection criterion for drought tolerance in wheat. *Aust. J. Agric. Res.,* **34**: 607-614.

Morgan, J.M. and A.G. Condon. 1986. Water use, grain yield and osmoregulation in wheat. *Aust. J. Plant Physiol.*, **13**: 523-532.

Morgan, J.M., R.A. Hare and R.J. Fletcher. 1986. Genetic variation in osmoregulation in bread and durum wheats and its relationship to grain yield in a range of field environments. *Aust. J. Agric. Res.*, **37**: 449-457.

Morison, J.I.L. 1987. Intercellular CO_2 concentration and stomatal response to CO_2. *In*: Stomatal Function. pp.229-251. Zeiger, E., G.D. Farquhar and I.R. Cowan (eds.). Stanford University Press, Stanford, California.

Morison, J.I.L. and R.M. Gifford. 1983. Stomatal sensitivity to carbon dioxide and humidity : a comparison of two C_3 and two C_4 grass species. *Plant Physiol*, **71**: 789-796.

Morison, J.I.L. and R.M. Gifford. 1984. Plant growth and water use with limited water supply in high CO_2 concentrations. II. Plant dry weight, partitioning and water use efficiency. *Aust. J. Plant Physiol.*, **11**: 375-384.

Muchow, R.C. 1985. An analysis of the effects of water deficits on grain legumes grown in a semi-arid tropical environment in terms of radiation interception and its efficiency of use. *Field Crops Res.*, **11**: 309-323.

Muchow, R.C., T.R. Sinclair, J.M. Bennett and L.C. Hammond. 1986. Response of leaf growth, leaf nitrogen, and stomatal conductance to water deficits during vegetative growth of field-grown soybean. *Crop Sci.*, **26**: 1190-1195.

Namken, L.N., C.L. Wiegand and W.O. Willis. 1974. Soil- and air-temperatures as limitations to more efficient water use. *Agric. Meteorol.*, **14**: 169-181.

Nerkar, Y.S., D. Wilson and D.A. Lawes. 1981. Genetic variation in stomatal characteristics and behaviour, water use and growth of five *Vicia faba* L. genotypes under contrasting soil moisture regimes. *Euphytica*, **30**: 335-345.

Nix, H.A. 1975. The Australian climate and its effects on grain yield and quality. *In*: Australian Field Crops. Vol I. Wheat and Other Temperate Cereals. pp.183-226. Lazenby, A. and E.M. Matheson (eds.). Angus and Robertson, Sydney.

O'Leary, G.J., D.J. Connor and D.H. White. 1985. Effect of sowing time on growth, yield and water-use of rain-fed wheat in the Wimmera, Victoria. *Aust. J. Agric. Res.*, **36**: 187-196.

O'Leary, M.H. 1981. Carbon isotope fractionation in plants. *Phytochem.*, **20** 553-567.

O'Toole, J.C. 1982. Adaptation of rice to drought-prone environments. *In*: Drought Resistance in Crops with Emphasis on Rice. pp.195-213. International Rice Research Institute, Los Banos, Philippines.

Orth, R.A., R.J. Baker and W. Bushuk. 1972. Statistical evaluation of techniques for predicting baking quality of wheat cultivars. *Can. J. Plant Sci.*, **52**: 139-146.

Park, R. and S. Epstein. 1961. Metabolic fractionation of C^{13} and C^{12} in plants. *Plant Physiol.*, **36**: 133-138.

Passioura, J.B. 1976. Physiology of grain yield in wheat growing on stored water. *Aust. J. Plant Physiol.*, **3**: 559-565.

Passioura, J.B. 1977. Grain yield, harvest index, and water use of wheat. *J. Aust. Inst. Agric. Sci.*, **43**: 117-120.

Passioura, J.B. 1982. The role of root system characteristics in the drought resistance of crop plants. *In*: Drought Resistance in Crops with Emphasis on Rice. pp.71-82. International Rice Research Institute, Los Banos, Philippines.

Passioura, J.B. 1986. Resistance to drought and salinity : avenues for improvement. *Aust. J. Plant Physiol.*, **13**: 191-201.

Philip, J.R. 1957. Evaporation, and moisture and heat fields in the soil. *J. Met.*, **14**: 354-366.

Planchon, C. 1979. Photosynthesis, transpiration resistance to CO_2 transfer, and water efficiency of flag leaf of bread wheat, durum wheat and triticale. *Euphytica*, **28**: 403-408.

Poskuta, J.W. and C.J. Nelson. 1986. Role of photosynthesis and photorespiration and of leaf area in determining yield of tall fescue genotypes. *Photosynthetica*, **20**: 94-101.

Quarrie, S.A. 1980. Genotypic differences in leaf water potential, abscisic acid and proline concentration in spring wheat during drought stress. *Ann. Bot.*, **46**: 383-394.

Rawson, H.M. and G. Hofstra. 1969. Translocation and remobilization of ^{14}C assimilated at different stages by each leaf of the wheat plant. *Aust. J. Biol. Sci.* **22**: 321-331.

Rawson, H.M., R.M. Gifford and P.M. Bremner. 1976. Carbon dioxide exchange in relation to sink demand in wheat. *Planta*, **132**: 19-23.

Rawson, H.M., J.E. Begg and R.G. Woodward. 1977. The effect of atmospheric humidity on photosynthesis, transpiration and water use efficiency of leaves of several plant species. *Planta*, **134**: 5-10.

Rawson, H.M., J.H. Hindmarsh, R.A. Fischer and Y.M. Stockman. 1983. Changes in leaf photosynthesis with plant ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny. *Aust. J. Plant Physiol.*, **10**: 503-514.

Rawson, H.M. and J.M. Clarke. 1988. Nocturnal transpiration in wheat. *Aust. J. Plant Physiol.*, **15**: 397-406.

Richards, R.A. 1982. Breeding and selection for drought resistance in wheat. *In*: Drought Resistance in Crops with Emphasis on Rice. pp.303-316. International Rice Research Institute, Los Banos, Philippines.

Richards, R.A. 1984. Glaucousness in wheat, its effect on yield and related characteristics in dryland environments, and its control by minor genes. *In*: Proc. 6th Int. Wheat Genetics Symposium, Kyoto, Japan, 1983. pp 447-451.

Richards, R.A. 1987. Physiology and the breeding of winter-grown cereals for dry areas. *In*: Drought Tolerance in Winter Cereals. pp.133-150. Srivastva, J.P., E. Porceddu, E. Acevedo and S. Varma (eds.) John Wiley and Sons, New York.

Richards, R.A. and J.B. Passioura. 1981. Seminal root morphology and water use of wheat. I. Environmental effects. *Crop Sci.*, **21**: 249-252.

Richards, R.A., H.M. Rawson and D.A. Johnson. 1986. Glaucousness in wheat : its development and effect on water-use efficiency, gas-exchange and photosynthetic tissue temperatures. *Aust. J. Plant Physiol.*, **13**: 465-473.

Richards, R.A. and T.F. Townley-Smith. 1987. Variation in leaf area development and its effects on water use, yield and harvest index of droughted wheat. *Aust. J. Agric. Res.*, **38**: 983-992.

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

Ritchie, J.T. 1972. Model for predicting evaporation from a row crop with incomplete cover. *Water Resour. Res.*, **8**: 1204-1213.

Roeske, C.A. and M. O'Leary. 1984. Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose bisphosphate. *Biochemistry*, **23**: 6275-6284.

Roy, N.N. and B.R. Murty. 1976. A selection procedure in wheat for stress environments. *Euphytica*, **19**: 509-521.

Russell, J.S. 1968. Nitrogen fertilizer and wheat in a semi-arid environment. 2. Climatic factors affecting response. *Aust. J. Exp. Agric. Anim. Husb.*, **8**: 223-231.

Salter, P.J. and J.E. Goode. 1967. "Crop Responses to Water at Different Stages of Growth". Commonwealth Agricultural Bureaux.

Schonfeld, M.A., R.C. Johnson, B.F. Carver and D.W. Mornhinweg. 1988. Water relations in winter wheat as drought resistance indicators. *Crop Sci.*, **28**: 526-531.

Seemann, J.R. and C. Critchley. 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta*, **164**: 151-162.

Seif, E. and D.G. Pederson. 1978. Effect of rainfall on the grain yield of spring wheat, with an application to the analysis of adaptation. *Aust. J. Agric. Res.*, **29**: 1107-1115.

Shantz, H.L. and L.N. Piemeisel. 1927. The water requirement of plants at Akron, Colorado. *J. Agric. Res.*, **34**: 1093-1190.

Shawcroft, R.W. and H.R. Gardner. 1983. Direct evaporation from soil under a row crop canopy. *Agric. Meteorol.*, **28**: 229-238.

Shimshi, D. and J. Ephrat. 1975. Stomatal behaviour of wheat cultivars in relation to their transpiration, photosynthesis and yield. *Agron. J.*, **67**: 326-331.

Sinclair, T.R., G.E. Bingham, E.R. Lemon and L.H. Allen. 1975. Water-use efficiency of field-grown maize during water stress. *Plant Physiol.*, **56**: 245-248.

Sinclair, T.R., C.B. Tanner and J.M. Bennett. 1984. Water-use efficiency in crop production. *BioSci.*, **34**: 36-40.

Singh, T.N., L.G. Paleg and D. Aspinall. 1973. Stress metabolism. III. Variations in response to water deficit in the barley plant. *Aust. J. Biol. Sci.*, **26**: 65-76.

Single, W.V. 1961. Studies on frost injury to wheat. I. Laboratory freezing tests in relation to the behaviour of varieties in the field. *Aust. J. Agric. Res.*, **12**: 767-773.

Slatyer, R.O. 1973. Effects of short periods of water stress on leaf photosynthesis. In: Plant Responses to Climatic Factors. pp.271-276. UNESCO, Paris.

Squire, G.R. and C.R. Black. 1981. Stomatal behaviour in the field. *In*: Stomatal Physiology. pp.223-245. Jarvis, P.G. and T.A. Mansfield (eds.). Soc. Exp. Bot. Seminar Series 8.

Stahl, R.S. and K.J. McCree. 1988. Ontogenetic changes in the respiration coefficients of grain sorghum. *Crop Sci.*, **28**: 111-113.

Stanhill, G. 1985. The water resource for agriculture. *Phil. Trans. R. Soc. Lond., Ser B.*, **310**: 161-173.

Stephens, C.G. 1962. "A Manual of Australian Soils" (3rd edition). CSIRO, Melbourne.

Synnott, K. 1944. "The Farmers' Handbook" (5th edition). NSW Dept. of Agriculture, Sydney.

Tanner, C.B. 1981. Transpiration efficiency of potato. *Agron. J.*, **73**: 59-64.

Tanner, C.B. and W.A. Jury. 1976. Estimating evaporation and transpiration from a row crop during incomplete cover. *Agron. J.*, **68**: 239-243.

Tanner, C.B. and T.R. Sinclair. 1983. Efficient water use in crop production : research or re-search? *In*: Limitations to Efficient Water Use in Crop Production. pp.1-27. Taylor, H.M., W.R. Jordan and T.R. Sinclair (eds.). ASA-CSSA-SSSA, Madison, USA.

Taylor, H.M. and H.T. Nguyen. 1987. Opportunities for manipulating root systems to reduce drought stress in wheat. *In*: Drought Tolerance in Winter Cereals. pp.285-299. Srivastava, J.P., E. Porceddu, E. Acevedo and S. Varma (eds.). John Wiley and Sons, New York.

Teare, I.D., E.T. Kanemasu, W.L. Powers and H.S. Jacobs. 1973. Water-use efficiency and its relation to crop canopy area, stomatal regulation, and root distribution. *Agron. J.*, **65**: 207-211.

Thorne, G.N. 1974. Physiology of grain yield of wheat and barley. Rothamstead Experimental Station Report for 1973, part 2 : 5-25.

Troughton, J.H. 1979. $\delta^{13}C$ as an indicator of carboxylation reactions. *In*: Encyclopaedia of Plant Physiology New Series. Photosynthesis II. Photosynthetic carbon metabolism and related processes. Vol. 6. pp.140-149. Gibbs, M. and E. Latzko (eds.). Springer-Verlag, New York.

Troughton, J.H., K.A. Card and C.H. Hendy. 1974. Photosynthetic pathways and carbon isotope discrimination by plants. *Carnegie Inst. Wash. Yearbook*, **73**: 768-780.

Turner, N.C. 1981. Designing crops for dryland Australia : can the deserts help us? *J. Aust. Inst. Agric. Sci.*, **47**: 29-34.

Unger, P.W. and B.A. Stewart. 1983. Soil management for efficient water use : an overview. *In*: Limitations to Efficient Water Use in Crop production. pp.419-460. Taylor, H.M., W.R. Jordan and T.R. Sinclair (eds.). ASA-CSSA-SSSA, Madison, USA.

Walker, G.K. 1983. Measurement of evaporation from soil beneath crop canopies. *Can. J. Soil Sci.*, 63: 137-141.

Waring, S.A., W.E. Fox and L.J.H. Teakle. 1958. Fertility investigations on the black earth wheatlands of the Darling Downs, Queensland. II. Moisture and evapotranspiration in relation to the wheat crops. *Aust. J. Agric. Res.*, 9:717-729.

Werk, K.S., J. Ehleringer, I.N. Forseth and C.S. Cook. 1983. Photosynthetic characteristics of Sonoran Desert winter annuals. *Oecologia*, **59**: 101-105.

White, J.W., J.A. Costello and J. Ehleringer. 1988. Correlations between carbon isotope ratio and yield of common beans in two environments. Submitted to *Crop Sci.*.

Wilson, D. 1975. Variation in leaf respiration in relation to growth and photosynthesis of *Lolium*. *Ann. Appl. Biol.*, **80**: 323-328.

Wilson, D. 1984. Identifying and exploiting genetic variation in the physiological components of production. *Ann. Appl. Biol.*, **104**: 527-536.

Wilson, D. and J.G. Jones. 1982. Effect of selection for dark respiration rate of mature leaves on crop yields of *Lolium perenne* cv. S.23. *Ann. Bot.*, **49**: 313-320.

Winter, K. 1981. CO_2 and water vapour exchange, malate content and $\delta^{13}C$ value in *Cicer arietinum* grown under two water regimes. *Z. Pflanzenphysiol.*, **101**: 421-430.

Winter, K., J.A.M. Holtum, G.E. Edwards and M.H. O'Leary. 1982. Effect of low relative humidity on $\delta^{13}C$ values in two C_3 grasses and in *Panicum milioides*, a C_3-C_4 intermediate species. *J. Exp. Bot.*, **33**: 88-91.

Wong, S.C., I.R. Cowan and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature*, **282**: 424-426.

Woodruff, D.R. and J. Tonks. 1983. Relationship between time of anthesis and grain yield of wheat genotypes with differing developmental patterns. *Aust. J. Agric. Res.*, **34**: 1-11.

Yoshie, F. 1986. Intercellular CO_2 concentration and water-use efficiency of temperate plants with different life-forms and from different microhabitats. *Oecologia*, **68**: 370-374.

Zur, B. and J.W. Jones. 1984. Diurnal changes in the instantaneous water use efficiency of a soybean crop. *Agric. and For. Meteorol.*, **33**: 41-51.

APPENDIX 1.

SEASONAL AND SITE-TO-SITE VARIATION IN CARBON ISOTOPE DISCRIMINATION AMONG WHEAT GENOTYPES

A1.1 INTRODUCTION

Before the measurement of carbon isotope discrimination (Δ) is adopted in plant breeding, it is essential that the measurement of Δ satisfies the requirements of a breeding programme. These requirements are that the measurement is rapid and accurate, that there be substantial genetic variation, that environmental variation can be minimised and that in the target environment, genotype x environment interactions are minor. Because Δ can be determined on any plant part at any time in the plant's life, much of the environmentally induced variation in Δ could be minimised by the identification of the most appropriate sampling time.

There are numerous potential sources of non-genotypic variability in the measurement of Δ. Those that can be readily overcome by technical or sampling means are those concerned with the composition of plant dry matter (O'Leary, 1981) and the size and storage of the dry matter sample (Farquhar et al., 1988).

Other sources of variation in Δ among plant organs result from temporal variation in the growth environment, i.e. environmental effects *per se*. Increased salinity (Guy et al., 1980; Downton et al., 1985; Seemann and Critchley, 1985; Guy and Reid, 1986), decreased soil water availability (Winter, 1981; Farquhar and Richards, 1984; Hubick et al., 1986) and increased vapour pressure deficit (Winter et al., 1982) all result in lower values of Δ in plant material. This effect is largely associated with the decreased stomatal conductance that accompanies each of these environmental perturbations.

Genotype x environment interactions were significant in the study on wheat by Farquhar and Richards (1984), and were a response to water stress. However, in the study presented in Chapter 2, genotype x environment interaction was not important, but then both environments tested were similar.

In this study, the magnitude and some possible causes of variation in Δ is examined among genotypes grown at several field sites around Australia. In particular, site-to-site and seasonal variation in water availability are examined as sources of non-genotypic variation in Δ of wheat.

A1.2 MATERIALS AND METHODS

To assess seasonal variation in Δ among genotypes, several organs were sampled at various times during the growing season from plants of 8 wheat genotypes sown in the 4-replicate trial at Moombooldool, New South Wales, in 1985 (Chapter 5). Six bread wheats (*Triticum aestivum* L.) and two durum wheats (*T. turgidum* L. *durum*) were chosen spanning an expected range in Δ of about 2 x 10^{-3} (Table 5.2). Cultural conditions for the experiment are described in Section 5.2.2.

Sampling for Δ measurements started on July 10 and proceeded at approximately 21 d intervals. For each harvest before anthesis, a subsample of the last fully expanded leaves was taken from each plot. These corresponded to, approximately, leaves 3.5, 5, 6.5, 8 and 9 (the flag leaf). Stem material from the third harvest was also measured. From a final hand harvest at physiological maturity (November 21), the peduncle (i.e. stem material from above the flag leaf node), grain and also the rachis were subsampled. For each organ a single measurement of Δ was made on the material sampled from each plot. Data presented for each organ are the means of the four replicate plots of each genotype.

Changes in soil water through the season were measured to a depth of 2m using neutron moisture meters as described in Section 5.2.6. Soil water content readings were first taken on July 10, 48 d after sowing, by which time the third leaf was fully emerged, and then every 21 d (approximately) thereafter until crop maturity in mid-November.

On several dates during the season, stomatal conductance data were obtained on all genotypes using a Delta-T Mk III diffusive resistance porometer. Data were obtained on uppermost fully expanded leaves only, between 1000h and 1500h (depending on cloud cover), on days with extended periods of bright sunshine and clear sky or (on one occasion) of uniform overcast. Data were obtained for both the adaxial and abaxial surfaces of all genotypes but only for surfaces facing upwards. For each genotype the mean conductance of 4 measurements on each surface was calculated and these were summed to give a leaf conductance.

Site-to-site variation in Δ among genotypes was measured in experiments on plant material from 2 broad sources.

(i) Unreplicated grain samples were obtained from trials which compared a range of genotypes in several environments. These samples were from 7 trials grown as part of the Australian Interstate Wheat Variety Trials Program (Table A1.2). In these trials, advanced breeders' lines from each State are grown, together with common check varieties, at several sites around Australia. Samples were obtained from 2 trials grown in 1983; Wongan Hills (Western Australia) and Rutherglen (Victoria). Samples from 5 sites from the 1984 series were obtained; Merredin (Western Australia), Urania (South Australia), Nangari (South Australia), Rutherglen (Victoria) and Condobolin (New South Wales). Growing-season rainfall varied considerably among these trials (Table A1.2).
(ii) Replicated peduncle samples were taken from 6 trials grown in southern New South Wales in 1985 (Table A1.2). Five of these trials were part of the Southern Wheat Variety Evaluation Project, conducted by the New South Wales Department of Agriculture, in which advanced breeding lines and currently-grown cultivars are compared at several sites throughout the region. Samples for Δ measurement were taken from trials sown at Cootamundra (in the eastern part of the wheat belt), Moombooldool (in the western part) and Goolgowi (on the western edge), average annual and growing-season precipitation declining from east to west. At both Moombooldool and Goolgowi, two trials were sampled; an early-sown trial designed to assess late-maturing genotypes and a later-sown trial designed to assess earlier-maturing genotypes. Only the late-sown trial was sampled at Cootamundra. A further independent trial was grown at Moombooldool in 1985 similar to those at Wagga Wagga and Yanco in 1984 (Chapter 2). Thirty-four genotypes were grown in this trial, 28 bread wheats (*T. aestivum*), 3 durums (*T. turgidum* L. *durum*) and 3 triticales (*Triticosecale* Wittmack). The genotypes were chosen for their diversity and will be referred to below as the Miscellaneous Trial. The Miscellaneous Trial was a 2-replicate randomized-block design, plot size being 8 rows (at 18 cm spacing) x 6m. The five Southern Wheat Trials were 3-replicate randomized-block designs with plots 8 rows (at 18 cm spacing) x 40m. The peduncle material measured for Δ from all six trials came from subsamples of approximately 0.6 m of plot row harvested at ground level immediately before machine harvesting. These cuts were also used to estimate harvest index in each plot.

Carbon isotope discrimination was measured on the plant dry matter samples from all experiments using the technique and equipment described in Section 2.2.3. The replicated measurements from the 1985 Miscellaneous and Southern Wheat Variety Evaluation Trials were analysed by analysis of variance. From these analyses, estimates of broad sense heritability, h_B^2, for Δ and harvest index for each trial were obtained using the following equation (Dudley and Moll, 1969),

$$h_B^2 = \frac{\sigma_g^2}{\sigma_e^2 + \sigma_g^2} \qquad \text{(A1.1)}$$

where σ_g^2 and σ_e^2 are the variances attributable to genotypic and environmental effects respectively. The Genotype Mean Square provides an estimate of $(\sigma_e^2 + n.\sigma_g^2)$, where n = the number of replications, and the Residual Mean Square provides an estimate of σ_e^2.

A1.3 RESULTS

A1.3.1. Seasonal variation in Δ among genotypes.

During the early part of the season, Δ values measured in different leaves were reasonably stable, with a mean value of approximately 19.6 x 10^{-3} for the last fully expanded leaves from the first four harvests (Fig. A1.1). Δ of stem material sampled at the third harvest had a mean value 0.3 x 10^{-3} lower than that of leaf material from the same harvest (data not shown). Over the latter half of the season the value of Δ dropped substantially in all genotypes. Δ values declined progressively below that measured in leaf 8 harvested in early September in the following order: Δ of Leaf 8 > Δ of Flag leaf > Δ of Rachis > Δ of Peduncle > Δ of Grain. This order corresponds exactly with time of formation of each organ. The mean Δ value measured in the grain at the final harvest was 15.25 x 10^{-3}.

The decline in Δ values over the latter half of the season was associated with a large decline in stomatal conductance in all genotypes (Fig. A1.2). The decline in stomatal conductance could be attributed to the effects of declining soil water availability and/or increasing vapour pressure deficit, the coincidence of these two factors in this environment being well illustrated in Figure A1.3.

All organs sampled throughout the season showed significant and substantial genotypic variation in Δ (Fig. A1.1). The range in Δ between

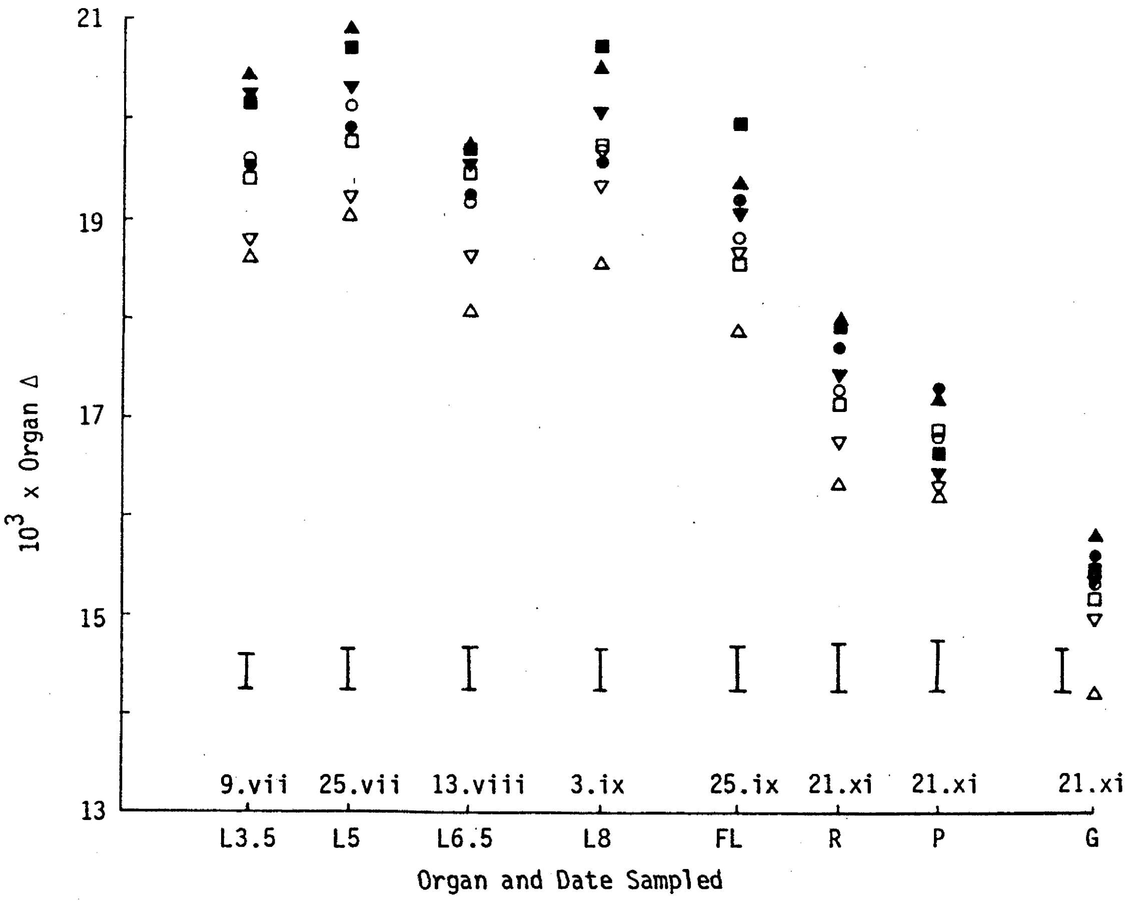


Figure A1.1. Carbon isotope discrimination, Δ, measured in different organs of eight genotypes over the course of the growing-season. The organs were sampled on the dates indicated. Organs sampled were leaf 3.5 (L3.5), leaf 5 (L5), leaf 6.5 (L6), leaf 8 (L8), the flag leaf (FL), rachis (R), peduncle (P), and grain (G). The leaf number shown is the mean last fully expanded leaf on plants sampled at the date indicated. Δ values presented are means of the four replicate plots of each genotype. The bars indicate the LSD (P=0.05) for each organ.

Symbols:

▲ Yallaroi, ■ Gutha, ● RAC 430, ▼ 820357, ○ Blade,
□ Hartog, ▽ Sundor, △ Quarrion

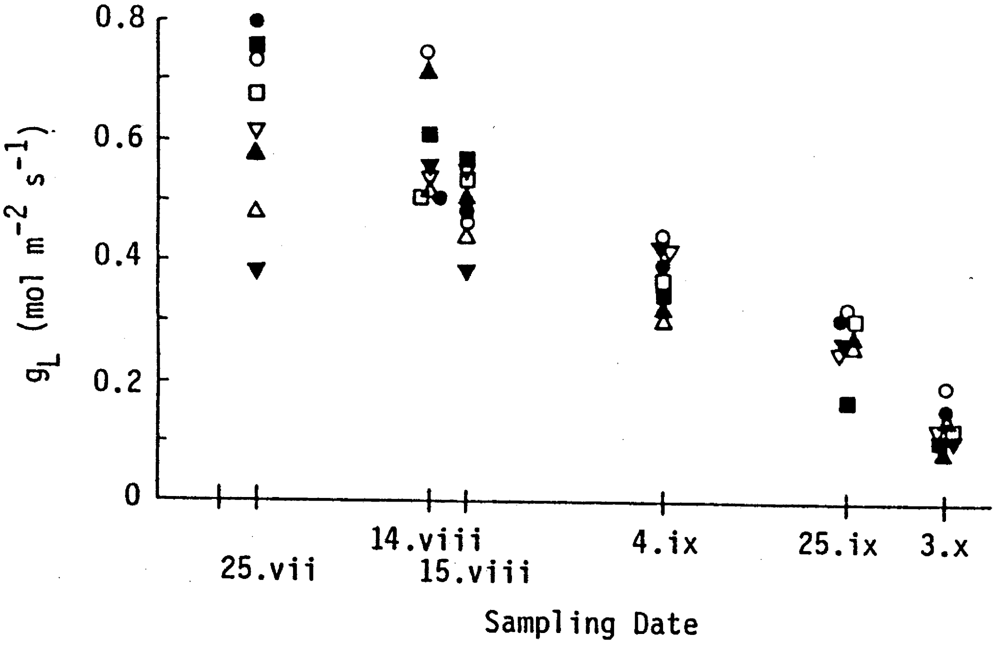


Figure A1.2. Genotypic and seasonal variation in leaf conductance. Measurements were taken on the dates indicated. Symbols as in Figure A1.1.

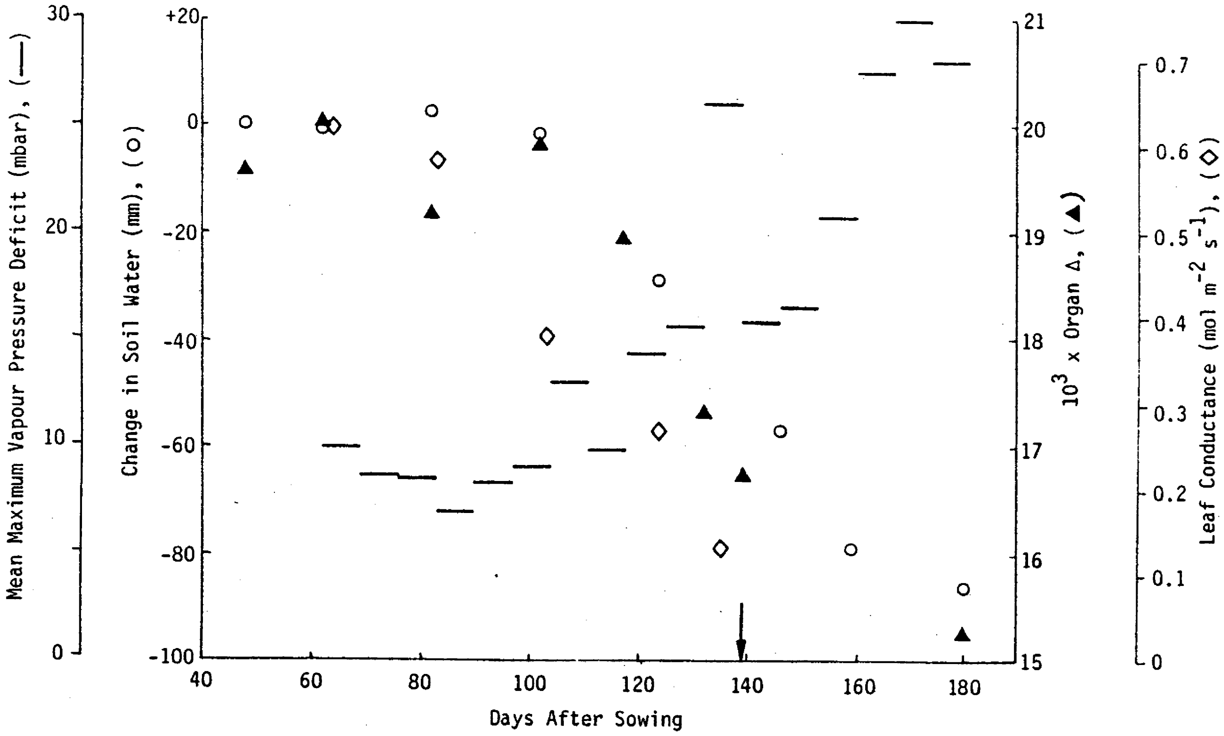


Figure A1.3. Seasonal changes in soil-water (open circles), leaf conductance (open diamonds), organ Δ (closed triangles) and weekly mean maximum vapour pressure deficit (horizontal bars). Values indicated for soil-water, leaf conductance and organ Δ are mean values for the eight genotypes. For the flag leaf, rachis and peduncle, the Δ values shown have been transposed to a date approximating full expansion of these organs, Δ having been measured at a later date. Mean anthesis date is indicated by the arrow.

genotype means was approximately 1.8 x 10^{-3} for each organ measured except the peduncle, for which the range in Δ was only 1.1 x 10^{-3}. The coefficient of variation for Δ of each organ sampled never exceeded 2.0%. Variability did increase slightly as the season progressed, as indicated by the LSD's shown for each organ in Fig. A1.1.

Genotypic ranking for the various plant parts measured in 1985 was fairly consistent throughout the season (Fig. A1.1). Gutha ranked high early in the season but declined later. RAC 430, ranked in the middle early, improved its ranking towards the end of the season. Changes in genotypic ranking late in the season were reflected in the correlation coefficients for relationships between Δ values measured in the different plant parts. While most correlation coefficients were significant, those involving the peduncle (and to a lesser extent the grain) were the poorest (Table A1.1).

A1.3.2. Site-to-site variation in Δ among genotypes.

Genotypic ranking between the two sites sampled from the 1983 Interstate Wheat Variety Trials was consistent; Δ values were significantly correlated ($r = 0.82$, $P<0.01$; Fig. A1.4). The mean Δ value of grain from Wongan Hills was 14.5 x 10^{-3}, 2 x 10^{-3} lower than that from Rutherglen (Table A1.3). This decrease is consistent with the difference in annual and growing-season rainfall between the two sites (Table A1.2) and also with differences in vapour pressure deficit between the two sites. In 1984 the range in site-mean Δ of grain was greater than in 1983, 3 x 10^{-3}, with Δ at Merredin and Nangari in 1984 being considerably lower than at Wongan Hills the previous season (Table A1.3). Growing season rainfall in 1984 was higher than average throughout the southern wheatbelt but there was considerable variation among sites (Table A1.2). This variation may have contributed to an apparent genotype x site interaction for grain Δ. This interaction cannot be tested for statistical significance, since the Δ measurements were on unreplicated samples. However, site to site correlations for grain Δ became weaker as the difference in growing-season rainfall between sites became greater (Table A1.3). In Table A1.3, site-mean Δ of grain is taken as an indicator of stress level, with sites being ranked accordingly. Δ tended to decline more in high Δ lines as stress level increased, the range in Δ being greatest at the wettest site, Rutherglen (Table A1.3).

Table A1.1. Correlation coefficients, r, for relationships between carbon isotope discrimination, Δ, measured in different plant organs over the course of the season. At P = 0.05, r = 0.35. At P = 0.01, r = 0.45. (df = 30).

1. Δ of Leaf 3.5	1.00								
2. Δ of Leaf 5	0.88	1.00							
3. Δ of Leaf 6.5	0.84	0.82	1.00						
4. Δ of Stem	0.85	0.85	0.84	1.00					
5. Δ of Leaf 8	0.81	0.85	0.81	0.81	1.00				
6. Δ of Flag Leaf	0.73	0.80	0.75	0.72	0.83	1.00			
7. Δ of Rachis	0.77	0.80	0.78	0.77	0.82	0.81	1.00		
8. Δ of Peduncle	0.42	0.47	0.52	0.45	0.38	0.50	0.70	1.00	
9. Δ of Grain	0.65	0.73	0.77	0.78	0.70	0.75	0.86	0.74	1.00
	1.	2.	3.	4.	5.	6.	7.	8.	9.

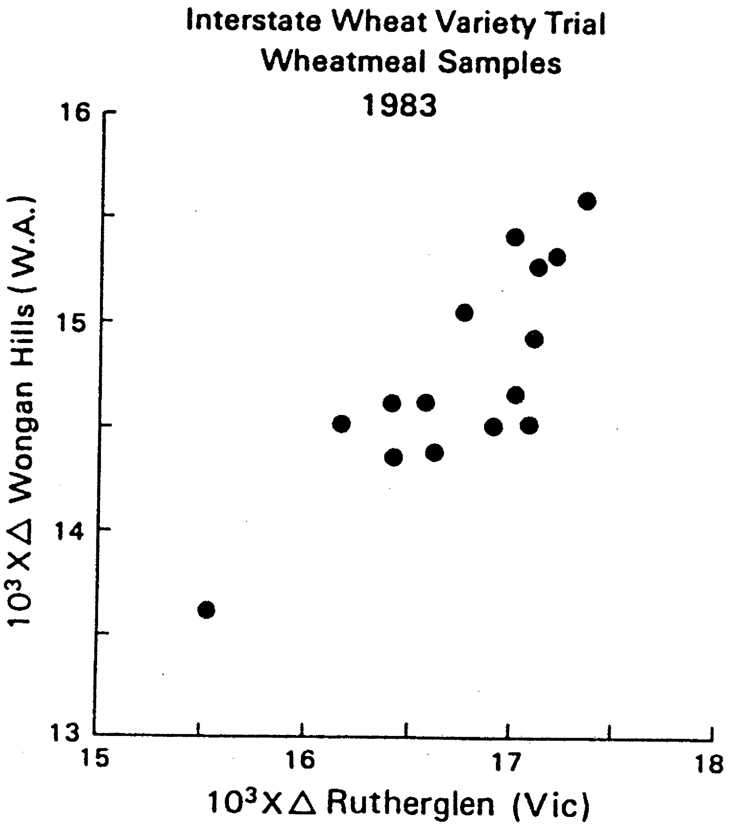


Figure A1.4. Relationship between grain Δ measured in wheatmeal samples of 15 genotypes grown at two sites, Wongan Hills, W.A., and Rutherglen, Vic., in the Interstate Wheat Variety Trials in 1983 ($r = 0.82$, $P<0.01$).

Table A1.2. Details of trials sampled for genotypic and site-to-site variation in carbon isotope discrimination. Rainfall figures in brackets are long-term averages for each site, where known.

Trial		Sowing Date	Annual Rainfall (mm)	April-November Rainfall (mm)	Site Mean Yield (g m^{-2})
Interstate Trials					
Rutherglen	1983	20.5.83	694(563)	570(414)	324
Wongan Hills	1983	22.6.83	449(345)	415(294)	275
Rutherglen	1984	17.5.84	595(563)	388(414)	392
Condobolin	1984	9.5.84	669(417)	357(266)	208[1]
Urania	1984	13.6.84	381(362)	357(306)	268
Nangari	1984	5.7.84	-	-	149
Merredin	1984	25.5.84	334(289)	262(229)	197
Southern Wheat Trials 1985					
Goolgowi Early		1.5.85	423(325)	308	371
Moombooldool Early		30.4.85	399(425)	327	254
Goolgowi Late		13.5.85	423(325)	308	407
Moombooldool Late		17.5.85	399(425)	327	331
Cootamundra Late		23.5.85	639(609)	481	472
"Miscellaneous" Trial		21.5.85	399(425)	327	407

[1] Grain yields at Condobolin in 1984 were affected by late frost. Genotype mean yields ranged from 80 to 320gm^{-2}.

Table A1.3. Summary of between- and within-site variation in carbon isotope discrimination, Δ, measured in grain of 15 genotypes grown at 5 sites in the 1984 Interstate Wheat Variety Trials. For each site, the site-mean Δ value ($\bar{\Delta}$) and the measured range in Δ is given. Correlation coefficients, r, for relationships between Δ of grain from the 15 genotypes at the different sites are also shown (at $P = 0.05$, $r = 0.514$; at $P = 0.01$, $r = 0.641$).

Site	$10^3 \times \bar{\Delta}$	Range in Δ	Correlation Matrix for Δ				
1. Rutherglen	16.39	1.51	1.000				
2. Condobolin	14.95	1.01	0.758	1.000			
3. Urania	14.43	1.23	0.630	0.492	1.000		
4. Nangari	13.64	1.15	0.221	0.145	0.618	1.000	
5. Merredin	13.39	1.16	0.261	-0.141	0.525	0.355	1.000
			1.	2.	3.	4.	5.

For the sites studied in 1985, growing season rainfall was again slightly above average, although heavy rain in November tended to inflate the figures given in Table A1.2. The mean Δ values for the late-sown Southern Wheat Trials and Miscellaneous Trial were higher than those for the early-sown Southern Wheat Trials, about 16.3 x 10^{-3} for the later trials and 15.7 x 10^{-3} for the early trials (Table A1.4). (The mean Δ value for bread wheats in the early-sown trial at Goolgowi was lower than that shown in Table A1.4. Three winter barleys were grown at Goolgowi which had substantially higher Δ values than any of the wheats grown there). At Cootamundra, the wetter of the 3 sites, the range in peduncle Δ was greater, 2.4 x 10^{-3}, and the mean Δ value higher, 17.6 x 10^{-3}, than for the other 2 late-sown Southern Wheat Trials.

There was significant genotype x site interaction for Δ in the late-sown Southern Trials (F ratio 1.88, $P<0.01$) but not in the early-sown Trials (F ratio 1.64, $P>0.05$). Broad-sense heritability for Δ ranged from 41% for the late-sown Southern Wheat Trial at Moombooldool to 75% for the early-sown trial at Goolgowi. These two trials had the highest (40) and lowest (15) numbers of genotypes respectively. In the other 4 trials broad-sense heritability for Δ ranged between 56% and 66%. These estimates of broad-sense heritability for Δ were similar to those obtained for harvest index (Table A1.4).

A1.4 DISCUSSION

Significant genotype x environment interaction for Δ was observed in the late-sown 1985 Southern Wheat Variety Evaluation trials and was also implied by the poor correlations between Δ of grain from the more contrasting sites sampled from the 1984 Interstate Wheat Variety Trials. There was no significant interaction in the early-sown Southern Wheat trials sampled, in which genotypes were grown in similar environments at Moombooldool and Goolgowi, or in the study of 27 genotypes grown in 2 similar but wetter environments at Wagga Wagga and Yanco in 1984 (Chapter 2). White et al. (1988) recently reported significant genotype x environment interaction for Δ measured in leaves when 10 bean lines were grown under contrasting irrigated and rainfed conditions at one site, but not when 2 similar rainfed sites were compared.

Broad-sense heritability estimates for Δ from the experiments reported here (average 60%) were also substantially lower than that from the study reported in Chapter 2 (80%). These relatively low heritability

Table A1.4. Summary of between- and within-site variation in carbon isotope discrimination, Δ, measured in the peduncle of genotypes grown in 6 trials in southern New South Wales in 1985. For each trial the number of genotypes grown (n), the mean Δ value ($\bar{\Delta}$), the measured range in Δ, the LSD at P = 0.05 for Δ, and estimates of broad-sense heritability for Δ, $h_B^2(\Delta)$, are given. Estimates of broad-sense heritability for harvest index, $h_B^2(HI)$, are also shown for comparison.

Trial	n	$10^3 \times \bar{\Delta}$	Range in Δ	LSD	$h_B^2(\Delta)$ %	$h_B^2(HI)$ %
Moombooldool Early	20	15.50	1.75	0.63	62	61
Goolgowi Early	15	16.04	2.71[1]	0.82	75	25
Cootamundra Late	26	17.61	2.43	0.63	56	46
Moombooldool Late	40	16.21	1.72	0.66	41	66
Goolgowi Late	26	16.46	1.96	0.61	64	69
Miscellaneous Trial	34	16.25	1.95	0.64	64	54

[1] Range in Δ for bread wheats only in Goolgowi Early trial 1.45×10^{-3}. Three winter barleys extended measured range to 2.71×10^{-3}.

estimates, as well as the significant genotype x site interaction for Δ, may have resulted from the organ chosen for the measurement of Δ in these experiments, the peduncle.

Although the peduncle was convenient to subsample, measurements of genotypic and seasonal variation in Δ in organs sampled from the 8-genotype experiment at Moombooldool indicate that, of the organs measured, the peduncle gave the most variable results and was the least representative of genotypic ranking for Δ throughout the season. Correlations between Δ of different organs were poorest for those involving the peduncle.

The increased variability found in the peduncle and the changes in genotypic ranking for Δ during the latter part of the season may largely be attributed to genotypic and plot-to-plot differences in the extent of soil water depletion near anthesis, i.e. at the time the peduncle was growing. Genotypic differences in the extent of soil water use to anthesis were large (Table A1.5), and were positively correlated with the Δ values calculated for plant material at anthesis (Fig. 5.2b; $r = 0.77$, $P<0.05$). This relationship is to be expected, given the positive relationships observed between anthesis dry matter production and Δ in this study (Fig. 5.2a; $r = 0.77$, $P<0.05$) and between final dry matter production and Δ at Wagga Wagga and Yanco the previous, wetter season (Fig. 2.2).

The positive correlation between Δ measured early in the season and soil water use to anthesis may have also led to the relatively small range in Δ measured in the peduncle compared to that measured in organs that grew earlier in the season. Greater soil water use by genotypes with higher Δ values would be expected to result in earlier and perhaps greater stomatal closure, and hence a relatively larger decrease in Δ, compared with genotypes with lower values of Δ. This may also explain the lower range in Δ at the drier sites of the Southern Wheat Evaluation Project compared to the range observed at Cootamundra and the greater range in Δ at Rutherglen in 1984 than at the other drier sites sampled in that season.

All these observations indicate that heritability should be maximised and selection for genotypic differences in Δ should be most effective if measurements of Δ are made on plant material from the early part of the season grown under well-watered conditions. Under such conditions, environmental variability in Δ associated with differences in

Table A1.5. Soil-water use (mm) since the first reading on July 10. Data are given for two dates, (September 24, October 16) spanning anthesis. Soil-water use to anthesis, calculated by linear interpolation, is also given.

Genotype	Soil-water use since first reading (mm)		
	Sept.24 (d.125)	Oct.16 (d.147)	Anthesis (d.139)
Yallaroi	32.2	59.0	49.3
Gutha	38.9	68.3	57.6
820357	29.5	54.2	45.2
RAC 430	33.4	61.1	51.0
Blade	31.0	62.5	51.0
Hartog	26.2	55.0	44.5
Sundor	22.1	49.7	39.7
Quarrion	18.9	46.4	36.4
LSD (0.05)	6.1	8.6	-

the rate of water use should be minimised. While such a strategy should help to maximise heritability for Δ, it should be noted that even in these experiments, the heritability estimates obtained were equivalent to those obtained for harvest index. Harvest index is becoming an increasingly accepted morphophysiological trait in breeding programs for wheat (Ellison et al., 1985) and other crop species. The heritability estimates for Δ also compare favourably with those for slow mature tissue respiration, a trait used to effect for improving herbage yields in ryegrass (Wilson and Jones, 1982). The relative stability of Δ compared to a related physiological characteristic, stomatal conductance, can be readily observed by comparing Figures A1.1 and A1.2. Jones (1987) notes that the use of stomatal conductance as a selection criterion has been severely limited by spatial and temporal variability in conductance measurements. This conclusion is supported by the results of the present study on wheat. Large temporal variability in conductance is indicated by the substantial changes in genotypic ranking for leaf conductance from one sampling time to the next (Fig. A1.2). Spacial variability resulted in few of the differences among genotypes in the mean conductance of either leaf surface at any sampling time being statistically significant (data not shown).

While the comparison between stomatal conductance and Δ emphasises the relative stability of the latter, the major aim of this study was to determine whether genotype x environment interaction may lead to significant variability in Δ. The results indicate that, in wheat, such interaction may be important under water-limited situations. This indicates that assessment of genotypic variation in Δ should be most effective under well-watered conditions and relatively early in plant development.

APPENDIX 2.

Summary of seasonal changes in soil water at Yanco and Wagga Wagga, 1984.

At both sites, measurements of soil water use were taken in the top 1.2m of the profile from plots of two spring wheat genotypes, Cleopatra and Yaqui 50E, grown in 3-replicate experiments immediately adjacent to the experiments reported in Chapter 2. The neutron attenuation technique was used to monitor changes in soil water. The data presented here are the average changes in soil water (mm) under the two genotypes since the first measurement dates. These were June 28 at Yanco and June 29 at Wagga Wagga. Mean anthesis dates at Yanco and Wagga Wagga were 11 October and 16 October, respectively.

Yanco		Wagga Wagga	
Date	Change in Soil Water (mm)	Date	Change in Soil Water (mm)
16 August	+49.4	16 October	- 23.9
5 September	+33.4	23 October	- 53.9
5 October	+ 6.3	15 November	-104.9
16 October	-46.5	11 December	-121.2
16 November	-79.7		

APPENDIX 3.

Genotypic variation in the response of CO_2-assimilation rate to changes in intercellular partial pressure of CO_2.

Summary of genotypic variation in the response of flag leaf CO_2-assimilation rate, A, to changes in intercellular partial pressure of CO_2, p_i, as external CO_2 partial pressure, p_a, was changed through 5.0, 15.0, 25.0, 35.0, 45.0 and 70.0 Pa. Measurements were taken on leaves of 14 wheat genotypes under constant conditions. These were : 1000µmol quanta PAR m^{-2} s^{-1}, 20°C leaf temperature and 1.2 kPa leaf-to-air vapour pressure difference. The data presented are the mean values from three replicate leaves of each genotype.

Table A3.1. Assimilation rate, A (µmol m^{-2} s^{-1}), and intercellular partial pressure of CO_2, p_i (Pa), at six levels of external CO_2 partial pressure, p_a (5.0, 15.0, 25.0, 35.0, 45.0 and 70.0 Pa) in flag leaves of 14 wheat genotypes.

Genotype†	p_a (Pa)											
	5.0		15.0		25.0		35.0		45.0		70.0	
	A	p_i	A	p_i	A	p_i	A	p_i	A	p_i	A	p_i
Gutha	1.46	4.43	11.5	10.99	20.2	17.92	25.1	25.17	28.3	33.10	31.8	56.12
Veery 3	1.23	4.50	11.7	10.84	21.1	17.66	26.1	25.34	30.6	33.62	33.9	54.93
Cranbrook	1.05	4.51	11.4	10.23	20.3	16.63	26.2	24.05	31.2	32.21	34.8	55.28
Yallaroi	1.02	4.50	9.8	10.91	18.2	17.79	22.4	24.50	26.6	33.36	29.0	54.92
RAC 430	1.38	4.43	11.8	10.52	20.7	17.51	26.2	24.68	30.8	33.49	32.9	54.51
K1056	0.90	4.54	10.4	10.59	18.9	17.28	24.3	24.08	28.3	32.08	33.7	52.53
820357	0.86	4.48	9.5	10.57	17.9	16.73	22.5	23.45	27.0	31.50	29.1	52.83
Blade	1.50	4.38	12.4	10.27	23.0	16.43	29.6	23.49	35.2	31.53	39.0	53.21
Hartog	1.10	4.51	10.8	10.72	19.3	17.58	23.7	24.64	29.0	32.83	33.7	54.24
Sunstar	1.27	4.49	13.3	10.35	23.5	16.96	30.6	23.91	35.8	32.49	39.6	55.22
Comet	1.48	4.39	11.4	10.54	21.2	16.97	27.3	24.33	32.3	32.15	35.6	54.89
Sundor	0.94	4.53	11.4	10.36	20.9	16.36	26.5	23.56	30.6	30.80	35.9	52.69
Quarrion	1.04	4.51	11.1	10.49	20.6	16.67	26.2	23.84	31.0	31.98	34.6	54.40
M3844	1.18	4.41	11.2	10.03	19.9	16.21	25.8	22.68	30.5	30.50	34.8	51.00
$\bar{x}$	1.17	4.47	11.3	10.53	20.4	17.05	26.1	24.08	30.5	32.25	34.2	54.05
LSD(0.05)	0.44	0.16	0.8	0.36	1.2	0.63	1.1	0.53	1.9	1.26	2.1	2.25

† All genotypes are *Triticum aestivum* L. except Yallaroi and 820357, which are *T. turgidum* L. *durum*. cv Comet is a hybrid wheat.

APPENDIX 4.

Summaries of variation in carbon isotope discrimination, water-use efficiency and components of water-use efficiency, among sixteen wheat genotypes grown under well-watered and water-stressed conditions in the glasshouse.

Table A4.1. Summary of variation in carbon isotope discrimination, Δ, and water-use efficiency on a total dry matter basis, W, and an above-ground dry matter basis, W^*, among 15 genotypes grown under well-watered conditions and harvested at ear emergence. Also shown is the date of harvest, water-use to the time of harvest, total dry matter production, TDM, above-ground dry matter production, AGDM, and root/shoot ratio for each genotype.

[1] Genotype	$10^3 \times \Delta$	W ($g\ kg^{-1}$)	W^* ($g\ kg^{-1}$)	[2] Water Use (kg)	TDM (g)	AGDM (g)	[3] Root/ Shoot	Harvest Date
1. Yallaroi	19.70	5.07	4.13	4.73 (4.22)	24.08	19.62	0.23	4.xi
2. Gutha	18.99	6.18	5.07	5.22 (5.22)	32.26	26.48	0.22	1.xi
3. Cranbrook	19.36	5.62	4.66	4.58 (4.58)	25.78	21.32	0.20	1.xi
4. Blade	20.10	4.86	4.01	4.87 (3.63)	23.74	19.50	0.21	7.xi
5. Comet	19.59	-	4.48	4.81 (4.29)	-	21.53	-	4.xi
7. Veery 3	19.47	5.23	4.41	4.79 (4.13)	25.08	21.10	0.18	4.xi
8. K1056	19.18	5.18	4.33	3.88 (3.88)	20.08	16.78	0.20	1.xi
9. Sunstar	19.72	4.89	4.22	4.35 (3.85)	21.28	18.30	0.16	4.xi
10. M3458	20.11	4.91	3.99	4.33 (3.79)	21.18	17.23	0.23	4.xi
12. RAC430	20.28	5.05	4.36	5.07 (3.93)	25.78	22.20	0.16	7.xi
13. Hartog	19.59	5.60	4.59	4.47 (4.47)	24.95	20.44	0.22	1.xi
14. M3844	18.92	5.17	4.41	4.50 (3.35)	23.43	19.97	0.17	7.xi
15. Millewa	19.19	5.13	4.22	4.16 (4.16)	21.41	17.60	0.21	1.xi
17. Sundor	19.00	5.55	4.65	4.65 (3.71)	25.98	21.66	0.20	7.xi
20. Quarrion	19.74	5.65	4.62	4.68 (3.70)	26.58	21.69	0.22	7.xi
$\bar{x}$	19.53	5.25	4.41	4.61	24.28	20.36	0.20	
LSD(P<0.05)	0.85	0.52	0.35	0.58	4.47	3.15	0.04	

---

[1] 820357 was not harvested at ear emergence. Genotype code corresponds to ranking of all 20 genotypes grown in the field.

[2] Figures in brackets give water use to November 1

[3] Root dry matter for cv Comet was mislaid prior to weighing

Table A4.2. Summary of variation in carbon isotope discrimination, Δ, and above-ground water-use efficiency, W^*, among 16 genotypes grown under well-watered conditions and harvested at maturity. Also shown is water use to the time of harvest, above-ground dry matter production, AGDM, grain yield and harvest index for each genotype.

Genotype	$10^3 \times \Delta$	W^* ($g\ kg^{-1}$)	Water Use (kg)	AGDM (g)	Grain Yield (g)	Harvest Index
1. Yallaroi	20.67	4.06	11.37	46.14	16.65	0.36
2. Gutha	19.40	5.17	11.19	57.88	16.47	0.28
3. Cranbrook	20.16	4.56	11.29	51.46	22.06	0.43
4. Blade	20.49	4.47	10.44	46.67	14.90	0.32
5. Comet	20.41	4.46	11.62	51.78	18.62	0.36
6. 820357	20.05	4.51	10.53	47.67	13.29	0.27
7. Veery 3	20.86	4.45	10.89	48.38	17.89	0.37
8. K1056	19.81	4.78	9.59	45.85	16.88	0.37
9. Sunstar	20.45	4.58	10.76	49.31	19.04	0.39
10. M3458	20.17	4.31	9.92	42.77	16.71	0.39
12. RAC430	20.25	4.73	10.86	51.42	19.34	0.37
13. Hartog	19.95	4.92	9.97	49.04	18.35	0.37
14. M3844	19.63	4.81	9.62	46.33	14.41	0.31
15. Millewa	19.43	4.41	10.88	47.94	18.92	0.39
17. Sundor	19.26	4.95	8.97	44.41	16.25	0.37
20. Quarrion	19.13	5.27	9.85	51.86	18.51	0.36
$\bar{x}$	20.01	4.65	10.48	48.68	17.39	0.36
LSD (0.05)	0.64	0.33	0.78	5.37	3.00	0.04

Table A4.3. Summary of variation in carbon isotope discrimination, Δ, above-ground water-use efficiency, W^*, water use and above-ground dry matter production, AGDM, among 16 genotypes grown under water-stressed conditions in the glasshouse. These results summarise data from harvests at ear emergence and maturity. Water use to ear emergence averaged 95% of final water use. Mean grain yield and harvest index of the two plants of each genotype harvested at maturity are also shown.

Genotype	$10^3 \times \Delta$	W^* ($g\ kg^{-1}$)	Water Use (kg)	AGDM (g)	Grain Yield (g)	Harvest Index
1. Yallaroi	16.94	4.69	1.23	5.74	0.70	0.12
2. Gutha	15.81	5.71	1.13	6.43	1.66	0.27
3. Cranbrook	16.29	5.95	1.17	6.98	2.31	0.32
4. Blade	16.57	5.38	1.28	6.86	2.19	0.31
5. Comet	16.21	5.42	1.23	6.68	1.85	0.28
6. 820357	16.88	4.91	1.14	5.57	1.43	0.24
7. Veery 3	16.37	5.63	1.07	5.99	1.35	0.21
8. K1056	15.80	5.72	1.19	6.82	2.17	0.31
9. Sunstar	16.79	5.52	1.23	6.81	1.91	0.27
10. M3458	15.98	5.49	1.15	6.27	1.40	0.23
12. RAC430	16.00	5.58	1.13	6.28	1.80	0.28
13. Hartog	15.85	5.73	1.18	6.78	2.27	0.33
14. M3844	15.97	5.86	1.22	7.15	1.92	0.27
15. Millewa	15.41	5.74	1.19	6.85	2.09	0.28
17. Sundor	15.73	5.63	1.19	6.66	1.80	0.26
20. Quarrion	15.27	5.92	1.18	7.02	1.06	0.16
$\bar{x}$	16.11	5.55	1.18	6.56	1.74	0.26
LSD(0.05)	0.70	0.38	0.11	0.69	0.65	0.10

APPENDIX 5.1.

Pedigrees of the twenty genotypes grown in the water-use efficiency experiments at Moombooldool in 1985 and 1986.

Triticum aestivum L.

cv. Cranbrook	WE//Ciano 'S'/Noroeste 66/3/Zambezi. Released 1984, W.A.
cv. Gutha	Gamenya//Gabo*3/Khapstein/3/Falcon*3/Chile 1B. Released 1982, W.A.
cv. Veery 3	CIMMYT Selection. Australian Wheat Collection, AUS20022.
cv. Blade	Mexico C3/2*Gabo//Kite. Released 1986, S.A..
cv. Sunstar	Condor/4/2*WW15/3/Steinwedel/WC356/2/La Prevision. Released 1983, N.S.W..
cv. Comet	F1 Hybrid : A line, Condor//Ciano 67/2*Olympic; R line, Kite/3/Dirk R' line/primepi. Released 1986, N.S.W.
cv. Hartog	Vicam 71//Ciano 'S'/Siete Cerros/3/Kalyansona/Bluebird Released 1982, QLD..
cv. Vulcan	Condor/Pitic//Condor sib. Released 1985, N.S.W..
cv. Millewa	Sonora 64/Yaqui 50E/Gaboto/Mexico 8156. Released 1978, Vic..
cv. Dollarbird	Wren/Gaboto//Kayansona/Bluebird. Released 1987, N.S.W.
cv. Skua	3Ag14/4*Condor. Released 1984, N.S.W..
cv. Sundor	Condor*4/3Ag14. Released 1984, N.S.W..
cv. Sunbird	Condor*4/3Ag14. Released 1986, N.S.W..
cv. Quarrion	Condor/TA3PNB3P//WW33G/3/Condor*2/WW33B. Released 1983, N.S.W..
RAC 430	Kite//Mexico C3/2*Gabo. Bred S.A..
K1056	CIMMYT Selection from N.S.W..
M3458	M2293/2*Cook. Bred N.S.W..
M3844	Condor/TA3PNB3P//WW33G/3/Condor*2/WW33G. Bred N.S.W..

Triticum turgidum L. *durum*

cv. Yallaroi	Kamilaroi sib//Guillemot 'S' sel. Released 1987, N.S.W.
820357	Kamilaroi sib//Guillemot 'S' sel. Bred N.S.W..

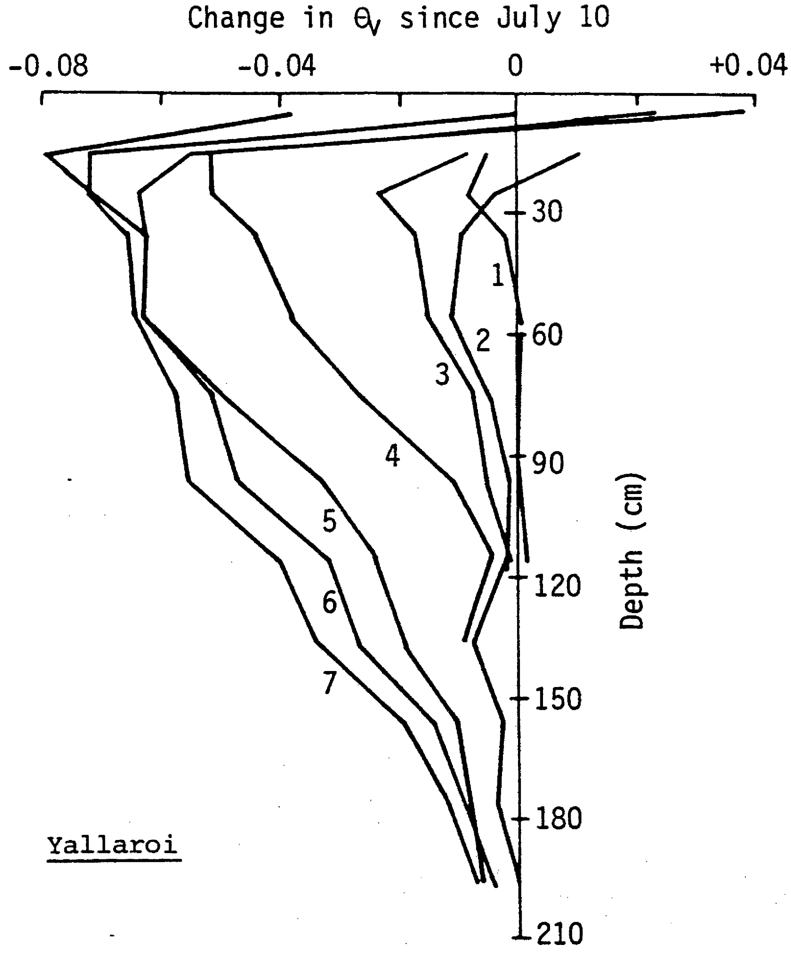
APPENDIX 5.2.

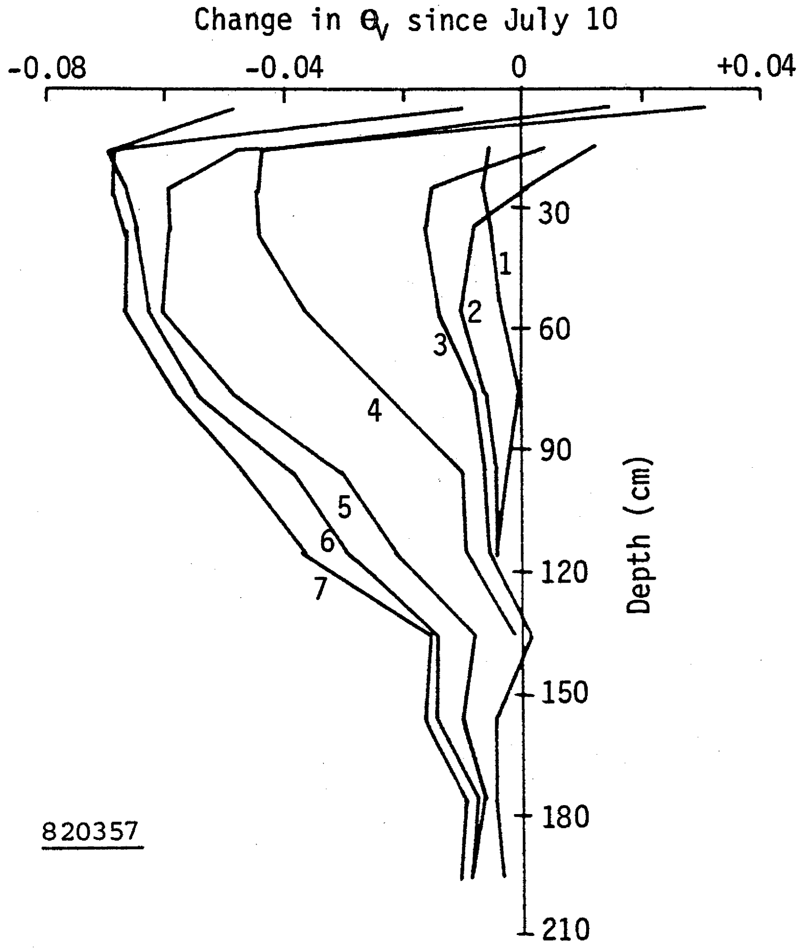
Patterns of soil water extraction for the eight genotypes grown at Moombooldool in 1985.

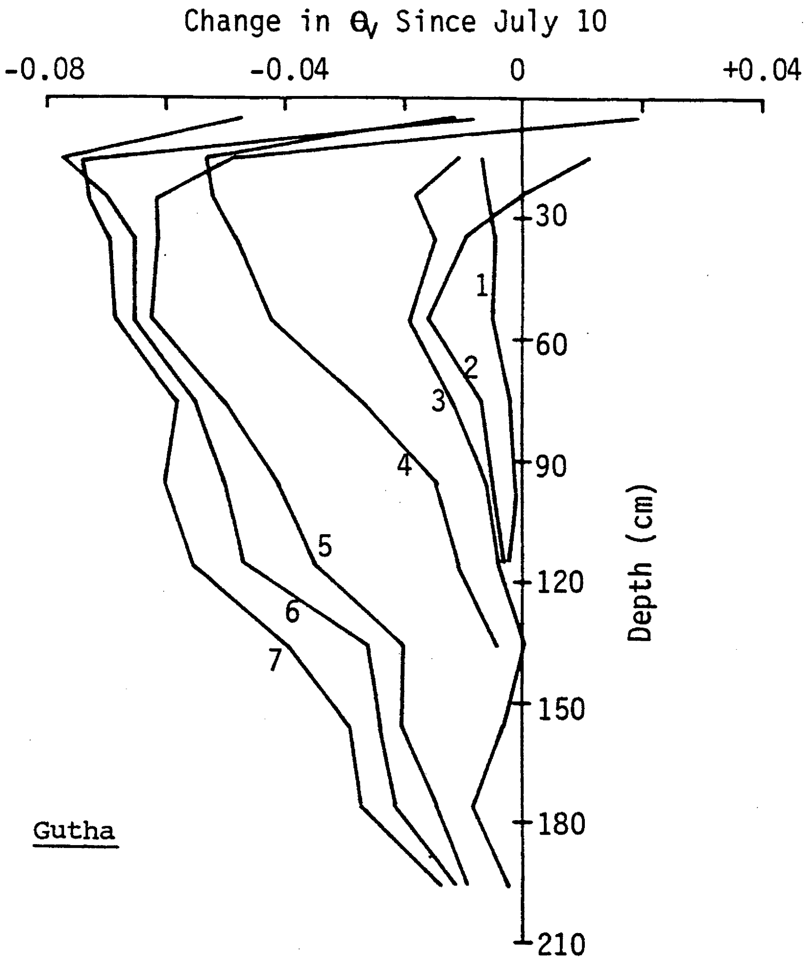
Data are presented graphically as changes in volumetric soil-water content (θ_v) since the first measurement on July 10. Soil water measurements were taken using the neutron attenuation technique as described in Section 5.2.6.. Numbers from 1 to 7 on each figure indicate sampling dates as follows :

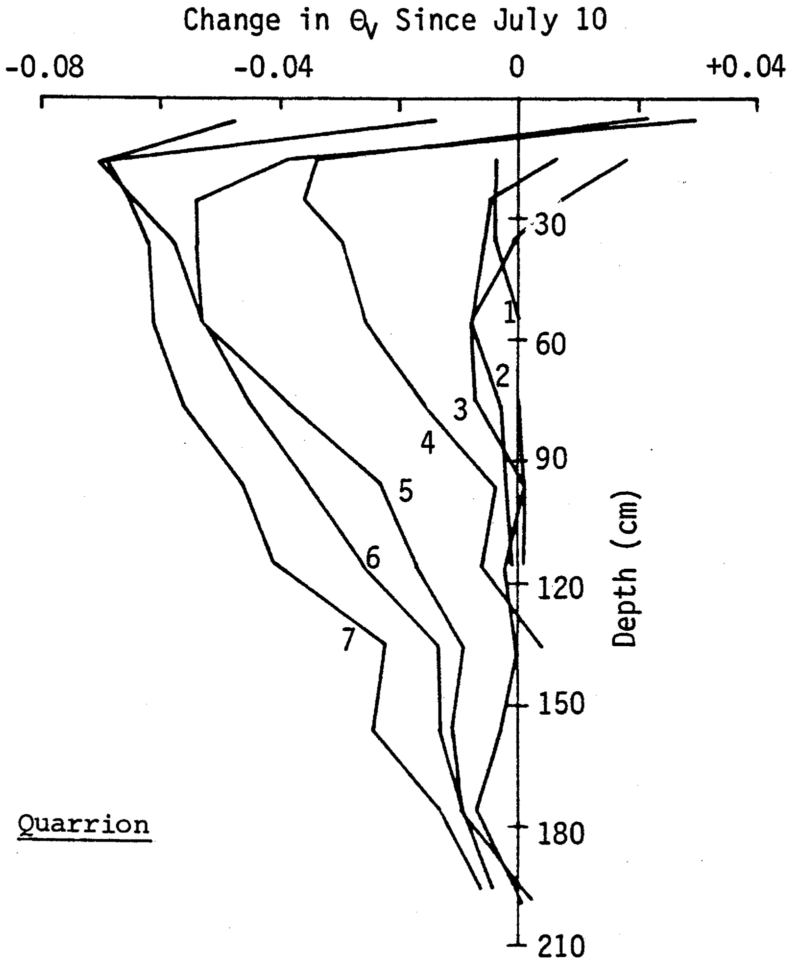
1. July 24; 2. August 13; 3. September 2; 4. September 24;
5. October 16; 6. October 29; 7. November 19.

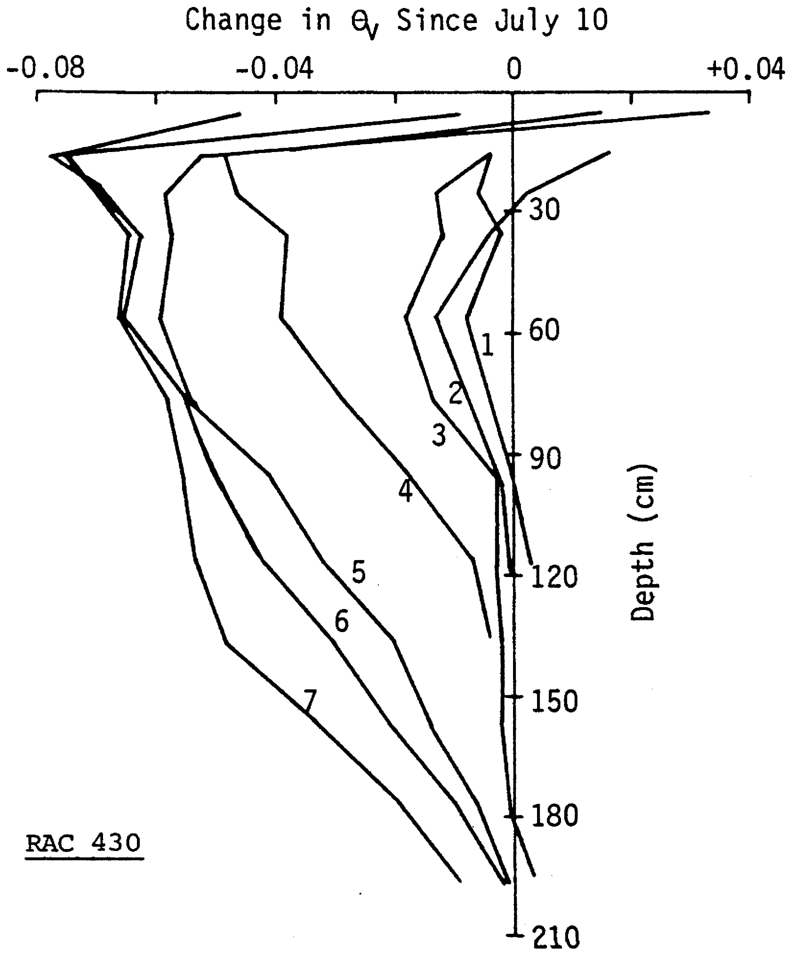
The large positive changes in θ_v in the top 0.15 m of the profile at the first three dates after the initial readings are omitted for clarity.

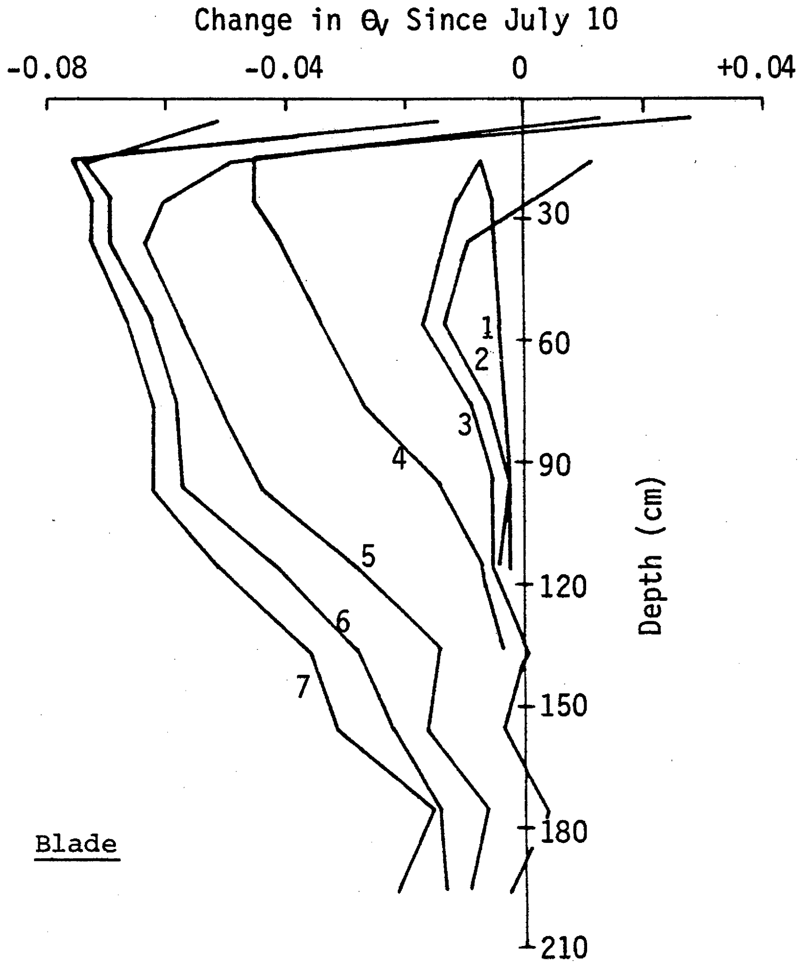

Change in θ_v since July 10
-0.08
-0.04
0
+0.04
30
60
90
120
150
180
210
Depth (cm)
1
2
3
4
5
6
7
Yallaroi

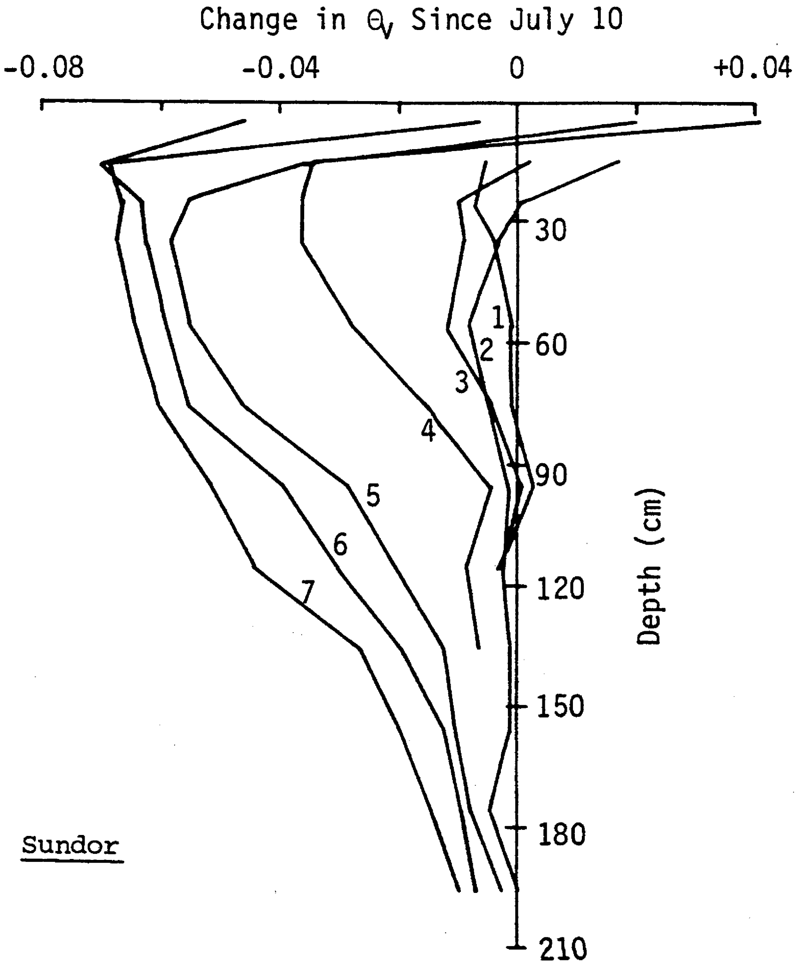

Change in θ_v since July 10
-0.08
-0.04
0
+0.04
30
60
90
120
150
180
210
Depth (cm)
1
2
3
4
5
6
7
820357

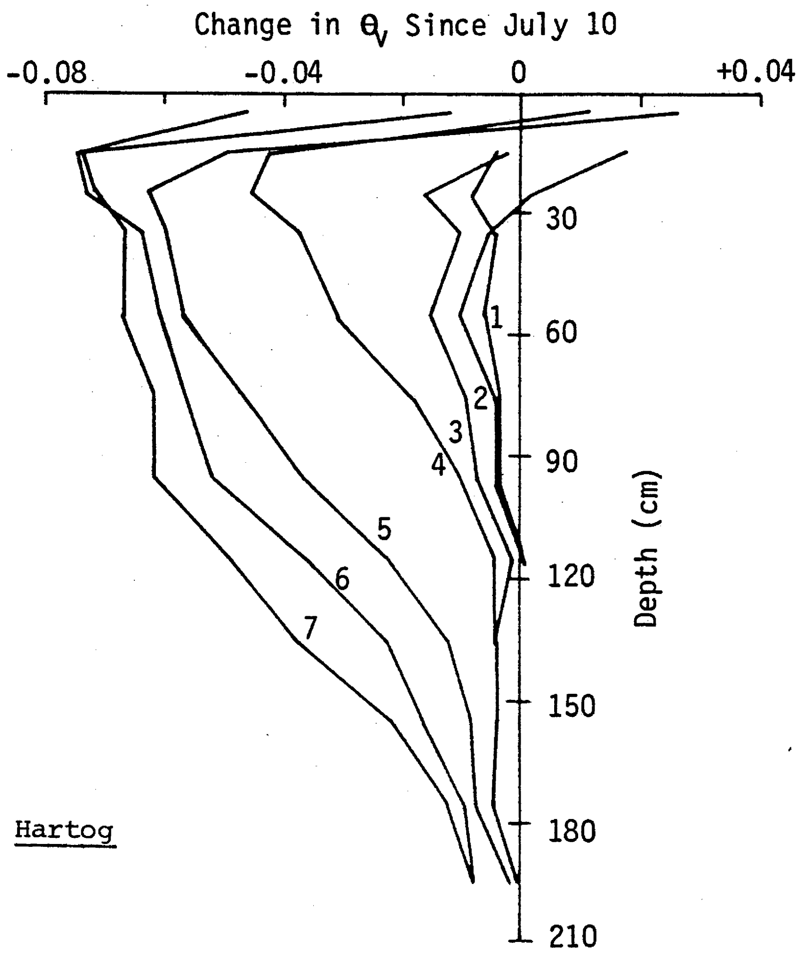

Change in θ_V Since July 10
-0.08
-0.04
0
+0.04
Depth (cm)
30
60
90
120
150
180
210
1
2
3
4
5
6
7
Gutha


Change in θ_V Since July 10
-0.08
-0.04
0
+0.04
Depth (cm)
30
60
90
120
150
180
210
1
2
3
4
5
6
7
Quarrion


Change in θ_v Since July 10
-0.08
-0.04
0
+0.04
30
60
90
120
150
180
210
Depth (cm)
1
2
3
4
5
6
7
RAC 430


Change in θ_v Since July 10
-0.08
-0.04
0
+0.04
30
60
90
120
150
180
210
Depth (cm)
1
2
3
4
5
6
7
Blade


Change in θ_v Since July 10
-0.08
-0.04
0
+0.04
30
60
90
120
150
180
210
Depth (cm)
1
2
3
4
5
6
7
Sundor


Change in θ_v Since July 10
-0.08
-0.04
0
+0.04
30
60
90
120
150
180
210
Depth (cm)
1
2
3
4
5
6
7
Hartog

APPENDIX 5.3.

Partitioning of Evapotranspiration into Components of Direct Soil Evaporation and Canopy Transpiration.

A major impediment to measuring crop water-use efficiency based on transpiration lies in successfully partitioning the two components of evapotranspiration (ET); direct soil evaporation (E) and transpiration by the plants (T). Direct measurement of E has been attempted in row-crops using shallow "mini-lysimeters" placed flush with the soil surface (Shawcroft and Gardner, 1983; Walker, 1983). Apart from the error associated with restricting root access to such lysimeters, their use in crops other than, say, maize or sorghum, where the between-row and within-row plant spacing is relatively large, is technically difficult. The procedure used to partition E and T in this study was similar to that used by Cooper et al. (1983), and revolves around the measurement of the proportion of incident radiation intercepted by the plant canopy, α.

Several workers (Ritchie, 1972; Tanner and Jury, 1976; Kanemasu et al., 1976; Al-Khafaf et al., 1978) have attempted to estimate E for predictive purposes using models of soil evaporation and extraction by plant roots. The models used by these workers are all similar, and consider evaporation from a bare soil surface as a two-phase process (Philip, 1957). During the first "energy-limited" phase, the soil surface layer is sufficiently wet for water to be transported to the surface at a rate at least equal to the potential evaporative demand. This is largely determined by the amount of energy reaching the soil surface. During the second "soil-limited" phase, the rate of evaporation from the surface depends on the water-transmitting properties, including the hydraulic conductivity. This phase has been modelled by Ritchie (1972) and subsequent workers as being a function of soil properties and the time since the soil surface was last wetted.

Cooper et al. (1983) argue that, for winter-sown wheat crops grown in a Mediterranean-type environment (such as northern Syria or southern Australia), where potential evaporation is relatively low for most of the crop growth cycle and the soil surface is frequently rewetted, E is largely determined by conditions in which the "energy-limited" phase of soil drying is dominant. Conditions favouring "soil-limited" evaporation would only dominate during the last phases of crop maturation. Further, they assume that radiant energy is the principal determinant of potential evaporative demand. Based on these simplifications, they suggest that the ratio E/E_s (i.e. the ratio of soil evaporation from under the crop, E, to that from bare soil, E_s) will depend on the proportion of radiant energy intercepted by the crop, α. Thus E can be determined from the equation

$$E = E_s(1-\alpha) \quad . \tag{A5.1}$$

Cooper et al. (1983) obtained cumulative and daily values for E by fitting curves to E_s vs time and leaf area index (G) vs time. They used the daily values of G derived from the latter curve and an estimate of K, the crop extinction coefficient, to calculate α on a daily basis using the equation

$$\alpha = 1 - e^{-KG} \quad . \tag{A5.2}$$

E_s and α were combined as in Equation A5.1 to obtain daily values of E. Daily values of T were computed by fitting curves to accumulated ET vs time, calculating daily ET and then subtracting E to obtain T.

For the 1985 study at Moòmbooldool, E and T were partitioned in a similar manner. Logistic functions (Hunt, 1982) were fitted to data of accumulated ET vs time for each experimental plot and also to the data of accumulated E_s vs time obtained using the mean values of soil water change from the four access tubes located in bare soil. In this study, α was measured directly on each plot on the same dates as the soil-water use measurements, as described in Section 5.2.5. Changes over time in $(1-\alpha)$ were described using logistic functions fitted to data from each plot to obtain daily values of $(1-\alpha)$. E and T were then estimated as above.

Examples of curves fitted to changes over time of accumulated ET are shown in Fig. A5.1 for four genotypes (Gutha, Yallaroi, RAC 430 and Quarrion) spanning the range in patterns of seasonal water use. Curves fitted to ET data for the remaining four genotypes are omitted for clarity. The curve fitted to E_s is also shown, along with accumulated Class A pan evaporation (data not fitted). Changes over time in $(1-\alpha)$ are shown for the same four genotypes in Fig. A5.2.

Crop ET was corrected by the addition of 31 mm for the first 48 d after sowing as described in Section 5.2.7.. T during this first 48 d was assumed to be 10% of ET for all canopies, i.e. 3 mm.

Estimates of T were not corrected for possible errors associated with the measurement of α. These measurements may have under-estimated total daily radiation interception, especially early in the season when G was less than approximately 2 (Hipps et al., 1983). This is because measurements were taken only during the middle of the day (between 1000 h and 1400 h) when radiation interception is least. The effect on estimated T would be less than that on cumulative radiation interception, since the under-estimation of α is greatest during the early morning and late afternoon, when the evaporative demand is relatively low, and during the early part of the season, when, again, evaporative demand is relatively low. Under-estimation of cumulative T to anthesis may have been of the order of 5-10% in this study.

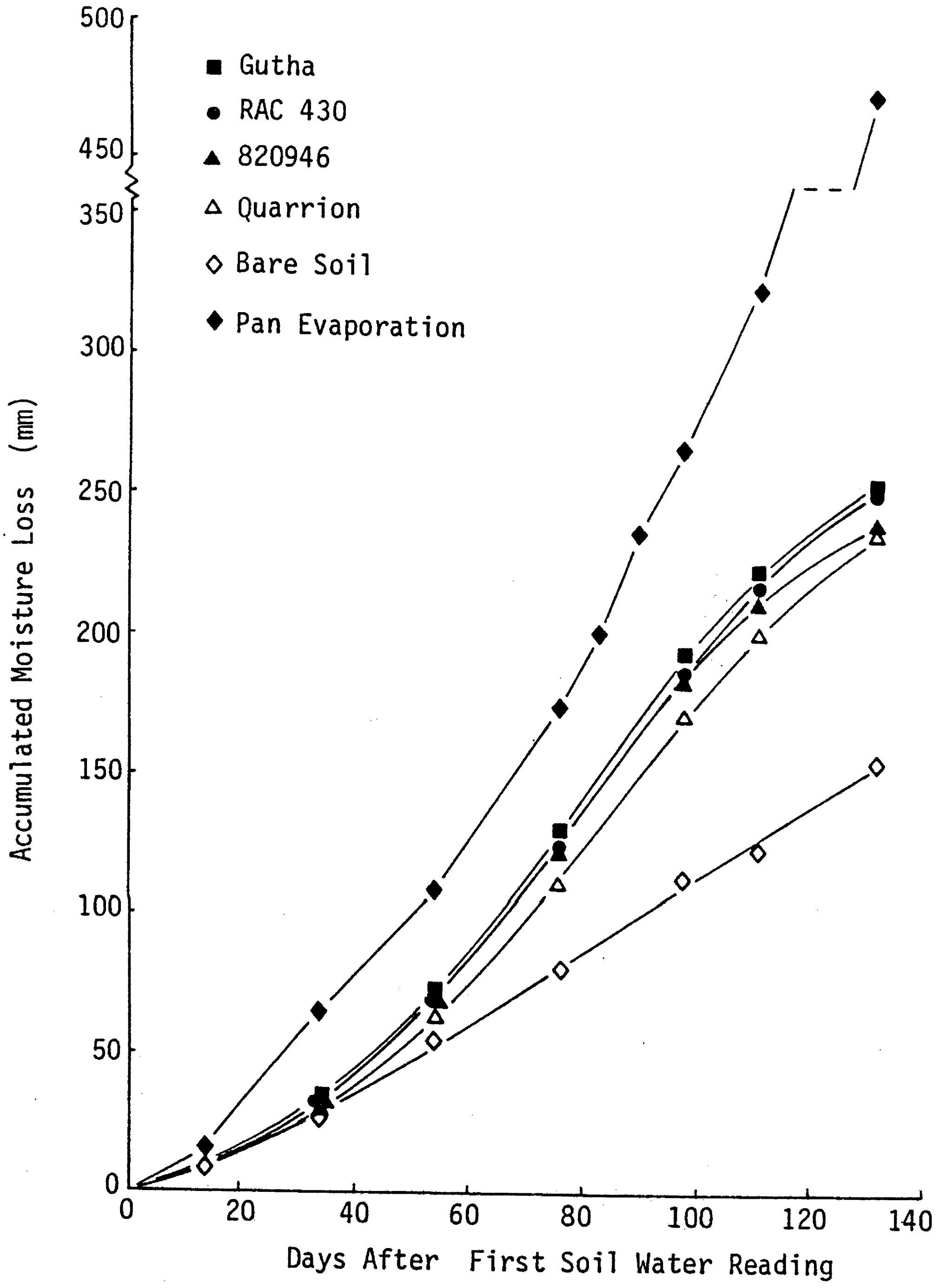


Figure A5.1. Accumulated moisture loss after the first soil readings for the bare soil and the genotypes Gutha, Yallaroi, RAC 430 and Quarrion. Accumulated Class A pan evaporation is also shown. Logistic functions were fitted to the data for each genotype and the bare soil. Coefficients describing these curves are given in Table A5.1.

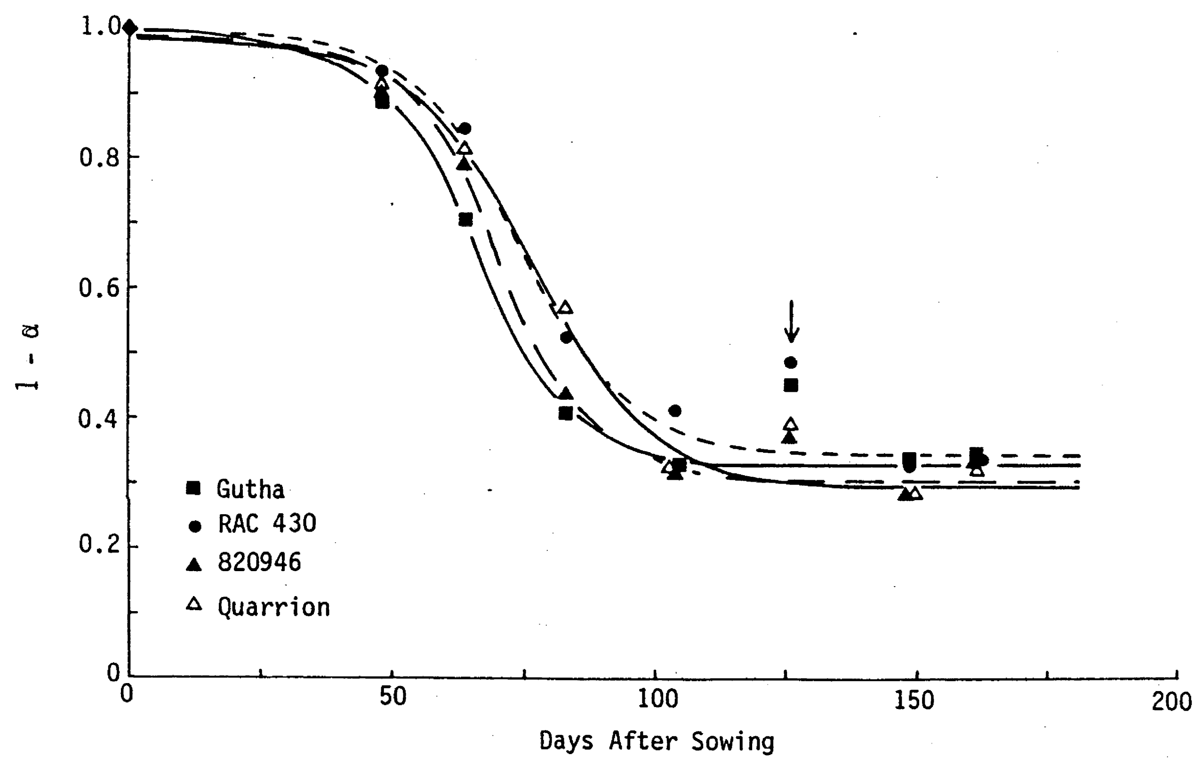


Figure A5.2. Changes with time in the proportion of incident radiation reaching the soil surface, (1-α), for four genotypes; Gutha, Yallaroi, RAC 430 and Quarrion. Logistic functions were fitted to the data for each genotype. Coefficients describing these curves are given in Table A5.1. The data set arrowed were collected on a day when leaves were visibly wilted and are not included in the simulations.

Table A5.1. Coefficients describing the logistic functions fitted to data on: (a) ET by each genotype and bare-soil evaporation, E_s, for the period between the first and final soil water readings; (b) penetration of incident radiation through the canopy, $(1-\alpha)$, for the period from sowing (100%) to maturity.
All curves were of the form $y = a + c/(1 + e^{(-b(x-m))})$, where y is the dependent variable (ET or $(1-\alpha)$), x is time in days (since sowing for $(1-\alpha)$ or since the first soil-water readings for ET and E_s), and a,b,c and m are coefficients. Curves were fitted, in the cases given below, to genotypic mean data sets using the Maximum Likelihood Programme developed at the Rothamstead Experimental Station, U.K..

	Genotype	a	c	m	b
ET	Yallaroi	-12.899	282.076	76.954	0.03946
	Gutha	-14.021	301.869	77.633	0.03813
	820357	-11.698	275.136	77.098	0.03886
	RAC 430	-13.287	302.226	80.343	0.03748
	Blade	-11.725	306.077	81.356	0.03774
	Hartog	-13.582	303.299	82.214	0.03628
	Sundor	-12.326	294.208	82.864	0.03663
	Quarrion	-13.703	295.467	84.004	0.03564
	Bare Soil	-50.327	294.033	87.715	0.01834
$(1-\alpha)$	Yallaroi	0.3072	0.6734	71.330	-0.11303
	Gutha	0.3299	0.6609	65.864	-0.10783
	820357	0.2886	0.6986	71.074	-0.09695
	RAC 430	0.3443	0.6517	74.587	-0.10567
	Blade	0.3608	0.6253	71.988	-0.11294
	Hartog	0.3048	0.7044	76.991	-0.06678
	Sundor	0.3084	0.6844	75.017	-0.09521
	Quarrion	0.2950	0.6903	77.179	-0.08738